HHS Public Access

Author manuscript

Trends Cancer. Author manuscript; available in PMC 2017 January 01.

Published in final edited form as:

Trends Cancer. 2016 January 1; 2(1): 6–19. doi:10.1016/j.trecan.2015.12.003.

Microfluidics: A new tool for modeling cancer-immune interactions

Alexandra Boussommier-Calleja[1], **Ran Li**[2], **Michelle B. Chen**[1], **Siew Cheng Wong**[3], and **Roger D. Kamm**[1,2]

Roger D. Kamm: rdkamm@mit.edu

[1]Department of Mechanical Engineering, MIT, Cambridge, MA, USA

[2]Department of Biological Engineering, MIT, Cambridge, MA, USA

[3]Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A-STAR), Biopolis, Singapore

Abstract

In recognition of the enormous potential of immunotherapies against cancer, research into the interactions between tumor and immune cells has accelerated, leading to the recent FDA approval of several drugs that reduce cancer progression. Numerous cellular and molecular interactions have been identified by which immune cells can intervene in the metastatic cascade, leading to the development of several *in vivo* and *in vitro* model systems that can recapitulate these processes. Among these, microfluidic technologies hold many advantages in terms of their unique ability to capture the essential features of multiple cell type interactions in three-dimensions while allowing tight control of the microenvironment and real-time monitoring. Here, we review current assays and discuss the development of new microfluidic technologies for immunotherapy.

Keywords

Microfluidics; immunotherapy; metastatic cancer; drug screening

Microfluidic models: developing immunotherapies against metastasis

Most conventional therapies have limited success in containing metastasis, which are responsible for 90% of cancer-related deaths. Recently, immunotherapies have shown promising results for reducing metastasis in melanoma or kidney cancer patients[1, 2]. This warrants further research to develop new similar immunotherapies by using improved models that can better recapitulate metastasis. We propose that microfluidics systems are particularly powerful tools to replicate the metastatic environment and should thus be exploited to study cancer-immune cell interactions. Here, we highlight the value of

Correspondence to: Roger D. Kamm, rdkamm@mit.edu.

microfluidic models for studying metastasis and the immune system and describe studies that employed microfluidics to study immune-cancer cell interactions. Finally, we discuss the future outlook of microfluidic technology applications in the field of cancer immunotherapy.

Microfluidic vs. traditional models for cancer and metastasis research

Microfluidic models have emerged in the past decade as important tools for cancer research. Various *in vivo*, *ex vivo* and *in vitro* types of experimental models have been traditionally employed to discover therapeutic targets and test new drugs for cancer (see Text Box 1). However, often, these experimental models have limited physiological relevance. *In vitro* systems, including microfluidic platforms, present an important advantage over animal studies with regard to studying cancer-immune cell interactions. Namely, *in vitro* studies can be performed using multiple cell types - immune, cancer and stromal cells - that are exclusively of human origin, while animal studies will inherently contain non-human host cells. In particular, human cancer cells are often introduced in immune-suppressed animals to minimize rejection by the host immune system. While rejection is attenuated, these models can also limit the study of certain cancer-immune cell interactions if key immune players are absent. Despite these limitations, mouse models remain tremendously powerful for cancer immunology research and humanized mouse models reconstituted with human dendritic cells, B or T cells are being developed[3].

Microfluidics is a rapidly expanding technology that relies on the use of small channels, ranging from tens to hundreds of micrometers in height or width for handling small fluid volumes[4]. Overall, microfluidic assays represent a dramatic improvement in physiological relevance over other *in vitro* models because they allow for precise control of the cellular, physical and biochemical microenvironment (see Text Box 2), making them a good compromise between *in vivo* and other types of *in vitro* studies (Figure 1). Microfluidic platforms, much like most *in vitro* assays, can be used to perform reductionist studies so that biological phenomena can be studied in a detailed and controlled fashion. Moreover, they can be easily designed according to the experimental requirements and display multiple compartments and channels. This compartmentalization enables excellent spatial control of cell distribution at physiological length scales. Uniquely, the presence of microfluidic channels permits precise control of flow, allowing the study of many important biomechanical processes such as shear stress. These channels can also be used to precisely establish physical (e.g., interstitial pressure) or chemical (e.g., cytokines) complex gradients in the microfluidic assay in a more precise and sustained manner than in macro-scale *in vitro* systems[5]. Microfluidic devices can contain cells cultured in 2D or in 3D embedded in hydrogels. Since these platforms are small, they require only minute amounts of cells and reagents that are often expensive, rare or difficult to obtain. More importantly, the distance from the biological samples in the device to the microscope objective is short and, therefore, it is easier to image all cells at high resolution, as opposed to imaging larger traditional 3D *in vitro* models.

There are, however, several limitations to microfluidic technology. For example, retrieving cells from microfluidic devices for subsequent biochemical studies is possible but not

straightforward [6]. The low cell number can also be a drawback if a large number of cells is need for downstream biochemical studies (e.g., by western blotting) or if secreted proteins need to be quantified (e.g., by ELISA). Of note, microfluidic platforms that include such miniaturized assays on-chip are currently being developed[7, 8]. The mechanical properties of the device materials (e.g. typically PDMS or glass coverslip) might also constitute an issue for some 3D studies, since these materials are much stiffer than the typical hydrogels in which cells are embedded. Thus, only cells that are far from the PDMS or glass surfaces should be analyzed. PDMS can also adsorb small hydrophobic molecules[9], which can be problematic for drug screening studies. Finally, care should be taken in extrapolating results from any *in vitro* study, including microfluidic ones, since they can never fully replicate all aspects of *in vivo* complexity.

Microfluidic: modeling the metastatic cascade

Microfluidic technologies have clear potential to advance cancer research. They are particularly relevant for studying the metastatic cascade, since metastatic spread occurs through several steps that are often difficult to resolve *in vivo*. While intravital imaging has proven extremely useful in the study of metastasis[6–7], it requires specialized expertise and equipment, and high image resolution is harder to attain in some visceral organs due to the depth of their location. Furthermore, some events of the metastatic cascade (e.g. intravasation) can be rare and thus difficult to image *in vivo*but this rate of occurrence can be more readily modulated *in vitro*. Each step of the cascade represents a promising target for therapeutic intervention. These steps include an epithelial-to-mesenchymal transition (EMT), invasion, intravasation, transport of circulating tumor cells (CTCs) in the bloodstream, extravasation in the distant organ, and recolonization at the metastatic site[12] (Fig. 2). Microfluidic assays have been designed to replicate certain aspects of several of these steps, such as EMT[13, 14], cancer cell invasion and adhesion[15–17], and intra-[18, 19] and extravasation[17, 20– 22] (see Text Box 3).

An essential feature of several of the metastatic steps is the interaction between cancer cells and blood or lymphatic vessels (Fig. 2C). For example, intravasation often requires cancer cells to transmigrate across the endothelium into the blood circulation, while extravasation consists of transendothelial migration from the bloodstream into the surrounding tissue. Thus, the endothelium is a crucial feature of the microenvironment to be included in microfluidic models of metastasis. To this end, some studies have used endothelial monolayers, wherein endothelial cells (ECs) are seeded on the walls of a micro-channel etched in PDMS (Fig. 2B)[17–20, 22]. Cancer cells are either seeded in the hydrogel for intravasation assays[18, 19] or directly perfused in the channel for extravasation[17, 20, 22] and adhesion studies[15–17]. Recent studies are increasingly employing microvascular networks (μVNs)[21, 23, 24], where the permeability, architecture and diameters of vessels are more similar to those found in *in vivo* microvascular beds (Figure 2A) [21]. These μVNs arise from the self-organization of ECs suspended in a hydrogel in the presence of fibroblasts[25, 26] or mesenchymal stem cells[27]. We and others have grown such μVNs in hydrogels flanked by channels in microfluidic platforms, where the lumens of the μVN open to the channels allowing the μVNs to be perfused (unlike similar studies performed in well-plates[24]), and in some cases, with cancer cells for extravasation studies[21]. These μVNs

represent a dramatic improvement over previous 2D extravasation models such as Boyden Transwell-chambers that consist of ECs seeded onto a permeable membrane inserted in a well-plate, since transwells do not allow for dynamic high resolution single cell imaging due to the long distance of the insert from the objective. In addition, μVNs replicate better the 3D vessel architecture and endothelial barrier function [21]. Studies have shown that dormancy can be induced by endothelial derived factors[24], thus demonstrating the need for microfluidic models that recapitulate interactions between ECs and tumor cells, using for example μVNs. As can be appreciated from Fig. 2C, these microfluidic studies reveal in detail the dynamics of cancer cells.

