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An introductory review of cell mechanobiology

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Abstract Mechanical loads induce changes in the structure, composition, and function of living tissues. Cells in tissues are responsible for these changes, which cause physiological or pathological alterations in the extracellular matrix (ECM). This article provides an introductory review of the mechanobiology of load-sensitive cells *in vivo*, which include fibroblasts, chondrocytes, osteoblasts, endothelial cells, and smooth muscle cells. Many studies have shown that mechanical loads affect diverse cellular functions, such as cell proliferation, ECM gene and protein expression, and the production of soluble factors. Major cellular components involved in the mechanotransduction mechanisms include the cytoskeleton, integrins, G proteins, receptor tyrosine kinases, mitogen-activated protein kinases, and stretch-activated ion channels. Future research in the area of cell mechanobiology will require novel experimental and theoretical methodologies to determine the type and magnitude of the forces experienced at the cellular and sub-cellular levels and to identify the force sensors/receptors that initiate the cascade of cellular and molecular events.

1 Introduction

Human beings experience diverse mechanical forces from a wide variety of sources. Gravity is an example of a ubiquitous force that acts on the body. Another example is tensile muscular forces that act on bones through tendons to move joints and permit human locomotion. Other examples of mechanical forces include compressive loads on cartilage and bones during walking and exercise as well as blood pressure and shear stresses on the vessels due to blood flow. Similarly,

lung tissue is stretched cyclically due to breathing and is exposed to mechanical forces from blood flow and surface tension while dermal tissues are subjected to tensile, compressive, and shearing forces. The heart is also continuously subjected to mechanical forces due to changes in blood volume and pressure. As a final example, one's senses of touch and hearing are initiated by forces as well.

It has been known for many years that tissues grow and remodel in response to changes in mechanical forces. A typical example is bone, which changes its shape, density, and stiffness when its mechanical loading conditions are altered (Turner and Pavalko 1998; Mullender et al. 2004). Similarly, blood vessels remodel themselves in response to altered blood pressure and shear stress (Owens 1996; Williams B 1998). It is the cells in tissues that are responsible for this remodeling in response to mechanical forces. It is now well recognized that mechanical forces play a fundamental role in the regulation of cell functions, including gene induction, protein synthesis, cell growth, death, and differentiation, which are essential to maintain tissue homeostasis. Conversely, abnormal mechanical loading conditions alter cellular function and change the structure and composition of the extracellular matrix (ECM), eventually leading to tissue or organ pathologies such as osteoporosis, osteoarthritis, tendinopathy, atherosclerosis, and fibrosis in the bone, cartilage, tendon, vessels, heart, lung, and skin (Ross 1986; Chicurel et al. 1998; Grodzinsky et al. 2000; Ireland et al. 2001; Riley et al. 2002; Ingber 2003; Bag et al. 2004; Borer et al. 2004; Eckes and Krieg 2004; Lammerding et al. 2004).

Although it is clear that cell response to mechanical forces is closely related to tissue physiology and pathology, it remains unclear how the cells sense mechanical forces and convert such "mechanical signals" into biological responses. This is further complicated by the fact that cells are highly dynamic and their complex structure changes in response to mechanical forces. Mechanobiology is an interdisciplinary study that is concerned with the cells' biological responses to mechanical loads and the mechanotransduction mechanisms by which these loads are transduced into a cascade of cellular and molecular events (Ingber 1998). Knowledge of

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these mechanotransduction mechanisms will aid in a better understanding of the physiological responses of various tissues, such as the effects of training or disuse on tendons and ligaments (Woo et al. 1982; Tipton et al. 1986). This comprehension will also help elucidate the pathogenesis of many diseases caused by mechanical loads such as tendinopathy (Wang 2005), and hence aid in developing new therapeutic approaches to treat these pathological conditions more effectively.

This article provides an introductory review of the mechanobiology of load-sensitive cells *in vivo*, including fibroblasts, osteoblasts, chondrocytes, endothelial cells, and smooth muscle cells (SMCs). We focus on a discussion of ECM gene and protein expression as well as the production of soluble factors by these cells in response to various mechanical forces *in vitro*. We also describe interactions between mechanical load and soluble factors and the effects of mechanical loading on tissue engineering constructs. This is followed by an overview of the cellular mechanotransduction mechanisms before concluding with future research directions in the area of cell mechanobiology.

