Modeling metastasis: engineering approaches to study the metastatic cascade

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Abstract

Tumor progression and metastasis requires a complex interplay between tumor cells and their surrounding environment. Conventional 2D and 3D tissue culture models lack the precision and spatiotemporal control required to accurately model the complexity of the tumor microenvironment and metastatic cascade. Advances in biomedical engineering have allowed us to generate precise and versatile model systems to elucidate mechanisms vital to tumor progression and metastasis. The incorporation of novel biomaterials creates a specific mechanical environment that has facilitated controlled studies of cancer cell mechano-transduction. In addition, microfluidic devices have not only allowed for the incorporation of flow and shear forces into vascularized tumor models, but also elucidated vital mechanisms of cancer cell migration that have shifted paradigms about the mode in which cancer cells initiate metastasis. Here, we review the latest developments in biomedical engineering approaches to model the tumor microenvironment and metastatic cascade. We discuss how these approaches have advanced the field of cancer biology and enhanced our understanding of the mechanisms driving metastasis. We initially focus on physical and mechanical aspects of the primary microenvironment that impact tumor cell invasion. We then transition to tumor cell migration using models of tumor extracellular matrix including confined migration. Finally, we review models of intravasation/extravasation and colonization of secondary sites.

1. Recapitulating hypoxic stress and oxygen gradients in the tumor microenvironment

Rapid changes in oxygenation in tumors has been recorded over time and changes over periods of minutes in vivo using electron paramagnetic resonance oxygen imaging [1–3]. Tumor hypoxia is a poor prognostic indicator in a variety of solid tumors [4]. Hypoxic oxygen levels inhibit prolyl hydroxylase enzymes which allows for the stabilization of hypoxia inducible factors and translocation to the nucleus as well as transcription of target genes modulating proliferation, metabolism, angiogenesis, and invasion. Interestingly, fate mapping of cancer cell exposed to hypoxia showed enhanced metastatic potential in breast cancer models [5].

Traditional 2D culture systems utilize ambient air containing 21% oxygen however, the highest levels of dissolved oxygen in the body are 14% oxygen in arterial oxygenated blood and within tissues oxygen levels are closer to 6%–8%. Partial pressures of oxygen measured in tumors in vivo ranges from <1% saturation to approximately 3% saturation with the periphery of tumors subject to more substantial fluctuation and tumor cores exhibiting more steady low oxygen saturation [1]. Oxygen gradients are also common within tumors and tissues due to diffusion barriers. Current models used to study hypoxia (<5% oxygen) such as hypoxic chambers flushed with premixed gases do not incorporate oxygen gradients.

The precise control of oxygen tension within biomaterials and microfluidic devices requires optimization of a number of variables, namely, the oxygen diffusion coefficient of materials used in the system including
hydrogels, the oxygen consumption rate of cells, and the dimensions of oxygen permeable and impermeable materials. Microfluidic device designs have incorporated channels for oxygen and nitrogen flow as well as oxygen producing or consuming chemical reactions in order to generate a gradient across cell-laden hydrogel structures [6, 7]. Such devices allow for the simultaneous observation of cells experiencing different oxygen tensions and have proven vital to dissecting molecular mechanisms of cellular response to hypoxia. Within oxygen gradients human cerebral microvessel endothelial cells exhibit nuclear localization of HIF2α at 3.5% oxygen tension, while levels at or below 1% oxygen were required for HIF1α activity [8]. The ability of oxygen gradients to induce anisotropic cell motility was demonstrated using a hypoxic gradient hydrogel polymerized using a redox enzyme that depletes oxygen to crosslink the gel (Figure 1(A)) [9]. This hypoxia-inducible hydrogel generated a steep oxygen gradient acellularly that could be maintained over long periods of time with the incorporation of cells. This innovative 3D culture system revealed that sarcoma cells migrate toward higher dissolved oxygen concentrations [10]. Other systems have generated oxygen gradients by balancing diffusion and cellular consumption of oxygen. Increasing collagen hydrogel thickness from 1 mm to 2 mm significantly lowered the partial pressure of oxygen at the bottom of the gel, an effect that was solely mediated by the metabolic activity of the cells as well as the passive diffusion of oxygen through the air:media interface and the media:gel interface [11].

