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Trends in Molecular Medicine

Feature Review

Can't handle the stress? Mechanobiology and disease

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Mechanobiology is a rapidly growing research area focused on how mechanical forces and properties influence biological systems at the cell, molecular, and tissue level, and how those biological systems, in turn, control mechanical parameters. Recently, it has become apparent that disrupted mechanobiology has a significant role in many diseases, from cardiovascular disease to muscular dystrophy and cancer. An improved understanding of this intricate process could be harnessed toward developing alternative and more targeted treatment strategies, and to advance the fields of regenerative and personalized medicine. Modulating the mechanical properties of the cellular microenvironment has already been used successfully to boost antitumor immune responses and to induce cardiac and spinal regeneration, providing inspiration for further research in this area.

Highlights

The physical architecture and mechanical properties of cells and their microenvironment, along with the ability of cells to sense and respond to mechanical inputs, are crucial mediators of tissue development, homeostasis, and repair.

Altered cell and tissue mechanics are a hallmark of many human pathologies and can serve as diagnostic and prognostic markers.

Many diseases are associated with perturbed mechanobiology functions, such as alterations in the mechanical microenvironment that trigger pathogenic cellular responses, or an impaired ability of cells to appropriately respond to mechanical stimuli.

Insights from mechanobiology research can result in improved *in vitro* models that better mimic physiological and pathological *in vivo* conditions.

Altering the mechanical properties of diseased or injured tissue can promote healing and regeneration and improve treatment efficiency.

The art of (mechanically) adapting

'It is not the strongest of the species that survives, nor the most intelligent. It is the one that is most adaptable to change.' Research over the past few decades has proven that Charles Darwin's statement was not only correct on the organismal scale, but that this principle also applies to the cellular and molecular scale. The human body is often referred to as the most intricate and efficient machine ever created. Generating such a complex machine involves the growth, differentiation, and morphogenesis of one of the basic building blocks comprising the body, the cell. During embryogenesis, a single cell grows into a multisystem organism. The importance of growth factors and morphogens in this process has long been recognized. By contrast, the crucial roles of physical forces and properties in successful tissue development have only recently begun to emerge [1]. However, the importance of the ability of cells to sense and respond to mechanical cues does not end with embryogenesis, as tissues rely on their individual cells to adapt and function in a dynamic and physically demanding environment. Human cells, tissues, and organs are constantly exposed to different mechanical forces, such as flow-induced shear stress in the vessels of the circulatory and lymphatic systems and compressive loads applied on cartilage and bone (Figure 1). The composition and mechanical properties of each tissue are carefully tailored to facilitate proper function. At the cellular level, mechanical inputs are transmitted by cell surface receptors, such as integrins and cadherins, as well as cytoskeletal filaments, such as actin and microtubules, and ultimately converted to biochemical signals through mechanosensitive proteins and organelles, which can trigger further signaling responses [2–4]. Throughout this review, we refer to the initial mechanotransduction step (i.e., the transduction of mechanical stimuli into biochemical signals) as 'mechanosensing'. Since it is often unclear which cellular events represent immediate mechanosensing processes, and which are further downstream, we refer to the consequences of mechanical inputs, including both immediate mechanosensing events and the resulting downstream processes, as cellular mechanoresponses (Box 1).

The integrated biochemical signals affect cellular 'decision-making' through activation of key signaling pathways and alterations in chromatin organization, epigenetic modifications, and gene

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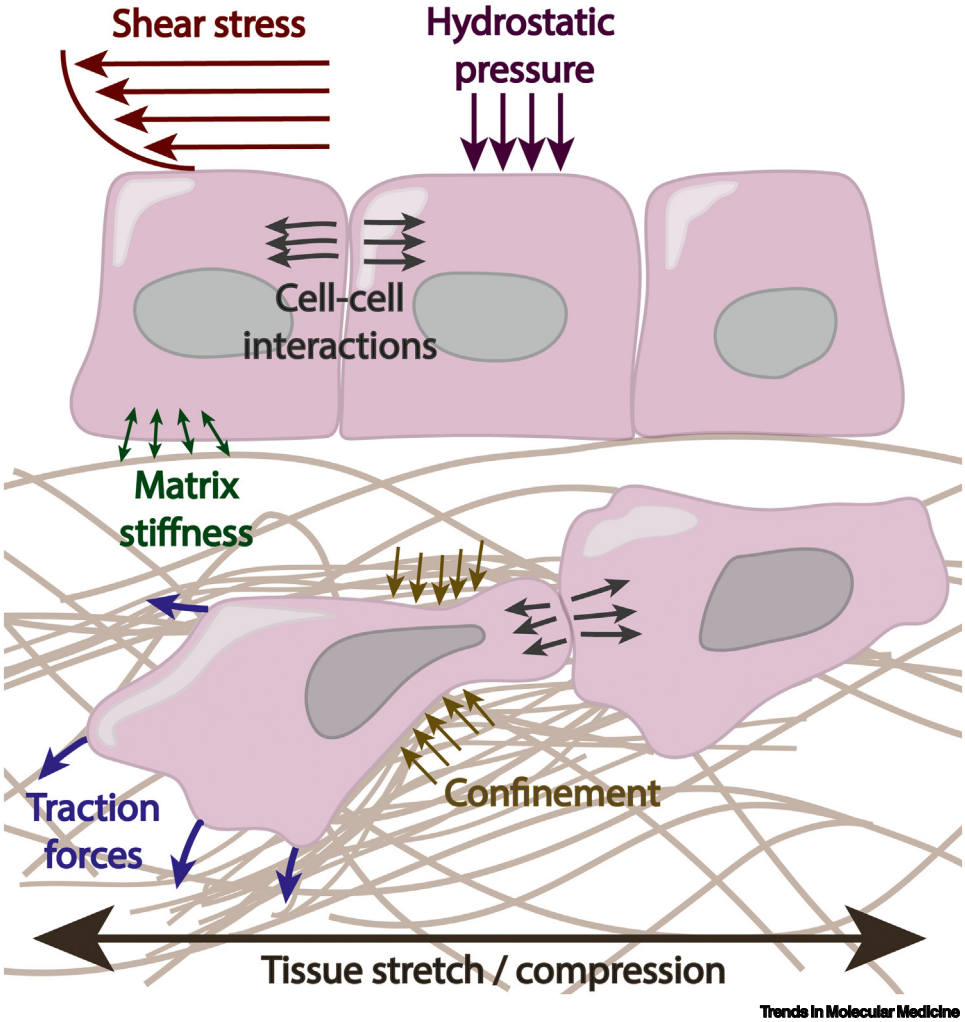