One of the major current limitations of *in vitro* metastatic models is their lack of organ-specificity[28]. This is particularly important as there is growing evidence that cancer cells communicate with their microenvironment[29], specifically with organ-specific cells[30], resulting in organ-selectivity in metastasis[31]. Hence, understanding organ-specific interactions might shed light on targeted therapeutic approaches for inhibiting metastasis in secondary organs. At the moment, microfluidics are most likely the best-suited *in vitro* systems for mimicking organ-specific environments because they permit precise control over the spatial distribution of different cell types that mimic the *in vivo* settings (see Text Box 2). To date, studies have added organ-specific cell types[32] or chemokines[17] to microfluidic models and replicated certain aspects of organ architecture[33] (e.g., an air-liquid interface for mimicking the pulmonary airways[34]). In this context, these models are highly amenable for increasing complexity, such as the addition of different immune cells for studying their role in metastasis.

Microfluidics: modeling immune-cancer cell interactions

The importance of the role of immune cells in cancer was revealed, amongst other pioneering studies, by William Coley in the late 1800s, when he observed tumor remission in patients with bacterial infections[35, 36]. This suggested that immune cells had the ability to control and inhibit tumors, leading him to inject bacteria into patients with cancer. More generally, immunotherapy aims to reduce cancer by inducing, reinforcing or suppressing an immune response. Since then, and particularly in the past several years, the field of immunotherapy has grown [37]. Although it was first observed that immune cells play an anti-tumorigenic role, it was later realized they can be “educated” by cancer cells to become tolerant to tumors and even promote tumor growth and metastasis[37]. Therefore, cancer immunotherapy takes one of several forms: 1) preventing cancer cells from escaping immunosurveillance; 2) targeting immune cells to prevent them from directly assisting cancer cells; or 3) activating or enhancing the capability of immune cells to fight cancer cells.

Cancer immunotherapy offers several unique advantages over other existing approaches. First, because these therapies specifically target certain immune-cancer cell interactions[38], they can have lesser off-target effects. Second, drug-resistance related problems are also minimized, since immune cells are less likely to acquire drug resistance than cancer cells[39]. Finally, treatment effects can be more sustained than with conventional regimens, due to immune memory[40]. Unlike radiation, chemotherapy, or surgery, immunotherapy is

simultaneously targeted and systemic and it can be used in combination with other therapies for enhanced results[40]. Finally immune cells such as macrophages have been shown to modulate (e.g. enhance or hinder) tumor responses to traditional anti-cancer therapies[41, 42]. Thus, targeting these immune cells could enhance cancer therapies.

We previously discussed the qualities of microfluidic tools that are key to model metastasis, some of which are also powerful for modeling the immune microenvironment. For example, immune cells infiltrating tissues are recruited from the bloodstream by inflammatory chemokine/cytokine gradients[43]. It is the ability to establish such precise gradients in microfluidic devices that makes them useful for studying immune cell responses *in vitro*[44]. In addition, many immune cells exist under flow conditions in the bloodstream that can be uniquely recreated through precise control of flow in microfluidic systems. These systems also allow the perfusion of vascularized networks with, for instance, immune cells, which could be key to replicate the dynamics of immune cells (e.g., adhesion or transendothelial migration) and recruitment to the primary or metastatic tumor site. The immune cell response is a spatially orchestrated process, occurring in a variety of different tissues (i.e. bone marrow, blood stream and target tissue), which can be captured in microfluidic models through spatial compartmentalization. Finally, immune cells are highly mobile and high resolution time-lapse imaging facilitated in microfluidic models is an important advantage for analyzing their dynamics in real-time[45].

In vitro 2D studies of immune-cancer cell interactions were crucial for discovering and understanding tumor associated antigens (TAA)[46]. Later, *in vitro* 3D studies were used to investigate immune-cancer cell interactions, such as immune cell infiltration of tumor spheroids. Importantly, these studies demonstrated important differences between 2D and 3D, with tumor cells cultured in 3D spheroids showing a decreased production of TAA and HLA class I (as also observed *in vivo*)[47]. This highlights the importance of performing these studies in 3D, which can be achieved easily using microfluidics.

To the best of our knowledge, only a limited number of recent studies have employed microfluidics to investigate immune-cancer cell interactions[18, 48–52], possibly because microfluidic models are not yet widely available to cancer researchers. There are, however, many animal studies that have revealed the paradoxical interactions of immune and cancer cells[53] during metastatic progression. On one hand, there is evidence that immune cells have the capability to attenuate metastasis[54–59], which provides insight into how to restore or enhance their ability to combat cancer. On the other hand, it has been shown that immune cells can also be pro-metastatic at different stages of cancer progression (reviewed by Kitamura et al[37]). These studies could be replicated in microfluidic devices in a reductionist and controlled fashion. Several immune cell types should be studied together, as there is evidence that their interplay is critical in the metastatic cascade. For example, neutrophils are known to assist with tumor cell extravasation following platelet aggregation around the tumor cell[60, 61].

Several studies have used microfluidic platforms to investigate the migration of splenocytes towards cancer cells through a network of micro-channels (Fig. 3A). These studies first showed that immune-deficient interferon regulatory factor-8 (IRF-8) knockout mice develop

more tumors with less immune cell infiltration than wild type animals[50], and subsequently used a microfluidic assay to investigate the underlying mechanism in greater detail. Specifically, they showed that immune spleen cells lacking IRF-8 do not migrate towards cancer cells, nor interact with them as efficiently as wild type immune cells[48–50], suggesting a mechanism to explain why IRF-8 knock-out cells fail to exert proper immune-surveillance leading to a heavier metastatic burden.

Recently, Vacchelli *et al*. identified a loss of-function mutation in the allele of the gene coding for formyl peptide receptor 1 (Fpr1) that correlated with poor survival in breast and colorectal cancer patients receiving adjuvant chemotherapy[62]. Results in mouse studies showed that cancer cells growing in Fpr1−/− hosts were resistant to anthracyclines and suggested that Fpr1 deficient immune cells failed to migrate towards dying cancer cells. Using a microfluidic platform similar to that described above (Fig. 3A), they tested this hypothesis by seeding cancer cells and immune cells in separate compartments and imaged over time the migration of immune cells towards the cancer cells in 2D through micro-channels. This confirmed that Fpr1 deficient immune cells interacted much less with dying cancer cells treated with anthracyclines than immune cells expressing functional Fpr1 (Fig. 3B), explaining why Fpr1 deficiency leads to defective anticancer immune response and less efficient chemotherapy.

In another recent study, a macrophage cell line (RAW 264.7) seeded in a 3D collagen gel in a microfluidic device was shown to assist and increase the intravasation of breast cancer cells through an endothelial monolayer[18], as has been observed *in vivo*[63]. In a separate study, Hsu *et al*. investigated the crosstalk between cancer cells, myofibroblasts and macrophages cultured in a 2D microfluidic device composed of different chambers enclosed by valves to control the release of conditioned media (CM) from myofibroblasts, macrophages or both cell types to the cancer cells cultured in a separate chamber. This study showed that CM from macrophage and myofibroblasts increased the migration of cancer cells. Interestingly, tumor necrosis factor- α (TNF-α) produced by macrophages counteracted the migration-promoting effects of myofibroblasts. This study provides insight into tumor-stroma interactions and also into the crosstalk between different types of stromal cells and combinatorial effects on cancer cells[51].

Huang *et al*. used a microfluidic device containing neighboring regions filled with hydrogels to study in 3D the dynamics of macrophages and cancer cells. While cancer cells did not invade the hydrogel that contained macrophages, more immune cells were seen invading the channel that contained cancer cells than control channels. Furthermore, cancer cells promoted macrophage proliferation[64]. Recently, Liu *et al*. co-cultured four different cell types (fibroblasts, macrophages, ECs and bladder cancer cells) in 3D in separate compartments. They observed the migration of macrophages towards cancer cells [48] and tested different chemotherapy treatments in the microfluidic device. They also showed that macrophages in co-culture expressed more Arginine-1, in an analogous manner to macrophage activation in the tumor microenvironment.