2 Cells and the ECM

2.1 Cell structure and function

All organisms are composed of cells, which are the basic structural and functional unit of life. All cells are composed of a plasma membrane, cytoplasm, nucleus, and other sub-cellular components. The plasma membrane consists of a phospholipid bilayer, with hydrophilic heads and hydrophobic tails, in which integral membrane proteins are embedded (Alberts 1989). The structure of the membrane allows cells to perform various necessary tasks: movement of substances in and out of the cells, antigen recognition, and maintenance of cell attachments, for example. The cytoplasm contains the cytoskeleton and various organelles, including the nucleus, ribosomes, endoplasmic reticulum, and mitochondria. These organelles carry out the basic functions of the cell, including gene expression and protein synthesis.

The human body has more than 200 different types of cells that vary in size, shape, and function. The characteristics of the different cells are determined by the nature of proteins, which carry out most of the cell activities. Cells arise in the body from stem cells and become specialized for one or more distinct functions such as contraction and secretion through a process known as cell differentiation (Ashworth 1973). The structural and morphological modifications are also accompanied by biochemical changes in cells during differentiation.

2.2 Load-sensitive cells

Many types of cells exist in a mechanical loading environment. For example, fibroblasts in the skin are subjected to tension, compression, and shear. Dense connective tissues like

tendons and ligaments are dominated by fibroblasts, which perform many vital functions during development and after maturity (Camelliti et al. 2005). These functions include organizing and maintaining connective tissues during development and repairing wounds during wound healing (Mathews 1975). Fibroblasts synthesize collagen types I and III and minor amounts of collagens IV, V, VI, elastin, laminin, proteoglycans and glycosaminoglycans (GAGs)(Camelliti et al. 2005), which are the structural constituents that constitute the ECM of many tissues, including tendons, ligaments, and skin. Finally, fibroblasts produce various factors such as cytokines [e.g., tumor necrosis factor- α (TNF- α)], growth factors [e.g., transforming growth factor- β (TGF- β)], and matrix metalloproteinases (MMPs), which are all involved in connective tissue maintenance, repair, and remodeling (MacKenna et al. 2000).

Chondrocytes are found in another type of load-bearing tissue, articular cartilage. Chondrocytes proliferate and differentiate in multiple stages to create the ECM that forms this complex tissue (Stockwell 1979; Erlebacher et al. 1995). At each stage of differentiation, chondrocytes express and secrete a distinct class of collagens, proteoglycans, and other ECM molecules (Karsenty and Wagner 2002; Mao and Nah 2004). At the proliferating stage, chondrocytes synthesize two major macromolecules: type II collagen and a large aggregating proteoglycan, aggrecan. These molecules interact with water through ionic and hydrogen bonding to form a stabilized load-bearing matrix that resists compressive and tensile forces. The cross-linked type II collagen fibrils allow cartilage to resist shear stress as well as compressive stress (Lane Smith et al. 2000). A number of minor forms of collagen, such as types III, VI, XII, XIV, and XVI, are also synthesized by chondrocytes (Aigner and Stove 2003).

It is known that bone bears tension, compression, and torsion *in vivo*. Therefore, bone cells such as osteoblasts are subjected to diverse mechanical forces. Osteoblasts are responsible for bone formation and are derived from mesenchymal cells found in bone marrow (Aubin and Triffitt 2002). These cells play a central role in creating and maintaining skeletal architecture. They secrete a mixture of ECM proteins, including type I collagen, which is the major component representing about 90% of the bone matrix, as well as proteoglycans and glycoproteins (Mackie 2003; Kartsogiannis and Ng 2004). In addition, osteoblasts produce growth factors such as TGF- β and bone morphogenetic proteins.

Forming the inner lining of the blood vessel, endothelial cells are influenced by two distinct hemodynamic loads: cyclical strain due to vessel wall distension and shear stress due to the frictional forces generated by blood flow. The structural and functional integrity of these cells are important in the maintenance of the vessel wall as well as circulatory function (Michiels 2003). In addition, these cells undergo morphological and functional alterations in response to cytokine signals, which may contribute to the pathogenesis of vascular diseases such as atherosclerosis (Toborek and Kaiser 1999). Endothelial cells secrete matrix products such as fibronectin, laminin, various types of collagens, and proteoglycans

(Sumpio et al. 2002). Amongst many other molecules, these cells express/secrete various growth factors such as platelet-derived growth factor (PDGF) and TGF- β .

