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In vitro three-dimensional cancer metastasis modeling: Past, present, and future*

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Metastasis is the leading cause of most cancer deaths, as opposed to dysregulated cell growth of the primary tumor. Molecular mechanisms of metastasis have been studied for decades and the findings have evolved our understanding of the progression of malignancy. However, most of the molecular mechanisms fail to address the causes of cancer and its evolutionary origin, demonstrating an inability to find a solution for complete cure of cancer. After being a neglected area of tumor biology for quite some time, recently several studies have focused on the impact of the tumor microenvironment on cancer growth. The importance of the tumor microenvironment is gradually gaining attention, particularly from the perspective of biophysics. *In vitro* three-dimensional (3-D) metastatic models are an indispensable platform for investigating the tumor microenvironment, as they mimic the *in vivo* tumor tissue. In 3-D metastatic *in vitro* models, static factors such as the mechanical properties, biochemical factors, as well as dynamic factors such as cell–cell, cell–ECM interactions, and fluid shear stress can be studied quantitatively. With increasing focus on basic cancer research and drug development, the *in vitro* 3-D models offer unique advantages in fundamental and clinical biomedical studies.

Keywords: cancer metastasis, microfluidic chip, three-dimensional *in vitro* model, chemotaxis

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1. Introduction

Over the past decades, the 5-year survival rates of cancer patients have increased dramatically, and the well-confined primary tumor can be effectively cured by surgery. However, the overall death rate of cancer remains stubbornly high, which is mainly due to secondary and recurring lesions. Once the tumor cells became metastatic, their disseminated nature could lead to metastasis, which causes more than 90% of cancer-related death.[1] Therefore, understanding, intervention, and eventually prevention of metastasis are the biggest challenges for effective cancer therapy.

Metastases are a series of cell-biological events that are determined by tumor cells and their microenvironments. The combined effect of the genetic and/or epigenetic alternations of tumor cells and the biochemical/biophysical microenvironments of the tumor induce incipient metastatic cells to develop into macroscopic metastases.[1,2] During the complex metastatic process, tumor cells at primary sites release vascular endothelial growth factor (VEGF) to initiate angiogenesis and lymphangiogenesis (Figs. 1(a) and 1(b)). Under increased stress and interstitial pressure, some tumor cells start to extravasate into distant tissues (Fig. 1(e)).[2,3] They detach from the primary sites, and then squeeze into the surrounding tissues (Fig. 1(c)) to finally intravasate into the nearby blood vessels (Fig. 1(d)). Some tumor cells can survive in blood circulation and are eventually arrested at a distant site, where they form a metastatic colony of cells after reinitiating their proliferative programs (Fig. 1(e)).[2,4,5]

The molecular mechanisms of metastasis have been extensively studied using two-dimensional (2-D) models.[6–11] Tumor cells are conventionally seeded onto collagen-coated 2-D plates or flasks for effective tumor cell culturing. There is no doubt that 2-D culture systems have enhanced cell biology and cancer mechanism studies.[7,12,13] To date, most insights into tumor cells, their microenvironments, and early-phase drug tests are accomplished using 2-D systems. However, 2-D culture systems cannot recreate the 3-D environments of cells *in vivo*. The unnatural 2-D substrates artificially reduce the living dimension of cells, which not only alter the native morphology and physiological processes of tumor cells, such as proliferation and migration, but also limit the biochemical and physical effects of the microenvironment.[11,14,15] Tumor cells in 2-D systems also have to adapt to an artificial flat, rigid surface such that their morphology, proliferation, differentiation, and gene expression are radically different from the natural 3-D environment.[11,14–17] Animal models are widely used

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in preclinical studies of tumor and drug screenings, but they cannot faithfully reflect human tissue-specific information and thus often fail to give predictive results in humans. Additionally, they are expensive and time consuming.[10,18] Therefore, scientifically rigorous 3-D *in vitro* cancer models will be invaluable for preclinical research.

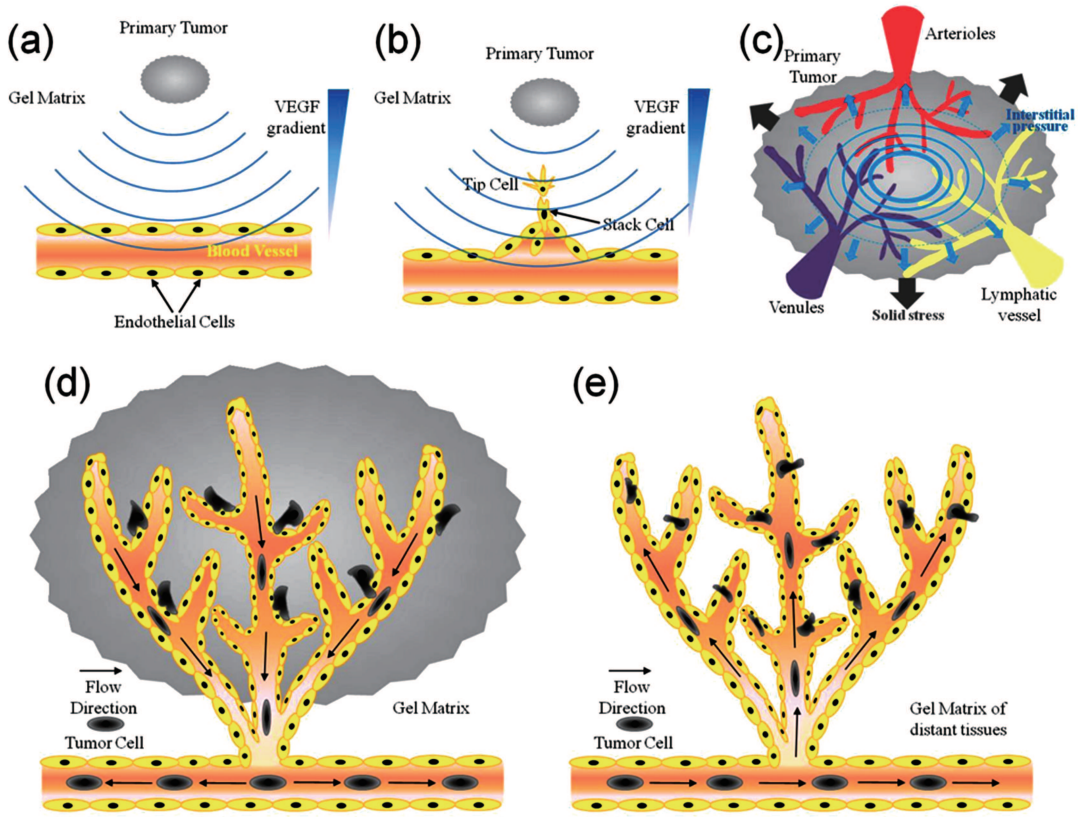


Fig. 1. Schematic diagram of the metastatic process. (a) and (b) The growth and proliferation of tumor cells leads to hypoxia and regulate the enhanced release of VEGF, which further alters the properties of endothelial cells and promote the initiation of angiogenesis and lymphangiogenesis. (c) The higher permeability of the newly formed blood vessels and lymphatic capillaries drive an abnormal interstitial flow, and the increased stiffness of primary tumors leading to solid stress *in vivo*. Both factors promote the detachment of tumor cells from the primary site and penetration into the ECM. (d) Tumor cells migrate along the chemo-attractant, break the basement membrane, and enter the nearby blood vessel. Some of the tumor cells circulating in the vascular system adhere to the blood vessels of the local tissues and eventually form a secondary tumor.[2,4,5]

Table 1. Comparison between 2D cultures, 3D *in vitro* cultures and *in vivo* models.

