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Mechanobiology and diseases of mechanotransduction

Donald E Ingber

The current focus of medicine on molecular genetics ignores the physical basis of disease even though many of the problems that lead to pain and morbidity, and bring patients to the doctor's office, result from changes in tissue structure or mechanics. The main goal of this article is therefore to help integrate mechanics into our understanding of the molecular basis of disease. This article first reviews the key roles that physical forces, extracellular matrix and cell structure play in the control of normal development, as well as in the maintenance of tissue form and function. Recent insights into cellular mechanotransduction — the molecular mechanism by which cells sense and respond to mechanical stress — also are described. Re-evaluation of human pathophysiology in this context reveals that a wide range of diseases included within virtually all fields of medicine and surgery share a common feature: their etiology or clinical presentation results from abnormal mechanotransduction. This process may be altered by changes in cell mechanics, variations in extracellular matrix structure, or by deregulation of the molecular mechanisms by which cells sense mechanical signals and convert them into a chemical or electrical response. Molecules that mediate mechanotransduction, including extracellular matrix molecules, transmembrane integrin receptors, cytoskeletal structures and associated signal transduction components, may therefore represent targets for therapeutic intervention in a variety of diseases. Insights into the mechanical basis of tissue regulation also may lead to development of improved medical devices, engineered tissues, and biologically-inspired materials for tissue repair and reconstruction.

Keywords: cytoskeleton; disease; extracellular matrix; integrin; mechanical forces; mechanotransduction; stress-activated ion channels; tissue engineering

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Introduction

The molecular biology revolution has led to advances in knowledge and new technologies that are revolutionizing the way in which clinical medicine is practiced. Completion of the Human Genome Project, massively parallel gene and protein profiling techniques, and powerful bioinformatics tools are just a few examples. Yet there is a huge disconnect between these 'genome-age' technologies and the reality of how diseases manifest themselves. From the time the first human looked, listened and felt for what is wrong with a sick friend, caregivers have recognized the undeniable *physical* basis of disease. The thrill in the chest of a patient with aortic valve disease, bounding pulse in the hypertensive and wheeze of the patient with emphysema all ignite reflexive clinical responses in the mind of the skilled physician, and sometimes even lead to immediate diagnoses.

But in the current genome euphoria, there appears to be no place for 'physicality'. This is especially worrisome given that abnormal cell and tissue responses to mechanical stress contribute to the etiology and clinical presentation of many important diseases, including asthma, osteoporosis, atherosclerosis, diabetes, stroke and heart failure. There is also a strong mechanical basis for many generalized medical disabilities, such as lower back pain and irritable bowel syndrome, which are responsible for a major share of healthcare costs world-wide. In fact, surgeons sometimes even use mechanical forces as therapeutics, such as when traction forces are used to accelerate bone healing. However, what is missing is how these physical interventions could influence cell and tissue function, or how altered cell or tissue mechanics may contribute to disease development.

In this article, I first review the fundamental role that physical forces and changes in tissue mechanics play in normal development and physiology. I then describe recent advances in our understanding of cellular mechanotransduction, the molecular mech-

Abbreviations and acronyms

ECM	extracellular matrix
ICAM	intercellualr adhesion molecule
PDGF	platelet-derived growth factor
RGD	argine-glycine-aspartate
3D	three-dimensional

anism by which cells sense and respond to mechanical signals. Finally, I explain how the clinical manifestations of many ostensibly unrelated diseases similarly result from abnormal mechanotransduction, and how this insight may lead to new avenues for therapeutic intervention.

Key messages

- Mechanical forces are critical regulators of cellular biochemistry and gene expression as well as tissue development.
- Mechanotransduction — the process by which cells sense and respond to mechanical signals — is mediated by extracellular matrix, transmembrane integrin receptors, cytoskeletal structures and associated signaling molecules.
- Many ostensibly unrelated diseases share the common feature that their etiology or clinical presentation results from abnormal mechanotransduction. Mechanotransduction may be altered through changes in cell mechanics, extracellular matrix structure or by deregulation of the molecular mechanisms by which cells sense mechanical signals or convert them into a chemical response.
- Molecules that mediate mechanotransduction may represent future targets for therapeutic intervention in a variety of diseases. Insights into the mechanical basis of tissue regulation also may lead to development of improved medical devices, engineered tissues, and biomimetic materials for tissue repair and reconstruction.

Mechanobiology

In biology and medicine, we tend to focus on the importance of genes and chemical factors for control of tissue physiology and the development of disease, whereas we commonly ignore physical factors. This is interesting because it was common knowledge at the turn of the last century that mechanical forces are critical regulators in biology (1). Wolff's law describing that bone remodels along lines of stress was published in 1892 (2). However, the advent of more reductionist approaches in the basic sciences, and the demonstration of their power to advance understanding of the molecular basis of disease, led to a loss of interest in mechanics.