Figure 1. Cells in tissues are exposed to numerous sources of mechanical forces that are sensed by the cells and that modulate cellular fate and function. (i) Fluid shear stress resulting from blood and lymph flow through the circulatory and lymphatic systems, as well as interstitial fluid flow. (ii) Hydrostatic pressure from the circulatory and lymphatic systems and/or interstitial fluid. The more fluid that filters into the interstitial space from the vasculature, the greater the hydrostatic pressure within the tissue. (iii) Cell–cell interactions, such as pushing and/or pulling forces from neighboring cells, or during collective migration or interaction with other cells, such as tumor-associated macrophages or during immune cell activation. (iv) Cells can sense differences in the stiffness, structure, and composition of the extracellular matrix via cell surface receptors, such as integrins and their cytoplasmic connections. Extracellular matrix stiffness is frequently altered in pathological conditions, such as tumorigenesis or fibrosis, modulating cellular functions. (v) Cells can experience physical confinement when migrating through small openings in the extracellular matrix or tight interstitial spaces, or from the presence of neighboring cells. (vi) Traction forces can be exerted from migrating cells on the extracellular matrix. (vii) Large-scale tissue deformation, for example contraction of muscle, stretching of skin, or compressive loads applied to cartilage and bone, result in tensile, compressive, and/or shear forces on the cells within the tissue.

expression [3,5,6]. For example, mechanical stimulation associated with exercise leads to skeletal muscle hypertrophy by inducing metabolic changes through the activation of mechanosensitive signaling pathways, such as Yes-associated protein/Transcriptional coactivator with PDZ domain (YAP/TAZ) and myocardin-related transcription factor (MRTF), resulting in increased protein synthesis and cell growth [7,8]. Tissues and organs not only have the ability to sense and respond to forces, but are also capable of generating them. With every beat, the heart pumps blood

Glossary

Basement membrane (BM): specialized, dense ECM that lines the basal side of endothelial and epithelial tissue, shaping it and providing biochemical and physical cues to the cells overlaying it. In cancer, it provides an initial barrier to invasion into neighboring tissues.

Cancer-associated fibroblasts (CAFs): key cellular components of the stromal TME that help promote metastasis through ECM remodeling and deposition, secretion of growth factors, and modulation of angiogenesis and immune system function.

Catch bonds: noncovalent bonds the lifetime of which increases with the application of tensile force (i.e., the bond strengthens under applied force).

Fluid shear stress: force per unit area acting parallel to the surface of a small volume element caused by local differences in flow velocity, for example, the velocity within a blood vessel and at the endothelial surface. For many fluids, fluid shear stress increases with the velocity gradient and viscosity of the fluid.

Glycolysis: evolutionary conserved metabolic process that does not require oxygen; energy is extracted from glucose through an enzymatic process that results in the production of the high-energy molecules ATP and reduced NADH.

Interstitial fluid pressure (IFP): interstitial fluid originates from blood capillaries and occurs in the space in between cells. It helps to oxygenate and nourish cells as well as remove waste products and is drained into the lymphatic system. IFP is regulated at the local tissue level because it is determined by factors such as capillary filtration mediated influx, lymph outflow, and tissue compliance (the ability of the tissue to expand).

Matrix metalloproteases (MMPs): family of calcium-dependent zinc-containing proteolytic enzymes capable of degrading ECM proteins and involved in a variety of cellular functions, including cell migration, proliferation, and differentiation, and mediation of apoptosis and the immune response.

Photoreactive/thermosensitive hydrogels: synthetic ECMs that pose chemical bonds that can be cleaved by exposing the matrix to light or a specific temperature, therefore allowing temporal control over the bulk mechanical properties of the matrix.

Box 1. Mechanobiology in a nutshell

Mechanobiology studies how mechanical forces are transmitted, sensed, and integrated by cells to affect their 'decision-making' and biological function. External mechanical inputs are transmitted to the cell through the ECM, a meticulously organized 3D network mainly comprising fibrous-forming proteins, such as collagen, which facilitate fast transport [173] and rapid adaptation to a dynamic environment [174–176]. The ECM can also transduce mechanical inputs into biochemical signals by force-induced release of signaling molecules or partial unfolding of the ECM molecules, which exposes cryptic binding sites [174–176]. Mechanical forces emanating from the ECM intersect with the cell at the plasma membrane, where they are sensed by specialized structures, such as focal adhesions, linking the ECM and actomyosin cytoskeleton. Focal adhesion proteins, such as talin, vinculin, p130Cas, paxillin, and focal adhesion kinase (FAK), contribute to both the force transmission from integrin transmembrane receptors to the cytoskeleton and initiation of mechanosensitive signaling pathways [4,177]. Cell–cell junctions, such as cadherins, can similarly transmit and sense mechanical forces exchanged between cells. Mechanical stimulation can also activate stretch-activated ion channels, such as Piezo1, which open in response to increase membrane tension at the plasma membrane or endoplasmic reticulum to allow influx/efflux of ions that can mediate further mechanoresponsive processes [2]. In recent years, piezo channels have been shown to possess key roles not only in physiological cellular mechanosensing, but also in cancer and heart disease [178]. Mechanically induced conformational changes in cytoskeletal proteins can further contribute to cellular mechanosensing. Nesprins and SUN proteins, located at the nuclear envelope, form the linker of nucleoskeleton and cytoskeleton (LINC) complex that connects the nucleus to the cytoskeleton and mediates the transmission of intra- and extracellular mechanical inputs from the cytoskeleton to the nucleus [179]. Emerging evidence suggests that the cell nucleus itself serves as a cellular mechanosensor [180]. Nuclear mechanosensing can arise from mechanically induced opening of nuclear pore complexes [181,182], affecting the shuttling of transcription factors and enzymes, such as Yes-associated protein/Transcriptional coactivator with PDZ domain (YAP/TAZ), myocardin-related transcription factor (MRTF-A), and histone deacetylase 3 (HDAC3) from the cytoplasm to the nucleus, membrane tension and calcium-dependent recruitment of cytosolic phospholipase A2 (cPLA2) to the inner nuclear membrane, where it can produce biochemical messengers promoting cell contractility and inflammatory responses [183–185], or physical deformation of chromatin, inducing changes in gene expression [186]. Collectively, these cellular mechanotransduction responses mediate behavioral changes, mechanical adaptation, and cell fate decisions.

throughout the body by integrating electric activity and tissue deformation to generate fluid pressure. The resulting blood flow induces directional **fluid shear stress** (see Glossary) on the endothelial cells lining blood vessels, causing them to align in the direction of the flow and undergo maturation [9].

