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Chapter 11

Engineering Strategies to Recapitulate the Tumor Microenvironment

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The tumor microenvironment is increasingly recognized as an important contributor to the processes of growth, invasion, and metastasis. Molecular and cellular targets within the microenvironment may thus offer new and fruitful targets for therapeutic intervention. However, progress in understanding and clinically leveraging this regulation has been limited by the complexity of the in vivo microenvironment, which does not easily allow clear dissection of specific regulatory effects. This in turn has created a strong need for in vitro engineered model systems that offer highly precise and independent control of a variety of extracellular parameters that can faithfully recapitulate the tumor microenvironment. In this chapter, we review recent progress in the development of such systems. We discuss microenvironmental signals that regulate tumor growth in vivo, focusing on the extracellular signals a cell receives in the most critical steps in

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cancer progression, including tumor initiation, growth, and metastasis. We then review strategies that have been developed to recreate in vitro important aspects of the cancer microenvironment. Building upon and adapting the lessons learned from tissue engineering, we then prospectively explore two specific paradigms that may enable improved dissection of in vitro signals: decellularized matrices and synthetic matrices.

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11.1 Introduction

Cancer remains one of the deadliest diseases in the United States, claiming more than 577,000 lives in 2012 alone. American men and women face lifetime probabilities of developing cancer of 45% and 38%, respectively [118]. Despite this high incidence and the pressing need for effective treatments, decades of intensive research have only produced a slow decline in mortality rates, suggesting an urgent need to revisit our traditional understanding of tumor progression and broaden the search for potential therapeutic targets.

An important goal in modern cancer therapeutics, including surgical resection, chemotherapy, and radiation therapy, is to selectively destroy or impair tumor cells while minimizing collateral damage to normal host cells. While radiation and systemic chemotherapy are certainly cytotoxic, their imperfect selectivity produces devastating side effects that frequently limit their deployment in the clinic. As a result, there has been an emphasis on improved characterization of tumor heterogeneity to identify the most important tumor-populating cells as well as the development of increasingly cancer-specific therapeutic approaches based on selective targeting of tumor cells based on their aberrant metabolism, receptor expression, or signal transduction.

In the quest to target tumor cells with increasing specificity, cell-intrinsic processes such as intracellular signaling cascades and cell proliferation have received much of the attention. However, mounting evidence indicates that the cell-extrinsic tumor microenvironment plays a critical role in controlling tumor behavior. Key components of the tumor microenvironment include theextracellular matrix (ECM), the solid-state biopolymeric scaffold that surrounds cells in tissue; stromal cells, which frequently

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regulate tumor growth through juxtacrine and paracrine signaling as well as ECM remodeling; and soluble factors such as growth factors and hormones. Just as tumors are frequently characterized by cell-intrinsic dysfunction, so too is the microenvironment often aberrant, which in turn suggests that the microenvironment may represent a relatively unexplored source of untapped therapeutic targets.

Because of the immense complexity of the microenvironment, it has been extremely challenging to determine how individual microenvironmental parameters contribute to tumor progression in vivo. For these reasons, there has been growing interest in engineering reductionist, in vitro model systems that mimic essential features of the in vivo microenvironment while allowing a level of reproducibility and control not achievable in vivo.

In addition to improving our mechanistic understanding of how the microenvironment regulates tumor progression, these reverse-engineered synthetic systems may offer new and unexpected opportunities for molecular screening and help to streamline the use of animal models for drug discovery and evaluation. This is analogous to the recent emergence of "organ-on-a-chip" platforms, which hold great promise as reductionist tissue-like units that could be integrated into microfluidic devices and used for high-throughput drug discovery [47]. Similarly, one could envision recapitulating key functional units of the tumor microenvironment on the microscale, incorporating tumor cells, and using the resulting cell-material platforms for discovery and screening. Such models may help bridge the gap between the culture dish and animal model and thereby improve the predictive power of these in vitro paradigms.

The goal of this chapter is to illustrate the ways the tumor microenvironment has been modeled in vitro and to describe how microenvironmental signals influence the many stages of cancer progression. We will begin with a brief overview of the roles of the ECM in controlling tumor progression, and then we will discuss in depth the many experimental systems used to model this complex environment in vitro. Finally, we will conclude with a discussion of how some next-generation microenvironmental platforms developed in the field of tissue engineering can be used and adapted for modeling the tumor microenvironment.

