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Cell Mechanobiology in Regenerative Medicine: Lessons from Cancer

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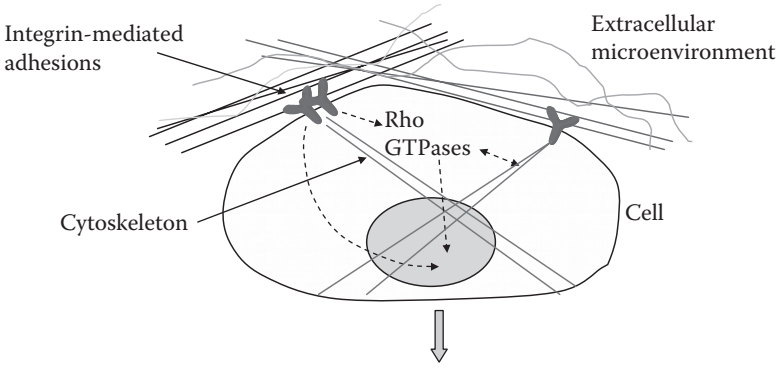
30.1 Introduction

The stem cell "niche" refers to the collective set of cell-extrinsic inputs that controls the functions of stem cells *in vivo*.[1,2] The key regulatory mechanisms within the niche include presentation of soluble and immobilized molecules such as growth factors and cytokines, direct interactions with other cells (e.g., stromal cells), and adhesion to the extracellular matrix (ECM). These diverse inputs are regulated and integrated in a temporally and spatially dynamic fashion to control self-renewal and differentiation, the two hallmark properties of stem cells. Traditionally, the field has approached this subject from a paradigm that is largely biochemical in nature, focusing on the regulatory roles of soluble and membrane-bound ligands on stem cell behavior. While it is clear that these inputs are indeed important, it is also increasingly being recognized that mechanical and other types of biophysical interactions between cells with their extracellular milieu can profoundly influence stem cell behavior. This idea is an extension of a broader awareness that many cell types can sense and apply forces to their surroundings,[3] and that the mechanical interactions of cells with their environment are critical regulators of function in physiology and disease, a concept now widely referred to as "cellular mechanobiology."[4,5] Early efforts in this area have demonstrated that, similar to other cell types in tissue, stem cells are also influenced by mechanical forces and that biophysical signaling can control stem cell self-renewal and differentiation.[6–8] These effects are mediated by intracellular signaling pathways that transduce force cues into biochemical signals that in turn drive fundamental cellular processes such as cell adhesion, motility, proliferation, and differentiation.[9,10]

Despite the growing interest in the mechanobiology of stem cells, our understanding of how these effects may be incorporated into a broader understanding of stem cell biology or leveraged to enhance stem cell-based therapies remains very limited. In addition, the mechanistic details of force transduction

processes in stem cells are still incompletely understood. By contrast, there is a comparatively more advanced literature on the effects of mechanical signaling on a variety of other non-stem cells. In particular, it is now well accepted that dysfunctional interactions between cells and their ECM play a significant role in the initiation and progression of some solid tumors,[11,12] and that mechanical forces can influence malignant transformation, migration, and proliferation of cancer cells in culture.[13,14] Several recent studies have illuminated the role of mechanical signaling from native and engineered ECMs in the initiation and spread of cancer, such as malignant transformation,[15,16] migration,[17] and proliferation.[18] Indeed, it is possible to conceptualize the various stages in the progression of cancer in the form of a "force journey" in which mechanical interactions with the environment influence cellular behavior in concert with genetic and epigenetic cues.[13] This raises the possibility that one might draw upon an understanding of tumor cell mechanobiology to formulate instructive analogies to stem cell mechanobiology, and that this in turn might offer important clues about mechanisms and therapeutic applications.

While the biology of cancer and that of stem cells may appear at first sight to be unrelated, there are in fact several important similarities (Figure 30.1). First, many of the molecular mechanisms known to process force cues are not unique to tumor cells and indeed are critical to the function of many normal cell types, including stem and progenitor cells. These include integrin-mediated adhesion to the ECM, establishment and stabilization of cell structure by the cytoskeleton, generation of cell–ECM tractional forces by actomyosin complexes, and regulation of cytoskeletal assembly and mechanics by Rho-family GTPases.[10,19] Second, many of the processes that contribute to tumor growth, such as cell motility, ECM remodeling, and assembly of angiogenic vessels, are often critical to the success of tissue engineering and regenerative medicine strategies.[80] Finally, the hallmark ability of stem cells to undergo either self-renewal or differentiation bears direct mechanistic relevance to tumors inasmuch as tumor