Located within blood vessels, SMCs are another type of vascular cell that are exposed to compression, shear, and cyclic stretch due to pulsatile blood pressure. These cells are responsible for the maintenance of vessel structure and function and for producing arterial ECM proteins, including collagens, and several proteoglycans. Also, SMC proliferation has been implicated in the pathogenesis of several vascular diseases such as atherosclerosis and hypertension (Jackson and Schwartz 1992; Ross 1993).

2.3 Extracellular matrix

As mentioned earlier, cells reside in the ECM. The ECM is composed of a mixture of precisely organized structural and functional proteins (e.g., collagens, glycoproteins, and elastin) and proteoglycans (Labat-Robert et al. 1990; Aumailley and Gayraud 1998; Bosman and Stamenkovic 2003). These ECM components not only provide structural support, mechanical strength, and attachment sites for cell surface receptors, but also act as a reservoir for many signaling molecules that modulate various cellular functions such as migration, growth, and differentiation. The ECM is dynamic and changes its structure and composition with time, which is dependent on its location within tissues and organs (Adams and Watt 1993).

The ECM regulates many cellular functions, including cell proliferation, migration, and differentiation (Lukashev and Werb 1998). The ECM of the artery wall, for example, is a highly organized network of various proteins that provide mechanical support and viscoelasticity, and regulate the functional behavior of vascular cells. The regulation of cell functions by the ECM is realized through interactions between matrix proteins and cell surface receptors.

The major component of the ECM is collagen (van der Rest and Garrone 1991; Cauty and Kadler 2002), of which at least 20 types have been characterized. Collagen is usually arranged in fibrils that resist tensile, shear, or compressive forces in tendons, arteries, bone, skin, and so forth (Silver et al. 1992). Collagens I, II, and III are the most abundant types in the human body (Hulmes 1992); dense connective tissues such as tendons and ligaments, for example, are mainly composed of collagens. Type I collagen is rod-like, with little extensibility and high mechanical strength, while type II collagen, which is mainly found in cartilage, forms striated fibrils (Eyre 2004). Type III collagen aggregates in fibers with type I and is found in tissues like tendons, ligaments, skin, and blood vessels. Type IV collagen is located in the basement membranes of all vascular structures and forms a mesh-like scaffold (Brown and Timpl 1995). Other types of collagens are present in varying amounts in different tissues, performing important functions such as fibril initiation, limiting fibril diameter (Mendler et al. 1989; Birk et al. 1990), and likely yet unknown functions (Aigner and Stove 2003).

Besides collagen, the ECM contains several classes of proteoglycans (Iozzo 1998). The major functional element of the proteoglycans is the GAG chain, which may be chondroitin sulfate, dermatan sulfate, or keratan sulfate (Poole 1986). Hyaluronan, a non-sulfated GAG, provides matrix structure and lubrication and is involved in cell movement and differentiation (Toole 2004), whereas aggrecan provides compressive stiffness to tissues (Schwartz et al. 1999; Luo et al. 2000; Aigner and Stove 2003). Proteoglycans are responsible for the spatial organization of the ECM in tissues such as skin, where they form complexes with other structural molecules. Proteoglycans are also present at the cell surface and act as receptor sites for various hormones and growth factors (Ruoslahti 1988).

In addition to collagen and proteoglycans, many adhesive glycoproteins, including fibronectin, laminin, vitronectin, and tenascin are present in the ECM (Labat-Robert et al. 1990). These glycoproteins can bind to collagen and proteoglycans as well as to the cell surface. Fibronectin promotes cell attachment to the underlying matrix and plays an important role in cell motility and differentiation (Mostafavi-Pour et al. 2003). Laminin and vitronectin bind to collagen and mediate interactions between cells and the ECM (Aumailley and Krieg 1996; Aumailley and Smyth 1998). Tenascins contribute to cell spreading and adhesion, and their expression is regulated by mechanical loading (Chowdhury et al. 2003). Elastin and fibrillin are insoluble, ubiquitous proteins of connective tissues that form elastic fibers (Yurchenco et al. 1994). Elastic fibers provide elastic recoil capabilities to these tissues as well as many biological signals to neighboring cells.