Although it has received much less attention than the genomics revolution, there has been a renaissance in the field of mechanobiology over the past two decades. Physiologists and clinicians now recognize the importance of mechanical forces for the development and function of the heart and lung, the growth of skin and muscle, the maintenance of cartilage and bone, and the etiology of many debilitating diseases. Exploration of basic mechanisms of sensation and autonomic control, including hearing, balance, touch, and peristalsis, also has demanded explanation in mechanical terms. At the same time, biologists have come to recognize that mechanical forces serve as important regulators at the cell and molecular levels, and that they are equally potent as chemical cues. For example, cell-generated tensional forces have been shown to regulate diverse functions, ranging from chromosome movements and cell proliferation to tissue morphogenesis, in addition to cell contractility and motility (3–5).

To explain mechanoregulation, we must take into account that living organisms, such as man, are constructed from tiers of systems within systems within systems (4, 6, 7). Our arms and legs, for example, are composed of several organs (e.g., bone, muscle) that are constructed by combining various tissues (e.g., bone, muscle, connective tissue, vascular endothelium, nerve). These tissues, in turn, are composed of groups of living cells held together by an extracellular matrix (ECM) comprised of a network of collagens, glycoproteins, and proteoglycans. Each cell contains a surface membrane, intracellular organelles, a nucleus, and a filamentous cytoskeleton that connects all these elements and is permeated by a viscous cytosol. Each of these subcellular components is, in turn, composed of clusters of different molecules. In other words, our bodies are complex hierarchical structures, and hence mechanical deformation of whole tissues results in coordinated structural rearrangements on many different size scales.

To understand how individual cells experience mechanical forces, we therefore must first identify the path by which these stresses are transmitted through tissues and across the cell surface. As in any three-dimensional (3D) structure, mechanical loads will be transmitted across structural elements that are physically interconnected. Thus, forces that are applied to the entire organism (e.g., due to gravity

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or movement) or to individual tissues would be distributed to individual cells *via* their adhesions to the ECM support scaffolds (basement membranes, interstitial matrix, cartilage, bone) that link cells and tissues throughout the body. This can be seen in specialized mechanosensory organs that recognize and respond to physical stimuli. In the vestibular apparatus, for example, the otoliths (dense calcareous crystals) mediate sensation of linear acceleration due to gravity by deforming a specialized bilaminar ECM. Local distortion of this ECM activates sensory neurons within adjacent hair cells by transferring mechanical forces across the cell surface and thereby inducing bending of cytoskeletal stereocilia that extend from the cell surface (8). ECM similarly mediates mechanical energy transfer to sensory cells within muscle stretch receptors (9).

The mechanical properties of the ECM also contribute significantly to the cellular mechanotransduction response. For instance, the high flexibility of the ECM of Pacinian corpuscle mechanoreceptor cells in skin ensures that rapid deformations will be sensed, whereas sustained stresses will dissipate before they reach the cell (10). This mechanism is used to filter out sustained signals due to continuous pressure or touch (e.g., when we sit and write on the computer for extend periods of time) — a common form of receptor adaptation. If the ECM is less flexible, then stresses will be transmitted to and through the cell, only to be dissipated through movements in the cytoskeleton, as observed in stereocilia in hair cells.

ECM plays a similar role in mechanoregulation in all solid tissues. These molecular scaffolds distribute stresses throughout tissues and focus these forces on sites of cell-ECM adhesion. Cells adhere to ECM through binding of specific cell surface receptors. The most ubiquitous and well characterized class of ECM receptors are known as 'integrins'. Over 20 different types of these dimeric protein receptors exist; their binding specificity (e.g., for collagen versus fibronectin) depends on the specific pairing combination of interacting α and β subunits (11, 12). The external portion of these transmembrane receptors binds to specific peptide sequences (e.g., RGD) in ECM molecules, while their intracellular domains physically associate with actin-associated proteins and thereby, form a molecular bridge between the ECM and the cytoskeleton. Integrins are not evenly distributed in the membrane, rather they cluster together within specialized anchoring complexes known as 'focal adhesions' (13).

Importantly, integrins provide a preferred site for mechanical signal transfer across the cell surface, when compared with other types of transmembrane receptors. This has been demonstrated directly by applying mechanical forces to surface membrane receptors of cultured cells (14–17). Cell surface integrins that link to the internal cytoskeleton provide a much greater degree of mechanical coupling across the cell surface as measured by an increased strengthening (stiffening) response when compared with transmembrane growth factor receptors, histocompatibility antigens, or metabolic receptors.