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11.2 Tumor-Microenvironment Interactions

The extracellular environment plays a critical role in virtually all aspects of tumor progression, invasion, and metastasis, including aberrant cell growth, enlargement of the primary tumor, tumor angiogenesis, migration of tumor cells away from the primary tumor, survival in the bloodstream and lymphatics, and finally metastasis to distal tissues. In this section, we will briefly review key microenvironmental factors that potentially influence tumor cell behavior at each stage of malignant progression, and how these factors are believed to influence this progression. Our overview is intended to provide brief background for the rest of this chapter, which will focus the reverse-engineering and modeling of these interactions in vitro. We would refer the reader to one of the many excellent reviews on the subject for a more comprehensive background [12,21,46,70,115].

11.2.1 Primary Tumor

During the initial stages of cancer development, a tumor consists of a few cells, which are typically undetectable with routine clinical technology. The origin of these cells remains controversial and may vary from tumor to tumor—for example, various lines of evidence support models in which tumors arise from tissue cells that have de-differentiated, from tissue stem cells with deregulated cell cycle control, or both. Whatever their source, tumor cells share the property of dysfunctional and inappropriately regulated growth [91,134]. As with most normal cells, the growth and survival of cells in solid tumors often depends on the engagement of the ECM [13]. The ECM is a complex macromolecular network that includes proteins such as collagen, fibronectin, and laminin as well as proteoglycans, polysaccharides, and other biopolymers [151]. In addition to dictating tissue structure and mechanics, the ECM provides adherent cells with important biochemical and biophysical inputs that control cellular growth; for example, altered presentation or removal of these inputs can stimulate proliferation or trigger apoptosis. Integrins are the best-characterized system of receptors through which cells adhere to the ECM [48]. Upon heterotypic dimerization and binding to a cognate peptide motif in an ECM protein, these transmembrane proteins cluster and

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induce assembly of intracellular adhesive complexes at the cell–ECM interface that mechanically couple the ECM to the cytoskeleton and can regulate the activity of a variety of mitogenic signaling pathways, including those mediated by ERK, Src, and FAK. Importantly, the expression level and complement of both ECM proteins and integrins are significantly altered in many tumors, which may serve to enhance cell growth, motility, and other behaviors relevant to tumor progression [64,104,114]. In fact, these alterations have been explored as potential targets for therapeutic intervention, such as monoclonal antibodies targeted against integrins [36] and tumor-enriched ECM proteins [97].

While integrins have long been understood to signal through classical receptor-mediated mechanisms, it has become clear over the past two decades that mechanical context in which integrins are engaged can strongly influence the resulting signaling. For example, it is now widely acknowledged that ECM stiffness can strongly regulate a wide variety of fundamental cell behaviors and that integrins play a critical, proximal role in stiffness-sensing. For example, increased matrix stiffness can strongly enhance cell division both in vitro and in vivo [55,67,132]. Tumors are often clinically identified by their increased stiffness [8,53], leading to the notion that this increase in stiffness may not only be a byproduct of tumor development, but that it may play a functional role in this progression [63,86,93].

As deregulated cell division continues, the tumor reaches a critical size, approximately 2–3 mm, beyond which the replication of tumor cells near the center of the tumor is limited by the diffusion of key nutrients through the tumor [26,28,139]. To overcome this critical bottleneck, the tumor secretes soluble factors, including vascular endothelial growth factor (VEGF) [23] and basic fibroblast growth factor (bFGF) [15,31,45,135], which serve to recruit endothelial cells from the host vasculature to form new vessels to supply the tumor. This process, known as tumor angiogenesis, allows for the delivery of oxygen and key metabolic precursors, facilitates the removal of metabolic waste products, and enables the tumor to grow substantially larger than the millimeter-scale size limits imposed by diffusion. More recently, it has become clear that tumor cells themselves may contribute directly to this process and thereby circumvent therapies that target host endothelial recruitment. For example, many tumor initiating cells

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may differentiate into endothelial cells that compete with host endothelial cells to populate angiogenic vessels [11,89,100,121,123].

Tumor cells often account for a comparatively modest fraction of the overall tumor mass [50], with the bulk of the tumor made up by fibrotic material and other deposited ECM, tumor-associated stromal cells such as immune cells, fibroblasts [82,141], and angiogenic endothelial cells. These tumor-associated cells often differ significantly from their counterparts found in normal tissue and indeed may contribute significantly to the progression of the tumor. Histopathological studies consistently show that the ratio of myofibroblasts to fibroblasts is enhanced at the edge of tumor, reminiscent of a wound healing response [7,107]. Often thought of as "activated fibroblasts," which may arise from the transformation of normal fibroblasts, these cells promote tumor growth through enhanced deposition of ECM and paracrine signaling through the release of growth factors and cytokines, such as stromal-cell derived factor 1 (SDF-1) [20,82].