2.4 Growth factors and cytokines

The synthesis of the ECM components is regulated by various growth factors/cytokines (Chiquet 1999). These include TGF- β s, various interleukins (e.g., IL-1, IL-6, IL-8), insulin-like growth factor-I (IGF-I), fibroblast growth factors (FGFs), vascular endothelial growth factor (VEGF), PDGF, connective tissue growth factor, and TNF- α . TGF- β exists in three mammalian isoforms: TGF- β 1, TGF- β 2, and TGF- β 3, which are known to stimulate the production and deposition of ECM proteins (MacKenna et al. 2000). High levels of TGF- β are found in pathological conditions such as cardiac fibrosis, chronic tendinosis, and osteoarthritis (Villarreal and Dillmann 1992; Kim et al. 1994).

Various interleukins, including IL-1, IL-6, and IL-8, participate in the development of many diseases. For example, IL-1 stimulates the expression of genes associated with inflammation and autoimmune diseases (Dinarelli 2002) while high levels of IL-6, a cytokine with various biological activities (Wolvekamp and Marquet 1990), are implicated in chronic inflammatory diseases such as rheumatoid arthritis and juvenile idiopathic arthritis (Nishimoto and Kishimoto 2004). IL-8 functions as a key mediator in diverse inflammatory disorders by promoting the recruitment, proliferation, and activation of vascular and immune cells (Baggiolini and Clark-Lewis 1992). Levels of this interleukin

are elevated in inflammatory tissue diseases such as rheumatoid arthritis, osteoarthritis, osteomyelitis, and periodontal disease (Hebert and Baker 1993; Zwahlen et al. 1993). TNF- α is another pro-inflammatory cytokine that is involved in the pathogenesis of both osteoarthritis and rheumatoid arthritis (Iannone and Lapadula 2003). Lastly, the potent growth factors FGFs, PDGF, and VEGF, which stimulate fibroblast proliferation and collagen synthesis, are involved in various pathological conditions such as chronic inflammation, atherosclerosis, arthritis, and tumors (Ornitz and Itoh 2001; Funo and Uramoto 2003; Verheul and Pinedo 2003).

2.5 Matrix metalloproteinases

The degradation of the ECM is carried out in part by MMPs, which are produced by many types of cells, including fibroblasts, chondrocytes, osteoblasts, and endothelial cells (Galis et al. 1994; Sternlicht and Werb 2001; Visse and Nagase 2003). MMPs represent a family of enzymes that selectively digest individual components of the ECM such as collagens, fibronectin, laminin, and proteoglycans (Galis and Khatri 2002; Visse and Nagase 2003). Based on their structure and substrate specificity, MMPs are divided into five classes, namely collagenases, gelatinases, stromelysins, membrane type MMPs (MT-MMPs), and others (McCawley and Matrisian 2001).

There is a high degree of sequence homology and structural domain conservation among the various MMP genes. MMPs are synthesized and secreted as inactive zymogens that are extracellularly activated by several proteinases such as trypsin and plasmin. The collagenases are primarily responsible for the degradation of native collagens, but can also degrade tenascin and aggrecan, while the gelatinases can degrade type IV collagen, fibronectin, laminin, aggrecan, elastin, and vitronectin (McCawley and Matrisian 2001; Overall 2002). Stromelysins and MT-MMPs, on the other hand, are capable of cleaving various ECM proteins, including proteoglycans, fibronectin, laminin, elastin, tenascin, aggrecan, and collagen.

Matrix metalloproteinases (MMPs) play an important role in a variety of normal and pathological conditions that involve matrix degradation and remodeling (McCawley and Matrisian 2000; Vu and Werb 2000; Sternlicht and Werb 2001). Since bone development requires the active remodeling of cartilage, MMPs also influence its development and homeostasis (Werb and Chin JR 1998). Vascular remodeling plays a role in many pathophysiological processes that require the degradation of the ECM. As such, MMPs contribute to the development and progression of vascular diseases as well (Lijnen 2003).

Besides their role in the regulation of ECM decomposition, MMPs are also implicated in the functional regulation of non-ECM molecules that include growth factors and their receptors, cytokines, cell surface proteoglycans, and a variety of enzymes (McCawley and Matrisian 2000, 2001; Seiki 2002; Mott and Werb 2004). They can degrade non-ECM substrates, such as pro-growth factors whose cleav-

age by MMPs results in active growth factors. For example, MMPs can release and activate latent growth factors and cytokines such as TGF- β , IGF, and VEGF (Fowlkes et al. 1994; Imai et al. 1997; Yu and Stamenkovic 2000; Mott and Werb 2004).