The study of how mechanical forces are experienced, integrated, and interpreted by cells to elicit precise biological responses has led to the emergence of a new field, mechanobiology, which encompasses cell and molecular biology, physiology, engineering, chemistry, and physics. Recent findings suggest that disturbed mechanobiology processes are implicated in a growing number of human pathologies, including two of the most common causes of death, heart disease and cancer. Mechanical inputs, such as flow-induced fluid shear stress and contractility, are crucial for cardiac development [10,11], and both genetic mutations and ischemic injury can alter the mechanical properties of the heart, resulting in diastolic and cardiac myocyte dysfunction [12]. Cancer was originally believed to be caused solely by genetic mutations affecting cell proliferation, differentiation, and survival. However, the recognition that mechanical factors, such as the stiffness, structure, and porosity of the local microenvironment, can promote tumorigenesis and modulate critical steps during cancer progression and metastasis has proven valuable in developing new and more effective treatment strategies [13–15], such as enzymatically reducing physical barriers formed by the extracellular matrix (ECM) surrounding the tumor to enhance the penetrance and effectiveness of chemo- and immunotherapies [16,17]. In this review, we highlight new insights into the various roles of mechanobiology in the progression of these two pathologies and discuss emerging roles for mechanobiology in mediating proper immune function. We further demonstrate how lessons learned from the study of mechanobiology, such as the ability to direct mesenchymal stem cell (MSC) differentiation [18,19] or promote tissue regeneration [20] by modulating matrix stiffness, are being implemented to generate new mechano-based therapeutic avenues, either as stand-alone treatments or in combination with existing therapies to boost their efficiency and specificity.

Programmed cell death protein 1 (PD1): inhibitory receptor expressed on the surface of T and B cells, natural killer cells, and some myeloid cell populations. PD1 suppresses the activation and function of potentially pathogenic self-reactive T cells, and PD-L1 can shield target organs and cancer cells from immune attack.

Slip bonds: noncovalent bonds the lifetime of which decreases with the application of force.

Titin: largest recorded protein in humans, also known as connectin and encoded by *TTN*. Titin is abundant in striated muscles and spans half the length of a sarcomere, determining the passive elasticity of muscle by acting as a molecular spring, maintaining the precise structural arrangement of thick and thin filaments, and serving as an adhesion template for the assembly of contractile machinery in muscle cells. Mutations in *TTN* are the most frequent cause of dilated cardiomyopathy.

Heart disease

The heart functions as a powerful pump, with well-defined mechanical properties that allow it to circulate blood rich in oxygen and nutrients to organs and dispose of waste to keep them functioning well. Cardiac morphogenesis is highly dependent upon mechanical forces and their proper sensing. For instance, flow-generated fluid shear stress and myocardial contractility are critical for chamber and valve formation, trabeculation, and ECM deposition in the atrioventricular canal, mediated through Klf-2a and Notch signaling [10,11,21,22]. Congenital heart disease (CHD) stems from improper cardiac morphogenesis and is the most common birth defect worldwide. Approximately 10% of CHD is caused by mutations in specific genes involved in cardiac cell specification or transcription factors with prominent roles in specifying ventricular identity [23]. In most cases, however, the molecular mechanisms causing CHD remain unknown, impeding proper diagnosis and the development of effective treatment options. Aberrant fluid shear forces caused by disrupted blood flow during cardiac morphogenesis are a driving factor for CHD through their effect on mechanosensitive signaling [10,11,21,22] and the expression of the miRNAs miR-143 and miR-21 during cardiac development, which modulate the proper spatial organization of the developing heart and have recently been studied as potential prenatal biomarkers of CHD [24,25].

The adult heart adapts in response to increased mechanical workload brought upon by physiological conditions, such as pregnancy or following physical exercise. In such cases, improved cardiac function is achieved through increased cardiac volume and wall thickness. This physiological hypertrophy results from the addition of sarcomeres, which increase both the length and diameter of cardiac myocytes. By contrast, chronic hypertension, aortic stenosis, myocardial infarction (MI), storage diseases, and genetic mutations can all lead to pathological hypertrophy. In this case, the enlargement of the heart is mediated through cardiomyocyte growth in only one direction; that is, either elongation, which leads to an increase in left ventricle volume without a corresponding increased wall thickness (eccentric hypertrophy), or widening of cardiac myocytes, which increases cardiac wall thickness without an increase (and often a decrease) in left ventricle volume (concentric hypertrophy), eventually leading to cardiac dysfunction and failure [26]. Unlike physiological hypertrophy, which is reversible, pathological hypertrophy is typically accompanied by interstitial and perivascular fibrosis, cardiomyocyte death, myofibroblast activation, and dysregulation of Ca^{2+}-handling proteins, resulting in a dramatic increase in myocardial stiffness at both the cellular and tissue level, mitochondrial dysfunction, increased reactive oxygen species, insufficient angiogenesis [27–29], and alterations in gene expression, mainly of developmental, metabolic, adhesion and ECM components or modulators [30]. The interstitial fibrosis and cardiac myocyte hypertrophy and disorganization associated with hypertrophic cardiac myopathy can result in arrhythmias, particularly atrial fibrillation, presenting a major clinical burden [31]. The increased tissue stiffness associated with pathogenic hypertrophy can also lead to functional impairment of cardiomyocytes [32], including disorganization of sarcomeres and myofibrils, which alters force generation [30,33,34]. Furthermore, increased cardiomyocyte resistance to deformation, resulting from elevated levels of desmin and α-actinin at Z-disks [35], residual actin–myosin cross-bridge formation [36], reduced **titin** phosphorylation mediated by the immune system [37–39], and hyperacetylated microtubules [40] can lead to increased myocardial stiffness, which can negatively affect both cardiac contraction and relaxation during the ventricular filling process, ultimately leading to heart failure.

Although devastating in the long run, fibrotic scar formation following cardiomyocyte loss associated with MI prevents ventricular rupture and, therefore, is crucial to maintaining cardiac structure and functionality after MI. Understanding the differences between physiological and pathological hypertrophy and finding ways to revert cardiac stiffening to physiological levels after it has served

its purpose have the potential to offer new therapeutic avenues. For example, pharmacological inhibition of Rho-associated protein kinase (ROCK), a key modulator of myosin contractility, in engineered heart muscle and connective tissue models reduces tissue stiffness, in part due to a downstream decrease in the expression of the collagen crosslinking enzyme lysyl oxidase (LOX) [41]. Indeed, inhibiting LOX in mouse models of MI reduced collagen deposition, augmented vascularization, improved cardiac function, and reduced cardiac scarring following MI [20,42]. Furthermore, ECM softening following LOX inhibition increased the prevalence of the ECM protein agrin, increased cardiomyocyte proliferation, and improved the regenerative potential in the heart [20,43]. Local delivery of recombinant human agrin to infarct sites improved cardiac function by reducing infarct size and fibrosis in a mammalian model for MI [44]. Transplantation of collagen sheets bound to the **basement membrane (BM)** protein laminin following MI reduced cardiomyocyte passive stiffness by upregulating the expression of the longer, more compliant N2BA isoform of the sarcomeric titin protein, and reducing the expression of the shorter, stiffer N2B [45], promoting the proliferation and adhesion of MSCs [46], reducing fibrosis, stimulating angiogenesis, and improving cardiac function in rat models of MI. These are just a few examples illustrating how modulating the physical microenvironment can improve cellular and cardiac function. Reverting cardiac functional deterioration may benefit from systemic analysis of other cardiac cell types and their potential contribution to pathological cardiac stiffness, including the role of macrophages, which can lead to increased myocardial stiffness and impaired diastolic function [47–49]. However, when modulating cardiac stiffness for regenerative and repair purposes, it will be important to avoid adverse long-term effects, such as wall thinning, and structural weakening due to a defective ECM structure, which can occur in the long run.