Regenerative medicine	Underlying cellular process	Cancer
Self-renewal and differentiation	Cell fate choice	Proliferation and tumorigenesis
Cell infiltration into tissue engineering scaffolds	Motility	Tumor invasion and metastasis
Vascularization and integration of newly formed tissue	Angiogenesis	Tumor angiogenesis

FIGURE 30.1 Similarities between cell–ECM mechanobiology of stem cells and cancer. A cell in its native microenvironment receives biophysical and biochemical inputs through integrin-mediated adhesions, initiating signaling cascades that direct the architecture and dynamics of the cellular cytoskeleton and in turn influence cellular contractility and force generation. These reciprocal relationships ultimately result in transcriptional programs effected by proteins such as the Rho family GTPases, thereby governing cell fate, motility, and angiogenesis. These fundamental cellular processes underlie phenomena of interest in regenerative medicine as well as in cancer.

growth frequently reflects profound dysregulation of cell-cycle progression, proliferation, differentiation, and death. This analogy has recently been articulated in a more literal way through the discovery of a privileged population of "cancer stem cells" within certain tumors, which often bear striking similarities to endogenous tissue stem cells.[20–24] The cancer stem cell concept argues that a subpopulation of cells within the tumor mass is largely responsible for sustaining tumor growth through continuous self-renewal, and that this process may be arrested if these cells can be directed toward an alternative fate choice (e.g., death or differentiation).

In this chapter, we seek to explore mechanistic and functional connections between tumor cell mechanobiology and stem cell mechanobiology, with the goal of using the former to guide understanding of the latter. We begin with a brief overview of the mechanobiology of stem cells and the molecular mechanisms that mediate the effects of mechanical signaling. We then focus on the mechanobiology of three critical cellular processes that have historically been investigated in the context of cancer but are equally applicable to stem cell biology: proliferation, motility, and angiogenesis. Finally, we offer a perspective on biomaterial systems that can enable investigation of stem cell and cancer mechanobiology in three-dimensional (3D) topologies, which are an important feature of many native tissue environments and are increasingly recognized to be critical to *in vivo* cell behavior.

30.2 Stem Cell Mechanobiology

Mammalian tissues exhibit a wide range of mechanical properties, ranging from soft tissues such as brain and fat to hard tissue such as cartilage and bone. In fact, there are often significant mechanical heterogeneities within a single tissue, as observed within the hippocampus of the brain.[25] The presence of these mechanical heterogeneities within the *in vivo* niche begs the question of whether they give rise to signals that can directly or indirectly modulate stem cell behavior, and this has recently begun to be addressed with the use of culture systems based on natural or synthetic polymeric matrices.[26–28] These material systems can be engineered to exhibit a wide range of elastic moduli, in contrast to traditionally used glass or plastic surfaces which are many orders of magnitude stiffer than most physiological tissues.

Several excellent reviews have covered the effects of mechanical signaling on stem cell fate,[6–8] so we will limit our focus to a few particularly illustrative examples. Dynamic mechanical loading is widely observed for mature tissues in the musculoskeletal system and vasculature, but has also been observed to be important in the early stages of development.[29] For example, application of force to the *Drosophila* embryo induces expression of *twist*, a gene central to the regulation of germ-layer formation and patterning.[30] Similarly, tensile forces in the cell cortex can promote the sorting of progenitor cells and organization of germ layers in the gastrulating zebrafish embryo.[31] At the cellular level, direct force application promotes myogenesis over adipogenesis in lung embryonic mesenchymal stem cells (MSCs),[32] downregulates pluripotency markers in mouse embryonic stem cells (mESCs),[33] and inhibits differentiation of human embryonic stem cells (hESCs).[34] Similarly, forces associated with shear flow, which have long been understood to be critical for the normal function of vascular endothelial and smooth muscle cells, are now recognized to also control the differentiation of stem cells into cardiovascular lineages[35] and the development of hematopoietic stem cells.[36,37]

The mechanical properties of the microenvironment have been shown to affect stem cell differentiation in dramatic ways even in the absence of directly applied forces. For example, when MSCs are shape-constrained through the use of micropatterned ECM islands and cultured in media permissive of multiple lineages, cells forced to adopt rounded shapes preferentially undergo adipogenesis, whereas cells allowed to spread more fully preferentially undergo osteogenesis.[38] Further, when MSCs are cultured on ECMs of varying stiffness under similar permissive media conditions, softer substrates (0.1–1 kPa) induce neurogenic differentiation, stiffer (8–17 kPa) substrates promote muscle formation, while the stiffest (25–40 kPa) substrates produce bone cells.[39] In other words, MSCs appear to differentiate into tissue types whose stiffness approximates that of the underlying ECM. In both cases, inhibition of actomyosin contractility abrogates ECM stiffness-dependent differences in MSC