3 Mechanobiological responses of cells

Mechanical loading of cells has been shown to affect cell proliferation, and ECM gene and protein expression, which depend on the cell source and loading conditions (Table 1). For example, in human tendon fibroblasts, when cells were subjected to uniaxial stretching with constant frequency and duration (0.5 Hz, 4 hr), but varying magnitudes of stretching (4 and 8%), cell proliferation, collagen type I gene expression, and protein production all increased in a stretch magnitude-dependent manner (Yang et al. 2004). Similarly, the cyclic biaxial stretching of tendon fibroblasts increased cell proliferation, which depended on stretching time (Zeichen et al. 2000). However, prolonged mechanical stretching can have inhibitory effects on cells. In human tendon fibroblasts, for example, when a 5% repetitive stretching magnitude at a frequency of 1 Hz was applied for 1 day, cell proliferation significantly increased. When the same conditions were applied for 2 days however, cell proliferation was inhibited (Barkhausen et al. 2003).

In addition, the response of anterior cruciate ligament (ACL) and medial collateral ligament (MCL) fibroblasts shows marked differences in mRNA expression levels of collagens type I and III after stretching (Hsieh et al. 2000). ACL fibroblasts responded to cyclic strains by expressing higher levels of type I collagen mRNA with no apparent changes in type III collagen mRNA, whereas MCL fibroblasts exhibited a significant increase in type III collagen, but not in type I collagen mRNA. Furthermore, differences in responses by fibroblasts from the two types of ligaments were detected between the two strain magnitudes: 7.5% strain induced a time-dependent increase in type III collagen mRNA levels in MCL fibroblasts, while 5% strain did not.

Adult cardiac fibroblasts also show differential responses to different stretching magnitudes. Compared to the unstretched cells, a single application of a 10% uniaxial stretch resulted in a threefold increase in collagen type III and fibronectin mRNA levels; however, a 20% stretch decreased the mRNA levels of type III collagen and fibronectin (Lee et al. 1999). Furthermore, a 10% stretch showed a slight increase in collagen type I mRNA levels (136% compared with the unstretched control), whereas a 20% stretch did not affect the collagen expression levels significantly. Mechanical loading of cardiac fibroblasts also increased the ratio of collagen type III to collagen type I (Carver et al. 1991).

Periodontal ligament fibroblasts (PDLFs) exhibit differential responses to varying levels of mechanical strain as well. A 5% cyclic biaxial stretch for 24 hours increased synthesis of both collagen type I (~ twofold) and fibronectin (~ threefold) (Howard et al. 1998). However, exposure to a 10% strain exhibited a similar response for fibronectin (~