Cancer

Cancer, one of the most prevalent diseases worldwide, is typically viewed as a disease of cells and their genes, in which cells acquire mutations that allow them to proliferate uncontrollably, thrive under extreme conditions, evade apoptosis and immunosurveillance, and colonize remote organs. However, fueled by collaborative research by clinicians, cancer cell biologists, engineers, and physicists, it is becoming increasingly clear that the physical properties of the cellular microenvironment, and of the cells themselves, impact all stages of cancer, from tumorigenesis to cancer cell invasion and metastatic outgrowth [50]. For example, the stiffness of the local microenvironment can modulate the behavior of mammary epithelial cells and the transition from normal acini formation to loss of polarity, uncontrolled growth, and invasion [51]. Accordingly, women with dense mammary tissue have a higher risk of developing breast cancer [50–53], and stromal fibrosis and increased tissue stiffness are pivotal drivers of metastatic progression [15,50,54–56]. A stiff and fibrous stromal environment promotes invasiveness by upregulating and activating the mechanosensitive calcium channel Piezo-1 [57] and altering mechanosensitive signaling pathways, such as receptor tyrosine kinase and MAPKs, Rho-ROCK, YAP, and integrin-linked kinase (ILK) [58,59], boosting tumor cell proliferation and inhibiting apoptosis. Epithelial–mesenchymal transition (EMT) is a crucial process in embryonic development and patterning that has been shown to be aberrantly reactivated during tumorigenesis to provide epithelial tumor cells with the ability to invade and disseminate by acquiring mesenchymal characteristics, such as reduced cell–cell contacts and enhanced cell–ECM interactions, which promote single cell migration and invasion, while its reversion favors metastasis [60]. Increased matrix stiffness was recently shown to induce EMT in a variety of cancer types by promoting EMT characteristics and alterations in mechanoresponsive signaling pathways, such as β-catenin and YAP/TAZ in an EPHA2/LYN/TWIST1-mediated manner [61,62]. ECM remodeling and alignment also has a key role in tumor progression, in which specific tumor-associated collagen signatures (TACS) are highly correlated with poor disease-specific and disease-free survival [63,64] (Figure 2). Perpendicular alignment of collagen fibers against the tumor boundaries enhances the distance cancer cells travel

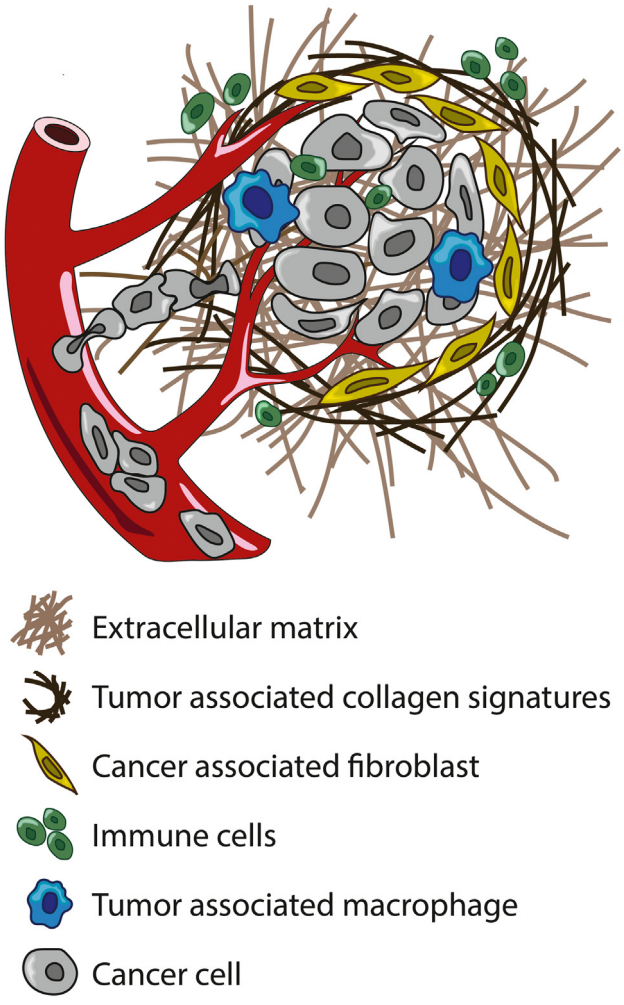

Figure 2. The tumor microenvironment (TME). Tumor cells manipulate both cellular and noncellular elements in their microenvironment to thrive under hostile conditions and facilitate tumor progression. The resulting TME comprises mainly hematopoietic immune cells, such as T cells, B cells, natural killer cells, dendritic cells, and tumor-associated macrophages (TAMs), which, in collaboration with resident stromal cells, such as cancer-associated fibroblasts (CAFs), have a key role in tumorigenesis and tumor progression through matrix remodeling and the generation of specialized tumor ECM structures, such as tumor-associated collagen signatures (TACS). The extracellular matrix serves as a scaffold and represents the noncellular component of the TME. The different elements of the TME interact through the different ECM components, cell–cell contacts, and the release of cytokines and chemokines, among others.

by increasing directional persistence and restricting protrusions along aligned fibers, resulting in a greater distance traveled [65].