Unregulated tumor growth, incomplete and disorganized angiogenesis, and an underdeveloped lymphatic system in the tumor mass leads to interstitial fluid pressures in the growing tumor that are often 50 times higher than that of normal tissue [43,66,119]. This interstitial pressure can lead directly to enhanced proliferation within the tumor, thus contributing to a positive feedback cycle in which enhanced pressures lead to enhanced growth, which further increases interstitial pressure [44,128]. In addition, increased pressure within the tumor can lead directly or indirectly to further angiogenesis through the regulations of VEGF or hypoxia-induced angiogenesis [42,75,102].

11.2.2 Metastasis

The majority of cancer deaths results from metastasis, which is characterized by the extrusion of tumor cells from the primary tumor, invasion of tumor cells into tissue, entry into the bloodstream and/or lymphatic system, transendothelial penetration of distal tissues, and establishment of a secondary tumor in that distal tissue [71,140].

In addition to its role in contributing to tumor growth, increased interstitial pressure may facilitate the migration of

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individual cells or small clusters of cells to break free from the tumor and migrate through the surrounding environment and, eventually, into the bloodstream. This increased intra-tumoral pressure may be transmitted to the surrounding healthy tissue, which can lead to thinning and/or rupture of that tissue's vascular basement membranes, facilitating the escape of tumor cells from the primary tumor [49,86].

If a tumor cell successfully escapes the primary tissue and enters the bloodstream, it is exposed to shear stresses in the vasculature, which can surprisingly enhance the ability of the cell to adhere to distal tissue endothelia, intravasate through the endothelial layer, and establish a secondary tumor [120]. Importantly, most metastatic tumors exhibit strong "tissue tropism," an observation first made by Paget more than a century ago [25,99]. This observation, frequently articulated as the "seed and soil" hypothesis, posits that the cells from specific tumors are calibrated to metastasize to and colonize in specific organs [84]. These and other observations have fueled the hypothesis that specific organs are especially hospitable for the establishment and metastasis of specific tumors by virtue of the physiological milieu they offer the tumor cells. In the years since Paget's observation, the biological basis for this tissue tropism has become better understood, with some lines of evidence pointing to the production of specific growth factors by the host organ [24] or the biophysical parameters of the host organ [57].

11.3 Engineering the Cancer Microenvironment

As illustrated above, extracellular signals in the tumor microenvironment collude with cell-intrinsic lesions to drive malignant progression. In vivo, cells must simultaneously integrate many extracellular signals at once, making it challenging to experimentally dissect how each signal individually contributes to phenotype. As discussed earlier, this need has spurred efforts to "reverse engineer" aspects of the in vivo environment in the form of simple, reproducible, and experimentally tunable paradigms in which individual extracellular cues relevant to tumor progression may be systematically manipulated.

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11.3.1 Natural Matrices

As noted previously, tumor cells must successfully migrate through tissue ECM as they dissociate from the primary tumor and locally invade the host tissue. Initial efforts to create culture paradigms to investigate and dissect this process have emphasized reconstitution of full-length (native) ECM proteins in three-dimensional hydrogels. Given that collagen is the most abundant ECM protein in connective tissues and the most abundant protein in the body as a whole, attempts at recreating a simple, environment-mimetic system featured the encapsulation of cells within reconstituted collagen I scaffolds [65]. In this paradigm, collagen I is isolated from animal connective tissue, e.g., rat tail or bovine skin, and concentrated in an acidic environment. To create a gel from this precursor, the solution is neutralized and warmed to 37°C, which induces fiber assembly and gelation. Though simple, this paradigm has yielded powerful insight into basic mechanisms of tumor cell motility, including the importance of matrix metalloproteases (MMPs) in the digestion of ECM prior to cell migration [109–111].

One of the major drawbacks to using a simple collagen I gel is that it lacks many of the potentially bioactive components of the basement membrane in vivo, including the glycoprotein laminin, which has been shown to specifically promote attachment of certain tumor and cell types [125], and the glycoaminoglycans that normally decorate collagen fibers in vivo. This in turn has spurred efforts to develop reconstituted in vitro matrix preparations that retain these important properties. However, the relative scarcity of basement membrane in typical tissue sources precludes its high-yield isolation. A key step in overcoming this limitation was the recognition that murine Engelbreth–Holm–Swarm (EHS) tumors produce large quantities of laminin- and collagen IV-rich ECM, which compose a substantial portion of many basement membranes. By decellularizing the EHS tumor and solubilizing the remaining ECM, it was possible to isolate large quantities of basement membrane for reconstitution studies. This material, which is now marketed as Matrigel™, forms a gel when warmed to 37°C [56]. Matrigel™ can support the growth of a wide range of tumor cell types [30,73,80,92,145–147] and bears some tumor-specificity; for example, Matrigel™ can support the 3D migration of fibrosarcomas but not non-transformed fibroblasts [58].