	Morphology	Proliferation	Motility	Stiffness of matrix	Gradient	Cell–ECM interaction	Cell–cell interaction
2D culture on surface	unable to form tissue-like structure	usually faster than 3D *in vitro*	migration is limited to one plane	depending on substrata (several GPa)	no gradient	interact with attached surface	possible in co-culture
3D *in vitro* culture	varies depending on the scaffold	depend on biophysical and biochemical properties of scaffold	migration in 3D scaffold	depending on matrix (hundred Pa to MPa)	depending on diffusion of nutrient through matrix	interact with surrounding scaffold	possible in co-culture
In vivo model	varies depending on the tissue	altered by tissues	migration in surrounded tissue	depending on tissues (hundred Pa-MPa (normal tissue), >10 Mpa (skeletal tissue))	depending on vascular supply and dynamic spatial gradient	interact with surrounding ECM	interact with endothelial, fibroblast, and myoepithelial cells

The 3-D culture models bridge the gap between *in vitro* and *in vivo* experiments.[7,8,13] They involve a microenvironment that entraps cells by two main mechanisms: 1) diffusion of bioactive molecules through the matrix, and 2) matrix-immobilized bioactive molecules. In 3-D culture models, tumor cells are grown within a scaffold (matrix) whose thickness is at least one order of cell diameter to mimic in situ tissues.[7,15,19,20] The *in vitro* extracellular matrix (ECM) mimics the *in vivo* tissues around the tumor cells.[7,10,19,21,22] The matrix can protect cells from environmental disturbances and facilitate the adhesion, extending processes, and migration of cells. The porous matrix provides optimal permeability for both nutrient and waste diffusion, and supports diffusion-mediated radial gradients.[16,18,23] Each factor of the tumor mi-

croenvironment can be manipulated in 3-D models, such as the ECM properties, gradients of soluble factors and oxygen, cell–ECM communication, cell–cell interactions, and shear stress effects.[24–28] 3-D *in vitro* models have several advanced features compared with both 2-D and *in vivo* models (Table 1).

In this review, the differences between 2-D and 3-D culture models are summarized, and the effects of physical parameters on cell behavior that can be quantitatively analyzed in 3-D metastatic models are introduced. These parameters include the structure of the microfluidic setup, mechanical properties of 3-D scaffolds, soluble factors gradients, fluid shear stress, as well as the interaction of tumor cells with their environment and neighboring cells.

2. The influence of mechanical parameters on metastasis

2.1. Microfluidic platform

Various 3-D culture models have been developed for cancer research, including self-aggregation, cellular multilayer, matrix-embedded culture, hollow-fiber bioreactor, microfabricated device, and *ex vivo* culture.[11,31,35] A reliable and efficient 3-D culture platform requires robustness, high throughput, reproducibility, and low cost.[11,29] As shown in Fig. 2, there are micropatterned chips that dicate the size and distirbution of integrin-mediated adhesions (Figs. 2(a) and 2(b)), sub-micrometer (Fig. 2(c)) and nanotopographic (Figs. 2(e) and 2(f)) arrays of silicon posts, and numerous designer hydrogels systems that are used to study the effects of ECM sitffness on cells (Fig. 2(d)). Moreover, microfluidic devices based on soft lithography are uesd to study polarization, migration, and chemotaxis of tumor cells under gradients (Figs. 2(g) and 2(h)).[97] Poly (dimethylsiloxane) (PDMS) based microfluidic devices scale down the 3-D culture platforms, since PDMS is a biocompatible material with high gas permeability.[30] Therefore, these microfluidic devices provide more efficient culture platforms to investigate complex mechanisms.[5] The microfluidic chips have been used for circulating tumor cell (CTCs) detection, 3D culture of single-cell and co-culture spheroids, high-throughput anti-cancer drug screening, and molecular diagnosis. They also have the potential for "organ-on-a-chip" testing.[8,31] These user-defined assays are established by incorporating microflow into complex 3-D scaffolds. They allow specific control of the microenvironment, establishment of precise, parallel, and stable gradients, and real-time observation of tumor cell migration.[31–35] However, several challenges remain that require future research, such as surface modification and mass production problems.

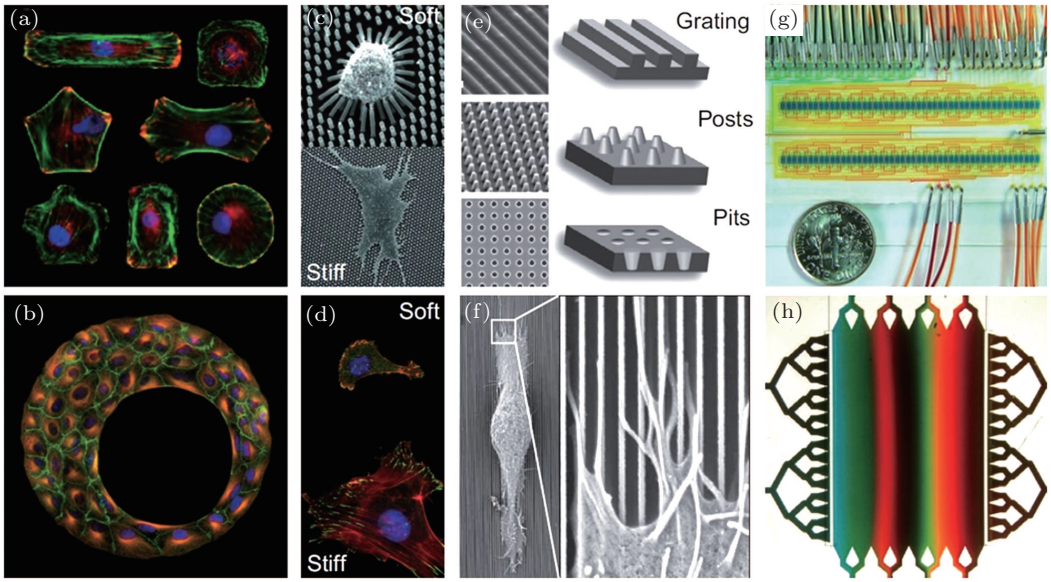


Fig. 2. (a) Design of microenvironments that capture the features of 3-D culture. Micropatterned substrates are presented to precisely control the cell shape and cytoskeletal architecture (b) as well as multicellular organization, without altering the physical or chemical attributes of the microenvironment. (c) Arrays of silicon posts of sub-micrometer diameter are used to study the effects of ECM sitffness on tumor cells. (d) Similary, numerous designer hydrogel systems can be differentially crosslinked to alter substrate stiffness and used to study the effects of ECM sitffness on cells. (e) and (f) Nanotopographic posts are also designed to sustain the size and geomery of cell-matrix adhesions. (g) and (h) In addition, microfluidic devices by which the soluble enviroment can be stringently defined for polarization study, migration, and chemotaxis of tumor cells under gradients are also designed.[97]