differentiation. More recently, ECM stiffness has been shown to regulate the proliferation of MSCs, with softer substrates inducing a quiescent state but not compromising the ability of cells to resume proliferation when transferred to stiff ECMs or to differentiate when treated with the appropriate factors.[40] Mechanosensitivity of stem cell differentiation has also been reported for tissues commonly regarded as protected from large external forces, such as the brain. For example, neural stem cells (NSCs) from the adult rat hippocampus differentiate optimally into neurons on soft substrates (~10 Pa), with stiffer substrates (~10 kPa) increasing glial differentiation.[41] This trend has subsequently been observed for hippocampal NSCs encapsulated in 3D alginate scaffolds[42] and for NSCs derived from other regions of the central nervous system,[43,44] although the precise relationship appears to depend on the tissue and the species source and the ECM ligand.

While the mechanistic details of the above effects remain to be completely elucidated, a large number of proteins and protein complexes have been implicated in the processing of force signals. The primary force sensors are often located in the plasma membrane—for example, G-protein-coupled receptors,[45] ion channels,[46,47] and integrins.[48] Indeed, the mechanosensitive growth and maturation of focal adhesions into structured complexes that contain a variety of cytoskeletal and signaling proteins represents one of the most important and well-studied ECM-mediated signaling pathways.[19,49,50] Another important class of proteins is the Rho family of GTPases, whose canonical members Rho, Rac, and Cdc42 serve as key control points for cytoskeletal assembly and dynamics.[51–53] These pathways directly influence the extent and nature of cell-generated forces, in part by regulating the assembly of actin stress fibers and bundles as well as the phosphorylation of nonmuscle myosin motor proteins that drive contraction of these structures.[54] Rho family proteins and actomyosin contractility have also been shown to mediate the mechanosensitive differentiation of MSCs and NSCs.[38,39,128] Together, these mechanosensitive pathways may contribute to the regulation of gene expression via transcription factors[55] as well as other indirect or epigenetic pathways[56] to direct, restrict, or impose selective pressure on stem cell fate choices.

30.3 Mechanobiology of Cell Proliferation

Self-renewal, the process by which a cell divides to generate daughter cells with developmental potentials that are indistinguishable from those of the mother cell, is one of the hallmark features of stem cells.[57] In other words, self-renewal involves mobilization of processes that promote proliferation concurrent with inhibition of differentiation into a less proliferative or terminally differentiated cell type. The factors that affect self-renewal of stem cells from different tissues and at different stages of development continue to be elucidated.[58] However, it is clearly recognized that the niche plays a central role in the maintenance of stem cells *in vivo*. It has been suggested that the subversion of these normal maintenance signals from the niche is one of the mechanisms through which cancer stem cells gain unlimited proliferative capacities.[59] Indeed, many of the signaling networks that are known to be essential for the self-renewal of stem cells, including the Notch, Wnt, and Hedgehog pathways, were originally identified as oncogenes based on their role in tumor formation.[60,61] This intimate connection between stemness and the proliferative properties of cancer raises the possibility that mechanisms identified as oncogenic in cancer might also facilitate stem cell self-renewal. Before exploring commonalities in signaling between the mechanobiology of tumor cell proliferation and the mechanobiology of stem cell self-renewal, we will discuss potential mechanisms that may underlie the mechanosensitivity of stem cell self-renewal.

There is evidence that some of the pathways that regulate self-renewal are sensitive to mechanical forces. The Wnt pathway is known to be important for the physiological adaptation of bone mass and structure to mechanical loading.[62] Both pulsatile fluid flow[63] and mechanical strain[64] have been shown to activate the Wnt/β-catenin pathway in bone cells, which results in nuclear translocation of β-catenin and increased proliferation. This pathway has also been implicated in tumorigenesis[65] and in controlling self-renewal of stem cells.[66] Similarly, mechanical forces have been shown to induce the expression of proteins of the Hedgehog family in smooth muscle cells[67] and chondrocytes.[68] The mechanosensitivity of these pathways has not yet been explored in the context of stem cell self-renewal.

There is a significant body of evidence supporting the role of mechanical forces in controlling proliferation, and it is becoming clear that several of these effects are communicated through cell–matrix focal adhesions. As we described earlier, these structures serve as organizing centers for both mechanotransductive and mitogenic signaling elements and grow and mature upon application of force. For example, focal adhesion kinase (FAK),[69] extracellular-signal-regulated kinase (ERK), and kinases of the Src family strongly promote proliferation and are all known to localize to focal adhesions.[49] Further, the Rho GTPases, previously mentioned for their role in organizing the cellular cytoskeleton, also play a direct role in controlling cell-cycle progression.[53,70,71] The effect of mechanical signaling on cell-cycle control was tested directly in a recent study in which cells from various tissues were cultured on variable-stiffness ECMs.[72] Compliant ECMs that mimic physiological tissue stiffness inhibited progression through the cell cycle (Figure 30.2), but highly stiff ECMs that mimic the stiffening associated with pathological matrix remodeling accelerated cell-cycle progression through various mechanisms including a FAK-Rac-cyclin D1 pathway. Rho GTPases have been shown to mediate the mechanosensitivity of mesenchymal stem cell differentiation in response to matrix elasticity[39] and cell shape.[38] Thus, mechanosensitive pathways known to be important in cancer and other cells may have direct roles in establishing self-renewal or directing differentiation.