Biomaterials and microfluidic devices incorporating oxygen gradients provide an opportunity to examine cellular crosstalk between cells experiencing hypoxic and non-hypoxic conditions. Furthermore, oxygen consuming hydrogels and devices allow for rapid oxygen depletion thus modeling acute hypoxia in a 3D environment. Traditional 2D culture systems in flasks and plates do not allow for study of acute changes in oxygen saturation due to the slow diffusion of oxygen through the air:media interface when using hypoxic chambers [19]. Tumors are subject to acute changes in oxygen saturation due to blood vessel compression and collapse. Furthermore, tumor cells are exposed to cycles of hypoxia and reoxygenation following angiogenesis [20]. Interestingly, transcriptional programs induced by cyclic hypoxia are distinct from chronic hypoxia. The development of engineered systems to model hypoxia reoxygenation cycles in a 3D scaffold would improve our ability to study mechanisms of adaptation by cancer cells and the effects of cyclic/intermittent hypoxia on invasion and metastasis.

1.2. Modeling mechanical stresses to study the induction of an invasive phenotype

The main contributors to the mechanical microenvironment of a tumor are the cells themselves, the extracellular matrix (ECM), and interstitial fluid [21]. ECM is deposited and modified by tumor and stromal cells resulting in a stiff network of ECM proteins, the mechanics of which prime tumor cells for invasion (figure 1(B)) [12]. Engineered synthetic and natural biomaterials have been carefully tuned to investigate the effects of bulk substrate stiffness on cancer cell phenotype. Collagen I, Gelatin, Matrigel, Fibrin, Alginate and Hyaluronan or hyaluronic acid (HA) are the most widely used natural scaffolds in cancer research. Using natural materials is more physiologically relevant however specific preparation conditions as well as batch-to-batch variability must be tightly controlled to ensure reproducibility of experiments. Synthetic polymers such as PDMS, Polyacrylamide, and Polyethylene glycol (PEG) allow for the most drastic differences in youngs modulus, but must be functionalized with adhesion sites as well as matrix metalloprotease (MMP)-cleavable sites to allow for remodeling. Modifying stiffness of polymeric hydrogels is achieved by altering crosslinking density, changing chemical properties of crosslinks, and varying the concentration of polymer. 3D models that have increased polymer concentration to alter stiffness present potentially confounding variables such as significant differences in pore size as well as an increase in integrin binding sites for natural biopolymers. Furthermore, collagen gels without any chemical modification are relatively soft (50 Pa for 1 mg ml\(^{-1}\) collagen up to 1 kpa for 10 mg ml\(^{-1}\)) [22]. In order to reduce these confounding effects and enhance the stiffening capacity of hydrogels, studies have focused on engineering different methods of crosslinking to alter stiffness of hydrogels. Methacrylation of gelatin, collagen, and hyaluronic acid has allowed for enhancing stiffness of 3D scaffolds by increasing crosslinking using short periods of UV light exposure. Glycation of collagen gels using ribose increases stiffness of collagen hydrogels [23] while the addition of recombinant enzymes found to naturally crosslink ECM fibers such as Lysyl oxidase and transglutaminase has also proven a successful technique to stiffen natural biopolymers [24, 25]. These studies have allowed for specific identification of mechanosensors in multiple cell types including tumor cells.