2.2. ECM and hydrogels

In living organisms, the majority of cells are embedded in the ECM, a bioactive hydrogel scaffold with very complicated compositions. ECM is primarily composed of crosslinked biomacromolecules, such as laminin, fibronectin, vitronectin, collagen, proteoglycans, and elastin.[39,40] These

molecules are secreted by cells and assembled into complicated networks. Fibrous proteins such as fibronectin, collagen, and laminin are the major components of the ECM backbone.[36–38] The physical properties of ECM, such as the composition, stiffness, topography, elastic behavior, fiber morphology, pore size, and non-linear deformability have been reported to be highly correlated with the growth of tumor cells. Specifically, they impact aspects of signaling pathways and cell behavior, particularly invasion, proliferation rate, as well as the overall mode of cell migration.[39–41]

The ECM consists of two parts, namely the basement membrane and interstitial matrix.[42,43] The basement membrane is composed of matrix proteins (such as collagen IV, fibronectin) and linker proteins. Linker proteins connect matrix proteins to form the compact porous membrane, which separates the epithelial cells from the stroma. The interstitial matrix is mainly composed of fibrillar collagens, which support the tissue structure and are involved in biochemical interactions, such as the interactions with chemokines and growth factors. To date, Matrigel and collagen are the most commonly used two types of ECM in *in vitro* studies.

Matrigel Matrigel (BD, USA) is a negatively charged basement membrane that can act as charged molecular sieves and affect cells indirectly by binding or sequestering cell growth differentiation factors. Currently, Matrigel is the most commonly used matrix for mimicking the *in vivo* basement membrane, which has complicated molecular compositions.[44] This commercially available basement membrane matrix is isolated from mouse tumors. It includes laminin, collagen IV, entactin, fibronectin, and growth factors. Matrigel is easy to use, since it is available in a liquid state at 4 °C and solidifies at 37 °C. Therefore, it is frequently used in *in vitro* 3-D studies of cancer cell motility, drug sensitivity, and controlled angiogenesis processes.[39]

Collagen There are several types of collagen in different tissues. However, the fibril forms in each collagen type are slightly different. Type I collagens are major polymers in interstitial matrix and important building blocks for normal fibrotic tissues. They are highly cross-linked and exhibit viscous and elastic behavior.[36,37] The viscoelastic and contractive properties of collagen can be quantitatively measured under an applied force such as tension, compression, shear, or a combination of these forces.[37]

Collagen I can easily be isolated from biological sources and can be customized. The mechanical properties of collagen I such as pore size, ligand density, cross link, and collagen fiber arrangement can be altered by either changing the protein concentration or introducing chemical crosslinking compounds.[41,42,45] Collagen I is biocompatible and biodegradable. It is widely used for both micro- and macroscale cell culture, and for studying cell motility and the role of the physical state of the 3-D matrix *in vitro*, and is a promising material for tissue engineering.[40,46]

The mechanical properties of the tumor cell microenvironment, such as the porosity, rigidity, and solubility, are influenced by both the basement membrane and interstitial matrix. These mechanical and physical factors can affect cell differentiation and migration directly through mechanical signaling transduction within cells as well as indirectly through regulating the diffusion of biochemical signals.[47–49] Therefore, collagen I or Matrigel is usually utilized to mimic ECM *in vitro* to explore the mechanisms of the interactions between tumor cells and their microenvironment.

2.3. ECM physical properties and functions

The physical properties of the microenvironment surrounding tumor cells are crucial for cell behavior, and can dominate the inductive processes associated with tissue morphogenesis, homeostasis, and regeneration.[36,44,50] However, it is discouraging that there are very few systematic studies on biophysical stimulations of ECM-cell interaction. This is probably due to the difficulty in conducting these studies on different body tissues with tremendous physical differentiation. Furthermore, pure ECM is not currently available for creating matrices *in vitro*. As such, natural hydrogels are adapted as ECM substitutes for *in vitro* studies, such as collagen, Matrigel, and laminin.

The detailed mechanism of the ECM-tumor cell interaction is still unclear. It has been demonstrated that ECM stiffness and position (in 2D or 3D model) are correlate with cell signaling and other functions.[35,39,51] Various protein densities, cross-linking state, matrix pore sizes, and fibril architecture can also affect the cell migration rate and invasive phenotype.[35,39,46,49,52–54]

According to creep mechanisms,[39,46] tumor cells might be able to migrate through the entangled matrix network by fiber dislocation only. The change of matrix protein density results in concurrent ligand density changes and alters the cell migration rate. It also potentially changes the pore size and fiber architecture of the matrix, which indirectly influences the invasive phenotype.

By increasing the cross-linking density of the matrix, parameters such as pore size, density, or spacing of adhesion sites are concomitantly decreased. Small pore sizes limit the cell migration rate and cell morphology by restricting movement of the cell nucleus along matrix fibrils.

Hydrogels with condensed and fine fiber architecture have small pore size and high ligand density. In this case, pericellular proteolysis may be the rate-limiting step in cell migration, since it is difficult for cells to escape through the pores within the hydrogels.

However, all of the above conclusions are obtained from hydrogels with constant concentration, and it is not entirely

the same *in vivo*. In future, systematic studies of ECM-tumor cell interactions should focus more on mimicking the *in vivo* microenvironment by using spatially non-random oriented and concentration gradient ECM.

2.4. Gradients

Chemokines and growth factors are secreted by tumor cells, leukocytes, and surrounding stromal cells. The distribution of these factors in the tumor microenvironment is spatially and temporally heterogeneous.[55–57] Chemokine- and growth factor-induced chemotaxis guide tumor cells along chemoattractant gradients *in vivo*. The chemoattractant gradients play critical roles in tumor progression and facilitate several crucial steps of metastasis, including tumor cell survival, proliferation, differentiation, angiogenesis, intravasation, extravasation, and metastatic colonization.[55–59]

Although both chemokines and growth factors can induce metastasis, their mechanisms are significantly different. Chemokines and chemokine receptors are downstream genetic events that cause neoplastic transformation. The chemokine gradient of the target organ milieu triggers the tumor cells with high expression of the unique chemokine receptor. This chemotaxis process repeats as tumor cells sense increasing concentrations of the ligand gradient. As a result, the cells move toward high chemokine concentration and stop when the concentration of the ligand saturates the receptors.[34,60–65] In the presence of growth factors, tumor cells display chemokinesis and cell motility is enhanced in random directions *in vitro*.[33,34,59,66,67] In addition to the chemokine gradient, the oxygen and cellular pH gradients are also particularly important for tumor migration.[32,68,69]

Several attempts have been made to mimic the chemoattractant gradient in the 3-D *in vitro* microfluidic model.[32] These approaches fill the gap between *in vitro* 2-D cell culture models and animal models. Hydrogel scaffold is used in 3-D models to mimic the ECM, in which stable linear chemoattractant gradients in the hydrogel scaffold are generated.[32–34] This 3-D mimicking platform offers a few advantages such as: (i) a well-established and maintained chemical gradient in space and time, (ii) capability for real-time assay for dynamic studies, and (iii) the ability to differentiate chemotaxis from chemokinesis. The diffusion of chemoattractants in hydrogel can be simulated using Ficks law[34]

$$\frac{\partial c}{\partial t}+\nabla\cdot(-D\nabla c)=0, \tag{1}$$

where c is the concentration of chemoattractants and D is the diffusion coefficient. Microfluidic linear gradients are subjected to the degree of diffusion. Precise control of the *in vitro* microenvironment enables quantitative analysis of different chemoattractants to cell migration. This 3-D microfluidic model has been used in the study of cellular chemotactic migration, angiogenesis, and cell–cell interactions. It can also help improve the study of fundamental signaling pathways that regulate cellular and subcellular behaviors.