Seminal work by Bissell and colleagues established that the tumor microenvironment plays a critical role in the formation and spread of tumors.[11,12,73] Later, Wang and colleagues showed that the stiffness of the ECM regulates the proliferative ability of normal cells, but that malignant transformation decreases this sensitivity to ECM mechanics, possibly allowing for anchorage-independent and uncontrolled proliferation.[74] This observation is reminiscent of the classical soft agar assay, in which cells are judged to be successfully transformed if they develop an ability to proliferate on soft, nonadhesive ECMs. The hypothesis that mechanics can mediate malignant transformation was tested directly in a landmark

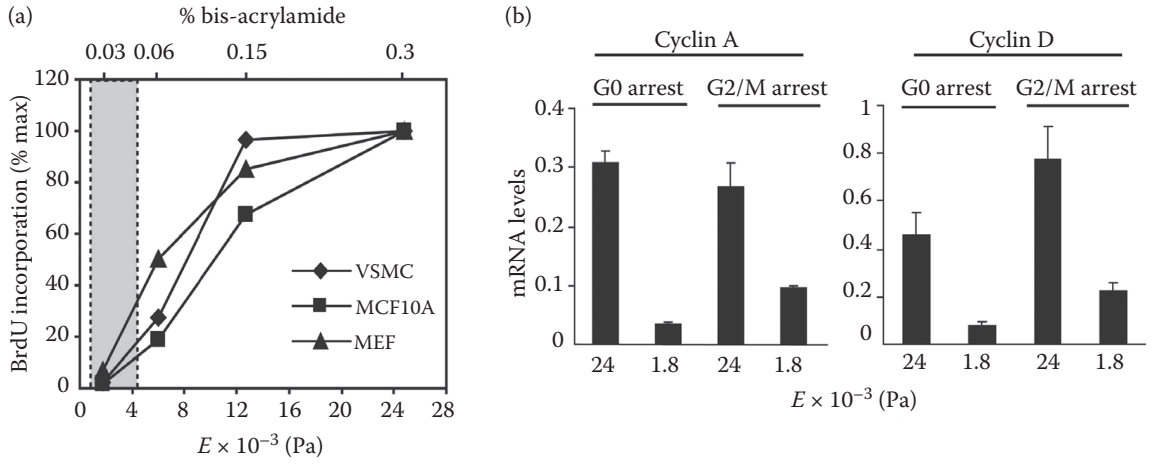


FIGURE 30.2 Mechanobiological control of cell-cycle progression. In this study, the effect of substrate stiffness on cell-cycle progression was assessed in mouse embryonic fibroblasts (MEFs), vascular smooth muscle cells (VSMCs), and MCF10A mammary epithelial cells. (a) Effect of substrate stiffness on cell proliferation. Increasing substrate stiffness results in a greater fraction of BrdU-positive cells for all cell types upon stimulation with mitogens. The shaded area highlights the range of elastic moduli measured in mouse mammary glands and arteries (data not shown). (b) Effect of substrate stiffness on expression of cell-cycle checkpoint genes. MEFs were synchronized at G0 (by 48 h serum starvation) or at G2/M (by treatment with 5 mg/mL nocodazole for 24 h) and then reseeded on hydrogels of varying stiffness and stimulated with 10% fetal bovine serum (FBS). Induction of cyclin A and cyclin D1 expression depended strongly on matrix stiffness regardless of whether cells entered G1 phase from G0 or G2/M, with higher stiffness substrates promoting increased cell-cycle progression. (Reproduced with permission from Klein, E.A. et al. *Current Biology* 2009, 19(18), 1511–1518.)

study by Weaver and colleagues, who showed that culturing nontumorigenic mammary epithelial cells on ECMs of tumor-like stiffness induces dysplasia, proliferation, and activation of oncogenic signaling pathways.[16] The recent finding that breast tumorigenesis is accompanied by crosslinking and stiffening of the collagenous matrix even in premalignant tissue verifies that this phenomenon is relevant to tumorigenesis *in vivo*. These effects are mediated by increased signaling through integrins and focal adhesions, and may be suppressed by the inhibition of lysyl oxidase (LOX).[15] A complementary set of studies with breast epithelial tumor cells in 3D collagen matrices has also elucidated the role of FAK, ERK, and Rho in the promotion of a proliferative and invasive phenotype in response to increased collagen density.[75,76] Our laboratory recently tested the link between ECM stiffness and the pathophysiology of malignant brain tumors *in vitro*.[18] When we cultured human glioblastoma multiforme (GBM) cells on