Table 1 Mechanobiological responses of cells

Response	Type of load/force/duration	Cell type	Significance	Reference
Increase in cell proliferation, collagen I gene expression, and collagen I protein	Uniaxial stretch, 0.5 Hz, 4 h with 4 and 8%	Human tendon fibroblasts	Stretch magnitude-dependent response	Yang et al. (2004)
Increase in cell proliferation	Cyclic biaxial stretch, 5%, 1 Hz, 6, 12, and 24 h	Human tendon fibroblasts	Stretch time-dependent response	Barkhausen et al. (2003)
Decrease in cell proliferation	Cyclic biaxial stretch, 5%, 1 Hz, 48 h			
Increase in collagen I mRNA, no change in collagen III mRNA	Cyclic equi-biaxial stretch, 5%, 1 Hz, 24 h	ACL fibroblasts	Cell source-dependent response	Hsieh et al. (2000)
Increase in collagen III mRNA, no change in collagen I mRNA	Cyclic equi-biaxial stretch, 5%, 1 Hz, 24 h	MCL fibroblasts		
Increase in collagen III and fibronectin mRNA	Uniaxial static stretch, 10%, 24 h	Adult cardiac fibroblasts	Differential regulation of and collagen III and fibronectin by specific strain patterns	Lee et al. (1999)
Decrease in collagen III and fibronectin mRNA	Uniaxial static stretch, 20%, 24 h			
Increase in collagen III and fibronectin mRNA	Equi-biaxial static stretch, 3%, 24 h		Differential regulation of collagen III and fibronectin by different types of loading	
Decrease in collagen III mRNA, no change in fibronectin mRNA	Equi-biaxial static stretch, 6%, 24 h			
Decrease in collagen III and fibronectin mRNA	Equi-biaxial compressive strain, 3–6%, 24 h			
Increase in collagen and fibronectin synthesis	Cyclic biaxial stretch, 5%, 0.5 Hz, 24 h	Periodontal ligament fibroblasts	Differential regulation of collagen type I and fibronectin by different tensile strains.	Howard et al. (1998)
Increase in fibronectin synthesis with no change in collagen synthesis	Cyclic biaxial stretch, 10%, 0.5 Hz, 24 h			
Increase in collagen I and fibronectin synthesis and secretion; increase in MMP-2 and TIMP-2 RNA	Cyclic equi-biaxial stretch, 10%, 0.5 Hz, 24 h		Differential response to different types of mechanical loading	He et al. (2004)
Decrease in the synthesis and secretion of collagen I and fibronectin, increase in MMP-2 RNA, no change in TIMP-2 RNA	Cyclic equi-biaxial compression, 10%, 0.5 Hz, 24 h			
Increase in procollagen production	Cyclic stretch, 20%, 1.5 Hz, 48 h	Pulmonary fibroblasts	Synergistic action of serum growth factors with mechanical loading	Butt and Bishop (1997) and Bishop et al. (1998)
Increase in procollagen mRNA	Cyclic stretch, 20%, 1 Hz, 48 h	Pulmonary fibroblasts	Substrate specific response	Breen (2000)
No increase in procollagen mRNA	Stretching cells grown on fibronectin-coated membranes			
Increase in collagen II and aggrecan mRNA	Cyclic stretch, 0.25 Hz, 24%, 3 h	Bovine chondrocytes	Involvement of integrins in signal transduction	Holmval et al. (1995)
	Cyclic stretch, 0.5 Hz, 24%, 24 h	Human chondrocytes		
Increase in collagen II and aggrecan mRNA	Hydrostatic pressure, 10 MPa, intermittent pressure	Bovine chondrocytes	Differential regulation of ECM by different types of load	Smith et al. (1996)
No increase in collagen II and aggrecan mRNA	Hydrostatic pressure, 10 MPa, constant pressure			
Inhibition of proteoglycan synthesis	Cyclic hydrostatic pressure, 5 MPa, 0.5 Hz, 1.5 h	Bovine chondrocytes	Differential regulation of ECM by different loading durations	Parkkinen et al. (1993)

Table 1 (Contd.)

Response	Type of load/force/duration	Cell type	Significance	Reference
Stimulation of proteoglycan synthesis	Cyclic hydrostatic pressure, 5 MPa, 0.5 Hz, 20 h			
Increase in proteoglycan and total protein synthesis	Dynamic compression, 3%, 0.1 Hz, 48 h	Cartilage explants	Synergistic effect with IGF-I, enhancement of IGF-I transport	Bonassar et al. (2001)
Increased cell proliferation	Cyclic stretch, 1 Hz, 48 h	Osteoblastic cells	Differential response to strain-rate	Kaspar et al. (2002)
No increase in cell proliferation	Cyclic stretch, 30 Hz, 48 h			
Decrease in DNA synthesis	Cyclic and static strain, 4%, 0.1 Hz, 4 h	Endothelial cells	Inhibition of DNA synthesis by mechanical strain	Woodell et al. (2003)
Increase in MMP-2 activity and expression	Cyclic strain, 10%, 1 Hz, 24 h	Endothelial cells	Stimulation of MMP expression by stimulation of p38 and ERK-dependent pathways	von Offenbergsweeney et al. (2004)
Increased collagen I synthesis	Cyclic stretch, 10%, 1 Hz, 24 h	Smooth muscle cells	Stretch directs L-arginine transport and metabolism to collagen synthesis	Durante et al. (2000)
Increase in collagen I, IV, and fibronectin mRNA	Cyclic stretch, 20%, 1 Hz, 24 h	Smooth muscle cells	Stretch-induced TGF- β 1 mediated matrix remodeling	Joki et al. (2000)
Increase in proteoglycans: versican, biglycan, and perlecan mRNA; decrease in decorin mRNA	Cyclic biaxial stretch, 4%, 1 Hz, 48 h	Smooth muscle cells	Coordinated ECM response to mechanical stimulation	Lee et al. (2001)
Increase in collagen and total protein synthesis	Cyclic stretch, 10%, 0.5 Hz, 24 h	Smooth muscle cells	Stretch-induced TGF- β 1 and angiotensin II-mediated matrix remodeling	Li et al. (1998)
Increase in MMP-2 and MMP-9 mRNA	Static strain, 5%, 1 Hz, 24 h	Smooth muscle cells	Selective response to different types of strain	Asanuma et al. (2003)
Decrease in MMP-2 mRNA	Cyclic strain, 10%, 1 Hz, 48 h			