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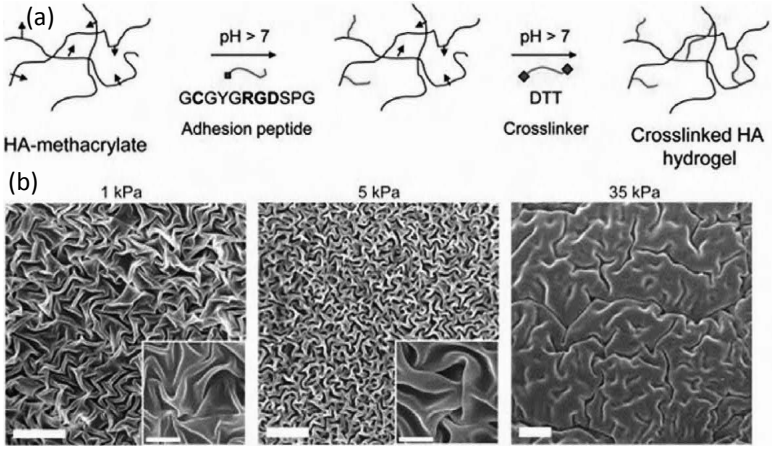

Figure 11.1 Brain-mimetic in vitro environments created from modified hyaluronic acid. (a) Hyaluronic acid is first treated with methacrylic anhydride to create HA-methacrylate. HA-methacrylate is then reacted with an integrin-adhesive peptide containing a cysteine, causing the free thiol and methacrylate to undergo a Michael-type addition resulting in covalent attachment of RGD-containing peptide to the HA backbone. The matrix is then cross-linked with a bi-functional thiol-containing molecule, such as dithiothreitol (DTT). (b) The resulting matrix, as seen with SEM, is a dense, folded sheet with a range of mesh sizes similar to native brain. Adapted from Ananthanarayanan et al., *Biomaterials*, 2011 [9]. Scale bar = 20 μm; insert scale bar = 5 μm.

While collagen and Matrigel™ may be suitable platforms for investigating tumors that arise in connective tissues may thus have close association with epithelial basement membranes, these materials are less appropriate for modeling other tissue types. For example, brain ECM is composed largely of the glycosaminoglycan hyaluronic acid (HA), which is nonfibrillar and plays important structural and biochemical roles in normal brain function and tumor progression. Importantly, the highly malignant brain tumor glioblastoma multiforme (GBM) shows enhanced deposition of HA and expression of its cellular receptor CD44. Moreover, CD44 expression correlates with both tumor-initiating capacity and poor patient prognosis, suggesting that the CD44-HA interaction plays an integral role in the progression of the disease [94,96,129,138]. Reconstituted HA hydrogels are used extensively in the fields of

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tissue engineering, regenerative medicine, and stem cell biology; however, it is only recently that HA has been used to study the migratory properties of tumors originating in the brain. Often HA is modified to yield cross-linkable moieties so that it may produce a robust gel with tunable mechanical properties [3,59,68]. While a wide variety of chemical strategies could conceivably be used to introduce cross-links, in practice many are based on modification of the carboxyl or hydroxyl groups on the HA backbone to yield a reactive moiety (e.g., acrylate or thiol groups), which may then be conjugated to one another with a bifunctional cross-linker (Fig. 11.1) [3,59,68].

11.3.2 Chemotaxis

Migration away from a host tumor by a cell or group of cells often is influenced by chemotaxis, which is defined as the directed migration of a cell or a collection of cells down a gradient of a soluble molecule known as a chemokine. In tumors, these chemokines often take the form of growth or motility factors secreted by endothelial cells, stromal cells, or tumor cells at the invasive front [103].

Chemotaxis has been extensively recreated in vitro [54], with the Boyden chamber assay serving as perhaps the most widely used paradigm for studying this phenomenon [9]. In this system, cells are cultured atop a porous, compliant matrix platform that allows transmigration of cells. This device is then placed in a larger container such as a well of a tissue culture plate that contains culture medium with a defined concentration of serum or one or more specific chemokines. The presence of the chemokine causes the cells to migrate through the matrix and then through the pores of the flexible membrane. The number of cells that cross the membrane and time required to do so serve as quantitative measures of the chemotactic strength of the chemokine. While this method is simple and easy to use, it does have some drawbacks: First, because the axis of migration is in the vertical dimension, this method does not readily permit high-resolution optical imaging of the transmigration process. Second, the chemotactic gradients established in this method are not, in general, quantitatively well-defined, and the population-based nature of the readout averages

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out cell-to-cell variations in the population and time-dependent changes in cell morphology during the migration process.