2.5. Shear stress

Shear stress is prominent in all mechanical forces during cancer metastasis. There are two major sources of shear stress: 1) from slow interstitial flow across the tumor microenvironment, and 2) from blood flow in the vascular microenvironment.[2,4] Shear stress generated by both blood and interstitial flow can impact cancer cell growth, proliferation, and behavior, which all contribute to the physiological and pathological processes of cancer metastasis.

2.5.1. Interstitial flow

Interstitial flow often associates with lymphatic drainage which returns plasma leaked out from the capillaries into the blood vascular system.[2] In living tissues, interstitial flow is constant and slow. It is driven by interstitial fluid pressure, which is caused by blood pressure, ECM composition, cell density, and tissue metabolism. When stroma is transitioning to a cancerous niche, the stiffness of the tumor and surrounding tissue increases, which further enhances tumor cell growth, proliferation, and migration. The increase in proliferation of tumor cells leads to hypoxia and abnormal interstitial flow within the tumor microenvironment. Vessel occlusion caused by both the increased interstitial flow and hypoxia up-regulates the release of VEGF, which initiates angiogenesis and lymphangiogenesis.[2–4] The newly formed blood vessels and lymphatic capillaries with higher permeability promote vascular leakage, which further increases interstitial flow, leading to an increase in angiogenesis and lymphangiogenesis again as a positive feedback loop.

Besides increasing angiogenesis and lymphangiogenesis, interstitial flow also induces tumor cell invasion by chemotaxis. The chemokines (such as CCR7 and CCL21) secreted by tumor cells are drained from the higher fluid pressure toward the lower pressure recruited lymphatic system to create weak chemokine gradients across the tumor.[2] Cells from high-pressure regions inside the tumor migrate chemotactically following the direction of interstitial flow and begin to invade to metastatic sites.

2.5.2. Blood flow

In the metastatic process, tumor cells release proteases that break down the basement membrane and intravasate through monolayer endothelium. Once in bloodstream circulation, the trajectories of CTCs are influenced by several chemical, physical, and mechanical factors, including immunological stress, blood cell collisions, blood flow pattern, and hemodynamic shear forces.[2,4,5,70–73] All of these factors can affect tumor cell survival and proliferation. In addition

to the influence on tumor cell viability, blood flow also alters the expression of proteins that affect the complex interplay (receptor–ligand interactions) between CTCs and endothelial cells, which is a crucial step for subsequent tumor cell extravasation and metastasis. Studies in experimental models demonstrate that the attrition rate of potential metastatic cells often exceeds 99% during the invasion-metastasis cascade.[1]

3. Cell–cell and cell–ECM interactions

3.1. Cell–ECM interaction

When cancer cells are invading the ECM, they can sense the physical properties of their surrounding environment and pull the ECM using intracellular contractile derived from actin–myosin interactions. Each kind of ECM has its own physical properties based on the specific components and structures.[74] The deformation of ECM is a combinational result of ECM stiffness and the forces exerted by cancer cells. Due to the interconnected mesh structure of the ECM, the tension or adhesion of tumor cells can be rapidly transmitted to distant sites. In softer ECM, tumor cells pull the fibril components of the ECM toward themselves, while in stiffer ECM, the intensive ECM fiber increases the contractility of tumor cells and facilitates focal adhesion of cells to ECM.[75–80] Mammary epithelial cells cultured in 3-D collagen at low concentration, which is softer, present a polarized acinar and ductal structure. However, with a higher concentration of collagen, which means higher stiffness, the cells lose polarization and become invasive.[74] Besides the tension and adhesion forces, the fiber orientation of the ECM and the chemical gradient formed in the ECM also alter cell tractions along the specific migration pathways and further influence the efficiency of cancer cell invasion.

3.2. Cell–cell interaction

Besides the interactions between tumor cells and ECM, there are strong interactions among tumor cells, which lead to different invasion morphology *in vivo*. There are three kinds of morphology that occur during metastasis: single-cell invasion, multi-cellular streaming invasion, and collective invasion (Fig. 3).[91]

3.2.1. Single-cell invasion

During the process of single-cell invasion, there is no cell–cell adhesion. The motive force of cancer cells comes from several sources, including the adhesion of the cell to the ECM, the contractility of the cell cytoskeleton, and the biochemical degradation of the ECM. There are two invasion modes in single-cell invasion: mesenchymal invasion and amoeboid invasion.

Mesenchymal invasion mode is similar to the movement on rigid 2-D substrates. Statistically, 10% to 40% of the cancer cells undergo the process of epithelial mesenchymal transition (EMT) and invade ECM in mesenchymal invasion mode.[81] During this invasion process, cancer cells first generate actin-rich protrusion, and then elongate their morphology through extension of the protrusion, which leads to cell polarity. They finally excrete proteases to degrade the ECM proteins.[82,83] Meanwhile, new protrusions attach to the ECM and further develop into large focal adhesions. The actomyosin contractile force of the cell lagging tail can be transmitted to other parts of the ECM, which then speed up cell body movements guided

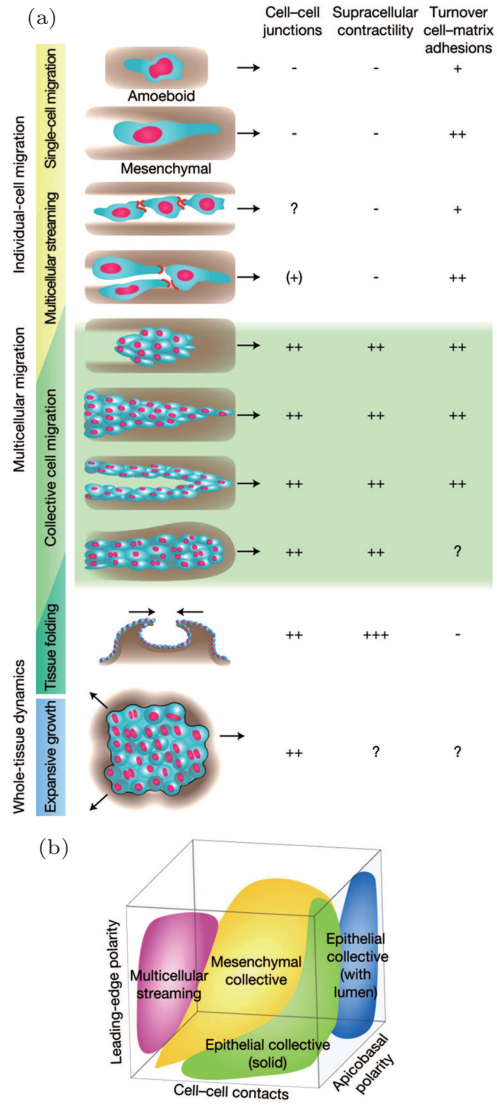


Fig. 3. (a) A schematic diagram of invasion patterns, including amoeboid or mesenchymal single-cell migration, multicellular streaming with or without weak junctional contacts, collective cell migration, tissue folding and expansive growth. Although each pattern can be described as a distinct process, overlapping or converting cell behavior can result in mixed or unstable phenotypes. The intensity range of proposed properties (from absent to strong) is indicated by minus and plus signs. (b) Relationships between leading-edge polarity, cell–cell adhesion, and apicobasal polarity in multicellular migration patterns. The parameters are plotted as a 3D cube to reflect the multiple ways in which transitions between modes of invasion can occur, with the origin in the lower left corner. A determinant not included in the diagram is the stability of cell–substratum interactions, which may also dictate leading-edge polarity and migration mode.[91]

by the cell leading edge.[84] During the protrusion extension process, activity of the RhoA protein and its effectors ROCK1 and ROCK2 are reduced to extend protrusions at the front of the cell. They are also helpful in promoting retraction of the lagging tail that neutralizes the overall inhibiting effect.[85–87]