The stiffness of the BM, a specialized ECM structure that migrating cancer cells must repeatedly overcome throughout their metastatic journey, poses an additional major determinant of metastatic potential. BM stiffness is governed, in large part, by its netrin-4:laminin ratio, which could serve as a predictor of metastatic potential in prone organs and, thus, also as a valuable tool in determining patient prognosis [66]. Invasive cells can breach the BM using **matrix metalloproteases (MMPs)**, along with the help of tumor-associated macrophages (TAMs) and **cancer-associated fibroblasts (CAFs)**; however, targeting MMPs as a potential anti-invasive treatment has repeatedly failed in clinical trials because cells can switch to alternative migration modes navigating pre-existing spaces [67]. Furthermore, cells are able to alternatively boost mitochondrial trafficking to the invasive cell membrane to fuel F-actin network formation, which physically breaches the BM [68]. A rapidly growing number of studies point to an intriguing crosstalk between cellular metabolism and mechanobiology processes. The increased metabolic

cost of cell invasion across the BM and the associated need for glucose and high levels of ATP are now well recognized [69,70]. Highly motile cancer cells exhibit a positive correlation between ATP production and collagen density [71], indicating an increased energy need and/or mechanosensitive adaptation of ATP production [72]. Recent mechanistic studies revealed important roles of cytoskeletal remodeling in controlling cell metabolism. Stiff cellular environments help maintain high levels of **glycolysis** by promoting the formation of thick actin bundles and stress fibers that spatially sequester the E3 ligase TRIM21, thus inhibiting the degradation of metabolic enzymes [73]. Conversely, disruption of the actin network can lead to release of aldolase from actin filaments, promoting glycolysis [74]

In many cancer types, malignant tumors are stiffer than benign ones, primarily due to increased cell proliferation and ECM deposition [75,76], making them easily palpable. The rapid growth of tumor cells causes the tumor to push against the surrounding stromal tissue, generating considerable mechanical stresses that often compress and partially block tumor-feeding blood and lymphatic vessels, reducing access of immune cells to the tumor [77,78] and resulting in hypoxia and a low pH in the tumor microenvironment (TME), which induce the production of immunosuppressive molecules that further limit immune cell infiltration into the tumor [79]. Hypoxic conditions also lead to hypoxia-inducible factor 1 (HIF1)-mediated alterations to cellular metabolism, increasing glycolysis and lactate production, and activation of mammalian target of rapamycin (mTOR) signaling, further effecting cancer cell growth, survival, and metabolism [80]. The heightened cellular metabolic state increases reactive oxygen species production, which effects autophagy, DNA integrity, and ECM composition [80]. The disturbed fluid homeostasis leads to an increase in **interstitial fluid pressure (IFP)**, practically nonexistent in normal tissue, in the tumor bulk and a steep drop in pressure toward the tumor periphery, pushing cancer cells and growth factors into the surrounding healthy tissue. For example, IFP can promote TGFβ1-mediated mechanotactic cancer cell invasion through fibroblast-driven local collagen fiber degradation and reorganization [81] and prevent the convection of therapeutic agents to the tumor bulk and reduce their retention [13].

Mechanical forces and secreted signals from the tumor, such as various growth factors, along with the local collagen microarchitecture activate resident tissue fibroblasts to become CAFs, which further stiffen the stromal environment by both depositing ECM proteins, including collagen, fibronectin, glycoproteins, and hyaluronic acid, and generating large contractile and compressive forces [82–87], resulting in a self-perpetuating pro-proliferative niche supporting cancer cell invasion [59,88,89]. TAMs can acquire fibroblast-like functions, depositing both collagen and mediators of collagen post-translational modifications and assembly, aiding tumor encapsulation and stiffening of the TME [90]. CAFs and TAMs also aid cancer cell invasiveness by breaching the BM [91], altering the microstructure of the environment to promote the migration of cancer cells [15,59,92–95], and interfering with both innate and adaptive anticancer immune responses [96,97] (Figure 2).

During invasion, intravasation, and extravasation, cancer cells must squeeze through tight interstitial spaces and vascular or lymphatic systems that are often substantially smaller than the diameter of the cell and its nucleus, the largest and stiffest organelle (Figure 2). Efficient migration in these environments is dependent upon the ability of cells to deform their nucleus [67,98]. Not surprisingly, published and preliminary evidence suggests that highly metastatic cells are often more deformable and/or have more deformable nuclei, resulting from reduced expression of the nuclear envelope proteins lamin A/C, promoting migration through such confined environments [99–103]. Lamins A and C are major components of the nuclear lamina that not only determine nuclear stiffness, but also modulate cortical and cytoplasmic stiffness through their interaction with the linker of nucleoskeleton and cytoskeleton (LINC) complex [104]. Depletion of lamin A/C

increases nuclear deformability, emerin mislocalization, and secretion of DNA-containing vesicles, thereby promoting malignancy [105]. Accordingly, lamin A/C and several LINC complex components are downregulated in breast and bone cancers [100,102,103,106]. Nuclear deformations can also occur when cells experience tight confinement, for example at the periphery of solid tumors [107]. Large nuclear deformations resulting from cell compression or confined migration can lead to nuclear envelope rupture, allowing uncontrolled content exchange between the nucleus and the cytoplasm, and DNA damage [108,109], which can promote genomic instability [110,111]. DNA damage can result from the local exclusion or efflux of DNA damage repair factors during nuclear envelope rupture [110] or the influx of cytoplasmic nucleases into the nucleus [107]. DNA damage can also occur from nuclear deformation alone, independent of nuclear envelope rupture, through deformation-induced replication stress [112].

The physical characteristics of the microenvironment affect not only the early stages of tumorigenesis and invasion, but also the subsequent colonization of distant organs. For example, collagen mineralization status in bone can modulate the metastatic potential of breast cancer cells, with breast cancer cells showing reduced adhesion and invasion capabilities when cultured on substrates containing physiological levels of collagen mineralization [113]. This can explain the clinical observation that reduced bone mineral content is associated with an increased likelihood of bone metastasis [114]. Intriguingly, tumor-secreted factors alter the bone microenvironment in regions prone to metastasis, suggesting that tumor cells perturb bone growth and microarchitecture before metastasis, thus preparing the soil for the seed [115,116].

These insights into the crosstalk between tumor cells and their physical microenvironment are beginning to be harnessed for new therapeutic approaches. The unique characteristics of the TME and reducing tumor fibrosis can be used to increase the vulnerability of cancer cells to treatment [88,89,117]. For example, enzymatic ablation of the ECM component hyaluronic acid, one of the primary contributors to the increased IFP and vascular collapse in pancreatic ductal adenocarcinoma (PDAC), normalized IFP and re-expanded the vasculature, allowing for improved anticancer drug penetration. Indeed, combined treatment with standard chemotherapy reduced the metastatic load and remodeled TME in mouse models, doubling overall survival [17]. The high collagen content of tumor ECMs makes it an attractive target for anticancer therapy. Inhibition of LOX-mediated collagen crosslinking reduced ECM content and tumor stiffness in several murine tumor models, leading to improved T cell migration and increased efficacy of the immune checkpoint blocker **programmed cell death protein 1 (PD1)** [16,59]. The abundance of collagen in the TME was recently taken advantage of to target cytokines to tumor sites and potentiate systemic antitumor immunity [117]. Fusing the antitumor cytokines IL2 and IL12 to a collagen-binding protein increased their retention at the tumor location, reducing systemic exposure and, in combination with anti-PD1, halting tumor growth in a melanoma mouse model [117]. In addition to being key mediators of cancer cell migration and invasion, integrins are an important constituent of the tumor immune evasion response [118,119], making them an additional potentially effective immunotherapy target. Indeed, the depletion of integrins β3 and β8 in combination with immunotherapy resulted in lasting immunotherapeutic effects due to reduced primary tumor growth and PD ligand 1 (PD-L1) expression in the TME [118] and increased T cell cytotoxicity [120] in multiple cancer models. Furthermore, whereas targeted depletion of CAFs resulted in increased tumor growth in various mouse models [15], inhibition of specific CAF activities or combining CAF depletion with chemotherapy or anti-CTLA-4 therapy resulted in improved responses in mouse models of PDAC, which is CAF rich and highly fibrotic [14,15].