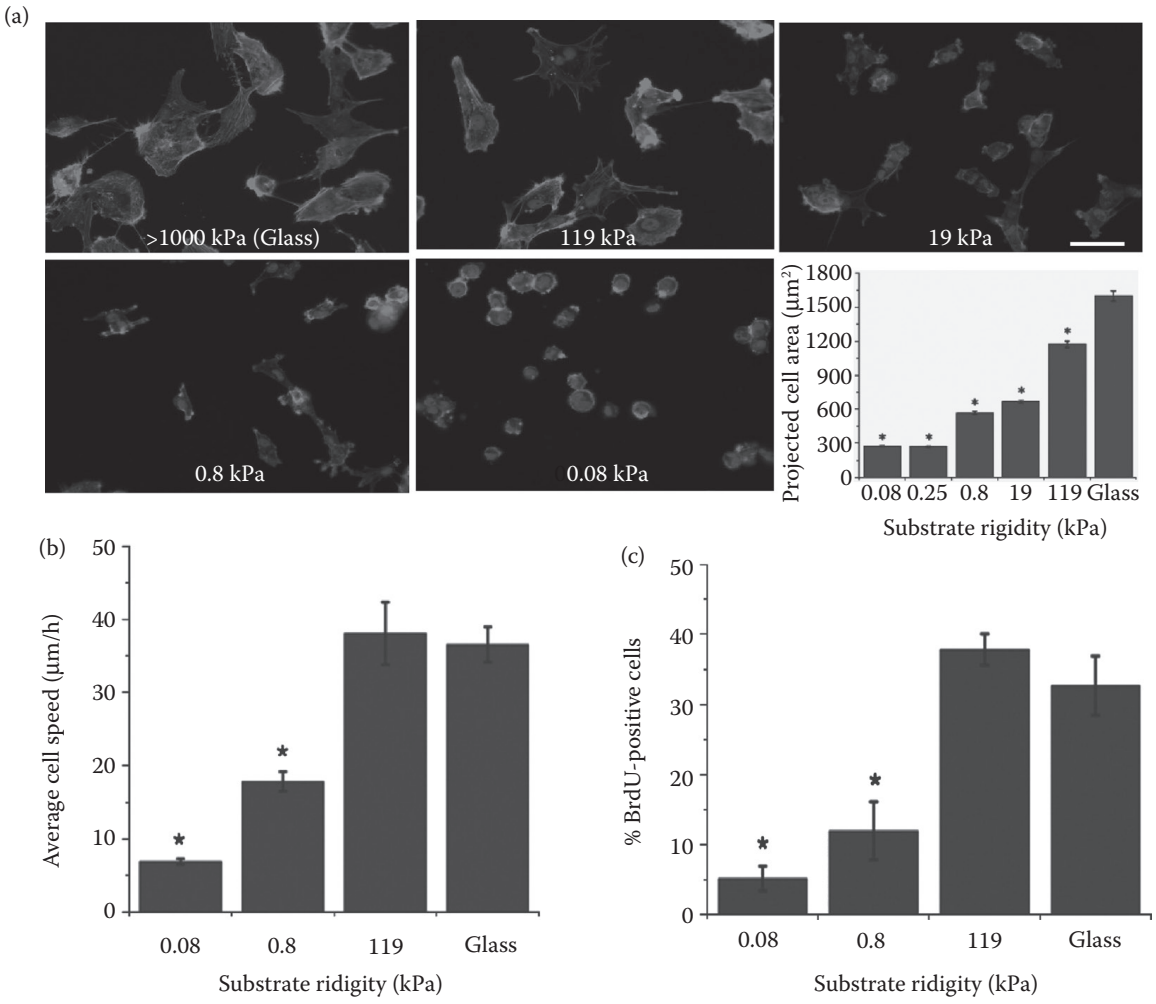


FIGURE 30.3 Mechanobiological control of glioma cell behavior. The effect of mechanics on the morphology, motility, and proliferation of U373 MG glioblastoma multiforme tumor cells was assessed by plating cells on variable-stiffness polyacrylamide substrates coated with fibronectin. (a) Effect on cell morphology and adhesion. Cell morphology shows a steep dependence on substrate stiffness, with cells spreading extensively and forming well-defined focal adhesions and stress fibers on glass or stiff substrates, but not on softer substrates. Immunofluorescence images depict nuclear DNA (blue), F-actin (green), and the proliferation marker Ki67 (red). (b) Effect on motility. Increasing substrate stiffness increases the speed of random cell migration. (c) Effect on proliferation. Substrate stiffness also influences proliferation, with a greater fraction of BrdU-positive cells seen on stiffer substrates. (Reproduced with permission from Ulrich, T.A. et al. *Cancer Research* 2009, 69(10), 4167–4174.)