Microfluidic models have been more widely used for studying immunology, outside of cancer research, as discussed extensively in other reviews[45, 65, 66]. For example, they

have been used to analyze T cell[67] or neutrophil migratory behavior under chemical gradients[68]; the migration of dendritic cells, macrophages and T cells through microchannels towards bacteria[44]; the rolling of neutrophils[69] and their extravasation through endothelium[70]; or the adhesion of T cells[71] or monocytes[72] [73] to ECs and their transmigration through an endothelial monolayer[73]. In addition, several "organ-on-a-chip" studies have developed microfluidic models for studying inflammation in the lung[74] or gut[75]. Another exciting application of microfluidics is immunophenotyping for characterizing the status of a patient's immunity (e.g. cytokine profiling, transcriptomics and proteomics of immune cells[76] [77]) to diagnose and tailor drug treatment[65], as reviewed elsewhere[45]. With the advent of cancer immunotherapy, the scope of these microfluidic studies should now be extended to include the study of immune cells in the context of cancer.

Outlook for future microfluidic applications in immunotherapy

Despite their tremendous experimental potential, to date, microfluidic studies have not been widely exploited to study cancer/immune cell interactions. Given their versatility and wide range of capabilities, we propose that microfluidic assays could be exploited for more complex modeling by using additional cell types and testing cancer– immune cell interactions in 3D, especially during and following trans-endothelial migration (Fig. 3C–D). For example, stromal cells such as cancer-associated fibroblasts should be included in future studies because of their critical role in modulating the immune response via the secretion of cytokines[46]. Similarly, organ-specific cells could be added, eventually leading to "body-on-a-chip" platforms, wherein multiple organs are mimicked in different compartments of a single microfluidic device and connected via channels, recapitulating the systemic nature of the metastatic process [66].

Microfluidic systems offer the potential for high throughput, quantitative clinical analyses. They could facilitate multiple tests on the same chip (ex., protein/gene expression, cell-cell interaction, cell phenotype, or migration). Some of this technology already exists, i.e. on-chip protein expression and cell isolation[52, 77–79]. Another promising application of microfluidics is in the development of cell-based vaccines for immunotherapy through manipulation of immune cells. In this context, Szeto *et al*. recently used a commercially-available microfluidic device to load B cells with desired antigens, which were then used to prime cytotoxic T lymphocytes both *in vitro* and *in vivo*. This was achieved by forcing the cells through a narrow channel, transiently disrupting their membranes and allowing intracellular delivery of proteins from the surrounding media through the membrane[80].

Microfluidic systems are uniquely positioned to facilitate the development of personalized medicine because of the small number of cells needed from the patient and the potential to obtain rapid and automated results. In the future, physicians could use patient-derived pluripotent stem cells, from which various cell types (e.g., ECs for μVNs) could be derived, to build patient-specific organ-on-the-chip microfluidic platforms. Using these patient-specific platforms, as well as tumor and immune cells obtained from patients, the efficacy of an immunotherapy on a specific cancer patient might be assessed prior to treatment. Moreover, microfluidic assays that can model individual metastatic steps could be used to

assess at which stage in the metastatic process a therapy is effective, so as to optimize the timing and location of its delivery. Another current application of microfluidic technology is the isolation of patients' CTCs[81]. Eventually, different components could be integrated in one microfluidic chip for increased throughput and automation, e.g. isolation of CTCs followed by diagnostic tests in a single microfluidic set-up.

Yet, challenges remain before microfluidic assays can be routinely used in the clinic [82, 83] (Outstanding Question Box) [84]. First, findings from these models need to be validated by direct comparison to *in vivo* results (e.g., in mice), especially when testing new drugs with possible systemic side-effects that cannot currently be assessed *in vitro*. This is particularly relevant to the study of the immune system, which relies heavily on interactions between numerous cell types dispersed throughout the body (even during metastasis[85]). Although these devices have better potential for high throughput testing than animal studies, many current microfluidic platforms depend heavily on high resolution imaging and similarly time-consuming image and data analysis[45]. New platforms should convert to automated data analysis and readouts for routine clinical use (e.g. colorimetric readouts, on-chip western blotting, or on-chip ELISA). Other challenges associated with microfluidic systems for translational immunology are manufacturability and scalability[66], limited by, for example, the predominant use of PDMS for making devices[84]. Indeed, these current "lab-on-a-chip" models are intended to facilitate time-consuming bench-top procedures, but are, after all, still "chips-in-a-lab"[86] that require external equipment (e.g. pumps, microscope) and much human intervention. Continuing efforts should be made to create integrated chips that include actuation devices, such as pumps for increased throughput, so that the translational potential of this technology can be exploited.

Lastly and most importantly, there needs to be an increased applicability of available microfluidic devices in biology and medicine. Sackmann *et al*. estimated that 85% of microfluidic related publications are published in engineering, as opposed to clinical or biological journals, mostly as proof-of-principle studies[84]. A more interdisciplinary approach needs to be taken via enhanced collaboration between technologists, scientists and clinicians to develop devices that are clinically relevant, inexpensive and easy to use.

Concluding Remarks

Microfluidic models have become valuable tools for studying the metastatic cascade. These models have also the ability to recapitulate key features of the immune microenvironment, such as chemical gradients, vascular flow and trans-endothelial migration. Thus, an opportunity exists to capitalize on the capability of microfluidic models to recapitulate interactions between cancer and immune cells as they occur *in vivo*, especially during metastatic spread.

We propose that cancer-immune cell interactions should be studied in organ-specific microfluidic models, since recent studies suggest that the role of immune cells in cancer progression, such as macrophages, may differ between organs[87]. Results of microfluidic studies should be systematically validated by *in vivo* experiments[83] (see Outstanding questions). Finally, future studies should analyze the role of several types of immune cells

simultaneously, as their interplay has been shown to be critical for some steps of the metastatic cascade[60, 61].

Microfluidic models can not only help characterize the immune-cancer cell interactions that have recently become interesting therapeutic targets, but also help in testing the efficacy of novel immunotherapies. Eventually, microfluidic systems could represent an important intermediate step, bridging current *in vitro* high throughput screens and animal studies and human clinical trials.

Acknowledgments

We want to thank Michel Boussommier-Calleja for drawing the figures of this manuscript. We would like to acknowledge the Cancer Research Institute for supporting Boussommier-Calleja A. through the Irvington Postdoctoral Fellowship.

References

1. Turcotte S, Rosenberg Sa. Immunotherapy for Metastatic Solid Cancers. Adv. Surg. 2011; 45:341–360. [PubMed: 21954698]
2. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat. Rev. Cancer. 2012; 12:252–264. [PubMed: 22437870]
3. Dranoff G. Experimental mouse tumour models: what can be learnt about human cancer immunology? Nat. Rev. Immunol. 2011; 12:61–66. [PubMed: 22134155]
4. Whitesides GM. The origins and the future of microfluidics. Nature. 2006; 442:368–373. [PubMed: 16871203]
5. Kim S, et al. Biological applications of microfluidic gradient devices. Integr. Biol. 2010; 2:584.
6. Patra B, et al. A microfluidic device for uniform-sized cell spheroids formation, culture, harvesting and flow cytometry analysis. Biomicrofluidics. 2013; 7:054114.
7. Thaitrong N, et al. Implementation of Microfluidic Sandwich ELISA for Superior Detection of Plant Pathogens. PLoS One. 2013; 8:e83231. [PubMed: 24376668]
8. Hughes AJ, Herr AE. Microfluidic Western blotting. Proc. Natl. Acad. Sci. 2012; 109:21450–21455. [PubMed: 23223527]
9. Regehr KJ, et al. Biological implications of polydimethylsiloxane-based microfluidic cell culture. Lab Chip. 2009; 9:2132–2139. [PubMed: 19606288]
10. Wyckoff J, et al. High-resolution multiphoton imaging of tumors in vivo. Cold Spring Harb. Protoc. 2011; 2011:1167–1184. [PubMed: 21969629]
11. Sahai E. Illuminating the metastatic process. Nat. Rev. Cancer. 2007; 7:737–749. [PubMed: 17891189]
12. Chambers AF, et al. Dissemination and growth of cancer cells in metastatic sites. Nat. Rev. Cancer. 2002; 2:563–572. [PubMed: 12154349]
13. Kuo C-T, et al. A spatiotemporally defined in vitro microenvironment for controllable signal delivery and drug screening. Analyst. 201410.1039/C4AN00936C
14. Kuo C, Te, et al. Modeling of cancer metastasis and drug resistance via biomimetic nano-cilia and microfluidics. Biomaterials. 2014; 35:1562–1571. [PubMed: 24269156]
15. Song JW, et al. Microfluidic endothelium for studying the intravascular adhesion of metastatic breast cancer cells. PLoS One. 2009; 4:e5756. [PubMed: 19484126]
16. Shin MK, et al. Integration of intra- and extravasation in one cell-based microfluidic chip for the study of cancer metastasis. Lab Chip. 2011; 11:3880–3887. [PubMed: 21975823]
17. Zhang Q, et al. A microfluidic-based device for study of transendothelial invasion of tumor aggregates in realtime. Lab Chip. 2012; 12:2837. [PubMed: 22648473]

