



In vitro models of tumor vessels and matrix: Engineering approaches to investigate transport limitations and drug delivery in cancer[☆]



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ARTICLE INFO

Available online 2 December 2013

Keywords:

Tumor microenvironment
Tumor angiogenesis
Tumor desmoplasia
Drug transport in cancer
Engineering models of tumor

ABSTRACT

Tumor–stroma interactions have emerged as critical determinants of drug efficacy. However, the underlying biological and physicochemical mechanisms by which the microenvironment regulates therapeutic response remain unclear, due in part to a lack of physiologically relevant in vitro platforms to accurately interrogate tissue-level phenomena. Tissue-engineered tumor models are beginning to address this shortcoming. By allowing selective incorporation of microenvironmental complexity, these platforms afford unique access to tumor-associated signaling and transport dynamics. This review will focus on engineering approaches to study drug delivery as a function of tumor-associated changes of the vasculature and extracellular matrix (ECM). First, we review current biological understanding of these components and discuss their impact on transport processes. Then, we evaluate existing microfluidic, tissue engineering, and materials science strategies to recapitulate vascular and ECM characteristics of tumors, and finish by outlining challenges and future directions of the field that may ultimately improve anti-cancer therapies.

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1. Introduction

Given its extensive socioeconomic impact, cancer continues to be a major focus of drug development and delivery research. Nevertheless, clinical success of anti-cancer therapies remains limited, and most

treatment strategies exhibit marginal efficacy, serious side effects, and the development of resistance. Moreover, complete tumor eradication is mostly impossible, and time until patient relapse or metastasis remains a tragic measure of clinical success. Targeted therapies interfering with specific genetic and molecular mechanisms of tumorigenesis have offered improvement relative to conventional cytotoxic therapy; however, cancer cells frequently evade therapy by assuming resistance mechanisms including secondary mutations and epigenetic modifications [0–2].

While many therapies directly target tumor cells, the microenvironment in which tumor cells reside is an equally important participant in

[☆] This review is part of the *Advanced Drug Delivery Reviews* theme issue on “Innovative tissue models for drug discovery and development”.

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disease progression. During health, normal “contextual cues” of the host microenvironment prevent the cancerous outgrowth of epithelial cells [3,4]. However, perturbation of this homeostasis, e.g., due to chronic inflammation, metabolic changes, or hormonal imbalance, enables the initiation and progression of malignancy [5–8] as well as the emergence of resistance [9,10].

In addition to directly affecting tumor cell behavior, microenvironmental conditions may promote recurrence by simply preventing effective transport of therapeutics. When anti-cancer drugs are systemically administered, steps of drug delivery include transport (1) within the circulation, (2) across blood vessel walls, and (3) through the interstitial space to the tumor [11,12]. Alterations of microenvironmental conditions interfering with any of these processes may affect drug bioavailability with consequences on efficacy.

The physicochemical properties of the vasculature and the interstitial extracellular matrix (ECM) are key regulators of anti-cancer drug distribution and efficacy [13]. As the primary conduits of perfusion, blood vessels determine the availability of drugs throughout the body and within individual tissues. However, heterogeneous microvascular function as present within tumors can compromise delivery and undermine the effects of therapeutic agents [13]. Enhanced permeability and retention (EPR) in leaky vessels has facilitated the targeting of macromolecular therapies [14–18]. Yet, the asymmetric distributions of oxygen or drugs within a tumor provide a conducive landscape for the evolution of resistance within heterogeneous populations of cancer cells [19]. Although vascular structure and function largely regulate the spatiotemporal distribution of drug, interstitial space can also affect transport rates [20]. In particular, excessive ECM deposition due to fibrotic remodeling (also termed desmoplasia) physically hinders diffusion of large anti-tumor molecules through the interstitium [20].

Despite the well-established physical principles governing biological transport phenomena, the opportunity to leverage these principles to improve therapeutic outcomes is limited. Conventionally, new anti-cancer compounds are first tested in 2D tissue culture, which provides homogeneous access to drug and neglects the 3D microenvironmental properties inherent to tumors. Additionally, even positive results from animal studies do not always translate to efficacy in humans due to species-dependent discrepancies in signaling and physiology [21,22]. The development of tissue-engineered model systems that accurately recapitulate human tumor with increasing physiological complexity may help to understand and test microenvironmental parameters affecting tumor response. Here, we review current understanding of the biological characteristics underlying tumor-associated changes of the vasculature and ECM properties, examine the consequences of these parameters for mass transport and drug delivery, and present emerging *in vitro* strategies that may provide new insights for improved anti-cancer therapies.

2. Tumor vasculature: biophysical changes and their relevance to drug delivery

2.1. Biological characteristics of tumor microvasculature

Since Judah Folkman's seminal observations in 1971 that tumorigenesis is associated with the ingrowth of abnormal blood vessels [26,27], vascular dysfunction has become an enduring theme in cancer biology and anti-cancer therapy. When compared to healthy vasculature, tumor vessels are leaky, fenestrated, tortuous, and dilated, with chaotic branching patterns including shunts and loops, as well as irregular hierarchy of vessels [28–33] (Fig. 1). The tumor vasculature comprises at least six types of vascular structures with distinct properties, including mother vessels (MVs) and several varieties of daughter vessels (capillaries, glomeruloid microvascular proliferations, vascular malformations) [34,35]. MVs are enlarged sinusoids resulting from pericyte detachment, basal membrane (BM) degradation, and endothelial thinning [35]. Despite this distension, MVs do not increase blood

flow, possibly due to their hyper-permeable membrane function [34]. Meanwhile, immature daughter vessels are fenestrated and lack functional perfusion. Vascular leakage, coupled to dysfunctional lymphatic drainage, results in an accumulation of interstitial fluid, which compromises the hydrostatic and osmotic pressure gradients that drive transvascular convection [36]. Collectively, this heterogeneous network of aberrant blood vessels yields erratic perfusion of diseased tissue, with important consequences for pathogenesis and therapy.

Hypoxia and acidosis are the most prominent consequences of deregulated vascular function. Compromised vessel characteristics lead to poor supply of oxygen and clearance of metabolic wastes, mediating sustained hypoxia ($<1\% \text{ O}_2$) and acidosis (as low as pH ~6.6–6.8 in some areas) [37–39]. These microenvironmental conditions directly promote tumor progression and new vessel formation by activating a variety of transcriptional programs [40–43]. In particular, stabilization of the alpha subunit of the hypoxia inducible factor 1 (HIF-1) transcription factor in low-oxygen conditions leads to an orchestrated program of hypoxic response [41,44–46] that includes the up-regulation of pro-angiogenic morphogens (including vascular endothelial growth factor [VEGF] and basic fibroblast growth factor [bFGF]). However, rather than promoting the formation of healthy blood vessels that could normalize tissue O_2 levels and pH, excess pro-angiogenic signaling activates a vicious cycle that undermines vascular stability by impairing vessel organization and permeability thus exacerbating these pathological conditions [47–49].