However, as the above examples illustrate, targeting specific physical aspects can have unintended negative consequences, owing to the pleiotropic mechanotransduction pathways.

For example, inhibiting myosin IIA contractility in glioblastoma reduced tumor invasion but enhanced tumor cell proliferation, and targeting of both myosin IIA and IIB was required to reduce tumorigenesis [121].

Immune response

Immunity is a coordinated response orchestrated by multiple cells over vast topography to combat pathogenic threats and maintain tissue homeostasis. The fast-acting innate immunity constitutes the first line of defense against pathogens. Innate immunity involves physical barriers, such as the skin and mucosal surfaces, and is mediated through cell-dependent mechanisms, such as phagocytosis and cytotoxicity, as well as secreted factors, such as cytokines/chemokines [122]. Adaptive immunity is the second line of defense when innate immunity is insufficient to control an infection. In contrast to innate immunity, adaptive immunity takes days to weeks to establish [123]. The adaptive immune response is pathogen specific and has memory, which leads to more effective responses during re-infection. Adaptive immunity comprises cell-activated responses championed by T cells, and humoral responses, which involve B cells and antibodies [123]. For both innate and adaptive immunity, it is increasingly being recognized that many immune cell functions and interactions with neighboring cells and the surrounding ECM are mediated by mechanical force and force sensing [124,125].

One set of key contributors to innate immunity are macrophages, which are dispersed throughout the body, removing pathogens and cellular debris by phagocytosis, and supporting tissue regeneration [126,127]. A multitude of mechanical cues affect the polarization state of macrophages, dictating their function as either proinflammatory (M1) or prohealing (M2). For example, reduced matrix stiffness and geometrical confinement of macrophages lead to reduced actin polymerization and nuclear translocation of MRTF-A as well as histone deacetylase 3 (HDAC3)-mediated epigenetic chromatin modifications, resulting in a shift from a proinflammatory program to an anti-inflammatory one with impaired phagocytosis [128–130]. Physiologically relevant forces caused by interstitial flow and hydrostatic pressure can regulate macrophage polarization through activation of integrin/Src-mediated signaling and the stretch-sensitive Piezo-1 ion channel [131–133]. The release of certain stress hormones, as well as some environmental pathogens, can alter macrophage stiffness and deformability, affecting their chemotactic and phagocytic abilities [131,134]. During phagocytosis, the shape and mechanical properties of the 'prey' further modulate macrophage function, with bigger and stiffer 'prey', particularly rod-shaped pathogens, more efficiently initiating phagocytosis [135].

T cells coordinate multiple aspects of adaptive immunity and maintain immunological memory and self-tolerance. At the same time, they are implicated as major drivers of many inflammatory and autoimmune diseases [136]. Central to T cell function are their T cell receptors (TCRs), which recognize diverse antigens from pathogens, tumors, and the environment. T cell activation requires the recognition of cognate peptide–major histocompatibility complexes (pMHC) on the surface of antigen-presenting cells (APCs) by TCRs. This mechanosensitive interaction is actin dependent and obtained by the T cell pushing against the APC. Once antigen recognition occurs, the T cell then pulls on the APC to establish close cell–cell interactions. Short-lived nonspecific TCR–pMHC bonds disassociate before achieving T cell activation, whereas longer lived specific TCR–pMHC bonds initiate signaling cascades resulting in massive rearrangement of the cortical actin cytoskeleton, forming three distinct actin networks that effectively stiffen the cells: (i) actin foci comprising branched filaments polymerized by Arp2/3 involved in TCR signaling; (ii) a dense peripheral branched actin filament network facilitating T cell spreading; and (iii) linear actin filaments organized into antiparallel concentric arcs by myosin IIA, facilitating increased T cell adhesion and signaling [137,138] (Figure 3). These actin networks largely function