Thus, integrins appear to function as cell surface 'mechanoreceptors' in that they are among the first molecules to sense a mechanical stress applied at the cell surface, and they transmit this signal across the plasma membrane and to the cytoskeleton over a specific molecular pathway. Cell-cell adhesion molecules, such as cadherins and selectins, may provide a similar mechanical coupling function between the cytoskeletons of neighboring cells (18–21). Interestingly, even forces that produce generalized cell distortion, such as apical fluid shear stresses in endothelium, eventually distribute the stress through the cytoskeleton and to integrins within the cell's basal focal adhesions, and to cell-cell adhesion molecules at the lateral cell borders (22, 23).

Force-induced changes in cell structure and mechanics

To understand the physiological mechanism by which cells respond to mechanical stress, we must first consider how forces impact the cell once they are transmitted across transmembrane adhesion receptors. When most of the readers of this article went to medical school, they learned that cells are composed of a viscous cytosol surrounded by a membrane, with a nucleus in its center. With this view of cell architecture, it is difficult to understand how mechanical forces could modulate intracellular structure or biochemistry. Over the past quarter century, however, our view of cell structure has changed completely. We now recognize that living cells contain a cytoskeleton. This is an internal molecular framework or lattice composed of three different types of molecular filaments (microfilaments, microtubules and intermediate filaments) that provides shape stability to the cell (24). However, the cytoskeleton is not simply a passive gel. All cells generate tensional forces through actomyosin filament sliding in their cytoskeleton. These tensional forces are resisted and balanced by external adhesions to ECM and neighboring cells, and by other molecular filaments (e.g., microtubules) that locally resist inward-directed tensional forces inside the cytoskeleton.

This type of force balance is a hallmark of an architectural system known as 'tensegrity', and computational models based on tensegrity theory can predict complex mechanical behaviors of mammalian cells (7, 24–26). Thus, the cell does not respond to mechanical stress like a 'balloon filled

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with molasses or jello'. Instead, the viscoelastic behavior of living cells results from collective mechanical interactions within the tensed molecular cytoskeleton. Cytoskeletal forces are also harnessed to transport organelles (e.g., mitochondria, synaptic vesicles) in the cytoplasm, to move chromosomes during mitosis, and as long recognized in muscle, to generate tensional forces that are important for cell contractility as well as movement. The effects of applied stresses on cell shape and mechanics will therefore depend on the material properties of the cytoskeletal filaments, their organization (architecture), and the level of isometric tension or 'prestress' in the cell, much like the mechanical responsiveness of whole muscle is governed by its structural organization and by its contractile tone. Because individual cells (both muscle and non-muscle) apply tractional forces on their adhesions, cultured cells spread and flatten on rigid ECM substrates, whereas they retract and round on flexible ECMs.

Mechanical determinants of cell and developmental control

What may be most surprising is that changes in microscale forces that alter the cytoskeletal force balance and modulate cell shape also control complex cell behaviors that are critical for development and tissue homeostasis. Cell growth, differentiation, polarity, motility, contractility and programmed cell death, all can be influenced by physical distortion of cells through their ECM adhesions. For instance, chondrocytes, hepatocytes, mammary epithelium, retinal epithelium, capillary endothelium, and fibroblasts can be switched from growth to differentiation in the presence of soluble mitogens by decreasing the stiffness or adhesivity of the ECM, and thereby promoting cell retraction and rounding (27–34). Adherent endothelial cells can be switched from growth to apoptosis by more fully restricting cell spreading (35). Varying the mechanical compliance (flexibility) of the ECM also influences the rate of cell migration (36) and the direction of motility can be affected by geometric cues from the ECM (37). Direct application of tensional forces to cultured endothelial cells similarly promotes capillary outgrowth in 3D collagen gels (38) and nerve cells respond to tensional forces exerted on their surfaces by extending nerve processes in the direction of the applied stress (39). Changing vascular smooth muscle cell shape through modulation of cell-ECM adhesion or alteration of ECM compliance also regulates its contractile response to vasoagonists, such as endothelin-1 (40, 41). In fact, individual cultured vascular smooth muscle cells display a bell-shaped, force-length relationship (40) that is highly reminiscent of the Starling curve exhibited by the whole heart. Cell shape-dependent changes in the sensitivity of the contractile machinery may ensure 'compliance matching' in muscle cells of the gastrointestinal tract, genitourinary system, pulmonary airways, blood vessels and heart, as well as in epithelial and connective tissues, so that the level of tension exerted by the cell precisely balances the mechanical stress transmitted through the surrounding ECM in response to tissue distortion.

In summary, these studies have revealed that the physicality of the ECM substrate and degree of cell distortion govern cell behavior regardless of the presence of hormones, cytokines or other soluble regulatory factors. Local alterations in ECM structure that influence cell shape and mechanics, such as thinning of basement membrane produced by increased ECM turnover (e.g., metalloproteinase activities), also appear to drive regional changes in cell growth and motility during tissue development (25, 42). Lung branching morphogenesis in the embryo can be selectively inhibited or accelerated by decreasing or increasing cytoskeletal tension, respectively, using pharmacological agents (43). Regional changes in ECM structure and associated changes in cytoskeletal mechanics similarly contribute to control of angiogenesis that is required for wound healing as well as tumor progression (44). In fact, cell-generated tensional forces appear to play a central role in the development of virtually all living tissues and organs (24, 25, 42), even in neural tissues, such as retina (45) and brain (46).