18. Zervantonakis IK, et al. Three-dimensional microfluidic model for tumor cell intravasation and endothelial barrier function. Proc. Natl. Acad. Sci. U. S. A. 2012; 109:13515–13520. [PubMed: 22869695]
19. Lee H, et al. A microfluidic platform for quantitative analysis of cancer angiogenesis and intravasationa). Biomicrofluidics. 2014; 8:054102. [PubMed: 25332739]
20. Jeon JS, et al. In vitro model of tumor cell extravasation. PLoS One. 2013; 8:e56910. [PubMed: 23437268]
21. Chen MB, et al. Mechanisms of tumor cell extravasation in an in vitro microvascular network platform. Integr. Biol. (Camb). 2013; 5:1262–1271. [PubMed: 23995847]
22. Riahi R, et al. A microfluidic model for organ-specific extravasation of circulating tumor cells. Biomicrofluidics. 2014; 8:024103. [PubMed: 24803959]
23. Ehsan SM, et al. A three-dimensional in vitro model of tumor cell intravasation. Integr. Biol. (Camb). 2014; 6:603–610. [PubMed: 24763498]
24. Ghajar CM, et al. The perivascular niche regulates breast tumour dormancy. Nat. Cell Biol. 2013; 15:807–817. [PubMed: 23728425]
25. Whisler JA, et al. Control of Perfusable Microvascular Network Morphology Using a Multiculture Microfluidic System. 2013 00.
26. Kim S, et al. Engineering of functional, perfusable 3D microvascular networks on a chip. Lab Chip. 2013; 13:1489–1500. [PubMed: 23440068]
27. Jeon JS, et al. Generation of 3D functional microvascular networks with human mesenchymal stem cells in microfluidic systems. Integr. Biol. (Camb). 2014; 6:555–563. [PubMed: 24676392]
28. Bersini S, et al. In vitro models of the metastatic cascade: from local invasion to extravasation. Drug Discov. Today. 2013 00.
29. Joyce, Ja; Pollard, JW. Microenvironmental regulation of metastasis. Nat. Rev. Cancer. 2009; 9:239–252. [PubMed: 19279573]
30. Fitzgerald DP, et al. Reactive glia are recruited by highly proliferative brain metastases of breast cancer and promote tumor cell colonization. Clin. Exp. Metastasis. 2008; 25:799–810. [PubMed: 18649117]
31. Fidler IJ. The pathogenesis of cancer metastasis: the “seed and soil” hypothesis revisited. Nat. Rev. Cancer. 2003; 3:453–458. [PubMed: 12778135]
32. Bersini S, et al. A microfluidic 3D in vitro model for specificity of breast cancer metastasis to bone. Biomaterials. 2014; 35:2454–2461. [PubMed: 24388382]
33. Benam KH, et al. Engineered In Vitro Disease Models. Annu. Rev. Pathol. Mech. Dis. 2015; 10:195–262.
34. Huh D, et al. Reconstituting organ-level lung functions on a chip. Science. 2010; 328:1662–1668. [PubMed: 20576885]
35. Budhu S, et al. The importance of animal models in tumor immunity and immunotherapy. Curr. Opin. Genet. Dev. 2014; 24:46–51. [PubMed: 24657536]
36. DeWeerdt S. Bacteriology: A caring culture. Nature. 2013; 504:S4–S5. [PubMed: 24352362]
37. Kitamura T, et al. Immune cell promotion of metastasis. Nat. Rev. Immunol. 2015; 15:73–86. [PubMed: 25614318]
38. Schietinger A, et al. Specificity in cancer immunotherapy. Semin. Immunol. 2008; 20:276–285. [PubMed: 18684640]
39. Qian B-Z, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. Cell. 2010; 141:39–51. [PubMed: 20371344]
40. Vanneman M, Dranoff G. Combining immunotherapy and targeted therapies in cancer treatment. Nat. Rev. Cancer. 2012; 12:237–251. [PubMed: 22437869]
41. De Palma M, Lewis CE. Macrophage regulation of tumor responses to anticancer therapies. Cancer Cell. 2013; 23:277–286. [PubMed: 23518347]
42. Hughes R, et al. Perivascular M2 Macrophages Stimulate Tumor Relapse after Chemotherapy. Cancer Res. 201510.1158/0008-5472.CAN-14-3587
43. Moser B, Willimann K. Chemokines: role in inflammation and immune surveillance. Ann. Rheum. Dis. 2004; 63(Suppl. 2):i84–ii89.

44. Gopalakrishnan N, et al. Infection and immunity on a chip: a compartmentalised microfluidic platform to monitor immune cell behaviour in real time. Lab Chip. 201510.1039/C4LC01438C
45. Junkin M, Tay S. Microfluidic single-cell analysis for systems immunology. Lab Chip. 2014; 14:1246–1260. [PubMed: 24503696]
46. Hirt C, et al. “In vitro” 3D models of tumor-immune system interaction. Adv. Drug Deliv. Rev. 2014; 79–80:145–154.
47. Feder-Mengus C, et al. New dimensions in tumor immunology: what does 3D culture reveal? Trends Mol. Med. 2008; 14:333–340. [PubMed: 18614399]
48. Agliari E, et al. Cancer-driven dynamics of immune cells in a microfluidic environment. Sci. Rep. 2014; 4:6639. [PubMed: 25322144]
49. Businaro L, et al. Cross talk between cancer and immune cells: exploring complex dynamics in a microfluidic environment. Lab Chip. 2012:13.
50. Mattei F, et al. A multidisciplinary study using in vivo tumor models and microfluidic cell-on-chip approach to explore the cross-talk between cancer and immune cells. J. Immunotoxicol. 2014; 11:337–346. [PubMed: 24597645]
51. Hsu T-H, et al. The migration speed of cancer cells influenced by macrophages and myofibroblasts co-cultured in a microfluidic chip. Integr. Biol. 2012; 4:177.
52. Huang N-T, et al. An integrated microfluidic platform for in situ cellular cytokine secretion immunophenotyping. Lab Chip. 2012; 12:4093. [PubMed: 22892681]
53. de Visser KE, et al. Paradoxical roles of the immune system during cancer development. Nat. Rev. Cancer. 2006; 6:24–37. [PubMed: 16397525]
54. Bidwell BN, et al. Silencing of Irf7 pathways in breast cancer cells promotes bone metastasis through immune escape. Nat. Med. 2012; 18:1224–1231. [PubMed: 22820642]
55. Eyles J, et al. Tumor cells disseminate early, but immunosurveillance limits metastatic outgrowth, in a mouse model of melanoma. J. Clin. Invest. 2010; 120:2030–2039. [PubMed: 20501944]
56. Paolino M, et al. The E3 ligase Cbl-b and TAM receptors regulate cancer metastasis via natural killer cells. Nature. 2014; 507:508–512. [PubMed: 24553136]
57. Granot Z, et al. Tumor entrained neutrophils inhibit seeding in the premetastatic lung. Cancer Cell. 2011; 20:300–314. [PubMed: 21907922]
58. Fidler IJ. Macrophages and metastasis--a biological approach to cancer therapy. Cancer Res. 1985; 45:4714–4726. [PubMed: 2992766]
59. Edris B, et al. Antibody therapy targeting the CD47 protein is effective in a model of aggressive metastatic leiomyosarcoma. Proceedings of the National Academy of Sciences. 2012; 109:6656–6661.
60. Labelle M, et al. Platelets guide the formation of early metastatic niches. Proc. Natl. Acad. Sci. U. S. A. 2014; 111:E3053–E3061. [PubMed: 25024172]
61. Labelle M, et al. Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis. Cancer Cell. 2011; 20:576–590. [PubMed: 22094253]
62. Vacchelli E, et al. Chemotherapy-induced antitumor immunity requires formyl peptide receptor 1. Science. 201510.1126/science.aad0779
63. Wyckoff JB, et al. A critical step in metastasis: In vivo analysis of intravasation at the primary tumor. Cancer Res. 2000; 60:2504–2511. [PubMed: 10811132]
64. Huang CP, et al. Engineering microscale cellular niches for three-dimensional multicellular co-cultures. Lab Chip. 2009; 9:1740–1748. [PubMed: 19495458]
65. Chen W, et al. Emerging microfluidic tools for functional cellular immunophenotyping: a new potential paradigm for immune status characterization. Front. Oncol. 2013; 3:98. [PubMed: 23626950]
66. Ramadan Q, Gijs MaM. In vitro micro-physiological models for translational immunology. Lab Chip. 2015; 15:614–636. [PubMed: 25501670]
67. Lin F, Butcher EC. T cell chemotaxis in a simple microfluidic device. Lab Chip. 2006; 6:1462–1469. [PubMed: 17066171]