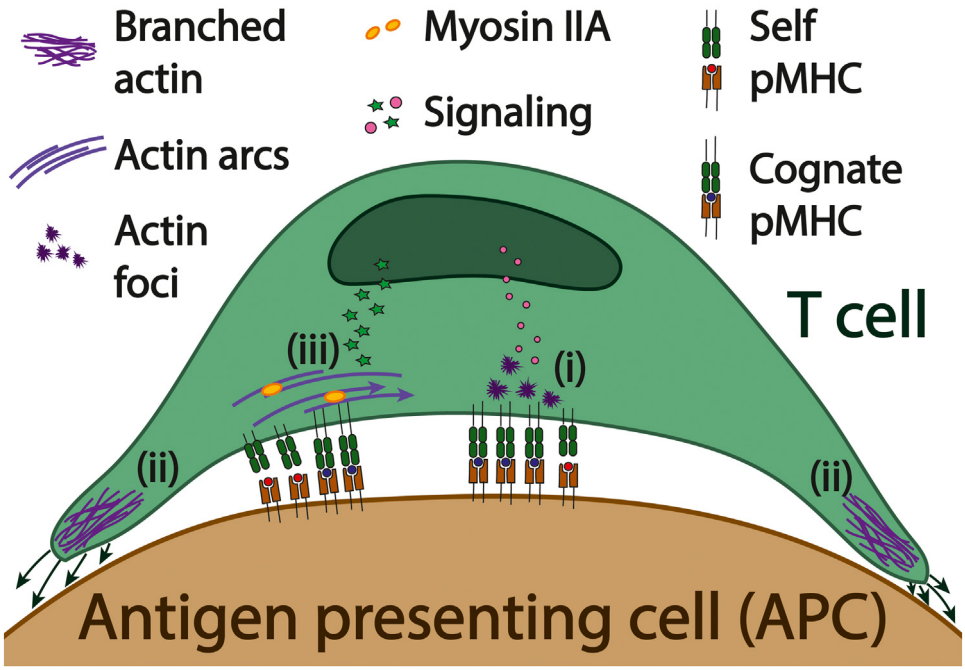

Figure 3. Mechanobiology of T cell activation. The interaction of T cells with antigen-presenting cells (APCs) is a crucial step in the adaptive immune response. Discrete cytoplasmic actin structures apply forces at different areas of the T cell–APC interaction, facilitating T cell activation. (i) Upon initial contact between a T cell and an APC, protrusive actin structures push into the APC to overcome the glycocalyx and create areas of close contact between the T cell and APC cell membranes. Subsequent retraction of these dynamic structures creates tension on the T cell receptor (TCR)–peptide–major histocompatibility complexes (pMHC) bonds that facilitates antigen discrimination between self and cognate pMHCs and TCR activation, because only strong, highly specific TCR–cognate pMHC bonds will withstand the applied forces. (ii) At the leading edge of the migrating T cell, actin-driven lamellipodial protrusions apply force on the interacting APC, allowing for TCR triggering and signal accumulation. This region contains a prominent branched actin network generated by the Arp2/3 complex activator WAVE2. The same mechanism is in play during early stages of the formation of a stable immune synapse, where initial synapse triggering induces spreading of the T cell on the APC surface. (iii) Myosin contractility drives retrograde flow of actin bundles that bend as they move inward and are cross-linked by myosin IIA. The resulting actomyosin arcs sweep TCR MHCs toward the center of the cell, promoting sequential triggering of many TCR molecules by a single agonist pMHC.

independently but collaborate to shape the function of the resulting specialized cell–cell interphase (immune synapse) [137–139]. The formation of the immune synapse is governed by the mechanical stiffness of the T cells and their actin dynamics: naïve T cells have a stiff actin cortex due to lower cofilin activity and high basal activity of the RhoA-ROCK/LIMK (LIM kinase) pathway [140]. By contrast, CD4+ activated T cells are more deformable, allowing for larger immune synapses formation, and greater proliferation, activation, and migration abilities in confined environments [141]. To prevent autoimmunity, immature T cells undergo positive and negative selection in the thymus to assure that they have correct force-dependent binding affinity for successful TCR–antigen interactions, in which nonspecific bonds can dissociate before achieving T cell activation. Positive selection is based on **slip bonds**, which are more likely to dissociate under force, whereas **catch bonds**, which strengthen under force, mediate negative selection, thus ensuring the eradication of T cells with a high probability to self-react [142].

Similar to T cells, B cells also rely on mechanical feedback to assess their interaction with antigens. B cells use myosin II-generated forces to physically acquire antigens from APCs, with only high-affinity interactions of the B cell receptor (BCR) and antigen forming sufficiently strong

Clinician's corner

The ability of cells and tissues to sense and adapt to mechanical inputs stemming from their surroundings is an important mediator of tissue homeostasis by effecting diverse cellular roles, including differentiation, migration, communication, and metabolism, as well as more global functions, such as mediating the immune response and several tissue regeneration and repair processes.

Disrupted mechanical adaptation is implicated in a growing number of human pathologies, from developmental defects, autoimmune diseases, musculoskeletal and cardiac myopathies to obesity and cancer.

Lessons learned from the study of mechanobiology in physiological and pathological conditions are starting to lead to new therapeutic approaches, either alone or in combination with traditional treatments. Reverting pathological tissue stiffening is emerging as a promising strategy to promote tissue healing and regeneration following various injuries, from MI to spinal injury. Targeting fibrosis associated with many tumors, such as pancreatic cancer, can increase the penetrance and effectiveness of existing anticancer drugs and immunotherapies. The unique properties and composition of a given cellular microenvironment can be taken advantage of for targeted delivery of healing agents, or, when manipulated *in vitro*, can drive the selective expansion and differentiation of specific cell populations, such as tumor-targeting T cells and patient-derived cardiomyocytes or muscle stem cells.

Biomaterials have long been recognized for their compatibility and functionality *in vivo*. Therefore, numerous synthetic and naturally derived biomatrices, such as collagen, gelatin, fibrin, alginate, hyaluronic acid, polylactide, and polyglycolide, are finding use as delivery carriers to improve the controlled delivery and efficacy of pharmaceutical agents and cells. At the same time, synthetic materials, such as polyethylene oxide (PEO), polyacrylic acid (PAA), poly-*N*-isopropylacrylamide, polyvinyl alcohol, and polyethylene glycol (PEG), which provide more precise control over the composition and mechanical properties and can be functionalized with

bonds for internalization, whereas weaker, low-affinity BCR–antigen bonds fail to allow internalization [143]. Dendritic cells (DCs), a family of APCs that serve as mediators between the innate and adaptive immune system, are highly mobile cells that patrol tissues in search of pathogens. DCs and other leukocytes, which migrate with their large nucleus facing forward, use their nucleus as a mechanical gauge to probe the pore size of their microenvironment and to choose the path of least resistance [144].

A better understanding of immune cell mechanobiology and the design of new culturing methods for immune cells can lead to more effective immune therapies. New culturing platforms, such as APC-mimicking [145] and hyaluronic acid-based [146] scaffolds, can induce select and large-scale activation of rare antitumor T cell populations, leading to reduced tumor growth and improved survival [145,146]. Fine tuning immune therapies could allow us to modulate the patient's own immune system to fight cancer, infection, and injury more efficiently and with less-detrimental effects.

Regeneration and tissue engineering

Whereas some tissues and organs in the adult human body can regenerate continuously or in response to injury, such as the epidermis, intestinal crypts, or the liver [147], other tissues, such as the heart or central nervous system (CNS), have only limited regenerative capacity, leaving them especially vulnerable to injury and disease. Recent findings suggest that changes to the tissue microenvironment can stimulate regenerative ability in multiple tissues, including the heart and CNS [20,148]. Cardiac and spinal injuries often result in scar formation, which is one of the key barriers to functional tissue regeneration and cell regrowth [149,150]. Indeed, reducing cardiac ECM stiffness induced cardiomyocyte proliferation, improving cardiac regenerative potential in mouse models of MI [20]. Understanding the difference in ECM composition and cellular response to injury between regenerative and nonregenerative organs [151,152], and through studying model organisms with more complete regenerative abilities [153,154], may open new avenues of regenerative medicine.