In addition to metabolic transport, impaired vascular function also compromises the homogeneous delivery of therapeutic agents, resulting in poor distribution of drugs throughout the tumor [50]. Low vascular function at the tumor interior prevents therapeutic access to large regions of tissue [30]. Homogeneous delivery is further undermined by the absence of hydrostatic and osmotic pressure gradients, which are necessary for interstitial convection to distribute large therapeutic agents [11,52]. In some cases, enhanced vascular permeability and retention (EPR) has been coopted as a mechanism for tumor targeting of large particles such as antibodies and micelles [14]. However, although high molecular weight drugs and drug carriers easily traverse the endothelial membrane of the tumor, they have poor penetration depth and achieve little benefit in regions distal to blood vessels [50,53,54]. Therefore, vascular normalization is an emerging theme for improving therapeutic delivery [55–57].

Whereas most vascular therapies emphasize the destruction of blood vessels to stunt tumor growth, some researchers hope to re-appropriate angiogenic drugs as adjuvant therapy in order to improve drug distribution [58]. By increasing perfusion in the tumor, this method may overcome major disadvantages of chemotherapy such as short half-life and small therapeutic index (range of concentration between efficacy and toxicity) [59]. In the past decade, numerous clinical trials have revealed the benefits of anti-angiogenic therapy as an adjuvant to chemotherapy or radiation therapy [59–61]. However, the dearth of pre-clinical models to recapitulate vascular transport remains a challenge for the development of new strategies for vascular normalization.

2.2. Effects of microvascular dysfunction on transport physics

Whereas conventional pharmacokinetic metrics (e.g. distribution volume, half-life, clearance rate) characterize the average availability of drug in tissues, these measurements only poorly approximate the effective distribution of agents within tumors. In fact, large regions of tumors lack adequate vascular perfusion and thus, can remain unaffected by treatment, even at high doses. This is particularly true of large molecules ($>10 \text{ kDa}$), which are not able to freely diffuse across the endothelium and through the tissue. Instead, convective forces govern the exchange of large compounds from the circulation (Box 1). In healthy tissues, these forces balance the influx and outflux at the arterioles and post-capillary venules to provide adequate perfusion. In the case of a tumor, however, vascular leakiness reduces hydrostatic pressure in the vessel while increasing osmotic pressure in the

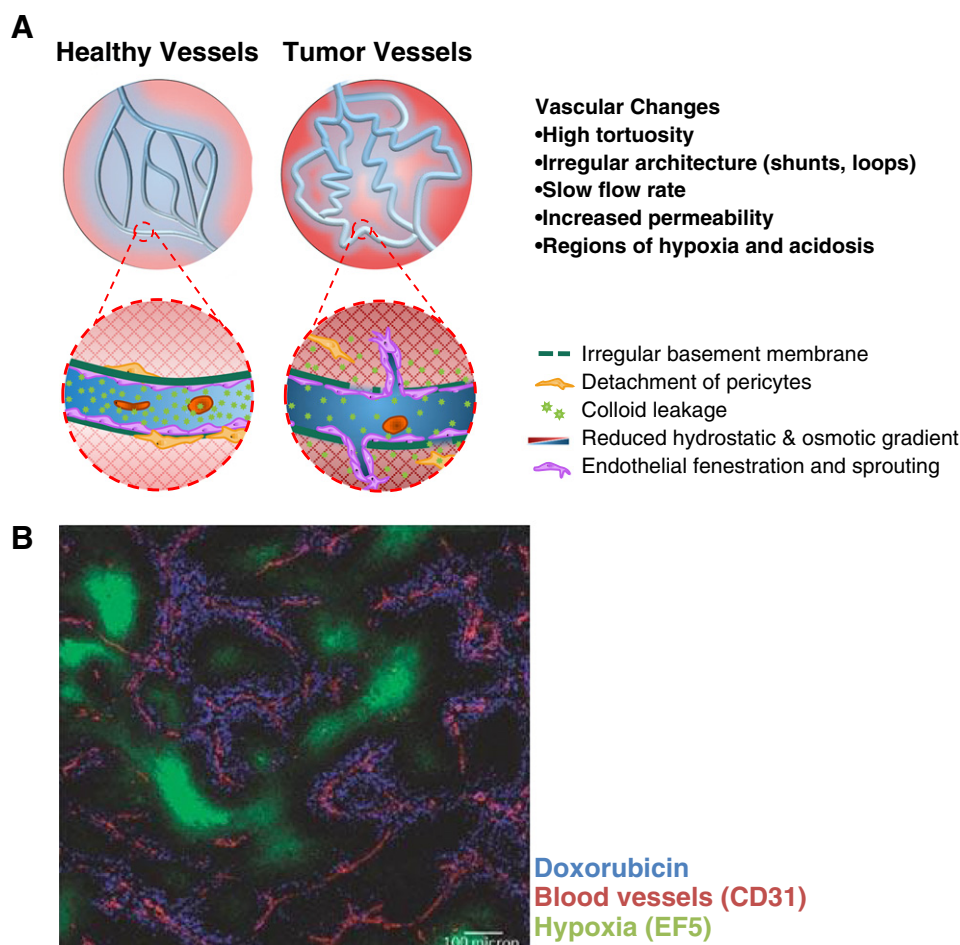


Fig. 1. Biophysical changes of tumor vasculature. A) Schematic illustration of the structural and functional changes in tumor vasculature, including tortuous vessels, chaotic branching, disrupted barrier function, and compromised hydrostatic and osmotic gradients. B) Aberrant vasculature undermines transport processes within diseased tissue, resulting in asymmetric distributions of oxygen and drugs to the tumor. This micrograph illustrates the penetration depth of doxorubicin and highlights the large, hypoxic regions of the tumor unaffected by therapy [54]. Reproduced with permission from Nature Publishing Group.

interstitium (approximately 29 ± 3 mm Hg in breast tumor, compared to -0.3 ± 0.1 mm Hg in healthy breast tissue) [62,63]. Together, these changes significantly diminish the bioavailability of drugs by reducing transcapillary hydrostatic gradients necessary for fluid exchange [64].