68. Halilovic I, et al. Neutrophil migration under spatially-varying chemoattractant gradient profiles. Biomed. Microdevices. 2015; 17:1–7. [PubMed: 25653054]
69. Sundd P, et al. “Slings” enable neutrophil rolling at high shear. Nature. 2012; 488:399–403. [PubMed: 22763437]
70. Han S, et al. A versatile assay for monitoring in vivo-like transendothelial migration of neutrophils. Lab Chip. 2012; 12:3861. [PubMed: 22903230]
71. Kim SK, et al. Inflammatory mimetic microfluidic chip by immobilization of cell adhesion molecules for T cell adhesion. The Analyst. 2012; 137:4062. [PubMed: 22822477]
72. Zhang B, et al. A standalone perfusion platform for drug testing and target validation in micro-vessel networks. Biomicrofluidics. 2013; 7:1–13.
73. Srigunapalan S, et al. A microfluidic membrane device to mimic critical components of the vascular microenvironment. Biomicrofluidics. 2011; 5:1–9.
74. Nesmith AP, et al. Lab on a Chip model of allergic asthmatic bronchoconstriction. Lab Chip. 201410.1039/C4LC00688G
75. Kim HJ, et al. Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. Lab Chip. 2012; 12:2165. [PubMed: 22434367]
76. Ma C, et al. A clinical microchip for evaluation of single immune cells reveals high functional heterogeneity in phenotypically similar T cells. Nat. Med. 2011; 17:738–743. [PubMed: 21602800]
77. Kotz KT, et al. Clinical microfluidics for neutrophil genomics and proteomics. Nat. Med. 2010; 16:1042–1047. [PubMed: 20802500]
78. Zhu H, et al. A microdevice for multiplexed detection of T-cell-secreted cytokines. Lab Chip. 2008; 8:2197–2205. [PubMed: 19023487]
79. Chen W, et al. Surface-Micromachined Microfiltration Membranes for Efficient Isolation and Functional Immunophenotyping of Subpopulations of Immune Cells. Adv. Healthc. Mater. 2013; 2:965–975. [PubMed: 23335389]
80. Lee S, zeto G, et al. Microfluidic squeezing for intracellular antigen loading in polyclonal B-cells as cellular vaccines. Sci. Rep. 2015; 5:10276. [PubMed: 25999171]
81. Hou HW, et al. Isolation and retrieval of circulating tumor cells using centrifugal forces. Sci. Rep. 2013; 3:1259. [PubMed: 23405273]
82. Cox MC, et al. Toward the Broad Adoption of 3D Tumor Models in the Cancer Drug Pipeline. ACS Biomater. Sci. Eng. 2015; 1:877–894.
83. van Duinen V, et al. Microfluidic 3D cell culture: from tools to tissue models. Curr. Opin. Biotechnol. 2015; 35:118–126. [PubMed: 26094109]
84. Sackmann EK, et al. The present and future role of microfluidics in biomedical research. Nature. 2014; 507:181–189. [PubMed: 24622198]
85. Labelle M, Hynes RO. The initial hours of metastasis: the importance of cooperative host-tumor cell interactions during hematogenous dissemination. Cancer Discov. 2012; 2:1091–1099. [PubMed: 23166151]
86. Streets AM, Huang Y. Chip in a lab: Microfluidics for next generation life science research. Biomicrofluidics. 2013:7.
87. Chen Q, et al. Macrophage binding to receptor VCAM-1 transmits survival signals in breast cancer cells that invade the lungs. Cancer Cell. 2011; 20:538–549. [PubMed: 22014578]
88. Choi SYC, et al. Lessons from patient-derived xenografts for better in vitro modeling of human cancer. Adv. Drug Deliv. Rev. 2014; 79–80:222–237.
89. Cheon D-J, Orsulic S. Mouse models of cancer. Annu. Rev. Pathol. 2011; 6:95–119. [PubMed: 20936938]
90. Liu S, Leach SD. Zebrafish models for cancer. Annu. Rev. Pathol. 2011; 6:71–93. [PubMed: 21261518]
91. Konantz M, et al. Zebrafish xenografts as a tool for in vivo studies on human cancer. Ann. N. Y. Acad. Sci. 2012; 1266:124–137. [PubMed: 22901264]
92. Deryugina EI, Quigley JP. Chick embryo chorioallantoic membrane model systems to study and visualize human tumor cell metastasis. Cell. 2009; 130:1119–1130.

93. Leong HS, et al. Invadopodia Are Required for Cancer Cell Extravasation and Are a Therapeutic Target for Metastasis. Cell Rep. 2014; 8:1558–1570. [PubMed: 25176655]

94. Unger C, et al. Modeling human carcinomas: Physiologically relevant 3D models to improve anti-cancer drug development. Adv. Drug Deliv. Rev. 2014; 79–80:50–67.

95. Qian B, et al. A distinct macrophage population mediates metastatic breast cancer cell extravasation, establishment and growth. PLoS One. 2009; 4:e6562. [PubMed: 19668347]

96. Kimlin LC, et al. In vitro three-dimensional (3D) models in cancer research: an update. Mol. Carcinog. 2013; 52:167–182. [PubMed: 22162252]

97. Polacheck WJ, et al. Microfluidic platforms for mechanobiology. Lab Chip. 2013; 13:2252–2267. [PubMed: 23649165]

98. Lee PJ, et al. An artificial liver sinusoid with a microfluidic endothelial-like barrier for primary hepatocyte culture. Biotechnol. Bioeng. 2007; 97:1340–1346. [PubMed: 17286266]

99. Shi M, et al. Glia co-culture with neurons in microfluidic platforms promotes the formation and stabilization of synaptic contacts. Lab Chip. 2013; 13:3008–3021. [PubMed: 23736663]

100. Trkov S, et al. Micropatterned 3-Dimensional Hydrogel System to Study Human Endothelial-Mesenchymal Stem Cell Interactions. J. Tissue Eng. Regen. Med. 2011; 4:205–215. [PubMed: 19998330]

101. Jang K-J, et al. Fluid-shear-stress-induced translocation of aquaporin-2 and reorganization of actin cytoskeleton in renal tubular epithelial cells. Integr. Biol. 2011; 3:134–141.

102. Huh D, et al. From 3D cell culture to organs-on-chips. Trends Cell Biol. 2011; 21:745–754. [PubMed: 22033488]

103. Polacheck WJ, et al. Interstitial flow influences direction of tumor cell migration through competing mechanisms. Proc. Natl. Acad. Sci. U. S. A. 2011; 108:11115–11120. [PubMed: 21690404]

104. Shin Y, et al. Extracellular matrix heterogeneity regulates three-dimensional morphologies of breast adenocarcinoma cell invasion. Adv. Healthc. Mater. 2013; 2:790–794. [PubMed: 23184641]

105. Sundararaghavan HG, et al. Neurite growth in 3D collagen gels with gradients of mechanical properties. Biotechnol. Bioeng. 2009; 102:632–643. [PubMed: 18767187]

106. Sung KE, et al. Control of 3-dimensional collagen matrix polymerization for reproducible human mammary fibroblast cell culture in microfluidic devices. Biomaterials. 2009; 30:4833–4841. [PubMed: 19540580]

107. Sung KE, et al. Transition to invasion in breast cancer: a microfluidic in vitro model enables examination of spatial and temporal effects. Integr. Biol. (Camb). 2011; 3:439–450. [PubMed: 21135965]

108. Bischel LL, et al. Microfluidic model of ductal carcinoma in situ with 3D, organotypic structure. BMC Cancer. 2015; 15:1–10. [PubMed: 25971837]

109. Acosta MA, et al. A microfluidic device to study cancer metastasis under chronic and intermittent hypoxia. Biomicrofluidics. 2014:054117. [PubMed: 25584114]

110. Wyckoff JB, et al. Direct visualization of macrophage-assisted tumor cell intravasation in mammary tumors. Cancer Res. 2007; 67:2649–2656. [PubMed: 17363585]

111. Pisano M, et al. An in vitro model of the tumor-lymphatic microenvironment with simultaneous transendothelial and luminal flows reveals mechanisms of flow enhanced invasion. Integr. Biol. 201510.1039/C5IB00085H

112. Kim J, et al. Cooperative actions of p 21 WAF 1 and p 53 induce Slug protein degradation and suppress cell invasion. 2014; 15:1062–1068.