Tumor cells respond to mechanical stress through transmembrane adhesion receptors, mechanosensitive ion channels, and associated downstream signaling. Integrin receptors facilitate transmission of mechanical signals to the interior of the cell via focal adhesion signaling. Subsequent actomyosin contraction and cytoskeletal rearrangement lead to changes in intracellular tension, stabilization of focal adhesions, and adaptive cell stiffening. In response to traction forces, cells deposit ECM proteins and remodel their surrounding matrix thereby further altering its mechanical properties [26].
Figure 1. Modeling the physical and mechanical stresses of the tumor microenvironment. (A) Model of oxygen saturation in hypoxia-inducible hydrogels depicting oxygen gradients across the gel and media. Schematic of oxygen consuming polymerization via laccase-mediated crosslinking [9]. Copyright © 2017, Springer Nature. (B) Mammary organoids embedded in collagen/basement membrane hydrogels of different stiffnesses. Immunofluorescent stains show degradation of the basement membrane and invasion into surrounding gel at higher stiffnesses. Reprinted from [12], Copyright (2005), with permission from Elsevier. (C) Fabrication of modular polyacrylamide hydrogels to assess mechanical memory. Cells primed in soft or stiff conditions migrate over a continuous surface with distinct stiffnesses. YAP (green) nuclear localization is seen on cells primed in stiff conditions. Reprinted from [13]. Copyright (2017), with permission from Elsevier. (D) Compressive stress model generated using a piston imparting solid stress onto cell layers through an agar cushion. Cells exposed to compressive stress enhance fibronectin deposition and collectively migrate [14]. (E) Melanocytes plated on nanopatterned substrates determine the optimal range of spacing between integrin attachment sites. β3-integrin (green) clusters and colocalizes with FAK (red) with spacing of 58 nm (top) but not 73 nm (bottom) [15]. John Wiley & Sons. Copyright © 2004 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (F) Second harmonic generation imaging of invasive breast cancer exhibiting TACS3 signature of aligned collagen fibers (green) perpendicular to the tumor periphery. Reproduced from [16], CC BY 2.0, © 2006 Provenzano et al; licensee BioMed Central Ltd. (G) Model of aligned collagen fibers to investigate mechanisms of contact guidance show elongation of cells on micropatterned lines and tension along the alignment axis indicated by the actin cytoskeleton. Reproduced from [17], CC BY 4.0. (H) Microfabricated confining pores demonstrate nuclear rupture occurring at regions devoid of Lamin C. From [18]. Reprinted with permission from AAAS.

Using modified HA hydrogels the ability of CD44 to modulate glioblastoma (GBM) cell adhesion and migration in response to stiffness was discovered. CD44 HA binding being a weaker bond than integrin-mediated adhesion suggests that the mechanosensing ability of CD44 is optimal for lower stiffness environments such as the brain microenvironment [27]. Interestingly, intermediate expression levels of CD44 allowed for the most efficient migration of GBM cells and correlated with poorest overall survival in GBM patients indicating that CD44-mediated regulation of tumor cell motility impacts clinical outcome [28].

In addition to mechanosensitive cell adhesion receptors, cancer cells express mechanically activated ion channels such as PIEZO channels that facilitate calcium influx, as well as other cations, from the extracellular...
space in response to membrane tension, stretching, and compression. Piezo channels are dysregulated in a variety of cancer types and largely regulate proliferation and migration [29]. A landmark study showed that matrix stiffness activates and upregulates PIEZO1 channels which promotes glioma proliferation through activation of integrin-focal adhesion kinase signaling [30]. Studies using tunable platforms to carefully control substrate stiffness have varied in the degree of stiffness change and due to the abundance of mechanisms of mechanosensing in tumor cells it is not surprising that studies have revealed cancer-specific effects of changing stiffness on proliferation, migration, and drug resistance [31–33]. Although not all cancer cells exhibit the same responses, many studies have found that cells on stiffer substrates upregulate expression of ECM and matrix modifying genes [34].

Platforms that allow for the generation of stiffness gradients have identified an entirely new mode of motility known as durotaxis. Multiple cell types including cancer cells migrate toward stiffer conditions, and the magnitude of the gradient directly correlates with anisotropy of cell migration along the gradient [35]. Furthermore, dynamic stiffening hydrogels using photoactivatable crosslinkers have permitted the discovery of mechanical memory, where changes in cellular signaling due to culture on stiff substrates are stable (figure 1(C)) [13]. Nuclear localization of Yap, a co-factor in the mechanosensitive Hippo pathway, persisted following exposure of cells to soft substrates that were primed in stiff conditions. The recent development of ‘switchable hydrogels’ that offer dynamic stiffening and softening provide a unique opportunity to further interrogate mechanisms regulating mechanical memory [36]. These mechanisms may underlie observations that tumor cells exposed to stiff environments retain their invasive phenotype even after being placed in an environment with different mechanical properties and thus, are more metastatic.