Various *in vitro* and *in vivo* studies confirm that mechanical forces directly regulate the shape and function of essentially all cell types (5). Individual bone cells increase deposition of bone ECM when exposed to mechanical stresses with high frequency and low strain *in vitro*, just as they do within whole bone (47), and differences in mechanical loading conditions can direct bone *versus* cartilage formation (48). Chondrocytes respond to compressive loading by altering production of proteoglycans that comprise cartilage matrix (49). Skeletal muscle cells increase their mass, upregulate expression of muscle-specific proteins and even organize into muscle fascicles *in vitro* when stretched with physiologically relevant load cycles (50, 51); heart cells increase secretion of atrial natriuretic factor (52). Skin epithelium, bone cells, fibroblasts, and embryonic heart muscle cells all increase their growth rates when they experience mechanical strain (53–56), whereas stretch induces differentiation in periodontal ligament cells (57). Endothelium sense fluid shear stresses and respond by altering their expression of proteins that are involved in lymphocyte binding (e.g., ICAM), tissue remodeling (e.g., PDGF) and handling oxidant stress, and some of these effects are mediated through

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activation of specific 'shear stress-response elements' in certain gene promoters (58, 59). Kidney epithelial cells respond to levels of fluid shear similar to those produced by urine flow in collecting ducts by increasing calcium influx (60, 61). Changes in gene expression and growth of bladder smooth muscle cells that are triggered by outlet obstruction appear to result from mechanical stretch secondary to overfilling of the bladder (62). Glomerular filtration rate is similarly controlled by alterations in vasomotor tone of preglomerular, glomerular, and postglomerular microvessels, as well as associated changes in mesangial cell contractility (63). During pregnancy, the onset of labor is triggered by distention of the uterus imposed by the growing fetus (64), and pulmonary epithelial cells increase secretion of surfactant when stretched *in vitro* (65), just as they do in a newborn when it takes its first breath.

Cellular mechanotransduction

But how do mechanical forces influence cellular biochemistry and gene expression so as to produce these varied effects on cell and tissue behavior? This mechanism is difficult to envision because it does not involve a classic 'stimulus-response' coupling as used by soluble hormones or secretagogues. In the case of hormonal stimulation, no molecular signal is present prior to stimulation and the relevant receptor binding sites are unoccupied. Then when the hormone stimulus is provided, it binds to its receptor and initiates an intracellular signaling response. In contrast, because cell shape is determined through a balance of mechanical forces (24–26), any external mechanical stimulus that impinges on an adherent cell is imposed on a pre-existing force balance, much like pulling an arrow back against a tensed bow-string. This is important because the pre-existing tensile stress (prestress) or tone in the cell can at times govern the 'response' to the mechanical 'stimulus' (66, 67).

In the case of adherent cells, forces applied at the macroscale also will result in changes in ECM and cytoskeletal mechanics on the microscale. For example, the vessel wall decreases its mechanical compliance (i.e., becomes more rigid) when it is physically distended due to increased blood pressure. Osmotic forces similarly tense and stiffen interstitial matrix, for example, in cerebral edema or following injury to the liver; pressure overload has a similar effect in the heart. These changes in ECM mechanics will not transfer force equally to all points on the surface of neighboring adherent cells. Rather, a tug on the ECM will be felt by the cell through its focal adhesions and hence, through its transmembrane integrin receptors that link to the cytoskeleton.

When integrins on the surface membrane of cultured cells are mechanically stressed, the cell responds by increasing recruitment of focal adhesion (cytoskeletal linker) proteins and mechanically strengthening itself against additional stress (14–17, 68–70). When the same stress is applied to other transmembrane receptors that do not mediate cell adhesion, there is very little response. Because integrins preferentially mediate mechanical signal transfer across the cell surface, the molecular components of the cytoskeletal scaffolds that connect to integrins within the focal adhesion will experience increased mechanical stress whereas soluble components in the nearby cytosol will not. For example, when large-scale deforming forces are applied to integrins, cytoskeletal filaments and linked intranuclear structures can be seen to realign along the applied tension field lines (71, 72). Application of fluid shear stress to the apical membrane of vascular endothelium similarly results in distortion of cytoskeletal filaments throughout the cell (73) as well as funneling of stress along this load-bearing network in the cytoplasm all the way to the cell's basal ECM adhesions (22, 23). Kidney epithelium senses shear stress through deformation of the primary cilium (60, 61). This is a single, specialized, cytoskeletal process that extends vertically from the apical cell surface and functions like a long lever arm for the whole cytoskeleton, much like stereocilia in hair cells of the inner ear.