113. Chaw KC, et al. Multi-step microfluidic device for studying cancer metastasis. Lab Chip. 2007; 7:1041–1047. [PubMed: 17653347]

114. Kim J, et al. Rapid apoptosis in the pulmonary vasculature distinguishes non-metastatic from metastatic melanoma cells. Cancer Lett. 2004; 213:203–212. [PubMed: 15327836]

115. Wong CW, et al. Apoptosis: An Early Event in Metastatic Inefficiency Apoptosis: An Early Event in Metastatic Inefficiency. 2001:1.

116. Zervantonakis IK, et al. Three-dimensional microfluidic model for tumor cell intravasation and endothelial barrier function. Proc. Natl. Acad. Sci. 2012; 109:13515–13520. [PubMed: 22869695]

TEXT BOX 1

Experimental models for cancer research

In vivo cancer and metastasis studies have predominantly been performed in mice[33], [71] and more recently, zebrafish[90]. Both can be readily manipulated genetically and allow for long term studies. However, animal studies raise ethical issues and exhibit species-related differences from human cancer. There are numerous established murine metastasis models (e.g. genetically engineered, xenograft or humanized models), as well as immune-suppressed mouse models, but these studies can be time consuming and expensive. Zebrafish allow for improved imaging, have shorter generation times and a large number of offspring. However, evolutionary, they are not as similar to humans as mice and lack some organs present in humans, e.g. mammary tissue, which limits the options for orthotopic transplantation. In addition, no immune-suppressed zebrafish exist, although immune suppression can be performed in adult zebrafish and embryos can be used wherein the adaptive immune response is not yet developed[91]. And while chick embryo models have been used to study cancer cell intravasation and extravasation, they have an under-developed immune system.[92, 93]

Ex vivo studies typically employ tissue explant cultures[70][94] or whole organ cultures from animals or humans. These studies allow for easier and higher resolution imaging than *in vivo*, particularly for visceral organs, but are limited in time due to tissue degradation. Organ cultures preserve the tissue architecture, while tissue explants can be patient-derived. For example, Qian et al imaged cancer cells extravasating as they interacted with macrophages into intact lungs dissected from mice[95]. However, these *ex vivo* models fail to replicate systemic signaling events (which are characteristic of immune responses) because tissues have been isolated from the organism.

In vitro studies generally allow for higher imaging resolution, a better control of cellular content and the use of cells that are exclusively human. They lack full physiological relevance, however, often requiring the use of serum and growth factors, and are not suitable for long term studies. In addition, *in vitro* studies tend to use immortalized cell lines, a disadvantage since they are genetically homogeneous, which fails to represent the cellular diversity found *in vivo*. Studies done in 2D fail to recapitulate the normal 3D microenvironment of cells in tissues and can lead to important disparities in cell migration or drug sensitivity[96]. *In vitro* 3D studies allow for multicellular structures with higher architectural complexity, which replicates direct cell-cell and cell-extracellular matrix interactions, as well as biomechanical cues found *in vivo* (e.g. hypoxia). It is possible to perform simple cell co-cultures in typical 2D or 3D studies (e.g. Boyden chamber), but with limited control over the size, number and distribution of compartments. In addition, the distances between compartments in these traditional *in vitro* systems are much larger than physiological length scales.

TEXT BOX 2

Microenvironmental control in microfluidic devices

Microfluidic tools possess key features that enable a better control of the microenvironment than other macro-scale *in vitro* models[97]. First, they allow for the design of precise channels and chambers, leading to a controlled compartmentalization and distribution of cells to recreate complex tissue architecture. For example Lee *et al*. mimicked the architecture of a liver sinusoid by creating a microfluidic cavity that was filled with hepatocytes[98]. Each microfluidic compartment can contain a specific cell population separately, allowing for well-defined co-cultures. Shi *et al*. cultured neurons in microfluidic chambers that were separated by pressure-regulated valves to control communication and neuronal synapse formation between them[99]. Trkov *et al*. varied the distance between channels containing endothelial or mesenchymal cells to study whether intercellular distances affected vascular sprouting[100].

Second, because they contain channels whose dimensions are precisely defined, they allow for tight control of flow and shear stress. For example, Jung et al assessed the effects of shear stress on renal cell actin reorganization and aquaporin-2 trafficking in a microfluidic channel[101]. The channels also allow for the guided delivery of cells or nanoparticles into the assay. In addition, valves can be added to direct and control flow, as Hsu *et al*. did to control the release of conditioned media (CM) from different cell types contained in separate compartments[51].

Third, the channels also permit local control of other environmental physical factors, such as pressure and strain or hypoxia, by connecting the micro-channels to sources of gas or to vacuum chambers. For example, a microfluidic device consisting of a porous membrane covered with cells placed between two apposed micro-channels was developed to mimic the alveolar-capillary interface in the lungs [34, 102]. Two larger flanking channels were connected to a vacuum pump that exerts a cyclical pressure on the central channel containing the porous membrane, thus cyclically stretching the cells as would occur during normal breathing.

Finally, the channels also enable physical or chemical gradients to be established and controlled. For example, Polacheck *et al*. generated an interstitial flow characteristic of solid tumors by creating a hydrostatic pressure drop across a collagen gel containing cells placed between two channels[103]. Alternatively, many studies have generated chemical gradients, as reviewed elsewhere[5]. Flow-based gradients generated in microfluidic assays exploit the features of laminar flow that is characteristic at micro-scales to better control diffusing molecules. In addition, the ability to create any desired network of channels enables the establishment of complex gradient profiles.

Importantly, microfluidics enables the culture of cells in 3D, with specified extracellular matrix composition [104], stiffness[105], and alignment [106]. In addition, growing 3D multicellular structures such as spheroids and organoids in defined microfluidic compartments allows for control of their shape, size and growth at the micro-scale.

TEXT BOX 3

Microfluidic studies of individual metastatic steps

Microfluidic studies have modeled EMT and invasion by analyzing the migration of cancer cells from spheroids into an underlying collagen gel[13, 14]. They demonstrated that the expression of the epithelial marker EpCAM decreased in migratory cells compared to those remaining in the spheroid, indicating that cells had undergone EMT, as occurs *in vivo*. Similarly, the transition of breast cancer cells to an invasive phenotype was shown to lead to collagen remodeling and was promoted by human mammary fibroblasts[107, 108]. Other microfluidic studies have shown that invasion of single cancer cell lines into hydrogels increases with increasing metastatic potential[16], either under hypoxia[109] or in the presence of C-X-C motif chemokine 12 (CXCL12) or matrix metalloproteinases (MMP) inhibitors[16].

Microfluidic intravasation studies of cancer cell transmigration through an endothelial monolayer have shown that TNF-α[18, 19] and macrophages increase tumor intravasation rates[18], as observed *in vivo*[110]. Intravasation through lymphatic monolayers was replicated using Boyden chambers with microfluidic channels, showing that luminal and transmural flow increased intravasation[111]. Ehsan *et al*. vascularized a spheroid of cancer cells in a well-plate[23], and showed that intravasation was enhanced under hypoxia and depended on the transcription factor Slug, consistent with *in vivo* results[112]. This should be replicated in future microfluidic studies, for enhanced capabilities, e.g. in the presence of perfusion of the μVNs.

Microfluidic adhesion assays of either single cancer cells or aggregates of cells onto an endothelial monolayer showed that E-selectin expression in human umbilical vein endothelial cells (HUVECs), CXCL12[15] and shear stress[22] are both capable of affecting adhesion.

Microfluidic assays have shown that extravasation of breast cancer cells generally occurs within 24 hours[20, 21] para-cellularly through transient gaps between the endothelial cells of a μVN. In contrast, extravasation of aggregates of tumor cells was shown to irreversibly disrupt an endothelial monolayer[17]. Extravasation rates were increased in the presence of osteo-like cells[32], replicating the *in vivo* organ-selectivity of breast cancer cells to bones. Extravasation also increased in the presence of CXCL12[22], and was blocked by AMD3100, a CXCR4 receptor antagonist currently in clinical trials[17]. Other microfluidic experiments have studied extravasation by analyzing the deformability of cells through narrow gaps (in 2D or channels filled with hydrogels[113]).

No long-term microfluidic recolonization assays have been reported that analyze events beyond extravasation, although the potential exists as it has been accomplished in presence of μVN in well plates[24]. This would be extremely useful as micrometastasis formation following extravasation is still poorly understood and has been shown to be the least efficient step in metastasis. Thus, it may be one of the most promising pharmacological targets for halting metastasis[114, 115]. Alternatively, a few extravasation studies quantified cancer cell proliferation or invasion in the ECM shortly

after extravasation[20], showing that CXCL12 exacerbated invasion[17]. At present, only Shin et al. have modeled several metastatic steps in the same microfluidic device by combining invasion of cancer cells out of a hydrogel and their subsequent adhesion to a monolayer of ECs for a more comprehensive model[16].