In addition to stiffness, ECM in the tumor microenvironment exhibits different profiles of relaxation with respect to time following the application or removal of strain. In this way, ECM protein polymers are considered viscoelastic. Therefore, strain applied to the ECM by cellular contraction or interstitial fluid pressure is followed by subsequent relaxation, albeit at different rates depending on protein composition and structure. The viscoelastic properties of natural and biomaterial polymers have been evaluated and characterized as plasticity or permanent deformation in response to an applied strain. Collagen I is highly elastic when compared to other materials such as Matrigel, fibrin, agarose, and alginate gels [37]. Chemically modified scaffolds derived from collagen I, RGD-alginate, and Matrigel have been used to study the effect of augmenting matrix relaxation time on tumor cells. Changes in stress relaxation time have been shown to alter matrix remodeling, focal adhesion maturity, actin stress fiber formation, and change invasive characteristics of tumor cells [38]. Specifically, quick stress relaxing collagen hydrogels enhanced 3D motility of sarcoma tumor cells and lead to enhanced pulmonary metastasis [39].

Tumor cell proliferation in the primary tumor contributes significantly to the bulk mechanical environment through the generation of solid stress [40]. Solid stress is a distinct physical property from stiffness and fewer models have been developed to measure solid stress in tumors. High resolution ultrasound imaging showed solid stress varied depending on tumor type, the microenvironment in which the tumor established, and interestingly, between size matched primary and metastatic tumors from the same cell line [41]. The impact of solid stress on intrinsic invasive behavior of tumor cells is less well established however, engineered models of solid stress using a piston to impart 5.8 mmHg of mechanical compression onto breast cancer cells showed that compressive stresses induce secretion of fibronectin, actin and cytoskeleton remodeling, and collective cell migration (figure 1(D)) [14]. The same model was used to show that pancreatic cancer cells are more migratory in response to compressive stresses secondary to upregulation of GDF15 and activation of Akt [42]. Outward forces, generated by solid stress, on the surrounding stroma lead to compaction of ECM proteins, alignment of collagen fibers, and constriction of blood and lymphatic vessels [43, 44]. Compression of vessels and draining lymphatics restricts proper interstitial fluid flow leading to an increase in interstitial fluid pressure. The forces generated by this process significantly impact cell behavior as well as the structure of the ECM [34, 45, 46]. Interstitial fluid pressure gradients generate a net outward flow of fluid from the tumor to the stroma [40]. Fluid pressure gradients are generated by altering the height of media reservoirs such that one contains more media than on the opposing side of the device or gel. Such a system was used to show that interstitial fluid pressure induced collective cell migration of breast cancer cells by activating an epithelial–mesenchymal transition program [46].

Increasing complexity of engineered platforms to assess mechanobiology have identified the importance of nanotopography of ECM substrates and intricacies of mechanical signaling [26]. The ECM is a heterogeneous mixture of fibrillar and nonfibrillar proteins with varying distributions of cell adhesion sites and physical structures. Fibrous hydrogels can be tuned to modulate polymer ultrastructure. Collagen hydrogels formed with higher collagen concentrations exhibit a higher fiber density and reduced pore size while gels formed at a higher pH and temperature contain smaller diameter fibers and pore sizes [47]. Increasing the ionic strength, the thrombin concentration or the pH during gelation of fibrin hydrogels results in finer fibrils with a decrease in gel permeability [48]. Furthermore, higher calcium levels cause...
lateral aggregation of the fibrin monomers generating thicker fibers. Patterning ECM coatings has identified intracellular mechanisms of actin reorganization and focal adhesion composition in response to specific spatial configurations of adhesion sites. Nanopatterning methods were used to determine a maximum distance (~60 nm) between individual integrin attachment sites for clustering, actin stress fiber formation, and maturation of focal adhesions, while greater distances interfere with focal adhesion maturation and cell attachment (figure 1(E)) [15]. However, spacing between cell adhesions of about 5–20 microns enhanced cell spreading due to efficient traction force generation [49, 50].

Mechanisms differentiating cell response to substrate stiffness, stress relaxation, and solid stress remain to be elucidated in systems where these mechanical properties are able to be decoupled. Similarly, controlling adhesion/degradation sites as well as 3D ultrastructure while altering mechanical properties remains a challenge in the field. In all, tumor cells experience a variety of physical forces and have various mechanisms of sensing changes in the mechanical properties of their surroundings. The physical properties of the tumor microenvironment induce phenotypic changes in tumor cells that impact proliferation, drug resistance and directional migration thereby significantly influencing early stages of the metastatic cascade.