fivefold increase), whereas the amount of type I collagen synthesized by the stretched cells did not differ from unstretched control levels, showing that these cells are capable of modifying their responses to varying magnitudes of tensional stress. PDLFs also responded differently to compressive strain. A 10% cyclic equi-biaxial compression of human PDLFs for 24 hours decreased collagen type I mRNA (threefold less compared to the control), decreased total protein by 50%, and decreased fibronectin by 50%. However, the same level of stretching increased collagen type I mRNA levels (fourfold more compared to the control) and total protein levels (threefold increase), while the fibronectin increases were not statistically significant (He et al. 2004). The results of collagen type I production in response to 10% stretching in the above two studies appear contradictory. The reason for this discrepancy may be attributed to the different substrates used in the two studies: silicone membrane coated with collagen type I (He et al. 2004) versus polyurethane polymer (Tecoflex) (Howard et al. 1998).

Cardiac fibroblasts also display a similar differential ECM synthesis in response to tensile and compressive strains. Equi-biaxial tensile strain at 3% increased mRNA expression levels of collagen type III and fibronectin by 1.5-fold; however, 6%

stretching decreased collagen type III mRNA levels while fibronectin levels remained unchanged (Lee et al. 1999). In contrast, 3–6% compressive equi-biaxial strains decreased fibronectin levels by 20 and 40%, respectively. In addition to changes in collagen and fibronectin levels, mechanical loading also induced changes in tenascin-C levels. For example, when 10% equi-biaxial stretching at 0.3 Hz was applied to chick embryo fibroblasts, tenascin-C mRNA and protein levels increased twofold within 6 h compared to the resting control (Chiquet et al. 2004).

Besides different types and magnitudes of mechanical loading, the nature of the underlying matrix also influences the cells' response to various mechanical loading conditions. For example, the mechanical loading of cardiac fibroblasts seeded in elastin-coated membranes increased procollagen mRNA levels twofold (Butt and Bishop 1997). In contrast, cells plated on a collagen type I matrix or hydrophilic substrates demonstrated a decrease in collagen production. As a similar example, pulmonary artery SMCs on a collagen type I substrate subjected to cyclic stretching showed a 24% decrease in collagen synthesis (Kulik and Alvarado 1993). However, some of these published results are contradictory because the comparisons did not take into account the ori-

gin of the cells (adult or fetal) or whether they were primary or established cell lines (Leung et al. 1976; Yasuda et al. 1996; Butt and Bishop 1997). Furthermore, the mechanical stretching apparatus, duration and magnitude of strain, culture media, etc., may have varied in each of these experiments. Regardless, these studies suggest the crucial role of the ECM in mechanotransduction.

One attempt to circumvent the differences in experimental set-up in the previous studies compared the effects of different substrates on procollagen mRNA levels in a single cell type. Pulmonary fibroblasts were cultured on membranes coated with fibronectin, laminin, or elastin and were then subjected to cyclic mechanical strain (Breen 2000). The cells grown on laminin and elastin-coated membranes showed increased expression of procollagen mRNA levels, but not the ones grown on fibronectin-coated membranes. These results suggest that the fibroblast response to mechanical strain is modulated by specific cell–ECM interactions.

Similar to fibroblasts, chondrocytes are also responsive to mechanical forces. Numerous studies show that the modulation of ECM synthesis in chondrocytes by mechanical loading depends on the magnitude, duration, and type of mechanical loading. When bovine chondrocytes were subjected to cyclic mechanical loading for 3 h at 24% strain at 0.25 Hz, mRNA expression levels of collagen type II and aggrecan increased (Holmvalld et al. 1995). However, constant hydrostatic pressure (10 MPa) applied for 4 h in primary cultures of bovine chondrocytes had no effect on collagen type II or aggrecan mRNA levels although intermittent pressure applied at 1 Hz increased aggrecan and type II collagen mRNA levels by 31 and 36%, respectively. Both intermittent and constant hydrostatic pressure, however, stimulated proteoglycan synthesis (Smith et al. 1996). On the other hand, the application of cyclic hydrostatic pressure (5 MPa) to cultured chondrocytes at frequencies of 0.05, 0.25, and 0.5 Hz for 1.5 h inhibited proteoglycan synthesis while the application of the same pressure at frequencies of 0.25 and 0.5 Hz for 20 h increased proteoglycan synthesis (Parkkinen et al. 1993).