In zebrafish, which can regenerate most of their organs, including the heart, embryonic-like intracardiac flow following cardiac injury activates the flow-sensitive transcription factor klf-2a and its downstream target Notch to promote early cardiac valve regeneration [153], suggesting that reactivation of developmental signaling pathways can offer a potential avenue to induce organ regeneration. For example, the mechanosensitive YAP/TAZ [155–157] signaling pathway has proregenerative effects in the heart, intestine, and liver; in skin, it can shift its role from maintaining homeostasis to promoting skin regeneration and healing [156]. However, when applying such a therapeutic strategy, caution must be taken, since many signaling cascades, such as YAP/TAZ, have diverse and tissue-specific functions and are activated by cell intrinsic and extrinsic cues [158]. Systemic reactivation that may benefit regeneration on the one hand can have off-target effects, such as promoting malignancy, on the other hand, thus impeding the intended therapeutic potential.

Insights into the mechanobiology of development and regeneration can be used to engineer biomaterials for therapeutic strategies to trigger repair and regeneration. By providing an appropriate scaffold or transient microenvironment, both existing and newly introduced cells can be stimulated to infiltrate the damaged tissue and then 'patch' damaged parts by forming new tissue. During brain development, neuronal maturation and differentiation are mediated by substrate stiffness [159], and the directionality of their path is perpendicular to the directionality of the strain of their substrate [148]. Using supportive scaffolds, such as porous collagen, to deliver embryonic neuronal stem cells to injury sites resulted in enhanced regeneration and improved motility in

biomimetic ligands, can be used to mimic the physical properties of tissues and to minimize potential immunogenic responses.

In the future, understanding the unique structure of the tissue microenvironment and its effect on resident cell function and interactions may enable us to harness the patient's own cells to regenerate and replace a dysfunctional or missing organ, without relying on organ donations and compatibility, and the fear of organ rejection.

experimental animal models of spinal cord injury [160]. **Thermosensitive hydrogels** mimicking the electromechanical properties of the myocardium can promote the successful delivery of mesenchymal and adipose-derived stem cells to infarct sites and boost their survival and differentiation to improve cardiac function [161]. Biomaterial comprising natural extracellular matrix proteins, such as collagen, elastin, and fibronectin, can be engineered to mimic the stiffness of specific organs [162]; in addition, the viscoelastic properties of these materials have been shown to be crucial in promoting tissue regeneration [163–165]. **Photoreactive hydrogels** have been successfully used to temporally control matrix stiffness [166], and offer the advantage of allowing the creation of spatially heterogeneous matrices, which can trigger downstream signaling cascades in specific cells at specific locations.

Importantly, to accurately engineer fully functional tissues, one needs to recapitulate not only the macroarchitecture of the tissue, but also local features of the microenvironment that help drive the proper organization and structure of cellular components, which are tightly linked to their function. The generation of complex, centimeter-sized tissues can be achieved by combining recent advances in 3D bioprinting, which allow for meticulous control of cellular geometry and density, and organoid-forming stem cells [167]. For example, recently engineered 'mini guts' contain features such as lumens, branched vasculature, and tubular intestinal epithelia with *in vivo*-like crypts and villus domains and are perfusable to allow for the removal of dead cells and to extend tissue viability [167,168]. Tissue folding and topographical patterning can be controlled by strategically seeding contractile cells within the ECM or altering the density and spacing of cells [169,170]. However, manipulating a single component in this cascade to achieve a functioning tissue is challenging, due to the multitude of feedback loops between cells and their environment, and the complexity of mechanical adaptation.

Concluding remarks

The growing recognition that all cells are highly sensitive to their physical microenvironment, and that changes in either the mechanical properties of the microenvironment, the local forces experienced by cells, or the ability of cells to sense their environment can contribute to various diseases is already starting to impact both clinical and basic research. In the research laboratory, these mechanobiology insights have motivated the use of engineered *in vitro* cell culture systems that better mimic the physical properties of the *in vivo* cellular microenvironment, from recapitulating 3D cell culture conditions [171] to matching the microstructure and viscoelastic properties of tissues, thus producing more robust and *in vivo*-like results [167,168] when studying normal and pathological cell function. Nonetheless, teasing apart the individual contributions of cellular mechanical adaptation (see Outstanding questions) and the interplay between physical and biochemical stimuli will necessitate further development of engineered experimental platforms and synthesized biomaterials. Engineered cell culture systems that promote normal cellular function by recapitulating physiological environments have also found use for the generation of cell-based therapies, including the expansion of muscle stem cells [172] and tumor-targeting T cells [145,146].

Notably, mechanobiology considerations are beginning to lead to new treatment strategies that manipulate ECM stiffness, which have already yielded promising results in improving the efficiency of cancer immunotherapy [16,59,117] and promoting organ healing and regeneration [20,41–43]. However, to fully transition these strategies from the bench to the clinic and to minimize adverse effects, a more refined understanding of the initiating factors leading to diseases associated with dysregulated mechanobiology must be gained, because some changes in the physical microenvironment may represent consequences, rather than causes, of the disease. Nonetheless, clinical applications inspired by mechanobiology insights, ranging from improved

Outstanding questions

How do cells integrate various mechanical and biochemical stimuli? Mechanical adaptation involves intricate crosstalk between many cellular and extracellular components, including positive and negative feedback loops. Thus, it is often difficult to pinpoint the exact path leading from mechanical input to biological response. Mechanical inputs can be sensed by many specialized cellular structures at various cellular locations, making it difficult to determine which effects are a direct consequence of force sensing, and which are downstream of other mechano-activated pathways, and how these inputs are being integrated to elicit specific physiological responses.

Can we use insights from mechanobiology to improve clinical outcomes and treat disease? Scientific discoveries demonstrating the importance of the physical microenvironment in mediating cellular and tissue function both in health and disease are increasingly being exploited not only in new, stand-alone therapeutic strategies, or to enhance the efficiency of traditional treatment, but also as powerful tools of disease diagnosis and stratification. These approaches will allow for improved medical monitoring of at-risk individuals, early disease detection, and more accurate and patient-specific treatments, presenting an enormous potential to decrease disease burden and improve survival. However, translating experimental insights into viable therapeutic strategies can prove difficult due to the complexity and extensive crosstalk between different cellular and extracellular components, and the potential off-target effects of systemic modulation of specific proteins/genes or molecular structures.

What is the triggering pathological event? When studying diseases associated with disturbed mechanobiology, the fact that the physical environment can both affect, and be affected by, mechanosensitive cellular signaling pathways can make it difficult to determine whether defects in cellular mechanosensing are the initiating factor for a given disease, or whether changes in the physical environment are in fact the cause for the altered cellular response.

diagnostic and prognostic applications, such as risk assessment in cancer based on tumor/stromal stiffness or the deformability of cancer cells [15,24,25,50–53,56], to targeting fibrosis or developing novel materials that stimulate nerve regeneration (see Clinician's corner) highlight the strength and immense potential of mechano-based therapeutics in preserving and promoting human health. In other words, helping cells to adapt to pathological physical changes may give them a needed leg up in evolution.

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Declaration of interests

None declared by authors.

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