1.3. Migration away from the primary tumor

The tumor microenvironment is characterized by distinct spatial distributions of ECM proteins such as alignment of fibrillar ECM proteins. Straightened, aligned collagen fibers perpendicular to the tumor boundary is independently prognostic of poor overall survival in breast cancer (figure 1(F)). The degree of alignment and directionality of aligned collagen fibers has therefore been classified into tumor associated collagen signatures (TACS) [51]. Although TACS have not been characterized in other tumor types, prostate and pancreatic cancers also exhibit patterns of aligned collagen fibers which correlate with more aggressive disease [52, 53]. Engineered platforms were used to show that cancer cells migrated along aligned collagen fibers [54]. Models of collagen fiber alignment can be achieved by solidifying collagen gels under conditions of high shear stress [55], pulling fibrous scaffolds following polymerization [54], and dragging physical objects through the scaffold during polymerization such as magnetic beads [56]. Although high degrees of alignment can be achieved using these models the dimensions of the fibers are difficult to control. Collagen fiber alignment generates physical tracts or paths of least resistance for tumor cells to migrate along [16]. Single cell and collective migration along collagen tracks from tumor spheroids has been observed using aligned collagen gel models as well as in preclinical models and patient tumor specimens [57]. In addition to physical tracks, alignment of collagen fibers provides guidance cues for anisotropic directional cell motility of tumor cells. Micropatterned ECM representing the aligned matrix was used to show that coordination between microtubules and actomyosin contraction regulates contact guidance sensing (figure 1(G)). Furthermore, this mechanism persists under low and high traction conditions where formins and focal adhesions are essential in stiff environments while Arp2/3-mediated actin branching mediates contact guidance sensing in low traction conditions [17, 58, 59].

Invasive tumor cells encounter confining physical environments throughout the metastatic process including migrating through dense ECM and transendothelial migration during intravasation/extravasation [60]. Microfabricated platforms consisting of an array of tightly controlled pore sizes were essential in discovering that migration through small pores (1–20 μm) required matrix modification or alteration of nuclear stiffness [61]. Micropatterned platforms showed that nuclear deformability was higher in malignant cells compared to benign cell lines [62]. These studies identified Lamins as critical structural components of the nuclear envelope that control nuclear stiffness and are depleted at sites of nuclear rupture when invasive cells are migrating through small pores (figure 1(H)) [18, 63]. Similar platforms identified nuclear blebbing in cells undergoing confined migration and described a mechanism where nuclear anchoring to the posterior of the cell creates a pressure gradient promoting nuclear influx and volume expansion prior to blebbing and rupture [64].

Nuclear rupture due to extremely confined migration of invasive tumor cells led to genomic instability which contributed to metastatic potential [18]. Furthermore, tumor cells known to have high metastatic potential are more proficient at migrating through confining channels. Thus, enriching breast cancer cells able to migrate through confining channels produced significantly more spontaneous metastatic lesions in the liver, lung, lymph node, and bone when compared to unsorted controls [65].

In all, these studies using engineered environments representing the confining and abnormal ECM surrounding the tumor demonstrate mechanisms by which structural aspects of the ECM impacts tumor cell behavior and how it contributes to the progression of metastasis.

1.4. Modeling the tumor vasculature

Solid tumors become vascularized quickly in response to generation of severe hypoxia and secretion of pro-angiogenic factors such as Vascular Endothelial Growth Factor (VEGF). There are two distinct well
established mechanisms which contribute to the formation of tumor vasculature; angiogenesis (sprouting from a pre-existing vessel) and vasculogenesis (de novo formation of vascular structures). Both occur in the context of the highly abnormal tumor microenvironment which contributes to a tortuous vascular structure. The primary advantage of using engineered and microfluidic models of the tumor vasculature is that they allow incorporation of fluid flow, which is vital to endothelial cell gene expression and function [66].

Direct write bioprinting methods allow for the patterning of vessels in tortuous constructs which mimic the tumor vasculature. Cells encapsulated in biomaterials are extruded and quickly crosslinked to allow for any pattern to be made while also maintaining circularity and smaller vessel sizes [67]. Evolution of nozzle design allowed for direct printing of hollow tubes that maintain patency and can be perfused immediately after crosslinking [68]. Alternatively, drop-based bioprinters are able to create ring structures using cell-laden bioink which also self-assembles into patent vascular channels [69]. The tortuous architecture of vessels has been modeled in devices where endothelial cells are seeded in pre-cast channels. Studies using these models confirm differential perfusion and shear forces felt by ECs within aberrant vascular structures (figure 2(A)) [70].