Outstanding Questions Box

- Can microfluidic models faithfull replicate key aspects of in vivo immune cell-cancer cell interactions?

  As more studies use microfluidic models to mimic and investigate the cross-talk between cancer cells and immune cells, it will be key to complement the *in vitro* results with similar *in vivo* experiments to confirm their validity.

- Are immune cell – cancer cell interactions organ-specific?

  There is mounting evidence that organ selectivity in metastasis is due to cross-talk between the cancer cells and the organs' unique microenvironment. Interactions between immune cells and cancer cells could similarly be affected by the organ-specific microenvironment, offering the potential for even more potent and targeted anti-metastatic immunotherapies.

- Can microfluidic assays reduce the need for animal tests for drug screening prior to human clinical trials?

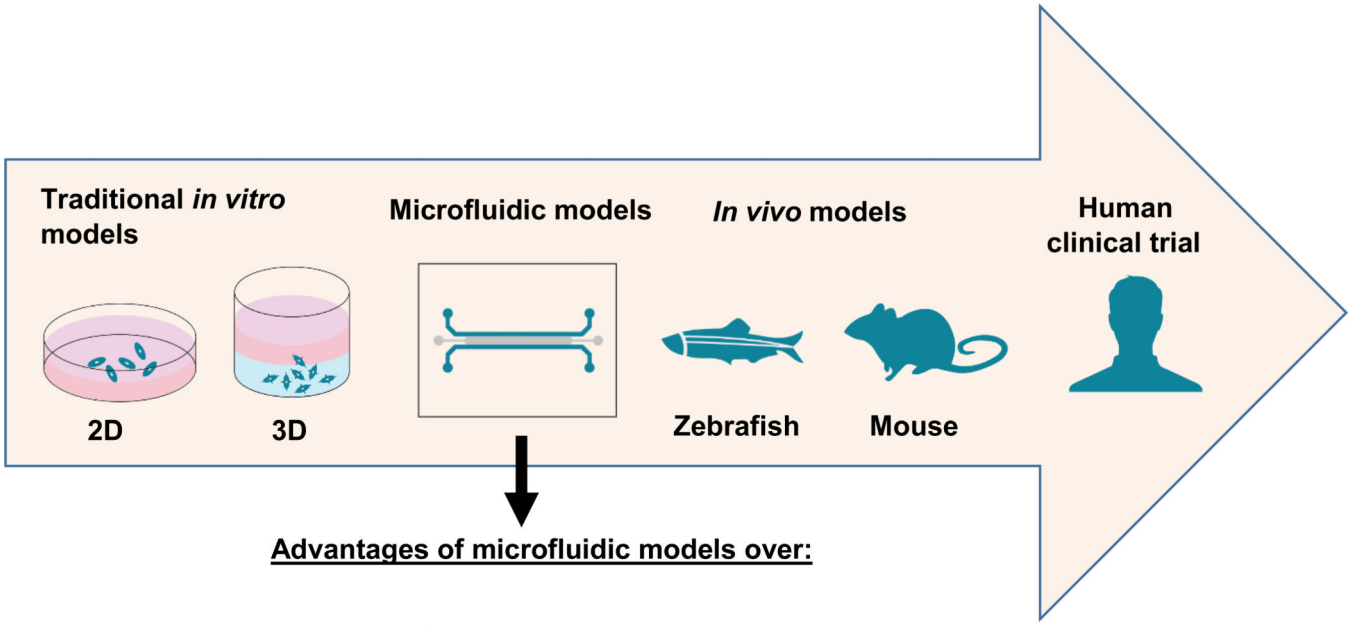
  Although microfluidic assays already present a dramatic improvement in physiological relevance over previous conventional *in vitro* models, more can be done to improve their validity as *in vivo* models, prior to their use for drug screening. In parallel, technological improvements are needed to automate and scale the models up for fast high throughput screening.

- Can microfluidic models become a clinical tool for personalized immunotherapy?

  Microfluidic devices require small amounts of reagents and cells, making them ideal platforms for systematic clinical application using patient-derived cells. This will depend both on the design of low-cost, high throughput systems, automation, and on progress in harvesting and expanding patient-derived cells.

Trends Box

- While most therapies fail to contain metastasis, immunotherapies have had promising success on patients with renal or melanoma cancer metastasis.
- Immune-cancer cell interactions are therapeutic targets for preventing metastasis. Recently, drugs targeting these interactions have been shown to prevent cancer progression and have received FDA approval. Many more immune-cancer cell interactions are being uncovered that should be better characterized using improved *in vitro* models.
- Recent advances in microfluidic technology have enabled the development of 3D co-culture models including a perfusable microvasculature. Microfluidic systems offer unique advantages for modeling the metastatic microenvironment and, in particular, immune cell – cancer cell interactions. They allow for tight control of the cellular, biochemical and physical microenvironment and high resolution time-lapse imaging.
- Despite enormous potential, challenges remain in the design of microfluidic models as drug screening platforms or clinical tools for personalized immunotherapy in cancer.



Traditional *in vitro*	Traditional *in vitro* and *in vivo* models	*In vivo* models
-Ability to directly perfuse vascularized structures	-Increased control of microenvironment -Improved imaging capabilities -Fewer cells and less drug needed	-Use of cells exclusively of human origin

Fig. 1. Microfluidic models as a compromise between traditional *in vitro* and *in vivo* models

The schematic shows the different *in vitro* vs. *in vivo* models available for studying biological processes and testing drugs prior to clinical human trials in order of increasing physiological relevance. The advantages of microfluidic models compared to other models are listed below. *Ex vivo* models are not shown here, but are discussed in Text Box 1.

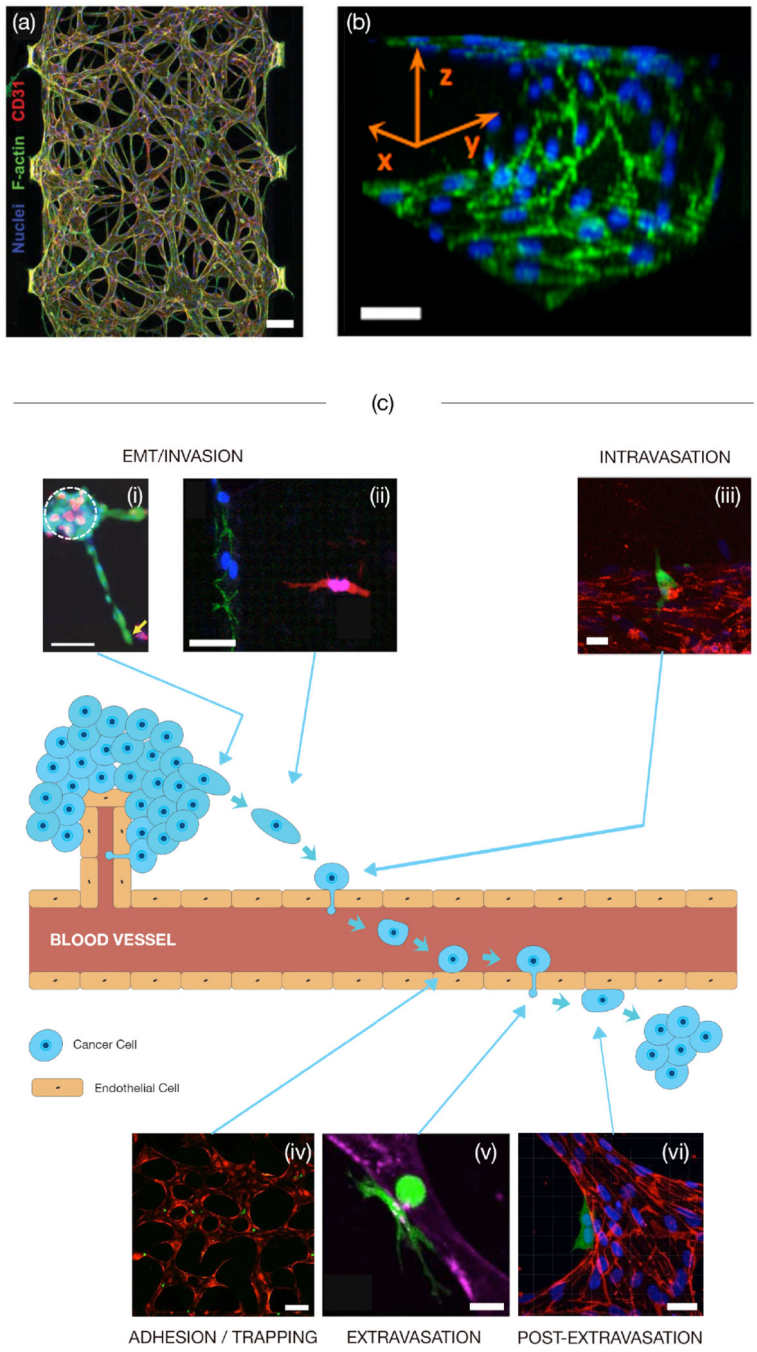


Fig. 2. Existing microfluidic platforms for modeling individual steps of the metastatic cascade