If the cytoskeletal filaments and associated regulatory molecules distort without breaking when integrins or specialized cytoskeletal extensions (e.g., stereocilia, primary cilia) are stressed, then some or all of the molecules that comprise these structures must similarly change shape. When the shape of a molecule is altered, its biophysical properties (thermodynamics, kinetics) change, and hence biochemistry (e.g., chemical reaction rates) will be altered (4, 74). This is important because many of the enzymes and substrates that mediate cellular metabolism (e.g., protein synthesis, glycolysis, RNA processing, DNA replication) are physically immobilized on the cytoskeleton and nuclear matrix (nucleoskeleton) (75, 76). In particular, many signal transduction molecules are oriented on the cytoskeletal backbone of the focal adhesion complex at the site of integrin binding; these include mechanically-gated ion channels, protein kinases (e.g., FAK, src), small GTPases, heterotrimeric G proteins, inositol lipid kinases, and certain growth factor receptors (77, 78).

Experiments confirm that local changes in biochemical signal transduction are produced when external forces are applied to integrins. The increased recruitment of focal adhesion proteins and associated cytoskeletal strengthening response that result when integrins are stressed (14–17, 79) are mediated by local activation of the small GTPase Rho and the

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protein tyrosine kinase, c-src (69, 70, 79). Mechanical stress application to integrins also stimulates rapid (within 10 msec) calcium influx in the neuromuscular synapse (due to rapid muscle twitching) (80), recruits the protein synthetic machinery to the site of force application (81), and activates cAMP signaling within the focal adhesion which eventually leads to stress-induced changes in gene transcription (82). Stress application through integrins induces endothelin-1 gene expression in endothelial cells and this response can be prevented by dissipating cytoskeletal tension (prestress) and hence, altering cell mechanics (66, 67). Again, application of similar mechanical stresses to other transmembrane receptors that are not adhesion receptors fails to produce these responses. Other signaling molecules that have been shown to be activated by mechanical stress in an integrin-dependent manner in various cell types include protein tyrosine kinases (FAK, src), Shc, ERK1/2, protein kinase C, PI-3-kinase, Akt, small GTPases (Rho, Rac), heterotrimeric G proteins, paxillin, SREBP1, hsp 27 and β-catenin (70, 79, 82–91).

Importantly, all cells also contain 'stress-sensitive' (mechanically-gated) ion channels that either increase or decrease ion flux when their membranes are mechanically stressed (92, 93). For example, specialized mechanosensory 'hair' cells of the inner ear detect sound through deflections of their stereocilia that result in the opening of mechanosensitive cation channels. Direction- and amplitude-dependent depolarizations caused by these deflections result in induced currents that are relayed to nerve fibers (94). The vestibular system relies on similar hair cells at the base of the semicircular canals to sense three-dimensional rotation through fluid flow; linear accelerations are sensed in the utricle and saccuole through deflection of mineral deposits (otoconia) within a specialized ECM (otolithic membrane) that again tugs on stereocilia within adjacent hair cells (95). Stretch-sensitive channels at the sensory neuron terminals located under the epidermis and hair follicles also mediate touch sensation, and related mechanisms are used for pressure and stretch sensation as well as proprioception (96, 97). However, even the function of these specialized mechano-electrical transducers appears to depend on their linkage to the cytoskeleton and hence, indirectly on integrin coupling to the ECM (93, 98) which stabilizes the entire cytoskeleton against shape distortion (24). For readers interested in molecular mechanisms of mechanotransduction, more detailed discussions can be found in various recent reviews (4, 5, 47, 85, 95, 98, 99).

In biology, we emphasize linear thinking and focus on local molecular binding and assembly events. But if all mechanosensing was carried out locally at the site where stresses impinge on the surface membrane (e.g., in the focal adhesion), then cells would be continuously activated by subtle variations in ECM structure within living tissues that are constantly exposed to physiological stresses. Because forces applied locally through integrins also produce coordinated deformation of molecular structures throughout the cytoskeleton and nucleus (71, 72), mechanochemical transduction could occur at distant or multiple sites in the cell. In fact, application of mechanical stress to integrins can produce the same focal adhesion signaling response (e.g., production of cAMP) in round *versus* spread cells (82), however, the cells that are globally distorted proliferate whereas the round cells undergo apoptosis (35). As described above, the global shape of the cell dictates its behavior (e.g., growth *versus* differentiation or apoptosis), and these effects are mediated through tension-dependent changes in cytoskeletal structure and mechanics (37, 100, 101). Thus, cells appear to 'think globally' in that large-scale mechanical distortion of cell shape and the cytoskeleton govern how the cell processes and integrates locally-elicited signals (mechanical as well as chemical) to produce a concerted behavioral response (74).