Another single-cell invasion mode is amoeboid invasion. This strategy is adopted from several species in the nature, such as the zebrafish macrophage, some stem cells, leukocytes, dendritic cells, dictyostelium, and discoideum. In this mode of invasion, receptors sense a chemokine gradient and trigger actin polymerization, which may spread through the cell cortex in a wave.[88] The Rock-dependent contractile force in cortical actin generates hydrostatic pressure and promotes the remodeling of cell shape.[89] Compared with the mesenchymal invasion, cells moving by amoeboid morphology can squeeze into the interconnected mesh structure of ECM without excreting the protease. Cell-matrix adhesion and proteolysis of amoeboid morphology cells are much weaker than that of mesenchymal morphology cells as a result of the uniform distribution of integrin.

3.2.2. Multicellular streaming invasion

Multicellular streaming invasion has been observed in orthotopic breast cancer, melanoma models neural crest cells, fibroblast, etc.[90] In multicellular streaming invasion mode, many single cells exhibit the same moving direction that is caused by oriented extracellular guidance structures or the chemokine gradient, and therefore multicellular streaming has a higher invasion speed. The cytoskeleton of each cell generates traction force in the ECM independently. However, the adhesion between cells is intermittent or less stable which leads to a loose tail-to-head cell–cell junction.[91]

3.2.3. Collective invasion

Collective invasion has been observed in Dictyostelium at slug stage, lateral line (zebrafish), border cells (Drosophila egg chamber), sprouting vessels, many epithelial and some other cancer types. Cell–cell junctions in single-cell, mutilcellular streaming, and collective invasion determine the invasion mode of cells in ECM. The collective invasion mode is similar to the collective form of mesenchymal invasion mode in which receptors of the leading cells sense the chemokine gradient and stretch the actomyosin-mediated protrusion. Together with the protrusions stretched by the cells at lateral regions of the group, the front cells generate traction force and contractility. At the same time, the front cells produce proteases to degrade the mesh structural proteins of the ECM and generate a path for subsequent cells.[92] During this process, the leading cells retain intact cell–cell junctions with the following cells, to maintain highly efficient paracrine cell–cell signaling. Cadherins, tight junction proteins, adhesion receptors of the immunoglobulin superfamily, gap junctions, and cytoskeletal synchronization participate in this process.[93–95] However, the precise molecular and mechanical mechanisms are still poorly understood and need to be explored in the future.

The geography, physical properties, and biochemical components of the ECM vary *in vivo*, thus various types of invasion morphology can be observed in a single tumor. The three kinds of invasion modes can convert to each other in certain situations. The ability of cancer cells to switch among different invasion modes brings a great challenge for preventing the invasion of cancer cells.

4. Current *in vitro* 3-D models

4.1. Enhanced invasion of metastatic cancer cells via the ECM interface

Currently, it is hard to conduct quantitative studies on multicellular and collective invasion since the current *in vitro* models need further development. Environmental heterogeneity, which facilitates tumor cell invasion has been systematically investigated via a combination of *in vitro* cell migration experiments and computer simulations.[96] This work focuses on setting up a 3-D ECM model and mimicking collective cell behavior. A double-groove equipment with a hollow cylindrical tunnel was designed (Fig. 4(a)). It combines joined force and different Matrigel curing times to generate an ECM interface for the invasion of MDA-MB-231 breast cancer cells (Fig. 4(b)). The invasion process of MDA-MB-231 on the interface can be separated into three steps. First, MDA-MB-231

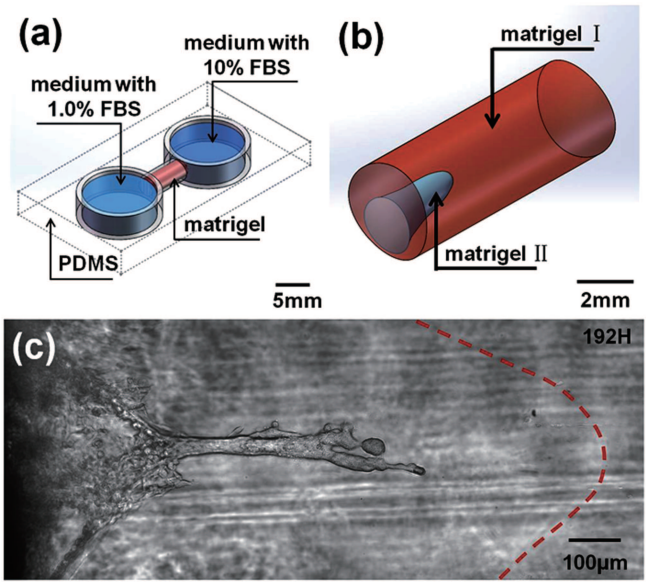


Fig. 4. (a) Diagram sketch of the PDMS chip. The horizontal cylinder channel between the two chambers is filled with matrigel (red). The left chamber is filled with the medium with 1.0% FBS, and the right chamber is filled with the medium with 10.0% FBS. (b) The picture shows the heterogeneous matrigel composed of matrigel I (red) and matrigel II (blue). The two sections form a funnel-like 3-D interface. (c) After the partial interface was filled with cells, the frontier cells escaped from interface confinement and produced finger-like invasions in the homogenous Matrigel, confirming the strong invasion of the MDA-MB-231 cells in heterogeneous gel space.[96]

invaded the Matrigel in a pattern of thin streamlines, which are mainly guided by the curved interface. Second, more cell streamlines appear at the interface, and cell–cell signaling help invaded cell streams communicate with each other and connect together forming cell networks. Finally, cell networks and a "finger" shape collective invasion are generated at the front (Fig. 4(c)). This phenomenon is induced by effects of the Matrigel interface structures, the cell–cell communication, and the guidance of a fetal bovine serum (FBS) growth factor gradient. In this model, the cell invasion microenvironment acts as a heterogeneous landscape that greatly affects cell invasive behavior and the overall cell invasion patterns. This research serves as a platform for further studies on the molecular and mechanical mechanisms of the collective invasion mode *in vitro*.