The abnormal structure of tumor vessels causes aberrant fluid flow thus altering shear stresses on endothelial cells lining vessels [76]. Although shear stresses felt by endothelial cells in the tumor vasculature are likely variable, fluid flow rate in tumor vessels can be up to three orders of magnitude less than vessels of the same diameter in uninvolved tissue [77]. Fluid flow-induced shear stress causes endothelial cells to elongate and align along the direction of flow. Shear stresses can range from 30 dyne cm$^{-2}$ in arteries to 4 dyne cm$^{-2}$ in veins [78]. Microfluidic models have been utilized to investigate signaling pathways regulating endothelial cell function to fluid flow in defined shear stress conditions. The same microfluidic models allow for temporal monitoring of vessel permeability under conditions of flow using diffusion of fluorescent Dextrans and measuring transendothelial electrical resistance. Shear stress's above 3 dyne cm$^{-2}$ drastically increase barrier function in microvascular networks following activation of a noncanonical Notch signaling pathway and fortification of cadherins junctions [79]. Furthermore, higher shear stresses (18 dyne cm$^{-2}$) promote arterial specification of endothelial vessels through Notch signaling in devices as well as zebrafish models indicating that not only does shear regulate vessel permeability but also endothelial cell phenotype. In addition to shear stress, oxygen tension and hypoxia regulate endothelial cell junction stability and vessel permeability. Moderate hypoxia (5% oxygen) enhances barrier function of blood-brain barrier endothelial cells under flow [80] while exposure of human umbilical vein endothelial cells (HUVECs) to 3% oxygen in microfluidic devices causes internalization of VE cadherin thus compromising barrier function and increasing permeability [6].

Although many engineered models of the tumor vasculature focus on endothelial cell function, fewer highlight contributions of vessel support cells, namely pericytes, to tumor vascularization. Vascularized microfluidic platforms used in tissue engineering applications have demonstrated that vessel support cells promote self-assembly of vessels, alter the overall vascular structure, and support long term stability of perfusable vascular structures either through paracrine factors or direct interaction [81, 82]. Furthermore, pericyte interaction with endothelial cells during vessel formation promotes vascular basement membrane production and deposition leading to more mature vessels [83]. Thus far, the majority of engineered model systems incorporating vessel support cells use fibroblasts, some of which closely associate with endothelial cells and start to express pericyte markers such as NG2, however, future studies would benefit from utilizing primary isolated pericytes or induced pluripotent stem cell (iPSC)-derived pericytes. Furthermore, these model systems have yet to be used to investigate the mechanistic role of pericytes in tumor vascularization and perfusion however, the Hughes group assessed the effect of different anti-angiogenic therapies on tumor vascularization using a model containing vessel support cells and found that while sorafenib disrupted the development of newly formed vessels, vincristine disrupted entire vascular networks [84]. In summary, rapid vascularization of solid tumors causes tortuous vasculature that is exposed to low shear stress and hypoxia thus, increasing permeability. Compromised barrier function in tumor vessels generates easy access points to the circulation for tumor cells.

1.5. Engineered systems for studying intravasation/extravasation

A vital early step in the metastatic cascade is gaining access to the circulation. In a physiological context endothelial cells form tight cell-cell junctions to regulate the diffusion of nutrients from the blood stream and the passage of infiltrating immune cells. In order to gain access to the blood circulation, tumor cells must penetrate the endothelial cell layer, this process is called intravasation. Similarly, tumor cells must then exit the blood circulation to set up micrometastatic lesions within secondary sites, a process termed extravasation [85].