In addition to simple mass transport equations, a variety of tools have been developed to quantitatively evaluate the effectiveness of mass transport in tissues. Dimensionless numbers are particularly useful to identify transport limitations [65]. For example, the Biot number compares the relative resistance attributed to the endothelial membrane versus the interstitial matrix. Likewise, the effectiveness factor describes the rate of transport compared to the rate of reaction. Both of these parameters can help identify limitations to drug penetration and efficacy. For example, in cases where transvascular transport limits therapeutic efficiency, combinations with permeabilizing agents may improve delivery, while other therapies limited by interstitial diffusion may benefit from stroma-targeted treatments. A modified effectiveness factor can incorporate the Biot number to identify the relative resistance due to vascular versus interstitial transport [66,67].

Similarly, the “observable modulus” evaluates penetration distance based on transport and reaction considerations [66]. This parameter defines a “dimensionless reaction rate” (the effectiveness factor * thiele modulus), and is defined as a function of the distance from the nearest vessel. This parameter allows the calculation of a penetration depth, or the distance at which the concentration drops below an effective dose. A low modulus indicates deeper penetration, and high modulus indicates poor distribution away from the capillary. The observable modulus has been used to determine the Krogh distance, or the range for

diffusion-limited oxygen transport before reaching anoxia and necrosis [66]. In the case of therapy, the same parameter can describe the effective therapeutic range of a drug and thus characterize and inform angiogenic therapy or EPR designs.

By evaluating the specific physical contributions to therapeutic resistance by transport or reaction kinetics, these quantitative methods may help identify major barriers of delivery and enable the rational design of dosing strategies, including in combination with drugs that target the vasculature or the stroma to optimize therapeutic efficacy. Nevertheless, many of these quantitative approaches are frequently neglected. This may be due in part to the inaccessibility of the various coefficients needed to determine the dimensionless parameters. Conventional methods to test vascular permeability, such as Evan's blue staining, provide little chance for rigorous quantification. Meanwhile, *in vitro* methods to measure endothelial permeability or tissue diffusivity, such as multilayer or spheroid cultures, may not accurately reflect *in vivo* parameters [54]. Therefore, new methods to accurately determine the parameters affecting vascular and interstitial mass transport will provide a valuable resource for the design of therapeutic strategies that target the tumor microenvironment [65].

2.3. Recapitulating tumor-associated vascular transport through *in vitro* models

Organotypic 3D culture models present an emerging opportunity to create controlled experimental conditions that faithfully reproduce aspects of the physiological environment. However, as sufficient mass

Box 1

Forces governing transvascular transport.

The Kedem–Katchalsky equation describes solute flux (J_s) as a combination of convective and diffusive forces across the endothelial membrane:

$$J_s = J_v(1 - \sigma_f)\bar{C}_s + PS\Delta C$$

where σ_f is the filtration reflection coefficient, $\bar{C}_s$ is the average molar concentration in the membrane, P is the permeability coefficient, S is the surface area, and ΔC is the change in concentration across the endothelium. The fluid flux (J_v) is derived from Starling's Law, which describes the contributions of hydrostatic (p) and osmotic (π) pressure gradients:

$$J_v = L_p S(\Delta p - \sigma_s \Delta \pi)$$

where L_p is hydraulic conductivity, S is surface area, and σ_s is the osmotic reflection coefficient, which ranges from 0 to 1 based on the membrane permeability to solutes. Based on these equations, transvascular solute transport is governed by four physiological constants describing solute and membrane characteristics:

$$\begin{aligned} \text{Hydraulic conductivity: } L_p &= \frac{J_v/S}{\Delta p} = \frac{\text{fluid flux}}{\text{hydrostatic pressure difference}} \\ \text{Permeability coefficient: } P &= \frac{J_s/S}{\Delta C} = \frac{\text{solute flux}}{\text{concentration difference}} \\ \text{Osmotic reflection coefficient: } \sigma_s &= \frac{\Delta p}{\Delta \pi} = \frac{\text{hydrostatic pressure difference}}{\text{osmotic pressure difference}} \\ \text{Filtration reflection coefficient: } \sigma_f &= 1 - \frac{J_s}{J_v \bar{C}_0} = 1 - \frac{\text{solute flux in membrane}}{\text{solute flux insolution}} \end{aligned}$$

Measurements for these parameters in healthy and tumor tissue are provided by [52]. These factors are affected by solute (size, charge, polarity) and membrane (pore size, pore density) properties [66].

transport within cell-laden biomaterials remains a significant limitation of 3D cultures, these approaches are typically performed in a microscale format and lack the recapitulation of tumor-inherent transport physics. New in vitro platforms that integrate functional vascular structures can help to overcome this limitation [68]. Biomimetic microvascular networks provide controlled experimental platforms to evaluate specific interactions between endothelial cells and tumor environment, improve the design of therapies targeting the vasculature, and assess the efficacy of new drugs or drug delivery strategies [69,70].

Two categories of microfluidic vascular models have been introduced. The first design, pioneered by the groups of Roger Kamm and Lance Munn, employs microfabricated silicone molds to confine biological hydrogels between parallel microfluidic channels (Fig. 2A). Endothelial cells cultured on the surface of these confined gels are able to sprout into the matrix and recapitulate aspects of angiogenic invasion, including sensitivity to shear stress, interstitial flow, and various angiogenic gradients [71–73]. These assays have been used to facilitate coculture with stromal or tumor cells and evaluate paracrine effects in angiogenesis [73]. However, these models are confined by thin geometries ($\sim 100 \mu\text{m}$) and are therefore sensitive to boundary effects due to cell and molecular interactions with the device surface. In addition, the endothelial cells initially form a monolayer against the gel region, rather than a fully-mimetic lumen structure. Therefore, more advanced models of microvascular structure and function have recently been developed.

This second type of platform comprises bona fide vascular structures fully embedded within 3D ECM. Such structures can be developed by molding biomaterials against a thin needle to define a vessel lumen and subsequently seeded with endothelial cells [74,75] (Fig. 2B). These vessels allow high throughput and facile fabrication, but they are restricted to simple linear geometries and are limited by the resolution of the needle. More complex channel configurations can be fabricated by patterning of a sacrificial resist that dissolves after gel crosslinking [76,77] (Fig. 2C), or by replica molding and soft lithography methods, originally developed by George Whitesides' group in silicone plastics [78,79]. Recently, advances in this technique allowed patterning

of cell-laden biological materials [80,81] and the perfusion of scaffolds with endothelialized microvascular channels [76,77,81,82] (Fig. 2D). Although lithographic techniques are limited to planar configurations, the bioprinted sacrificial molds were capable of generating a lattice of interconnected vessels [77]. Finally, modular assembly of cell-laden beads permits the formation of contiguous endothelial architectures, although the geometries of these networks are less defined than the other approaches [83,84]. Together, these platforms enable unprecedented access to questions in vascular biology and vascularized tissue function [85].