4.2. A 3-D *in vitro* metastasis model

Metastasis is a multi-step cell-biological process referred to as the invasion-metastasis cascade, and is driven by genetic and/or epigenetic alternations within the tumor, the corporation of neoplastic stromal cells, and the tumor microenvironment. Therefore, an *in vitro* model composed of intravasation and extravasation sections was generated (Fig. 5(a)) to mimic the metastatic process and quantitatively investigate the effect of mechanical parameters on metastasis, including multiple gradients, shear stress, and other interaction and adhesion between cell–cell and cell–ECM. The intravasation section (Fig. 5(b)) has successfully been used for studying the guidance of the FBS gradient during cell invasion *in vitro*. The intravasation model is composed of two medium channels and one ECM hydrogel channel (Fig. 5(b)). After hydrogel was injected into and cured in the channel, the fluorescent dye dextran-rhodamine and distilled water were injected into the upper and lower channels separately to establish a gradient that can simulate the FBS gradient. The gradient profiles (Fig. 5(c)) are represented by the dextran-rhodamine fluorescent intensities at specific locations. The results indicated that the gradient could be maintained for up to 24 h. Additionally, MDA-MB-231 cells were placed in the lower chamber and eventually invaded the ECM hydrogel. In the homogenous 10% serum environment, tumor cell invasion was mainly driven by population stress (spatial stress) (Fig. 5(d)). While in the gradient environment (1%–10% serum gradient) (Fig. 5(e)), cell invasion was apparently driven by growth factor attraction, which also resulted in an increase in the average cell invasion speed.

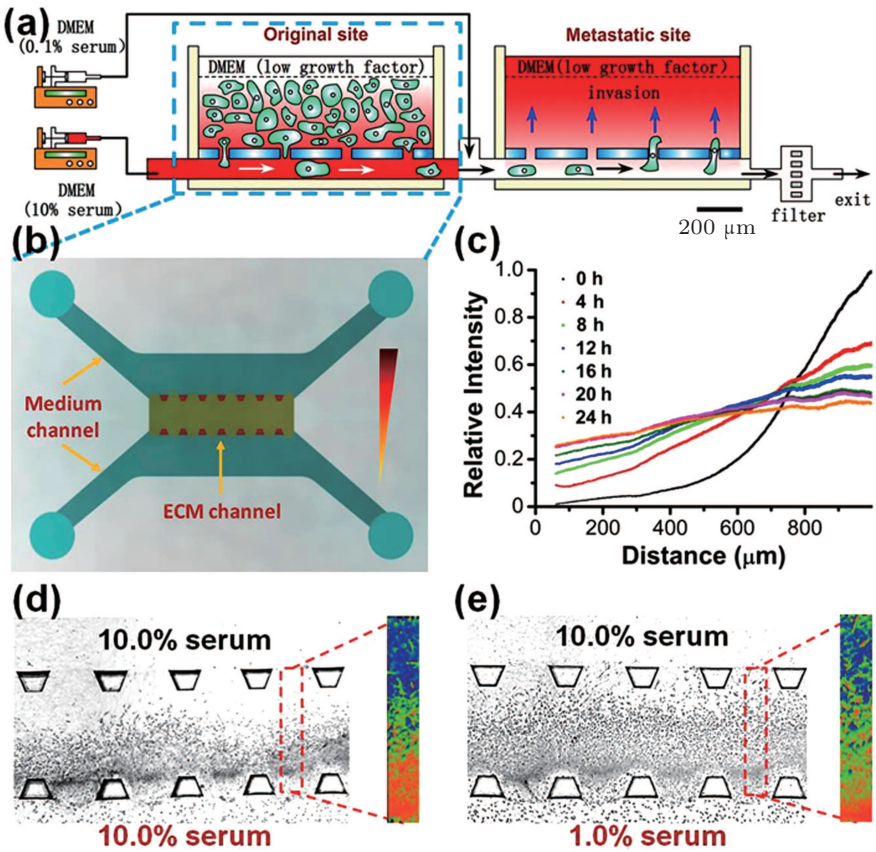


Fig. 5. (a) Schematic diagram of a full *in vitro* metastasis model. The model is composed of intravasation and extravasation sections. (b) The scheme shows that the intravasation model is composed of two medium channels and an ECM channel that is designed for chemo-attractant gradient studies. (c) Quantitative analysis of the dextran-rhodamine gradient in the time- and space-dependent establishment in ECM. The gradient remained stable after 24 h. (d) and (e) MDA-MB-231 cells invade the ECM in a homogenous 10% serum and gradient environment (1%–10% serum gradient) separately, indicating that MDA-MB-231 cells are sensitive to the FBS chemical gradient and move towards higher concentrations.

5. Conclusions and perspectives

The 3-D *in vitro* models function as a more physiologically relevant, human tissue specific, consistent, and reliable 3-D microenvironment for cancer metastasis research, which is fundamentally lost in the conventional planar 2-D petri dish system. These biologically relevant models encompass the complex interplay of several key biophysical and biochemical factors in cell–cell and cell–ECM interactions, such as gradients of soluble factors, microstructure and mechanical properties of the ECM, cell force, interstitial, and shear flow, which can regulate cell proliferation, differentiation, migration, and apoptosis. As such, 3-D *in vitro* models will shift the paradigms of semiquantitative/quantitative metastasis studies and will make predictive precision better. In addition, 3-D models have the potential to work as high-throughput, low-cost, highly reproducible, and point-of-care devices for cancer diagnosis and chemotherapeutic drug testing, which are currently under development for the design of personalized medicine.[19–22]

Although the emerging 3-D models for cancer diagnosis and therapy are a very promising technology, they also bring new challenges to the current technology and research platforms for future studies.

(I) Longer culturing times and larger 3-D volume Current 3-D models can usually only keep cells active in a small volume of ECM (less than 1 mL for 1–2 weeks), while many metastasis processes can only be displayed in large volumes and months at a time. The limitations come from the difficulties in controlling gas exchange, diffusion of soluble nutrients, and chemical agents in a solid 3-D matrix. To solve these problems, a profound understanding of long-term cell behavior, new mechanical designs, and synthetic biopolymer scaffolds are needed.[98–100]

(II) Micro-manipulating and imaging methods for 3-D models Conventional microscopy, biologist eyes become almost blind in three dimensions because they can only work in a very short work distance (less than 1 mm). In 2-D they can see cell mobility, cell morphology, sub-cellular organelles and even protein activity, but in 3-D even identifying the cell nucleus can be problematic, as well as tracking cell mobility. Two-photon microscopy allows a relatively larger penetration depth, but its scanning light intensity limits its applicability for 3-D model visualization. Light sheet microscopy and optical tomography techniques have emerged in recent years, and are especially suitable for imaging large 3D samples. Therefore, they partially mitigate these problems, however; further improvement will be required.[101–103]

Unlike the common requirement of observing cells in a 3-D matrix, cell manipulation is less common but necessary. Current cell manipulation tools such as the microinjection pipette, optical tweezers, and magnetic tweezers work well in 2-D but whether they will work in a 3-D matrix is not fully understood. Tools that can manipulate specific cells inside a 3-D matrix without disturbing its surrounding cells do not yet exist.

(III) Force measurement and characterization of micro/nano-mechanical properties Mechanical forces modulate cell adhesion and motility and thus metastasis in the 3-D extracellular matrix. Precise measurement of these forces and the micro/nano- mechanical properties of the matrix are essential to understanding their interplay in both physiological and pathological processes. Tools like atomic force microscope (AFM) using stiff cantilever and 2D traction microscopy using soft pillar arrays have been utilized to study surface mechanical properties and forces that act on substrates. However, these cannot measure 3-D traction forces exerted by cells encapsulated in ECM. By simultaneously 3-D tracking hundreds to thousands of embedded fluorescence labeled beads in matrix, measurement of matrix deformation or force exerted by cells is possible, although major challenges still exist.[104,105]

(IV) Universal platform and standard protocols Unlike well-established 2-D models that have a universal set-up, standard protocols, and sophisticated automated processes, current 3-D models are task-oriented and their set-up protocols are highly laboratory specific. This limits their accessibility to the broader researcher and makes research results less comparable between research groups. Fortunately, techniques like 3-D cell printing[106–108] will improve such situations tremendously.