There are other simple methods to establish chemokine gradients, such as the release of a chemoattractant from a microcapillary tube [112,124]. This method allows for direct visualization of the cell during chemotaxis; however, the gradient established is neither well-defined nor at steady state. Other simple methods to study chemotaxis include the Zigmond and Dunn chambers [149,150], in which two wells of differing chemokine concentration are connected by a bridge containing cells to establish a gradient, and the under-agarose method, where two solutions are separated by a block of agarose in which cells migrate under the agarose toward the solution of higher chemokine concentration. While more amenable to live cell microscopy than the Boyden chamber assay, these methods suffer from lack of reproducibility and the use of an ill-defined gradient [78]. Furthermore, all of the methods described thus far only established gradients at scales much larger than the cell.

Microfluidic devices, which enable control of flow at the microscale, offer impressively high parallelization and yield extremely precise chemical gradients (Fig. 11.2). One of the first microfluidic systems for the investigation of chemotaxis was developed by the laboratory of George Whitesides, and was subsequently adapted to cancer cells [51,72,133]. In this innovative study, cells were cultured on a microfabricated polydimethylsiloxane (PDMS) device in which cells were exposed to two distinct flow streams, one with and the other without the chemotokine CXCL12. Under laminar flow, two distinct fluid streams have a predictable, steady-state mixing pattern, permitting the investigation of chemotaxis in a well-defined chemokine gradient. This study revealed that cells were only responsive to the presence of CXCL12 gradient when EGF was present in the media. However, an important potential drawback of this system is that it exposes cells to shear stress, which can exert potentially confounding effects, such as altering cell proliferation and stiffness, complicating the interpretation of such experiments [78]. As this technology has progressed, microfluidic devices have also been developed that establish well-defined gradients based on diffusion rather than convection, which reduces the amount of shear stress experienced by cells [1,14].

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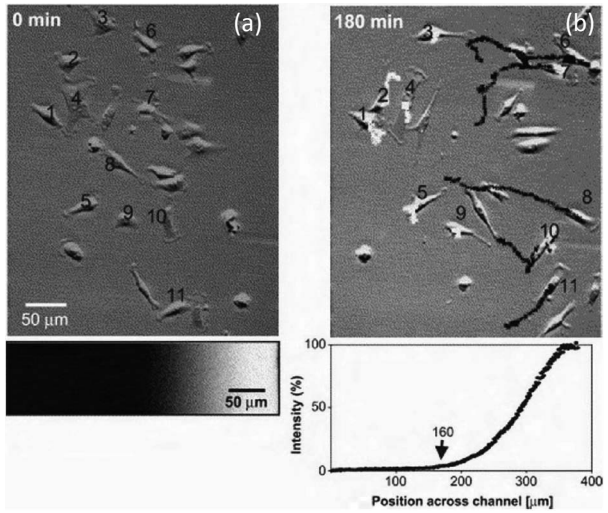

Figure 11.2 Controlling MDA-MB-231 cell migration with a non-linear gradient of epidermal growth factor (EGF). MDA-MB-231 breast cancer cells were followed with phase-contrast microscopy over a period of three hours in a field with a non-linear gradient of EGF concentration. (a) Initially, cells were evenly distributed across the field of view. (b) After three hours, a percentage of the cells migrated persistently towards higher concentrations of EGF (b; black lines). Figure adapted from Wang et al., *Exp. Cell Res.*, 2004 [133].

Combining chemotactic cues with other matrix-associated cues promises to yield insight into the complex factors guiding tumor cell migration. For example, recognizing the importance of both the dimensionality of the matrix and the role of specific adhesion ligands in cell migration, it was shown that cells cultured in a modified microfluidic device containing 3D matrices of either collagen or Matrigel™ were able to respond to chemotactic gradients even in the presence of insoluble, ECM-based signals [2,18,39,40,105,113]. Engineering further complexity into these devices and improving the accessibility of them should greatly enhance the understanding of chemotaxis in tumor environments.

11.3.3 Interstitial Pressure and Flow

The effect of interstitial pressure and fluid flow on tumor cell migration has been convincingly established [44], which has fueled

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efforts to engineer in vitro model systems in which interstitial pressures and flows can be established and controlled. These approaches have exploited both gravity-driven and pump-driven pressure heads. In an especially elegant example of the former approach, Shields and colleagues cultured various breast cancer cell lines in the within a thin (~1 mm) layer of Matrigel™, placed this cell/gel composite in a Boyden Chamber, and then imposed a gravitational flow by placing culture medium atop the Matrigel™ [117].

Many systems have also been developed that use pumps to establish pressure gradients through matrices. By using a peristaltic pump to move fluid vertically through a circular collagen slice, Swartz and colleagues created a radially symmetric flow profile through the gel and discovered that dermal fibroblasts aligned perpendicularly to the flow direction [79]. Microfluidic devices can also be designed to establish interstitial flows, for example, by creating a device with a collagen matrix sandwiched between two reservoirs of culture media and applying a high pressure to one of the reservoirs. Breast carcinoma cells were found to migrate along the streamlines imposed by the flow field [90].