In vitro microvascular platforms are particularly suited to quantify physiological parameters governing mass transport and drug delivery [75,81]. For example, microfabricated vascular structures permit measuring endothelial permeability in authentic lumenized channels as a function of pericyte coverage, and in response to biochemical factors known to interfere with endothelial cell signaling [81]. Furthermore, microvascular platforms offer exquisite opportunity to measure cell viability as a function of Krogh distance and metabolic consumption at specific cell densities [77,80] and to monitor transvascular and interstitial transport of therapeutic agents. Finally, they provide increasingly accurate models of the tumor environment, allowing tumor–stromal cell cross talk in the assessment of efficacy [73,86–89]. Taken together, engineering models of vascular transport facilitate pre-clinical studies of drug delivery with human cells within a biomimetic tissue environment.

3. Tumor desmoplasia and ECM remodeling: biophysical changes and their relevance to drug delivery

3.1. Biology of tumor desmoplasia and ECM remodeling

As tumors progress from a benign to malignant stage, they recruit cancer-associated fibroblasts (CAFs) that can modulate drug response through altering ECM physicochemical properties (Fig. 3). CAFs, which include myofibroblasts, deposit abundant amounts of fibrillar ECM molecules including collagen I and fibronectin [90]. These ECM compositional changes entail structural and mechanical alterations of the ECM

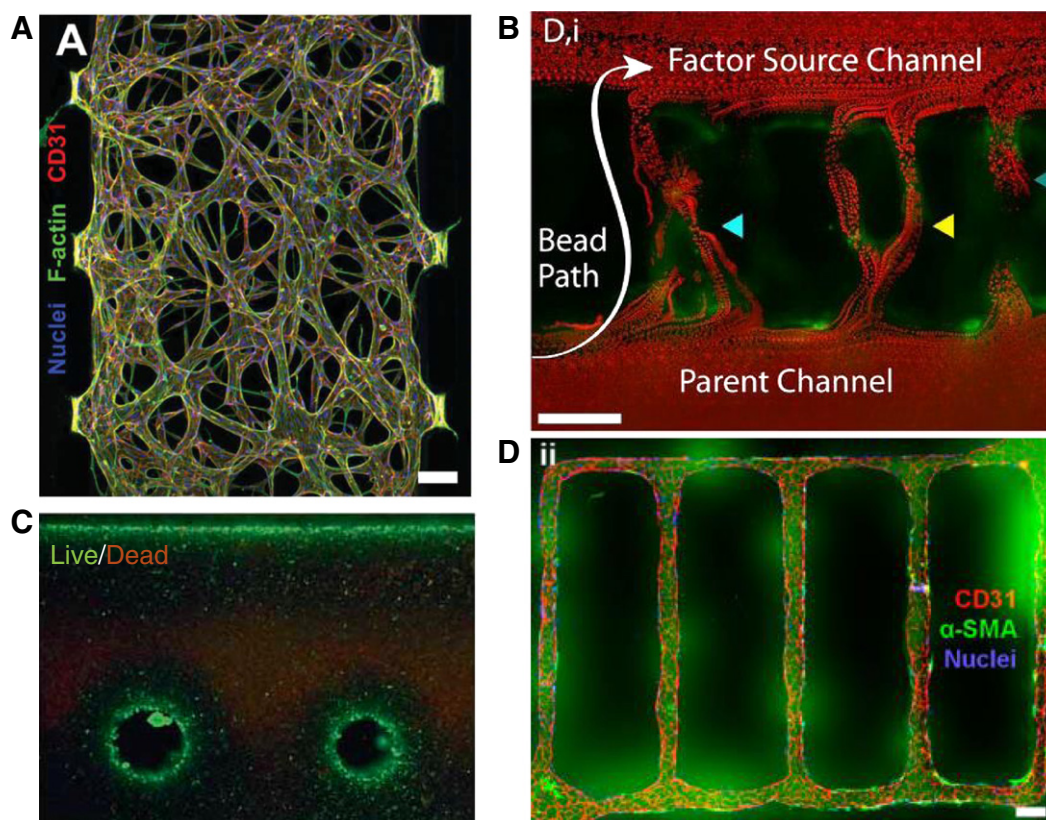


Fig. 2. Examples of microengineered vascular structures. A) Vascular structures created by culturing endothelial cells in confined gels within a microfluidic platform are useful for evaluating endothelial cell response to angiogenic gradients, shear stresses, or paracrine interactions [73]. B) Anastomosis between parallel vessels enables the autonomous formation of capillary-scale networks fully embedded within 3D biological matrices. The original vessel structures were fabricated by the pull-through method [75]. C) Live/dead staining illustrates dependence of cell viability on spatial differences in oxygen and nutrient supply caused by diffusion-limited transport from perfused vascular channels. Vessels were produced by molding a hydrogel against a bioprinted sacrificial scaffold [77]. D) Vascular networks enabling control over endothelial cell (CD31) interactions with pericytes (α -SMA) and membrane permeability (fluorescein leakage) permit complex studies of vascular biology in healthy and disease conditions. Networks were generated in hydrogels by soft lithography [81]. Images were reproduced with permission from the Royal Society of Chemistry (A) and the National Academy of Sciences, USA (B–D).

[91–93]. For example, CAFs mediate the partial unfolding of fibronectin, which increases both the stiffness of individual fibronectin fibers [94,95] and their ability to bind other ECM molecules such as glycosaminoglycans (GAGs) and collagen [96]. Along with elevated collagen crosslinking (e.g., by lysyl oxidase, non-enzymatic glycation, or transglutamination [97,98]) and GAG concentration [92], these pronounced changes in ECM density and intermolecular interactions globally enhance tumor stiffness with direct consequences for tumor progression.