Implications for clinical medicine

These new insights into mechanobiology suggest that many ostensibly unrelated diseases may share a common dependence on abnormal mechanotransduction for their development or clinical presentation. Mechanotransduction may be altered through changes in cell mechanics, ECM structure or by deregulation of the molecular mechanisms by which cells sense mechanical signals or convert them into a chemical response. In fact, physicians in almost every branch of medicine and surgery care for patients who have ailments that may be viewed as diseases of mechanotransduction, as discussed below and summarized in Table 1.

Although the question of how cells determine their shape and mechanics may seem esoteric, the reality is that it has important clinical implications. For example, leukocytes physically deform when they pass through pulmonary capillary beds (102) and inflammatory agents that increase cytoskeletal stiffness in circulating neutrophils induce leukocyte sequestration in the lung (103). The ability of tumor cells to resist traumatic destruction in the vasculature, and hence their ability to metastasize and survive in distant capillary beds, depends on their flexibility (104). The effectiveness of delivery of therapeutic cytotoxic lymphocytes into tumor tissues similarly can vary with their stiffness (105). Mechanical stretching of kidney mesangial cells through integrins due to glomerular hypertension represents a common final pathway for glomerulosclerosis (106–107), and

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Table 1. Diseases of mechanotransduction

Cardiology	Angina (vasospasm)	C T
	Atherosclerosis	T M
	Atrial fibrillation	M
	Heart failure	C T M?
	Hypertension	C T M?
	Intimal hyperplasia	C T M?
	Valve disease	T
Dermatology	Scleroderma	T
Gastroenterology	Achalasia	C
	Irritable bowel syndrome	C M?
	Volvulus	C T
Nephrology	Diabetic nephropathy	C T M?
	Glomerulosclerosis	C T M?
Neurology	Cerebral edema	T
	Facial tics	C
	Hydrocephalus	T C?
	Migraine	C M?
	Stroke	C T
	Stuttering	C
Oncology	Cancer	C T M?
	Metastasis	C
Opthalmology	Glaucoma	C T M?
Orthopedics	Ankylosing spondylitis	C T
	Carpal tunnel syndrome	C T
	Chronic back pain	C T
	Dupytren's contracture	C T
	Osteoporosis	T M
	Osteoarthritis	T
	Rheumatoid arthritis	T
Pediatrics	Collagenopathies	T
	Congenital deafness	C T M
	Mucopolysaccharidoses	T
	Musculodystrophies	C T M
	Osteochondroplasias	C T
	Polycystic kidney disease	T M
	Pulmonary hypertension of newborn	C T M?
Pulmonary medicine	ARDS	C T M
	Asthma	C T M?
	Emphysema	T
	Pulmonary fibrosis	T
	Pulmonary hypertension	C T M?
	Ventilator Injury	C M
Reproductive medicine	Pre-eclampsia	C T M?
	Sexual dysfunction (male & female)	C M?
Urology	Urinary frequency/incontinence	C M?

A partial list of diseases that share the feature that their etiology or clinical presentation results from abnormal mechanotransduction. The right column indicates whether the mechanical basis of the disease or condition is likely due to changes in cell mechanics (C), alterations in tissue structure (T), or deregulation of mechanochemical conversion (M); '?' indicates situations where deregulation of mechanochemical conversion is likely but remains to be demonstrated.

altered cell mechanics contributes to the clinical presentation of asthma and other pulmonary diseases (108). Even some of the genetic causes of deafness involve mutations in cytoskeletal proteins, such myosin, espin and mDia, that alter hair cell mechanics (109–111), and certain patients with autoimmune ear disease have antibodies directed against β-actin (112). Mutations in specialized ECM proteins and deletion of integrin α8β1, which is found in hair cells, also hinders stereocilia maturation and hair cell differentiation (113).

Systemic and pulmonary hypertension, persistent pulmonary hypertension of the newborn, bronchopulmonary dysplasia, asthma, achalasia, preeclampsia, urinary frequency, irritable bowel syndrome, and many causes of chronic back pain, are all based on muscle cell hypercontractility. Dupytren's contracture is characterized by hypercontractility of ligamental fibroblasts (114), whereas glaucoma (115) and hydrocephalus (116) result from physical constrictions that obstruct fluid flow in the eye and cerebrospinal space, respectively. Recent studies suggest that genetic mutations or malfunction of cytoskeletal proteins, ECM molecules or integrins that alter cell and tissue

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mechanics can lead to impaired vascular smooth muscle and cardiac muscle contractility, as well as various forms of heart disease (119–121). In fact, most of the molecular causes of heart failure appear to disrupt the biomechanical balance between the cytoskeleton, membrane, and ECM (122). In contrast, decreased smooth muscle cell contractility results in urinary stress incontinence (123), as well as defects in male and female sexual function (124, 125). Abnormal muscle tone also can lead to destabilization of the skeleton (126) and contribute to skeletal and joint diseases. For example, axial muscular dysfunction has been implicated in the development of joint pathology in ankylosing spondylitis (127).