11.3.4 Mechanical Properties of the Tumor

In addition to active processes such as fluid flow and chemical stimulation, tumor cells respond to static mechanical properties of their microenvironment, including the stiffness and dimensionality of the surrounding matrix. In the past decades, many systems have been developed that can recapitulate important features of a tumor's mechanical environment in vitro.

In one of the first attempts to systematically study effects of ECM stiffness on tumor proliferation, it was discovered that *h-ras*-transformed fibroblasts could rescue the suppression of proliferation normally observed on soft ECMs [132]. This study took advantage of polyacrylamide-based matrices, in which stiffness is varied by adjusting the amount cross-linker, bis-acrylamide, while maintaining a constant acrylamide content. The surface is then chemically conjugated with a specific ECM protein to promote cell adhesion. Subsequent studies have used the same principle to vary the stiffness of PDMS gels [38]. PDMS gels are hydrophobic, but can be activated for protein adsorption by treatment with plasma.

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Using PDMS as a cell culture system significantly simplifies the ECM attachment step but suffers from some loss of control of coating density. Polyacrylamide hydrogels and PDMS are still widely used for many experiments today. While the polyacrylamide system has long been assumed to afford orthogonal control over ECM stiffness and biochemistry, this has recently been called into question by the suggestion that cross-linker concentration alters surface porosity, and that this in turn influences cell behavior by affecting the density of conjugation points of the ECM protein to the polyacrylamide surface [130].

An important drawback of these approaches is that they only study the response of cells in two dimensions. The presence of a 3D ECM has not only been shown to influence the growth rate of tumor cells, [27] but it has also been shown capable of reversing a malignant and invasive phenotype in a breast cancer model [131,136,137]. Proper 3D matrix cues have also been shown to play an important role in maintaining the proper differentiation state of many cells, including hepatocytes [6]. Recent advances in 3D culture technology have allowed for the creation of defined 3D tissues in collagen gels, which has led to the discovery that the geometry of these tissues can affect the epithelial-to-mesenchymal transition [37,77]. Furthermore, the biophysical properties of the 3D matrices, especially stiffness and pore size, can be adjusted by a variety of techniques, including double-emulsion polymerization [88,122] and micropatterning [87]. High-throughput methods to systematically manipulate these and other properties offer promise for disentangling the complex inputs that cells receive from 3D matrices [95].

11.3.5 Tumor-Associated Fibroblasts

As noted previously, a significant portion of a solid tumor consists of tumor-associated fibroblasts, suggesting that these cells play a role in disease progression. Co-culturing tumor cells with normal fibroblasts has revealed that fibroblasts produce many soluble factors that promote tumor cell invasion [7,82,141]. Fibroblasts may also independently promote tumor cell migration away from the tumor through ECM remodeling. Using an in vitro mimic of tumor invasion in which fibroblasts and cancer cells were cultured

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simultaneously upon a layer of collagen gel, it has been observed that fibroblasts serve as leader cells—that is, they degrade and remodel the ECM to leave tracks that tumor cells migrate along as they leave the host tumor [32,116].

11.3.6 Angiogenesis

As described earlier, diffusion of material to and from nearby blood vessels is a critical bottleneck to the growth of solid tumors. Thus, the recruitment of new blood vessels from the host vasculature (angiogenesis) is key to continued tumor growth and has been extensively studied and modeled in vitro.

Recently, assays that simulate the formation of blood vessels in vitro have become increasingly popular. In these assays, endothelial cells are cultured on an ECM substrate—typically gels formed from fibrin, collagen I, Matrigel™, or a synthetic polymer such as polyacrylamide—and over time the cells are observed as they migrate, proliferate, and self-organize into structures that resemble vessels, including the formation of tight junctions between cells [4,52,60,98]. Similarly, cells encapsulated within 3D matrices also are able to self-organize and form 3D branched structures [33,34]. Modeling of angiogenic sprouting is often accomplished by culturing cells on latex or dextran "carrier beads," embedding them into a hydrogel (e.g., fibrin) and observing the rate at which the cells leave the beads and penetrate into the gels (Fig. 11.3) [76,148]. Recent work has focused on establishing highly defined gradients of soluble or ECM-bound endothelial chemoattractants to study angiogenesis. Angiogenesis can be spatially patterned by confining pro-angiogenic VEGF and anti-angiogenic VEGF antibodies in agarose hydrogels, which can create highly defined, and temporally stable regions of an ECM hydrogel amenable to angiogenesis [148]. Similar control of pro-angiogenic and anti-angiogenic growth factors can be achieved using microfluidics [22]. In addition to spatially and temporally controlling angiogenesis with soluble signals, it has been shown that patterning PEG hydrogels with light to contain spatially localized zones of the integrin-adhesive peptide RGD or bound VEGF can restrict the zone where tubule formation occurs [61].