variable-stiffness ECMs coated with fibronectin, we found that ECM stiffness strongly regulates cellular morphology, motility, and proliferation (Figure 30.3). Increasing ECM stiffness resulted in a higher fraction of dividing cells, as determined by bromodeoxyuridine (BrdU) incorporation. Thus, proliferative signals generated by mechanosensitive pathways have been shown to influence the formation and progression of cancer, and bear investigation in the context of stem cell self-renewal. Mechanoregulation of self-renewal is important not just in niche-mediated maintenance of adult stem cell populations, but also for engineering stem cell therapies where control of cell fate is essential.

30.4 Mechanobiology of Cell Motility

Cell motility is a fundamental process that contributes to development, tissue homeostasis, wound healing, and a wide variety of pathological processes.[77,78] In embryonic development, movements of single cells and multicellular sheets contribute to segregation and patterning and establish the highly specified architecture of developing tissues.[77] Migration of progenitor cell populations is essential in tissues that undergo continuous regeneration during adult life such as the skin, intestinal epithelia, and the brain, where large-scale movements of neural progenitors along defined paths are observed.[78] Further, cell migration is essential during all phases of tissue repair and regeneration, including recruitment of leukocytes as part of the inflammatory response, reentry of cells into the wound area, and revascularization of the tissue.[79] Similarly, cell migration is essential for the success of regenerative therapies such as scaffold-based tissue engineering.[27,80] Indeed, cell infiltration into the scaffold has long been recognized as an important consideration in the design of tissue engineering scaffolds. This has spurred a significant interest in optimizing pore size within scaffolds, for example, for bone tissue engineering,[81] to allow sufficient cell penetration without compromising bulk mechanical properties. Similarly, significant efforts have been devoted to the development of synthetic matrices that can be proteolytically degraded by migrating cells.[82–84] Despite these advances in scaffold engineering, the field could benefit from a greater understanding of the mechanisms that govern cell motility in synthetic ECMs to efficiently design tissue engineering scaffolds for regenerative medicine.

Cell migration on two-dimensional (2D) substrates has been described as a physically integrated molecular process in which the cell undergoes cycles consisting of morphological polarization and membrane extension, attachment at the leading edge, contraction of the cell body, and finally detachment of the trailing edge.[85] In this mode of motility, known as mesenchymal motility, the cell must be able to physically exert force on the substratum through cell–matrix adhesions. This depends not only on the strength of these adhesions[86] but also on the mechanical compliance of the substrate, which determines the response to cell-applied forces. It has now been clearly established that the migration speed of a variety of cell types depends on the elasticity of the underlying substrate.[87–89] For example, we recently showed that the average speed of random migration of glioma cells significantly increases when the matrix stiffness is increased (Figure 30.3).[18] This trend was also observed for glioma cells cultured on variable-stiffness hydrogels composed of hyaluronic acid, thereby extending our previous observations to a brain-mimetic ECM platform.[129] Inhibition of nonmuscle myosin II-based contractility ablates this stiffness sensitivity and rescues motility on soft substrates, indicating a tight balance between protrusive and contractile forces within cells. The phenomenon of "durotaxis" describes cell motion in response to variations in substrate stiffness, with many cell types displaying a trend to migrate toward stiffer regions.[90,91] Therefore, engineering the mechanical properties of the matrix may enable better infiltration of stem cells into scaffolds for tissue engineering applications.

Several novel insights into the mechanisms of cell migration have been deduced from recent studies on tumor invasion and metastasis.[92,93] Perhaps the most intriguing of these is the recognition that tumor cells can exhibit several different modes of motility, differing not only in their average speeds but also in their requirement for cell–ECM adhesions, contractile force generation, and ECM remodeling via proteolysis. As tumor cells invade the surrounding matrix, they often exhibit mesenchymal motility, which is typically accompanied by pericellular proteolysis by secreted and membrane-associated enzymes such

as matrix metalloproteases (MMPs). These enzymes can degrade the surrounding matrix to clear steric barriers against migration. However, in the absence of proteolytic abilities, or when proteolysis is specifically blocked by pharmacological agents, tumor cells have been observed to switch to an "amoeboid" form of motility in which cells depend primarily on contractile forces generated by the actomyosin cytoskeleton to extrude themselves through existing pores and channels in the ECM.[94–96] Amoeboid motility is often viewed as independent of protease activity and the strength of cell–matrix adhesions, permitting tumor cells to escape strategies directed against mesenchymal motility. These findings have obvious clinical relevance in therapies targeting cancer metastasis, but they are also relevant for tissue engineering. The exact nature of stem cell motility in tissue engineering scaffolds will dictate whether strong cell–ECM adhesions are required, or whether degradability by cellular proteases is an important design requirement. Further investigation of these questions should facilitate the formulation of more precise strategies for engineering stem cell behavior in synthetic scaffolds.