References

[1] Valastyan S and Weinberg R A 2011 *Cell* **147** 275
[2] Wirtz D, Konstantopoulos K and Searson P C 2011 *Nat. Rev. Cancer* **11** 512
[3] Swartz M A and Fleury M E 2007 *Annu. Rev. Biomed. Eng.* **9** 229
[4] Koumoutsakos P, Pivkin I and Milde F 2013 *Annu. Rev. Fluid Mech.* **45** 325
[5] Zhang Z and Nagrath S 2013 *Biomed. Microdevices* **15** 595
[6] Caron M M, Emans P J, Coolsen M M, Voss L, Surtel D A, Cremers A, van Rhijn L W and Welting T J 2012 *Osteoarthritis Cartilage* **20** 1170
[7] Wenner W 1998 *Grand Valley Rev.* **18** 6
[8] Page H, Flood P and Reynaud E G 2013 *Cell Tissue Res.* **352** 123
[9] Baker B M and Chen C S 2012 *J. Cell. Sci.* **125** 3015
[10] Gurski L A, Petrelli N J, Jia X and Farach-Carson M C 2010 *Oncology Issues* **25** 20
[11] Sung K E, Su X, Berthier E, Pehlke C, Friedl A and Beebe D J 2013 *PloS One* **8** e76373
[12] Meng X, Leslie P, Zhang Y and Dong J 2014 *Springer Plus* **3** 80
[13] Pampaloni F, Reynaud E G and Stelzer E H K 2007 *Nat. Rev. Mol. Cell Biol.* **8** 839
[14] Meyer A S, Hughes-Alford S K, Kay J E, Castillo A, Wells A, Gertler F B and Lauffenburger D A 2012 *J. Cell Biol.* **197** 721
[15] Liu W, Bergenstock M K, Lau W, Sun W and Liu Q 2008 *Proceedings of the 2nd World Congress on Tissue Engineering and Regenerative Medicine* 2008
[16] Corall S, Haraszti T, Bartoschik T, Spatz J P, Ludwig T and Cavalcanti-Adam E A 2014 *Comput. Mech.* **53** 499
[17] Wu J, Voytik-Harbin S L, Filmer D L, Hoffman C M, Yuan B, Chiang C S, Sturgis J and Robinson J P *Proc. SPIE* 4621 (*Three-Dimensional and Multidimensional Microscopy: Image Acquisition and Processing IX*, 52, May 17, 2002)