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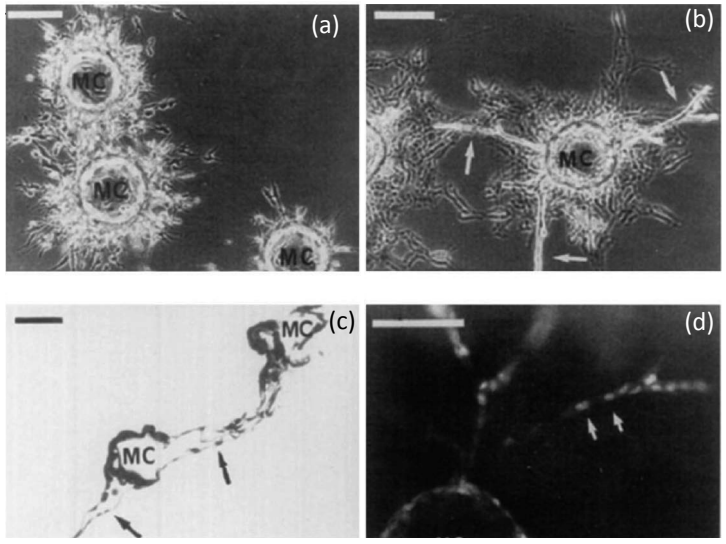

Figure 11.3 Modeling angiogenesis with cell-laden carrier beads embedded in fibrin gels. Human endothelial cells were cultured on carrier beads (MC), embedded in fibrin hydrogels, and observed after three days. (a) Absent addition of growth factors, cells migrated away from the bead but did not form capillaries. The addition of basic fibroblast growth factor (bFGF) resulted in migration away from the bead and formation of vessel-like constructs (white arrows) as observed by phase contrast microscopy (b), methylene blue staining (c), and fluoresecent labeling of nuclei with bis-benzimide (d). Adapted from Nehls and Drenckhahn, *Microvasc. Res*., 1995 [76]. Scale bars = 100 μm.

11.3.7 Extravasation

Model systems have also emerged to recapitulate an important and clinically undetectable early step in cancer progression: the extravasation of circulating tumor cells from the bloodstream through the endothelium. The simplest and most widely used system is a parallel plate flow chamber system in which endothelial cells are cultured on the glass coverslip that serves as a model endothelium [16,17,35]. A second glass coverslip is suspended above the epithelial layer and sealed at the edge to provide a closed flow chamber. Finally, inlet and outlet valves attached to a peristaltic pump are added to the chamber. Perfusion of cell

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culture medium containing suspended cancer cells through the chamber allows for observation with light microscopy of the cells as they adhere to, roll along, and finally extravasate through the endothelium.

11.4 Future Directions and Conclusions

Clearly, efforts to engineer the tumor microenvironment have produced tremendous insight into the role of individual microenvironmental cues in regulating cancer progression. However, the weaknesses of these approaches are derived from their strengths, in that cells in vivo do not experience environmental cues in isolation but rather as a cacophony of simultaneous inputs. By engineering additional complexity into these model systems, it will be possible to maintain the reproducibility of experiments while presenting cells with increasingly engaging environments.

11.4.1 Next-Generation Matrices

The most obvious room for improvement in the engineering of the tumor microenvironment is in the design of matrices that more closely mimic the ECM in which the tumor grows in vivo. Tissue engineers have developed an impressive collection of both synthetic and natural matrices that allow for increasingly precise and multiplexed manipulation of the extracellular environment. Perhaps one of the most powerful set of ECM systems in tissue engineering, given their ability to capture en masse the complex biochemical and architectural features of the ECM that guide cells to produce functional organs, are decellularized matrices. In these systems, ECMs are generated by removing cells with detergent treatment from a whole organ [5,83]. In the context of tumor biology, one could envision using this approach to remove cells from a primary or secondary tumor while retaining salient physical and chemical cues encoded or embedded in the matrix. A key limitation to the use of decellularized matrices is batch-to-batch variability, as every tumor environment is likely to differ in subtle but functionally important ways. Additionally, these matrices also may contain poorly defined quantities of soluble and matrix-bound factors, which may confound the interpretation of results from these studies. Despite these limitations, decellularized