Roger Kamm's group designed a microfluidic system where intravasation of human cancer cells could be recorded in real time [86]. Simultaneously, endothelial cell permeability could be monitored throughout the
process as well. They showed that macrophages play a vital role in facilitating intravasation of fibrosarcoma cells by producing Tumor Necrosis Factor alpha (TNFα) to increase vessel permeability. This study built the framework for downstream investigations of macrophage influence in cancer cell intravasation. Importantly, they showed that neutralizing TNFα produced by macrophages reduced intravasation events. This mechanism was confirmed years later in vivo in spontaneous murine tumor models using intravital imaging [87]. This same microfluidic model was used to assess the impact of the bone microenvironment on the ability of cancer cells to extravasate by incorporating differentiated osteocytes into collagen gels adjacent to endothelial channels. Cells that had extravasated exhibited distinct gene expression profiles including upregulation of proteases which degraded the glycocalyx of the endothelial cell layer. The bone microenvironment specifically enhanced extravasation of breast and bladder cancer cells, but not ovarian cancer cells [88]. A more complex iteration of the Kamm device was used to study the effect of monocyte
phenotype on extravasation of breast cancer cells from microvasculature constructs. Here, perfused capillary beds were formed in a fibrin hydrogel and monocytes or cancer cells were flown through the vascular bed to visualize extravasation in real time [71]. Using this device, the group was able to distinctly characterize monocytes that had extravasated (inflammatory CCR2+ compared to those that had not (patrolling CCR2−). Furthermore, they were able to establish that intravascular monocytes reduced extravasation of breast cancer cells (figure 2(B(i))). They found that monocytes did not impact vessel permeability rather, many extravasating tumor cells were in direct contact with monocytes implying paracrine signaling significantly impacts extravasation (figure 2(B(ii))). Additionally, the microvasculature construct device was utilized to demonstrate the impact of hypoxia on breast cancer extravasation, by putting the entire device in hypoxic conditions, the authors were able to show that hypoxia significantly increases extravasation events, and that this process is mediated by HIF1α signaling in the breast cancer cells themselves (figure 2(C)) [72]. The single channel model was improved upon by adding curvature to the engineered microvessel, while also incorporating shear force as well as a 3D collagen hydrogel scaffold. By analyzing time lapse videos of tumor cells intravasating through the engineered vessel, Wong et. al. was able to characterize behavior of intravasating cells and showed that tumor cells divide directly before breaking through the endothelial barrier [89].

A completely different mechanism by which pancreatic cancer cells initiate metastasis, termed vessel ablation was described using a microfluidic system containing a perfused vessel and cancerous duct in a 3D hydrogel system. Using time lapse imaging over long periods of time, Nguyen et. al. were able to characterize the process of vessel ablation to show that the tumor cells disrupt the endothelial cells and co-opt the vessel (figure 2(D)). This model was used to show that the mechanism behind this phenomenon in pancreatic cancer was ALK7 signaling [73]. The group proposed that this method of vessel ablation could result in high numbers of circulating tumor cells and subsequent metastases seen in pancreatic cancer. Histological evidence of this mechanism has been observed in pancreatic cancer as well as ovarian cancer [90, 91].

1.6. Modeling colonization of secondary sites

The first step of colonization of secondary sites during the metastatic cascade is extravasation from vasculature within distant sites. Breast, prostate, lung, kidney, and thyroid cancers show propensities to metastasize to bone [92]. The bone microenvironment is comprised of two distinct niches, the endosteal niche (adjacent to the mineralized matrix) and the perivascular niche (adjacent to vasculature within the bone marrow). Other cell types that make up the bone microenvironment are osteocytes, osteoblasts (bone matrix depositing cells), osteoclasts (bone matrix resorbing cells), and various immune cells/hematopoietic progenitor cells. The physical environment of the endosteal niche in uniquely stiff due to the material properties of its major component, mineralized collagen fibers. Arrays of aligned collagen fibrils interspersed by apatite-based crystals dictate the mechanical properties of the bone matrix making it suitable for mechanical loading [93]. Osteocytes are mechanically sensitive cells which sense differences in interstitial fluid flow during mechanical loading and regulate bone remodeling through crosstalk with osteoblasts and osteoclasts. A microfluidic model of the bone microenvironment interrogated the effect of mechanical loading on breast cancer cell extravasation using a channel lined with HUVECs adjacent to a channel containing osteocytes exposed to oscillatory fluid flow. In agreement with observations that exercise reduced tumor burden in cancer patients, exposure of osteocytes to oscillatory fluid flow significantly reduced the number of circulating breast cancer cells able to extravasate [94]. Incorporation of interstitial fluid flow using a niche-on-a-chip model of the bone perivascular microenvironment also decreased the proliferation rate of breast cancer cells that successfully engrafted (figure 2(E)) [74]. Many studies of the bone microenvironment incorporate hydroxyapatite into hydrogel structures to investigate the impact of the material on cellular function [95–98]; however, few have used models of physiologically relevant mineralized collagen fibers. Claudia Fischbach’s group reported a model that mineralizes collagen fibers by immersing them in calcium, phosphate, and polyaspartic acid. They found that morphology of breast cancer cells including cell lines that preferentially metastasize to bone was much more compact on mineralized collagen due to reduced focal adhesion based force generation [99]. In addition to altering cancer cell phenotype, multiple studies have indicated that the bone microenvironment promotes resistance to chemotherapies in lung and prostate cancers although the underlying mechanisms remain unknown [100, 101].