30.5 Mechanobiology of Angiogenesis

Vascularization is crucial for the viability of engineered tissue replacements.[97] The therapeutic potential of stem cells in medicine hinges on the ability to generate functional replacements of diseased cell types in the body; however, the efficacy of any stem cell-based therapy will ultimately depend on the extent of vascularization, innervation, and functional integration of the newly formed tissue. Since oxygen and nutrient supply and waste removal depend critically on the vasculature,[98] angiogenesis represents an important step in the success of regenerative therapies using stem cells. It is not surprising, therefore, that a significant amount of work in the development of scaffolds for tissue engineering has been focused on the controlled delivery of growth factors that promote angiogenesis.[27,99,100] Although soluble signaling via growth factors from the vascular-endothelial growth factor (VEGF)[101] and angiopoietin[102] families represent the primary mechanisms governing angiogenesis in mammalian tissue, it has also been recognized that solid-state biochemical and physical signals from the ECM play an important role.[103,104]

Angiogenesis is also clearly an important step in the progression of cancer.[105,106] As a tumor grows and spreads, it outstrips the capacity of diffusion to supply the oxygen and nutrients needed for continued proliferation and expansion. Some tumors acquire the ability to circumvent this limitation by directing the host vasculature to extend new blood vessels. This "angiogenic switch" has received increasing attention in recent years as a potential point for therapeutic intervention to limit the growth of tumors. Indeed, antiangiogenic interventions such as a monoclonal antibody against VEGF (e.g., bevacizumab, commercially marketed as Avastin) have shown clinical success in the treatment of colorectal cancer in combination with chemotherapy.[107] These successes have spurred interest in the diverse mechanisms that promote angiogenesis, including the role played by ECM-mediated mechanical signaling.[104]

Initial work in the mechanobiology of angiogenesis concerned the effects of mechanical signaling on the growth of endothelial cells. For example, it was found that fibronectin density governs cell shape and cell fate, directing proliferation when cells are spread on high fibronectin density substrates, but triggering apoptosis on rounded cells on low-density substrates.[108] The connection between cell shape and cell fate was established conclusively in a landmark study by Ingber, Whitesides, and colleagues, who used microcontact-printed fibronectin ECMs to control cell shape independently of matrix density and soluble factors, and showed that cell shape can independently drive proliferation, differentiation, and death.[109] Further work has focused on the development of microvasculature, such as the formation and structure of capillary networks, as a function of ECM density and stiffness. For example, it has been shown that the density of the collagenous matrix in which endothelial cells are cultured influences their ability to form branched capillaries with small lumens, resembling those found *in vivo*.[110] Similarly, the density of fibrin matrix surrounding endothelial cells cultured on beads has been shown to govern the extent of capillary network formation.[111] Both these results implicate cellular force generation due to actomyosin contractility as an important process through which cells sense and respond to mechanical forces in their environment. In addition to these angiogenic effects, mechanical signaling

is also known to be important in force-dependent neovascularization via enlargement and elongation of existing blood vessels. For example, in an *in vivo* model of wound healing, neovascularization was found to depend on the ability of cells to stress and contract the collagenous matrix.[112]

Mechanistically, the transduction of mechanical force into angiogenic signals is known to partly follow the canonical routes of force transduction outlined previously, including the generation of cytoskeletal tension through the actomyosin apparatus and the activity of GTPases such as Rho.[113] In addition, it has recently been discovered that there may be direct crosstalk between force-mediated signaling and the classical VEGF signaling pathways that govern angiogenesis. In a recent study, it was determined that p190RhoGAP, an endogenous inhibitor of Rho GTPase activation, controls capillary network formation both *in vitro* and *in vivo* by sequestering transcription factors that govern sensitivity to VEGF via expression of the VEGFR2 receptor gene.[55] Further, p190RhoGAP activity may be decreased by increasing the stiffness of the substrate, resulting in increased Rho activation as well as promotion of VEGFR2 gene expression and VEGF-based angiogenesis. Thus, study of the mechanobiology of angiogenesis has revealed several interesting regulatory effects and their mechanisms. These studies can inform the design of material scaffolds and clinical protocols, which, by promoting angiogenesis and vascularization, might enable better integration of stem cell-derived engineered tissues *in vivo*.