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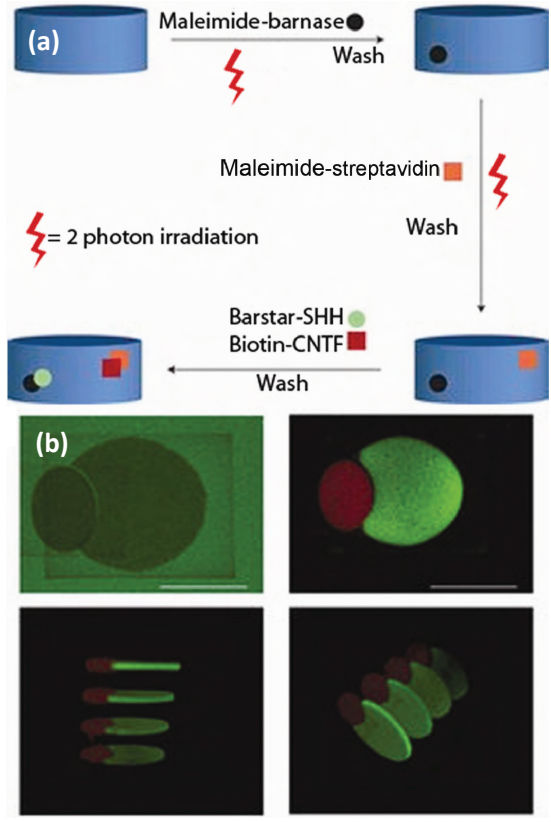

Figure 11.4 Simultaneous patterning of multiple growth factors using two-photon deprotection of thiol groups. A coumarin-based modality protects thiol groups that were grafted onto the backbone of an agarose hydrogel (a). The hydrogel was incubated in a solution containing a maleimide-modified barnase group. Two-photon absorption was used to remove the protecting group, exposing free thiols to react with maleimide functional groups resulting in the covalent attachment of barnase to the agarose backbone. The unreacted maleimide–barnase complex was removed by thorough washing, and the process was repeated using a maleimide-streptavidin complex. By introducing the growth factors sonic hedgehog (SHH) and ciliary neurotrophic factor (CNTF) attached to barstar or biotin, the highly specific binding partners of barnase and streptavidin, respectively, there was high-precision localization of the two growth factors in three dimensions (b). Adapted from Wylie et al., *Nature Materials*, 2011 [144]. Scale bar = 100 μm.

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matrices represent an important and relatively untapped resource in cancer biology and indeed are already beginning to be used in the study of cancer angiogenesis [10].

Alternatively, the tissue engineering field has produced an impressive cadre of modified natural matrices and fully synthetic matrices with many orthogonally tunable properties. Recent work has shown the ability to pattern stiffness in hyaluronic acid and polyacrylamide hydrogels [68,142]. Similarly, three-dimensional patterning of ligands has been shown in many different types of matrices using two-photon patterning and photocaging of specific reaction sites tethered to the polymer backbone (Fig. 11.4) [41,74,143,144].

A wide array of synthetic matrices has also been developed that afford an impressive amount of functionality and customizability [126]. Generally, these matrices involve the use of a bioinactive polymer backbone that can be functionalized in a variety of ways and conjugated with ligands and cross-linked to control matrix stiffness. These polymer scaffolds may be functionalized by including cell adhesive domains such as the peptide sequence RGD [81,85], or by including cell-degradable peptide sequences [62,101,106]. Additionally, by controlling the starting polymer subunit and the polymerization conditions, one can actively control the material properties of these matrices, including scaffold pore size, structure, and stiffness [19,69,108]. Further spatiotemporal complexity can be engineered into the matrix by adding moieties that can undergo photopolymerization or photodegradation [29,126,127]. This rich degree of design flexibility should enable researchers to custom tailor the synthetic matrix to accurately model the complex features of individual tumor environments.

Finally, to fully capture the complexities of the in vivo microenvironment, it will be important to develop strategies for integrating these complex matrices with other reverse-engineered systems representative of the cancer environment. Once this is achieved, it is conceivable that these model tumors will provide the basis for high-throughput drug discovery platforms, along the lines of motivations for current organ-on-a-chip efforts. In an ambitious view, successful application of this technology will not only immensely accelerate the drug discovery process but should also significantly reduce discovery costs and increase the efficiency of the translational pipeline.

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11.5 Conclusions

The tumor environment is a complex milieu that contains many extracellular signals that contribute to tumor growth, progression, angiogenesis, and metastasis. Over the past few decades, enormous effort has been devoted to developing reductionist platforms that have allowed investigators to dissect the contributions of individual physical and biochemical factors to tumor progression. Much work remains to integrate these technologies to produce truly tumor microenvironment-mimetic platforms, which should continue to facilitate both basic scientific investigation and high-throughput drug discovery.

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