Examples of a microvascular a) network or b) monolayer developed in microfluidic assays[26, 116]. c) Schematic showing the principal stages of the metastatic cascade, accompanied by confocal images of the corresponding steps reproduced in different microfluidic devices. Note that we show intravasation for a single cancer cell, but future models should also consider intravasation from a multi-cellular tumor mass, as has been done in a non-microfluidic 3D model [23]: i) Spheroid of GFP-MCF-7 labelled cells to model epithelial to mesenchymal transition (EMT), where the dashed circle shows the body

of the spheroid and the arrow points to cells migrating away from it[13]; ii) Invasion of a single RFP- HT1080 cell towards a monolayer of GFP-HUVECs[116]; iii) Intravasation of a single GFP-MDA-MB-231 cell through a monolayer of RFP-HUVECs. [19]; iv) adhesion of GFP-MDA-MB-231 in a vascular network of RFP-HUVECs[21]; v) extravasation of a GFP-MDA-MB-231 through a channel of a vascular network stained for phalloidin in pink[21]; and vi) GFP-MDA-MB-231 in a perivascular position in the ECM after extravasation through the vascular network of RFP-HUVECs (with blue DAPI nuclear staining)[21]. Images from Kim *et al*.[26]., Kuo *et al*.[13], and Lee *et al*.[19], were reproduced with permission via the Rightslink Copyright clearance center.

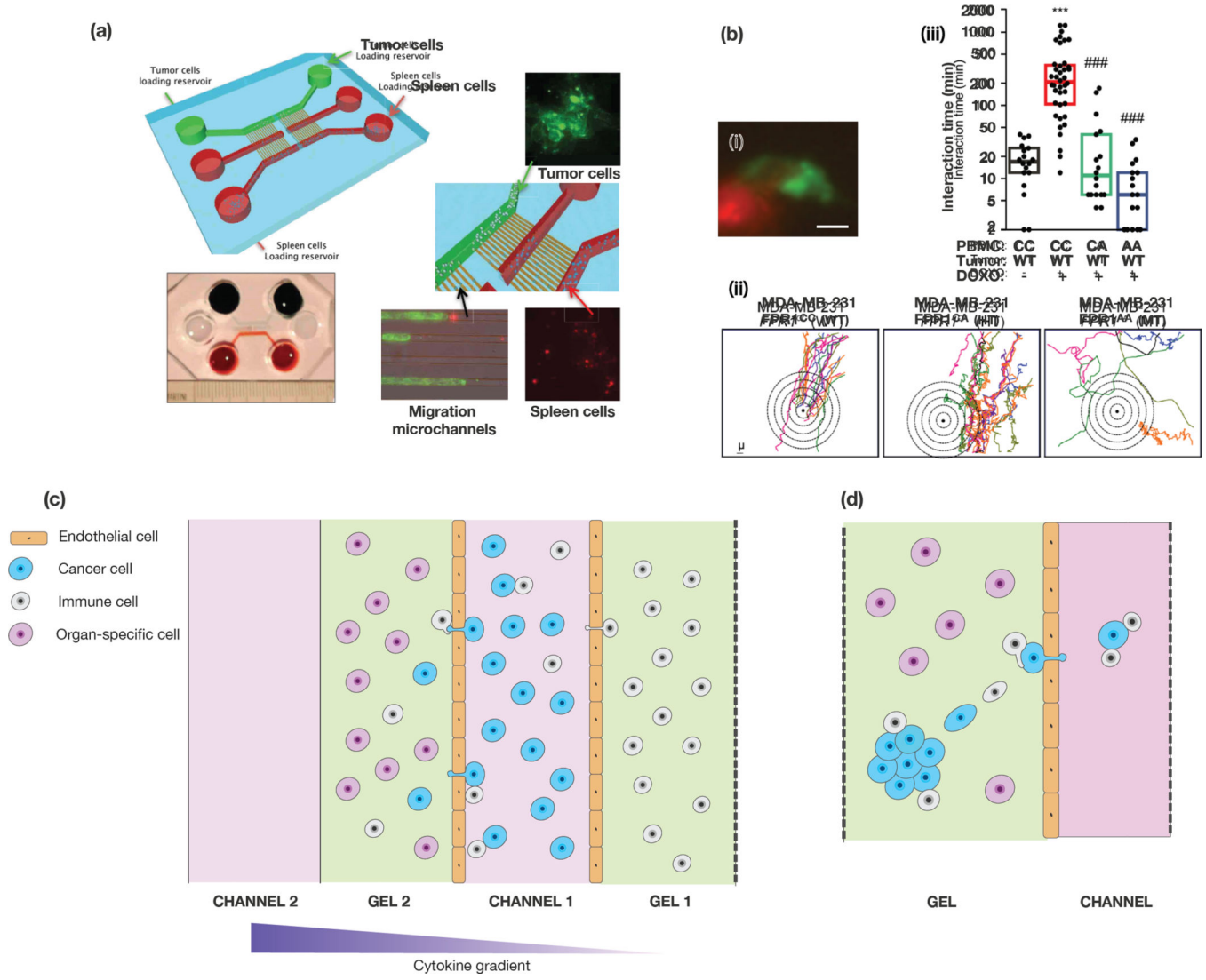


Fig. 3. Example of microfluidic models for studying interactions between cancer and immune cells

a) Agliari *et al*. designed a microfluidic platform wherein tumor cells and spleen cells are compartmentalized across a network of microchannels[48]. The migration of immune cells (red) towards tumor cells (green) is visualized. b) Vacchelli *et al*. used a similar platform to investigate the interactions between immune cells deficient in FPR1 and dying cancer cells[62]: (i) image of a dendritic cell (green) in contact with a dying cancer cell (red); (ii) tracks of immune cells recorded over time as they migrate in the microfluidic platform towards the cancer cell (black central dot). The immune cells either had no mutation (WT, FPR1 ^{CC}), were heterozygous (FPR1 ^{CA}) or homozygous for the FPR1 mutation (FPR1 ^{AA}); (iii) quantification of interaction time between immune cells and cancer cells imaged in the microfluidic platform. The + or – below the graph indicates whether the immune cells (peripheral blood mononuclear cell, PBMC) had the FPR1 mutation (CA or AA) or not (CC), and whether cancer cells were treated with doxorubicin or not. Immune cells deficient in FPR1 interacted less with dying cancer cells treated with doxorubicin than functional

immune cells. c-d) Proposed microfluidic designs that would mimic immune-assisted cancer cell extravasation. c) The schematic shows different channels of a microfluidic platform, some of which are filled with a gel, others with media. Channel 1 is lined with endothelial cells, and mimics the bloodstream. Cancer cells are perfused in channel 1 and extravasate through the endothelial monolayer into gel 2, filled with organ-specific cells, which mimic the metastatic organ microenvironment. Immune cells could either i) be seeded in gel 1 to be recruited and intravasate into the “bloodstream” (i.e. channel 1); ii) be directly perfused in channel 1 along with cancer cells; or iii) placed in gel 2 so as to replicate cancer-immune cell interactions during circulation, adhesion or extravasation, respectively. Note that immune cells could help cancer cells extravasate either from the bloodstream or from within the tissue (both options are represented). A cytokine gradient could be established between channels 1 and 2 to guide the migration of immune cells towards the target tissue, as occurs *in vivo*. d) For intravasation studies, immune cells could be directly seeded into the gel channel with spheroids to observe how they might assist cancer cell dispersion from a tumor, migration through matrix, transendothelial migration into the “bloodstream” (i.e. channel) and circulation in the bloodstream. Dashes indicate that the proposed designs are only a region of the entire microfluidic device. Images from Agliari *et al*.[48], and Vacchelli *al*.[62], were reproduced and modified with permission from the authors and via the Rightslink Copyright clearance center.