Other common sites of metastasis include the lungs, liver, and brain; each site exhibits unique physical and biochemical characteristics however, they are all highly vascularized tissues. The lungs and brain are very high in oxygen and nutrients making them ideal locations for tumor cells to grow. Metastatic colonization of the brain requires passage through the blood-brain-barrier, which exhibits much higher barrier function compared to other vessel structures in the body and is viewed as the main obstacle to cells colonizing the brain microenvironment. Thus, several engineered brain microenvironment models are centered around the blood brain barrier by using brain endothelial cells and incorporating other cell types that constitute the
brain microenvironment and contribute to barrier function such as pericytes, astrocytes, and neurons. A microfluidic model of the blood brain barrier demonstrated higher propensities of lung and breast cancer cells to penetrate the barrier compared to melanoma and liver cancers (figure 2(F)) [75]. The mechanical environment in the brain is relatively soft, with an elastic modulus ranging from 2 to 6 kPa [102], as most of the ECM is comprised of HA. HA hydrogels of varying stiffness (0.2 kPa to 4.5 kPa) confirmed that metastatic breast cancer proliferation and migration significantly increased on stiffer gels, an effect which could be inhibited by blocking Focal adhesion kinase activation [103]. Various other engineered models of the brain microenvironment have been used to model neurological disorders and drug delivery to the brain; however, they provide excellent systems to study brain metastasis [104].

Due to its primary function in drug metabolism, the complex endothelial network in the liver comprised of sinusoidal endothelial cells lacks basement membrane and is naturally permeable allowing cancer cells to easily extravasate [105]. Breast, prostate, colon, and skin cancers frequently metastasize to the liver underlying the need for sophisticated models to optimize treatments for tumors colonizing the liver. Specialized cell types in the liver are hepatocytes, Kupffer cells (macrophages), sinusoidal endothelial cells, and hepatic stellate cells (fibroblasts). Various liver-on-a-chip models exist that incorporate primary, immortalized, or IPSC-derived hepatocytes as well as non-parenchymal cells to represent the cellular heterogeneity in the liver [106–108]. A liver microphysiologic system recapitulated dormancy phenotypes of breast cancer cells, and identified the non-parenchymal cells as the cell type perpetuating the non-proliferative environment for metastatic breast cancer cells [109]. The same platform was used to investigate this mechanism further by characterizing exosomes secreted by the hepatic niche and identifying a microRNA signature which stabilized epithelial phenotype in breast and prostate cancer cell lines to induce dormancy [110].

2. Future directions

In summary, engineering model systems have significantly contributed to our current understanding of the complex tumor microenvironment and metastatic cascade. Development of systems that incorporate the entirety of the metastatic cascade from acquisition of invasive characteristics through colonization of secondary sites would allow further dissection of mechanisms driving metastasis; however, choosing cell types to incorporate in such a model is crucial to our understanding of metastatic progression in humans. Currently many of the above discussed studies utilize immortalized cell lines, and could be improved through the use of primary cells or tissue specimens. IPSC-derived cells provide an additional alternative as they are being increasingly used in engineered systems and compared functionally and genetically to their primary counterparts. Moreover, the increasing evidence of genetic and functional heterogeneity of tissue specific cell types warrants incorporation of these cells into the engineered model systems to improve physiological relevance. Tumor-derived ECM scaffold hydrogels present an opportunity to model the tissue specificity of primary and metastatic sites. Indeed, current hydrogel model systems lack the diversity of ECM proteins in the tumor stroma. Continued improvement of engineered model systems of the tumor microenvironment and metastasis will identify novel druggable targets to treat metastatic cancer patients.

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