[18] Fischbach C, Kong H J, Hsiong S X, Evangelista M B, Yuen W and Mooney D J 2009 *Proc. Natl. Acad. Sci. USA* **106** 399
[19] Doyle A D, Petrie R J, Kutys M L and Yamada K M 2013 *Curr. Opin. Cell Biol.* **25** 642
[20] Tibbitt M W and Anseth K S 2009 *Biotechnol. Bioeng.* **103** 655
[21] Longati P, Jia X, Eimer J, Wagman A, Witt M R, Rehnmark S, Verbeke C, Toftgard R, Lohr M and Heuchel R L 2013 *BMC Cancer* **13** 95
[22] Li L and Lu Y 2011 *J. Cancer* **2** 458
[23] Fallica B, Maffei J S, Villa S, Makin G and Zaman M 2012 *PloS One* **7** e48024
[24] Stegemann J P, Hong H and Nerem R M 2005 *J. Appl. Physiol.* **98** 2321
[25] Richard J and Shemin S F 2012 *Regen. Med.* **2** 1
[26] Wang L and Carrier R L 2011 *Biomimetic Topography: Bioinspired Cell Culture Substrates and Scaffolds*, in: *Advances in Biomimetics* (INTECH Open Access Publisher)
[27] Rhee S 2009 *Exp. Mol. Med.* **41** 858
[28] Pathak A and Kumar S 2011 *Integr. Biol. (Camb)* **3** 267
[29] Lee P, Lin R, Moon J and Lee L P 2006 *Biomed. Microdevices* **8** 35
[30] Gomez-Sjoberg R, Leyrat A A, Houseman B T, Shokat K and Quake S R 2010 *Anal. Chem.* **82** 35
[31] Sung K E, Su G, Pehlke C, Trier S M, Eliceiri K W, Keely P J, Friedl A and Beebe D J 2009 *Biomaterials* **30** 4833
[32] Shiwa T, Uchida H and Tsukada K 2012 *Am. J. Biomed. Eng.* **2** 175
[33] Saadi W, Wang S J, Lin F and Jeon N L 2006 *Biomed. Microdevices* **8** 109
[34] Kim B J, Hannanta-anan P, Chau M, Kim Y S, Swartz M A and Wu M 2013 *PloS One* **8** e68422
[35] Fischer R S, Myers K A, Gardel M L and Waterman C M 2012 *Nat. Protoc.* **7** 2056
[36] Cukierman E, Pankov R and Yamada K M 2002 *Curr. Opin. Cell Biol.* **14** 633
[37] Yamada S, Yamamoto K, Ikeda T, Yanagiguchi K and Hayashi Y 2014 *Biomed. Res. Int.* **2014** 302932
[38] Norman J J, Collins J M, Sharma S, Russell B and Desai T A 2008 *Tissue Engineering Part A* **14** 379
[39] Wells R G 2008 *Hepatology* **47** 1394
[40] Artym V V, Yamada K M and Mueller S C 2009 *Extracellular Matrix Protocols, Volume* 522 *of the Series Methods in Molecular Biology* pp. 211–219
[41] Frantz C, Stewart K M and Weaver V M 2010 *J. Cell. Sci.* **123** 4195
[42] LaPlaca M C, Vernekar V N, Shoemaker J T and Cullen D K 2010 *Three-dimensional Neuronal Cultures Methods in Bioengineering:* 3*D Tissue Engineering* (Norwood: MA: Artech House) pp. 187–204
[43] Ebong I M 2007 *Three-dimensional Extracellular Matrix Hydrogel Environments for Embryonic Stem Cell Growth* (Georgia: Georgia Institute of Technology)
[44] Dewitt D D, Kaszuba S N, Thompson D M and Stegemann J P 2009 *Tissue Engineering Part A* **15** 2785
[45] Ulrich T A, Jain A, Tanner K, MacKay J L and Kumar S 2010 *Biomaterials* **31** 1875
[46] Schedin P and Keely P J 2011 *Cold Spring Harb. Perspect. Biol.* **3** a003228
[47] Ward M, McCann C, de Wulf M, Wu J Y and Rao Y 2003 *J. Neurosci.* **23** 5170
[48] Nemir S and West J L 2010 *Ann. Biomed. Eng.* **38** 2
[49] Kong Y P, Carrion B, Singh R K and Putnam A J 2013 *Sci. Rep.* **3** 3474
[50] Rehfeldt F, Engler A J, Eckhardt A, Ahmed F and Discher D E 2007 *Adv. Drug. Deliv. Rev.* **59** 1329
[51] Trappmann B and Chen C S 2013 *Curr. Opin. Biotechnol.* **24** 948
[52] Raeber G P, Lutolf M P and Hubbell J A 2005 *Biophys. J.* **89** 1374
[53] Dikovsky D, Bianco-Peled H and Seliktar D 2008 *Biophys. J.* **94** 2914
[54] Sharma S, Cross S E, French S, Gonzalez O, Petzold O, Baker W, Walczak W, Yongsunthon R, Baker D and Gimzewski J K 2009 *J. Scanning Probe. Microscopy* **4** 7
[55] Raman D, Baugher P J, Thu Y M and Richmond A 2007 *Cancer Lett.* **256** 137
[56] Fosslien E 2008 *Annu. Clin. Lab. Sci.* **38** 307
[57] Strieter R M 2001 *Nat. Immunol.* **2** 285
[58] Kakinuma T and Hwang S T 2006 *J. Leukoc. Biol.* **79** 639
[59] Anderson A R A, Chaplain M A J, García-Reimbert C and Vargas C A 2000 *Math. Comput. Model* **32** 1141
[60] Iijima M and Devreotes P 2002 *Cell* **109** 599
[61] Brown C E, Vishwanath R P, Aguilar B, Starr R, Najbauer J, Aboody K S and Jensen M C 2007 *J. Immunol.* **179** 3332
[62] Aldinucci D and Colombatti A 2014 *Mediators Inflamm.* **2014** 292376
[63] Richmond A 2008 *Clin. Cancer Res.* **14** 621
[64] Mantovani A, Savino B, Locati M, Zammataro L, Allavena P and Bonecchi R 2010 *Cytokine Growth Factor Rev.* **21** 27
[65] Engl T, Relja B, Marian D, Blumenberg C, Muller I, Beecken W D, Jones J, Ringel E M, Bereiter-Hahn J and Jonas D 2006 *Neoplasia* **8** 290
[66] Brychtova V, Vojtesek B and Hrstka R 2011 *Cancer Lett.* **304** 1
[67] Rao S, Tata U, Lin V and Chiao J C 2014 *Micromachines* **5** 13
[68] Gerweck L E and Seetharaman K 1996 *Cancer Res.* **56** 1194
[69] Paradise R K, Whitfield M J, Lauffenburger D A and van Vliet K J 2013 *Exp. Cell Res.* **319** 487
[70] von Sengbusch A, Gassmann P, Fisch K M, Enns A, Nicolson G L and Haier J 2005 *Am. J. Pathol.* **166** 585
[71] Haier J and Nicolson G L 1999 *Clin. Exp. Metastasis* **17** 713
[72] Mitchell M J and King M R 2013 *Front. Oncol.* **3** 44
[73] Lee J S, Panorchan P, Hale C M, Khatau S B, Kole T P, Tseng Y and Wirtz D 2006 *J. Cell Sci.* **119** 1760
[74] Schedin P and Keely P J 2011 *CSH Perspect Biol.* **3** a003228
[75] Wozniak M A, Desai R, Solski P A, Der C J and Keely P J 2003 *J. Cell Biol.* **163** 583
[76] Paszek M J, Zahir N, Johnson K R, Lakins J N, Rozenberg G I, Gefen A, Reinhart-King C A, Margulies S S, Dembo M and Boettiger D 2005 *Cancer Cell* **8** 241
[77] Provenzano P P, Inman D R, Eliceiri K W and Keely P J 2009 *Oncogene* **28** 4326
[78] Chen C S, Mrksich M, Huang S, Whitesides G M and Ingber D E 1997 *Science* **276** 1425
[79] Wang H B, Dembo M, Hanks S K and Wang Y 2001 *Proc. Natl. Acad. Sci. USA* **98** 11295
[80] Discher D E, Janmey P and Wang Y 2005 *Science* **310** 1139
[81] Thiery J P 2002 *Nat. Rev. Cancer* **2** 442
[82] Friedl P and Wolf K 2003 *Nat. Rev. Cancer* **3** 362
[83] Friedl P 2004 *Curr. Opin. Cell Biol.* **16** 14
[84] Ridley A J, Schwartz M A, Burridge K, Firtel R A, Ginsberg M H, Borisy G, Parsons J T and Horwitz A R 2003 *Science* **302** 1704
[85] De Wever O, Nguyen Q D, van Hoorde L, Bracke M, Bruyneel E, Gespach C and Mareel M 2004 *FASEB J.* **18** 1016
[86] Vial E, Sahai E and Marshall C J 2003 *Cancer. Cell* **4** 67
[87] Etienne-Manneville S and Hall A 2001 *Cell* **106** 489
[88] Vicker M G 2002 *FEBS Lett.* **510** 5
[89] Sahai E 2005 *Curr. Opin. Genet. Dev.* **15** 87
[90] Roussos E T, Balsamo M, Alford S K, Wyckoff J B, Gligorijevic B, Wang Y, Pozzuto M, Stobezki R, Goswami S and Segall J E 2011 *J. Cell Sci.* **124** 2120
[91] Friedl P, Locker J, Sahai E and Segall J E 2012 *Nat. Cell Biol.* **14** 777
[92] Nabeshima K, Inoue T, Shimao Y and Sameshima T 2002 *Pathol. Int.* **52** 255
[93] Hegerfeldt Y, Tusch M, Brocker E B and Friedl P 2002 *Cancer Res.* **62** 2125
[94] Hidalgo-Carcedo C, Hooper S, Chaudhry S I, Williamson P, Harrington K, Leitinger B and Sahai E 2011 *Nat. Cell Biol.* **13** 49
[95] Ewald A J, Huebner R J, Palsdottir H, Lee J K, Perez M J, Jorgens D M, Tauscher A N, Cheung K J, Werb Z and Auer M 2012 *J. Cell Sci.* **125** 2638
[96] Zhu J, Liang L, Jiao Y and Liu L 2015 *PloS One* **10** e0118058
[97] Baker B M and Chen C S 2012 *J. Cell Sci.* **125** 3015
[98] Hutmacher D W 2010 *Nat. Methods* **9** 90
[99] Hutmacher D W, Horch R E, Loessner D, *et al.* 2009 *J. Cell Mol. Med.* **13** 1417
[100] Seliktar D 2012 *Science* **336** 1124
[101] Sharpe J, Ahlgren U, Perry P, *et al.* 2002 *Science* **296** 541
[102] Verveer P J, Swoger J, Pampaloni F, Greger K, Marcello M, Stelzer E H 2007 *Nat. Methods* **4** 311
[103] Huisken J, Swoger J, Del Bene F, Wittbrodt J, Stelzer E H 2004 *Science* **305** 1007
[104] Legant W R, Miller J S, Blakely B L, Cohen D M, Genin G M and Chen C S 2010 *Nat. Methods* **7** 969
[105] Bloom R J, George J P, Celedon A, Sun S X and Wirtz D 2008 *Biophys. J.* **95** 4077
[106] Huh D, Hamilton G A and Ingber D E 2011 *Trends. Cell Biol.* **21** 745
[107] Mironov V, Boland T, Trusk T, Forgacs G and Markwald R R 2003 *Trends. Biotechnol.* **21** 157
[108] Othon C M, Wu X, Anders J J and Ringeisen B R 2008 *Biomed. Mater.* **3** 034101