In other conditions, mechanotransduction may be compromised as a result of changes in ECM formation or remodeling. Many genetic diseases and developmental disabilities, including various osteochondrodysplasias, mucopolysaccharidoses and collagenopathies are essentially disorders of connective tissue structure and mechanics (128, 129). In one form of muscular dystrophy, a mutation in an ECM protein (laminin $\alpha 2$) leads to both the muscular degeneration and sensineural hearing loss that are observed in many patients with this disease (130). A mutation in a fibrillar collagen gene (COL11A1) produces chondrodyplasia when homozygous, and both osteoarthritis and hearing loss when heterozygous. Patients with Stickler syndrome and Marshall syndrome are also heterozygous for mutations in this gene (131). Abnormal fibrillin deposition in patients with Marfan's syndrome alters the vascular endothelial cell response to hemodynamic stresses and results in aortic dissection due to local weakness of the vascular wall (132). Accumulation of abnormal ECM also contributes to development of abnormal tissue mechanics and clinical compromise of function in patients with scleroderma, pulmonary fibrosis, vascular hypertension, and diabetic nephropathy, whereas emphysema is characterized by enhanced ECM breakdown. Although rheumatoid arthritis has an inflammatory basis, joint pain and reduced movement are also due to the breakdown of the cartilage matrix. In fact, angiogenesis inhibitors that prevent cartilage matrix dissolution by inhibiting capillary invasion can significantly suppress the clinical and histological symptoms of rheumatoid arthritis in an animal model without evidence of immunosuppression (133). Changes in ECM structure that alter tissue mechanics and provide a constitutive stimulus for cell growth may even contribute to cancer initiation and progression (134–136). For example, overexpression of an ECM-degrading enzyme in transgenic mice results in formation of malignant tumors (136, 137). The 'angiogenic switch' that initiates tumor angiogenesis and is required for cancer formation (138) also appears to be controlled by metalloproteinases that structurally remodel ECM (139).

Other diseases result directly from deregulation of transmembrane mechanical signaling. Atrial fibrillation may be caused by abnormal conversion of mechanical stress gradients (e.g., secondary to volume overload) into intracellular gradients of electrical activity as a specific peptide inhibitor of stress-activated ion channels can prevent the heartbeat from losing its rhythm (140). Dystrophin, the gene product that is mutated in Duchenne's muscular dystrophy is part of the specialized focal adhesion (dystroglycan) complex that mechanically couples the cytoskeleton to ECM in skeletal muscle (141). Mutations in various load-bearing molecules in muscle, including other adhesion complex proteins, integrins, or ECM proteins, lead to development of similar muscular dystrophies (130, 141–143). Moreover, cells with these mutations exhibit abnormal responses to mechanical stress, as well as altered cell and cytoskeletal mechanics (143–145). Kidney duct epithelial cells from transgenic mice that lack functional polycystin 1, and hence develop autosomal dominant polycystic kidney disease, fail to increase calcium influx in response to fluid shear stresses when applied at levels similar to those that occur *in vivo* (60, 61). If collecting ducts utilize a mechanical control mechanism similar to that of blood vessels which increase their diameter when hemodynamic shear stresses rise (58), then loss of this normal homeostatic mechanism could lead to unregulated duct expansion and hence cyst formation. Osteoporosis also may be caused by aberrant mechanotransduction since similar bone loss can result from mechanical unloading, for example, due to extended bed rest or exposure to microgravity (146). Interestingly, certain osteoporosis drugs specifically target integrin receptors that mediate mechanotransduction (147). Other conditions that may result from stretch-activated signaling cascades include development of intimal hyperplasia induced by stent placement in coronary arteries or by replacement of constricted vessels with arteriovenous grafts (148), and ventilation-induced lung injury (e.g., ARDS) (149).

Recognition of the importance of mechanics and cellular mechanotransduction for tissue development also may help to explain the focal incidence of disease. Although high cholesterol and LDL promote atherosclerotic plaque formation, these plaques preferentially form in regions of disturbed blood flow (e.g., near vessel branches) (58). Thus, if one could understand how cells sense flow, it might be possible to prevent plaque formation in the future. Local changes in tissue structure also may explain why genetic diseases, including cancer, often present focally (e.g., retinoblastoma usually only occurs in one eye). In other words, changes in tissue mechanics may

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Table 2. Mechanical therapies

Acupuncture
Anti-arrhythmic drugs
Anti-spasmodic drugs
Bone fracture healing
Botox
Cardiac perfusion
Distraction osteogenesis
Inotropic drugs
Lung ventilation
Massage therapy
Muscle relaxants
Orthodontics
Physical therapy
Rho-kinase inhibitor (fasudil)
Stents
Surfactant
Tissue engineering (manufacturing process)
Tissue expansion (e.g., breast)
Vasodilators
Ventilator therapy
Wound closure (e.g., vacuum-assisted)

A partial list of clinical therapies that are currently in use or in development whose action is largely based on altering cell and tissue mechanics, or directly altering cellular mechanotransduction (see text for details).

contribute significantly to the epigenetic basis of disease.