Despite this overall increase in matrix deposition and cross-linking, tumor cells simultaneously mediate the degradation of the ECM, in part by secreting enzymes such as matrix metalloproteinases (MMPs). In addition to generating tumorigenic ECM fragments [99], elevated MMP activity affects the local (microscale) physical properties of the tumor ECM. For example, MMPs can govern degradation and reorganization of fibrillar collagen, while enhancing interactions with other ECM components through exposure of cryptic binding sites [100–102]. Nevertheless, it has to be kept in mind that non-proteolytic functions of MMPs may be similarly important. For example, MMP-9 has been associated with smooth muscle cell adhesion, migration, and cell-mediated collagen contraction, independent of its proteolytic function [102,103]. The diverse roles of MMPs in tumorigenesis have previously been reviewed by Egeblad [104] and Page-McCaw [105].

Alterations of ECM composition, structure, and mechanics stimulate malignancy and drug resistance by inducing manifold changes in cell signaling [106–109]. In general, cell–ECM interactions facilitate the clustering of integrin adhesion receptors leading to cytoskeletal reorganization and cell contractility [107,110]. These changes activate signaling cascades that play critical roles in cell fate decisions, tumor

progression, and drug resistance (e.g., Rho/Rock, ERK/MAPK, and PI3K) [111–114]. For example, integrin signaling alters the co-activation of certain growth factor receptors (e.g., epidermal growth factor receptor [EGFR]) [115] which can impact targeted therapies designed to interfere with the related signaling cascades (e.g., cetuximab and panitumumab targeting EGFR, PLX4720 and PLX403 interfering with BRAF kinase activity) [116,117]. Changes in ECM composition and exposure of cryptic binding domains partially modulate these signaling cascades by affecting integrin selectivity [118,119]. Likewise, increased stiffness-mediated integrin signaling plays a major role in promoting cell contractility, which directly stimulates malignancy by fueling tumor cell proliferation and migration [120]. Moreover, matrix stiffness alters the behavior of tumor-associated stromal cells to drive disease progression. For example, increased stiffness promotes the pro-tumorigenic capability of mesenchymal stem cells [113,121], compromises endothelial barrier functions [122,123], and alters macrophage functions [124]. Finally, stiffness-dependent changes in cell contractility are critical to the myofibroblastic differentiation of stromal fibroblasts [96], thus, providing a positive feedback mechanism that further stimulates desmoplastic remodeling. For these reasons, stromal markers have the potential to serve as important indices that can help to assess and predict patient diagnostics and outcomes [125–132].

In addition to the direct mechano-biological effects, physicochemical changes in the ECM can impact soluble factor signaling [133]. Specifically, the increase of ECM components with growth factor-binding domains affects the bioavailability of these molecules. However, this process is complicated by the reciprocal interplay between multiple ECM molecules. For example, interactions with GAG (e.g. heparin)

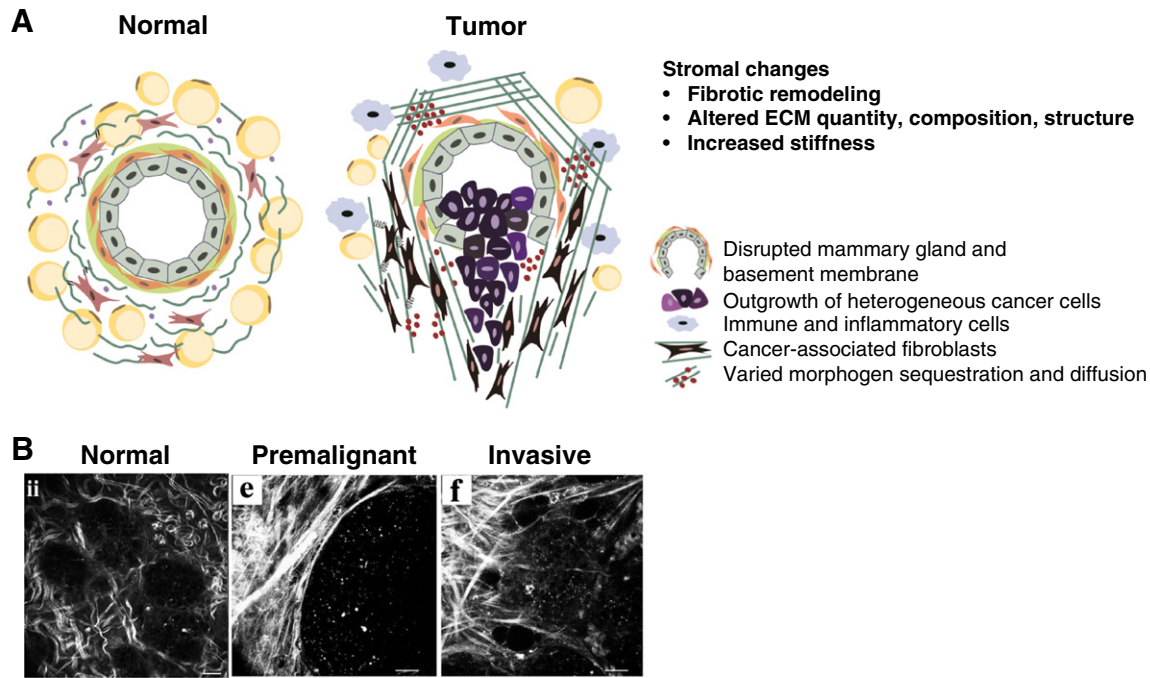


Fig. 3. Biophysical changes of mammary tumor stroma. A) Schematic illustration of the structural and functional changes in tumor-associated mammary stroma. Tumor-derived cytokines not only promote recruitment of inflammatory cells, but also cancer-activated fibroblasts, which stiffen tumors by thickening, linearizing and aligning fibrillar ECM components. These changes alter the transport of growth factors and cytokines, but also of therapeutic compounds with consequences on cancer cell drug resistance. B) Visualization of collagen structure in mammary tissue of normal and tumor-bearing mice via second harmonic generation imaging. Coiled and disorganized collagen fibers are present in normal mammary tissue. Upon tumorigenesis, thicker and linearized collagen fibers develop, which are tangentially and radially oriented along the boundary of premalignant and invasive mammary tumors, respectively [148]. Reproduced with permission from Biomed Central.

modulate fibronectin conformation and expose cryptic binding sites involved in growth factor sequestration [134]. Likewise, changes in cell contractility can expose these latent binding sites and thereby cause the release of morphogens from their ECM depots (e.g. post-translational activation of latent transforming growth factor β [TGF- β]) [135].

Box 2

Forces governing interstitial transport.