30.6 Perspective: Three-Dimensional Material Systems for Investigating Mechanobiology

A large amount of the existing knowledge on cell–ECM interactions has been derived from *in vitro* studies using cells cultured on 2D surfaces. Although these studies have revealed a great deal about the mechanisms of cell adhesion, migration, and force transduction, it is becoming increasingly recognized that cells in their native 3D ECM exhibit behavior that is distinct from that seen in 2D.[114,115] For instance, cell–matrix adhesions in 3D display strikingly different morphology, effects on matrix organization, and protein recruitment patterns compared to those observed in 2D.[116] These fundamental differences in cell–ECM contacts result in a functionally different behavioral phenotype for cells in 3D matrices. This fact has been recognized for the last two decades in the context of the formation and growth of tumors,[11,117,118] and is beginning to be apparent in the context of stem cell self-renewal and differentiation. For example, hESCs cultured in a medium conditioned by fibroblast feeders were shown to undergo self-renewal in 3D scaffolds of crosslinked hyaluronic acid, but not on 2D surfaces of the same material.[119] Similarly, directed differentiation of mESCs into hematopoietic lineages has been shown to be more efficient in 3D culture.[120] Since mechanical communication between cells and the ECM is largely channeled through cell–ECM adhesions, it follows that force sensing and transduction and the concomitant effects on cellular physiology should also depend on the dimensionality of the matrix.[121] For example, we recently delineated the effects of one important aspect of 3D culture—cellular confinement in narrow spaces—by building a novel microfabricated polyacrylamide gel system, where tumor cells confined within narrow channels migrated faster than in wide channels or on flat surfaces of the same ECM stiffness, due to more efficient polarization of cell-generated traction forces.[130] Therefore, it is essential that cell–ECM mechanical signaling be explored in physiologically relevant 3D models.

Traditional approaches to study cell–ECM biology in 3D have focused on natural ECM proteins that form gels under physiological conditions, for example, collagen I and Matrigel. While these materials do partially recapitulate the rich biochemical milieu to which cells are exposed in native environments, they offer a fairly limited range of mechanical properties. Further, the mechanics, microstructure, and biochemistry of these gels are intimately linked, in that changing the bulk density of the gel-forming proteins simultaneously varies all the above properties, making it difficult to attribute observed differences in cell behavior unambiguously to chemical or mechanical stimuli. Further, many of these native biomaterials are inappropriate for stem cell-based regenerative medicine, because they are typically derived from animal sources and therefore suffer from batch-to-batch variability and pose unacceptable

risks with respect to pathogenicity and immunogenicity. Therefore, there has been a significant drive toward the development of semisynthetic and synthetic 3D model ECMs that can be used to study cancer and stem cell biology and might potentially be appropriate for therapeutic use.[27,28,122–124] Several synthetic polymer systems have been developed that can be crosslinked to varying extents, and by inclusion of full-length proteins or short peptides, can mimic the native ECM and also permit independent variation of matrix stiffness and adhesive functionality. Taking a cue from the recent tissue engineering efforts,[125,126] we recently developed a system for studying cell–matrix mechanobiology in 3D based on mixtures of collagen I and agarose, a biologically inert polysaccharide that forms a filamentous meshwork and serves to stiffen collagen gels with modest effects on their fibrous architecture.[127] This hybrid system allows the study of cell mechanobiology in 3D while uncoupling the effects of matrix structure and mechanics from biochemistry. Studies of invasion of spheroids of glioma cells implanted in these gels revealed that increasing agarose concentrations created increasingly stiff gels but progressively slowed and eventually stopped invasion. This result was somewhat surprising, given that increasing stiffness was found to increase glioma cell motility on 2D surfaces (Figure 30.3). However, it appears that steric barriers created by the agarose meshwork present an obstacle to cell migration in 3D and limit the ability of the cells to contract and remodel collagen fibers, combining to prevent glioma invasion.[131] This study illustrates clearly that some aspects of cellular behavior, such as the dependence of motility on the porosity and degradability of the matrix, can only be captured in 3D environments. Therefore, the development of material systems that can increasingly mimic native 3D ECM while retaining independent control of various design parameters such as stiffness, porosity, biochemical functionality, and degradability is crucial for facilitating studies on the mechanobiology of stem cells and cancer.

30.7 Conclusions

Biophysical interactions of stem cells with the extracellular milieu in their native niches as well as in engineered tissue constructs represent an important class of inputs governing cell behavior. Some of the mechanisms by which cells detect and process these inputs are conserved among many cell types, including stem cells, normal cells, and tumor cells. Therefore, a comparative study of these mechanisms may allow us to leverage our knowledge of the mechanobiology of normal cells and cancer to accelerate our understanding of the processes that control stem cell fate and design more effective strategies for regenerative medicine.

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