Understanding of the relation between structure and function in living tissues and of fundamental mechanisms of cellular mechanotransduction may therefore lead to entirely new modes of therapeutic intervention (Table 2). In fact, surgeons already use mechanical therapies to promote tissue growth and remodeling. Examples include the use of surfactant to promote lung development in premature infants (150), mechanical ventilation with low tidal volume to decrease morbidity and death in patients with acute lung injury and acute respiratory distress syndrome (ARDS)(151), expandable stents to physically prevent coronary artery constriction (152), tissue expanders to increase the skin area available for reconstruction of large surface defects, and devices for tension application for distraction osteogenesis, orthodontics, bone fracture healing, craniofacial surgery, cosmetic breast expansion and closure of non-healing wounds (153, 154). These devices are believed to act through alterations in microscale forces (e.g., cell stretching) that activate cellular signal transduction (153, 154). The therapeutic value of physical therapy, massage, and muscle stimulation is also well known. But even the effects of acupuncture therapy on pain control and other clinical symptoms appear to result from physical manipulation (twisting) of the needles that produces ECM distortion and associated integrin-dependent changes in cellular mechanotransduction (155).

The finding that abnormal cell contractility is a common feature in many diseases may explain why a toxin that modulates cell tension — Botulinum A (Botox) — is being tested as a treatment for a wide range of ailments, including stroke paralysis, migraine headaches, facial tics, stuttering, lower back pain, incontinence, carpal tunnel syndrome and tennis elbow, in addition to being a high priced cosmetic (156). Another chemical inhibitor of cell tension that targets Rho-associated kinase, a molecule that both mediates mechanosignaling through integrins and regulates cytoskeletal contractility (69, 70), also has been found to prevent disease progression in experimental models of glaucoma (157) and intimal hyperplasia (158). Importantly, one form of this compound, fasudil, appears to be useful for treatment of systemic hypertension (159) as well as angina due to myocardial ischemia in humans (160). Conventional vasodilators, muscle relaxants, inotropic agents and anti-spasmodic drugs similarly prevent clinical symptoms based on their ability to modulate cell mechanics, and anti-arrythmics directly modulate mechano-electrical conversion in heart cells. The function of cardiac perfusion devices is also purely mechanical. However, even complex developmental processes, such as angiogenesis, can be controlled by altering cell and tissue mechanics, for example, using drugs that target the cytoskeleton (161, 162), integrins (163) or the ECM (164–166). Some of these drugs have entered human clinical trials for angiogenesis-dependent diseases, such as cancer and macular degeneration. Thus, someday it may be possible to treat a huge range of diseases using drugs that specifically target molecules that contribute to mechanoregulation. In the field of tissue engineering, mechanical force regimens also have been integrated into device fabrication protocols. Engineered tissues, including artificial blood vessels, skeletal muscle, cardiac muscle and heart valve, greatly increase their mechanical strength and clinical efficacy if preconditioned using force regimens prior to implantation (167–171). Design and fabrication of synthetic 'biomimetic' biomaterials and nanotechnologies that mimic the mechanical as well as chemical properties of natural tissue structures may revolutionize the medical device industry in the future.

Conclusion

The current focus in medicine is on the genetic basis of disease. However, it is not necessary to correct the underlying genetic defect in order to treat clinically relevant symptoms or relieve the pain and morbidity of disease. Moreover, most of the clinical problems that bring a patient to the doctor's office result from changes in tissue structure and mechanics. Although these physical alterations have been commonly

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viewed as the end-result of the disease process, recent advances in mechanobiology suggest that abnormal cell and tissue responses to mechanical stress may actively contribute to the development of many diseases and ailments. Thus, it might be wise to search for a physical cause when chemical or molecular forms of investigation do not suffice.

These observations also raise the possibility that the molecules that mediate mechanotransduction, including ECM molecules, cell surface adhesion receptors, cytoskeletal components, and related signal transduction molecules may represent future targets for therapeutic intervention in a variety of diseases. The value of macroscale forces as therapeutics has already been demonstrated by surgeons, however, the potential clinical value of developing approaches to selectively control microscale forces may be even greater. Pursuit of the relation between structure and function at the molecular scale in living cells and tissues also may lead to the development of entirely new biomaterials and microdevices for repair and replacement of injured tissues. Thus, if we are to advance patient care in the twenty first century, we need to do more than delineate the genetic causes of disease; we also must reintegrate mechanics into our understanding of the molecular basis of disease.

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