Solute transport through interstitial space results from the sum of convective (J_C) and diffusive (J_D) fluxes:

$$J_s = J_D + J_C = -D\nabla C + v_f f C$$

where D is the effective diffusion coefficient, C is the solute concentration, f is the retardation coefficient, and v_f is the interstitial fluid velocity, determined by the solution to the Brinkman equation for flow through porous media:

$$\mu \nabla^2 v_f - \frac{1}{K} v_f - \nabla p = 0$$

where μ is the fluid viscosity, K is the hydraulic conductivity, and p is the hydrostatic pressure difference between the vascular and lymphatic vessels. In most cases, K , D , and f are approximately equivalent to the hydraulic conductivity (L_p), permeability (P), and 1 minus the filtration reflection coefficient ($1 - \sigma_p$) for membrane transport (see Box 1). Parameter values can be found in [11,66].

3.2. Effects of tumor-associated ECM on transport physics

Transport of therapeutic molecules through interstitial tissue is dependent on convection and diffusion [20]. However, the combination of leaky vasculature and dense ECM increases interstitial fluid pressure (IFP) [136,137] and inhibits convection-mediated transport. Consequently, drug-delivery within tumor stroma primarily depends on diffusion [20]. However, dense cellular and matrix components represent diffusion barriers that hinder transport through the interstitium [138,139]. Specific parameters that regulate diffusion efficiency through the stroma include 1) diffusion distance, 2) available volume fraction of pores (accessible space where molecules can pass through), 3) tortuosity of pathway, 4) hydrodynamic resistance, and 5) ECM affinity of the molecule of interest [66] (Box 2). All of these parameters are affected by tumor stroma remodeling. For example, desmoplasia-mediated enhancement of ECM density and structural changes decrease the available volume fraction of pores and increase the tortuosity of the void space, both of which reduce the rate of diffusion through the stroma [140,141]. Therefore, properties of both the drug (e.g. size, charge, configuration, etc.) [142] and the ECM (e.g., composition, viscoelasticity, geometrical arrangement, and/or electrostatic properties) should be considered when approximating an agent's bioavailability [138,140,143–145].

The specific structure of ECM components also modulates diffusion fluxes of therapeutic molecules. Analysis of collagen fiber orientation via second harmonic generation imaging (SHG) revealed that increased malignancy is associated with collagen fiber re-orientation. While collagen in initiating tumors is characterized by isotropic orientation, progression leads to tangential and ultimately radial alignment in expanded and invading tumors, respectively [146,147] (Fig. 3B). These changes in ECM fiber network orientation can promote diffusion anisotropy without affecting the overall diffusion coefficient of the drug [148].

For example, fibers tangentially aligned to the tumor boundary could redirect drug diffusion away from the tumor and, therefore, impair therapy efficacy during the initial stage of tumorigenesis. Theoretically, radially aligned fibers should mediate the opposite effect; however, at this stage tumor cells may have developed resistance phenomena rendering them unresponsive to therapy.

Finally, changes in ECM composition can affect delivery by altering hydrodynamic and electrostatic interactions of the drug with the ECM. Generally, tumor-mediated elevation of ECM viscosity increases the hydrodynamic resistance of molecules and hence slows down their movement through the interstitial space [66,138,141,143,149]. These differences are aggravated by changes in ECM charge distribution (e.g. due to increased association with negatively charged sulfated GAGs including heparan sulfate [133,143]). More specifically, electrostatic repulsion or attraction of charged particles can reduce the available volume fraction of pores and/or cause local sequestration of the drug. Furthermore, electrostatic interaction may not only be relevant to ECM–drug interactions per se, but can also alter the conformation of ECM molecules (e.g. as outlined above for fibronectin–heparin interactions [150]). These variations, in turn, can impact their ability to bind morphogens ultimately exaggerating asymmetric drug distribution within tumors.

The potential opportunity for enhancing drug transport by modifying tumor matrix was demonstrated in a mouse model of pancreatic cancer, where gemcitabine delivery was improved through depletion of desmoplastic stroma by inhibition of hedgehog signaling [151]. However, Infinity Pharmaceuticals' phase II clinical trials for saridegib (IPI-926) in combination with gemcitabine were halted last year due to increased mortality, suggesting that this strategy may require an improved understanding of tumor–stromal interactions in human patients. Consequently, tumor-associated ECM remodeling critically impacts diffusive transport, yet most drug testing approaches neglect the contribution of the above-described parameters. Physiologically relevant 3D tumor models recapitulating the physicochemical properties of the ECM that interfere with diffusion fluxes offer promise to selectively define coefficients modulating drug transport dynamics in tumors.

3.3. *In vitro* models recapitulating ECM-mediated changes in tumor transport

Natural biomaterials can be employed to assess tumorigenesis as a function of altered ECM characteristics. Matrigel®, a basement membrane mixture isolated from murine Engelbreth–Holm–Swarm sarcoma, is currently most widely used. However, inherent batch-to-batch variability, incomplete controllability of physicochemical parameters (e.g. narrow range of stiffness), and lack of covalent crosslinks critical to the basement membrane barrier function [94,152–154] represent limitations of this material. Collagen type I is a defined biological scaffold that can be synthetically modified to provide a wider range of physicochemical properties. For example, collagen stiffness can be adjusted through covalent crosslinking by non-enzymatic glycation [155]. However, this procedure can impact cell behavior by preventing enzymatic degradation of the matrix and by accumulating advanced glycation end products, both of which can affect biochemical signaling [156].

Synthetic biomaterials have emerged as attractive alternatives to modulate stiffness in a more controlled manner. ECM-coated polyacrylamide (PA) gels allow adjustment of stiffness independent of ligand concentration. This platform has been critical to the identification of mechanoregulatory signaling mechanisms of tumorigenesis [120]. However, PA gels cannot be remodeled by cells, and the fabrication method further limits their use to 2D studies which may affect critical cell functions [109,157]. Moreover, altering crosslinking density can affect the surface porosity of PA gels, which may cause conformational changes of the ECM coating that modulate cell behavior [158].

Poly (ethylene glycol) (PEG)-based materials can address some of these challenges. For example, remodelable PEG-based hydrogels have been developed to study the independent effects of stiffness and peptide ligand density on lung cancer cells in a 3D context [159] (Fig. 4A). Moreover, photodegradable or photoreversible crosslinks may be introduced to enable temporal control of substrate stiffness *in situ* [160,161]. By allowing dynamic physicochemical changes in the ECM, such platforms can provide important new insights to tumor development. For example, studying proteolysis-mediated variations of cellular traction could reveal the temporal effects of substrate stiffness on cell fate decisions as recently shown with hyaluronic acid-based degradable materials [162] (Fig. 4B). Furthermore, integration of growth factor binding sites could mimic the effects of matrix-binding on morphogen or drug distribution [163].

While the above approaches rely on changing the material chemistry, ECM structural changes can also be introduced by varying the scaffold fabrication conditions. One relatively simple approach to modify collagen fibril characteristics is by adjusting the gelation temperature, pH, and/or material concentration [164]. Additionally, the collagen isolation technique is a critical consideration, as fibers from acid-solubilized collagen may mimic *in vivo* microarchitecture, but these gels also contain telopeptides that may compromise biocompatibility relative to pepsin-digested collagen [165,166]. Furthermore, the random orientation of spontaneously formed collagen fibrils does not recapitulate fiber alignment in tumors. One method to orient collagen fibers is by casting collagen gels into PDMS molds and exposing them to tensile stress axially [167] (Fig. 4C). However, this loading method may also modulate the behavior of the embedded cells, and interpretation of results is complicated by non-linear cellular response to combinations of mechanical forces and ECM properties [168]. Electrospinning of artificial ECM scaffolds may help to overcome some of these challenges and allow the selective manipulation of ECM structural features at relevant length scales [169–172].

Fibronectin represents an important tumor-associated ECM component whose conformation can be quantified and recapitulated using biomaterial-based approaches. FRET-based analysis as established by the Vogel lab has provided quantitative insights into tumor-mediated fibronectin unfolding [95,173]. This unfolding impacts integrin selectivity by altering the spatial separation between the RGD adhesion and PHSRN synergy sequence. One elegant approach to study the consequences of protein conformation is to modify synthetic, otherwise non-adhesive, hydrogels with RGD and PHSRN peptides either alone or in combination [168]. Alternatively, conducting polymer substrates have been employed to unfold native fibronectin. Applying differential voltages to poly (3,4-ethylenedioxythiophene) doped with *p*-toluenesulfonate (PEDOT:TOS) permits selective manipulation of the conformation of adsorbed fibronectin [150,169] (Fig. 4D). Such systems have been used to elucidate that fibronectin conformational changes influence cell adhesion and secretory patterns for both stromal and tumor cells [170,171]. Yet, fibronectin does not occur in isolation, but is part of complex ECM scaffolds, which can be partially mimicked with fibroblast-derived 3D ECMs. These matrices are generated by detergent-based decellularization and recapitulate various physicochemical aspects of native ECMs including fiber alignment, composition, and density [114,172]. Furthermore, they allow the delineation of effects mediated by healthy and tumor-associated ECMs and have revealed differential cell morphology, orientation, and migration, as well as drug response in healthy versus disease conditions [173].

In order to be useful in drug discovery and screening, the *in vitro* platforms described above must support high-throughput testing of complex libraries of bio-chemical–physical cues. Recently, sophisticated high throughput methods were introduced to screen for combinational effects of ECM physicochemical parameters on cells incorporated in 3D hydrogels [174]. These high throughput microarrays, combined with topographically-controllable biomaterial-based scaffolds, afford a more comprehensive understanding of the

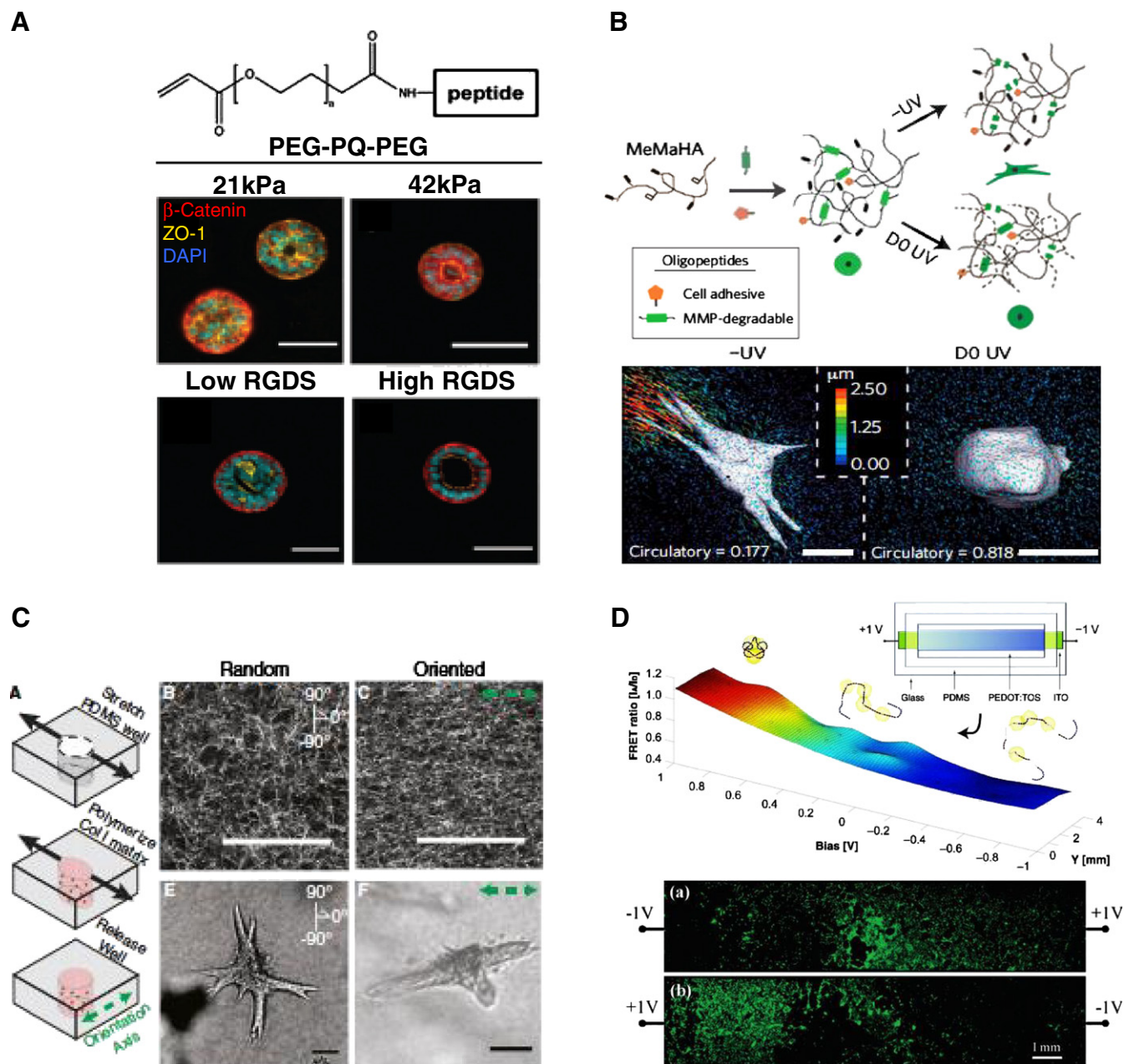


Fig. 4. Examples of engineering approaches to recapitulate tumor-associated ECM characteristics. A) Covalently cross-linked PEG-based hydrogels with controlled stiffness and ligand concentration have been used to investigate the impact of these parameters on lung cancer cells [159]. B) Tunable hyaluronic acid-based 3D hydrogels containing proteolytically cleavable crosslinks revealed a role of ECM degradation-mediated cellular traction in cell fate decisions [162]. C) Studies with stretchable PDMS molds containing fibrillar collagen gels revealed that axially oriented collagen fibers promote epithelial co-orientation, which is prevented by randomly oriented collagen fibers [167]. D) Tumor-mediated changes in fibronectin conformation can be simulated with conducting polymers. Applying an appropriate potential to the cytocompatible conducting polymer poly (3,4-ethylenedioxythiophene) doped with *p*-toluenesulfonate (PEDOT:TOS) yields conformation gradients of adsorbed fibronectin that can be confirmed by FRET analysis and that influence cellular adhesion (green calcein staining) [150,175].

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integrated effects of the tumor microenvironment on drug delivery and efficacy.

4. Future directions

Artificial cell culture platforms have traditionally benefited from the ability to isolate individual biological parameters in a controlled experimental environment, but many challenges exist when moving toward increasingly realistic models for replicating tumor pathology and therapy. Specifically, such biomimetic cultures are confined by

boundaries of space, time, and complexity. Addressing these limitations will help to accurately study cancer biology and translate the findings to clinical practice.

Spatial resolution is a critical parameter of engineered tissues. Although microfabrication technologies afford unprecedented access to cell-scale precision, integrating this resolution into meaningful tissue or organ-level organization is nontrivial. For example, artificial tumors grown in confined structures may undergo differential morphogenesis relative to human disease. In addition, most microfluidic geometries present boundary artifacts, including mechanical and chemical

interactions along the walls of the device that may contribute to this end [175–176]. Moreover, engineered tumors are often isolated systems that lack an organism-level context. Recent integration of multiple organ compartments into body-on-a-chip devices has started to simulate aspects of organism physiology, including drug metabolism and toxicity of anti-cancer drugs [177,178]. These in vitro models provide a first glimpse at holistic consequences of tumor growth or therapy; however, they still neglect critical physiological characteristics, including cellular transport through the blood contributing to tumor progression or metastatic homing. For example, the molecular communication between breast cancer cells and bone marrow-derived progenitor cells may contribute to pre-metastatic niche formation in the target organ [179]. Systemic in vitro models that connect diverse organ compartments through engineered vascular systems may facilitate a more comprehensive understanding of the mechanisms underlying metastasis and define future treatment strategies [180,181].

The temporal scale of in vitro models introduces a second experimental obstacle. Culture conditions of days to weeks must simulate emergent phenomena of months and years. This challenge is especially relevant in the evolution of drug resistance, where population drift relies on many generations of cell division [182,183]. In vitro platforms may approximate these effects of tumor heterogeneity by cell sourcing from primary tumors or by seeding mixed cell populations representing different stages of tumor development [184]. In addition to drug resistance, morphological changes in tissue stroma, including vascular and ECM remodeling, often require longer durations than conventional experimental techniques. This motivates the use of artificial factors such as phorbol 12-myristate 13-acetate (PMA) to rapidly stimulate cellular response [185,186], or the use of low density matrices to accelerate remodeling and migration [187,189]. Although useful experimental tools, these artifices may compromise the integrity of the in vitro system. Computational models of tumorigenesis offer an alternative strategy for recapitulating the time-scale of pathogenesis [190]. For example, multiscale computational models of the tumor microenvironment can be used to predict changes in tumor malignancy as a function of stroma-dependent selective pressure [19]. However, in order to mimic physiological growth rates, these models rely on the accurate measurement of cell kinetics. By providing controlled platforms to measure these critical coefficients, in vitro tumor models may inform the development of accurate in silico models of progression and response.

Physiological complexity, including matrix composition, cellular components, or spatiotemporal presentation of soluble factors, introduces additional compromises for in vitro tissue culture. Notably, increasing complexity often undermines the controllability of the system. For example, although in vitro tumor models enable human-specific drug screening, tumor cells have a broad spectrum of characteristics depending on originating organ, stage or malignancy, and patients' genetic background, additional health complications, and life style. Therefore, short-term culture of cell lines may not cover organ-, patient-, and stage-specific features of tumors. Furthermore, genomic and epigenomic instability leads to diverse clonal populations within a single tumor that cross-communicate [191,192], and this signaling is lost given the uniformity of conventional cell culture lines. Advances in personalized medicine have begun to appreciate the unique molecular signature of individual patients. Integration of patient-derived biopsies within engineered models may allow personalized drug screening prior to treatment. Closely approximating physiological complexity is an asset of engineered tumor models, but remembering these limitations will help guide the interpretation and translation of data collected with these systems.

5. Conclusion

By integrating cancer biology and engineering approaches, realistic tumor models may be developed that have the potential to advance current anti-cancer therapies. More specifically, this approach will help to

better define qualitative and quantitative parameters currently complicating drug transport in tumor-associated blood vessels and stroma. In the future, however, further improvements are needed to enhance the relevance of these models. For example, integration with organ-on-a-chip devices may not only help to recapitulate systemic aspects of the disease, but also to better define the spatiotemporal relationships and patient-inherent complexity complicating drug transport in the clinic. Together with current advances in cancer (epi)-genomics and computational modeling engineered tumor models offer promise to bridge the gap between bench to bedside research and help translate new therapeutic approaches to the clinic.

Acknowledgements

Funding was provided by a NSF graduate research fellowship for P. DelNero, the Clinical and Translational Science Center at Weill Cornell Medical College as well as the National Cancer Institute (R21CA161532, R01CA173083, and by the Cornell Center on the Microenvironment & Metastasis through Award Number U54CA143876). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health.

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