The mechanobiological response of osteoblastic cells to mechanical loading has also been studied (Murray and Rush-ton 1990; Jones et al. 1991; Brighton et al. 1992). These studies have demonstrated that dynamic cell stretching stimulates osteoblastic cell proliferation and collagen I secretion, where cell proliferation depends on the stretching cycle and frequency (Neidlinger-Wilke et al. 1994; Kaspar et al. 2000, 2002). Combined with an appropriate number of cycles, an optimal frequency (1 Hz) as stimulus for bone cell proliferation was observed; however, a higher frequency (30 Hz), in combination with a higher number of cycles, reduced the cell number to the control level (Neidlinger-Wilke et al. 1994; Kaspar et al. 2000, 2002). The response of osteoblasts to the chronic low frequency strain showed a 3.4 fold increase in collagen type I mRNA (Harter et al. 1995). Moreover, it has been shown that mechanical forces regulate the contractile gene transcription in osteoblasts (Wang et al. 2002).

Vascular endothelial cells are yet another type of cell shown to be responsive to mechanical forces. Cyclic mechanical stretching, for instance, induces cell reorientation (Wang

et al. 2001), remodels the actin cytoskeleton (Wang and Grood 2000), and elevates the TGF- β 1 mRNA expression parallel to the increase in total collagen and fibronectin synthesis (O'Callaghan and Williams 2000). A recent study reported the inhibitory effects of stretching on DNA synthesis in bovine aortic endothelial cells. Compared with the non-stretched control, 4% cyclic stretching at 0.1 Hz and 4% static stretching caused a 40 and 70% decrease in DNA synthesis, respectively (Woodell et al. 2003). This study indicates that the response of endothelial cells to mechanical loading is different from that of other types of cells such as tendon fibroblasts (Yang et al. 2004), osteoblasts (Neidlinger-Wilke et al. 1994), and vascular SMCs (Li et al. 1997), where these cells increased proliferation in response to appropriate magnitudes of cyclic mechanical stretching.

Besides stretching, vascular endothelial cells *in vivo* are also subjected to shear stress due to blood flow. It was shown that the application of physiological levels of shear stress (16 dynes/cm²) induced the release of basic FGF (bFGF) (Gloe et al. 2002). The release of bFGF during shear stress was abolished when an integrin antagonist was present, suggesting that the stress-induced release of this growth factor is mediated by cell–matrix interactions. Endothelial cells also express shear stress-dependent endothelin-1, a potent vasoconstrictor, and nitric oxide synthase, which produces the vasodilator nitric oxide (Ziegler et al. 1998). Finally, endothelial cells are responsive to hydrostatic pressure. For instance, bovine pulmonary artery endothelial cells subjected to hydrostatic pressure exhibited cytoskeletal rearrangement, cell proliferation, and the release of bFGF (Acevedo et al. 1993). The effect of fluid shear stress on endothelial cell function has been reviewed extensively (Davies 1995; Malek and Izumo 1995; Resnick et al. 2003; Kakisis et al. 2004).

Another type of load-sensitive cell is the SMC. When grown under static culture conditions, SMCs are randomly oriented; however, when these cells were subjected to cyclic uniaxial stretching, they reoriented to a uniform alignment at an angle of about 65° to the stretching direction (Dartsch and Hammerle 1986). This cell reorientation response depends on the stretching magnitude (Dartsch et al. 1986) and is consistent with other types of cells that align in the direction of the minimum substrate deformation (Wang et al. 1995, 2001; Wang and Grood 2000). In addition, mechanical stretching influenced the induction of ECM proteins in various types of SMCs. For example, when rabbit vascular SMCs were grown on elastin sheets and subjected to cyclic stretching, the cells increased production of collagen types I and III and proteoglycans (Leung et al. 1976). Numerous studies also reported that cyclic stretching of SMCs stimulated collagen and non-collagenous protein synthesis (Sumpio et al. 1988; Li et al. 1998; Feng et al. 1999; Durante et al. 2000; Joki et al. 2000; Lee et al. 2001). Additionally, a recent study has shown that uniaxial, but not equi-biaxial, strain on mesenchymal stem cells (MSCs) induced a transient increase in collagen I expression and increased SMC markers without changing the expression of differentiation markers of the other cell types (Park et al. 2004). This study suggests that uniaxial strain, which mimics the type of mechanical strain

experienced by the SMCs, may promote the differentiation of MSCs into SMCs.

Mechanical loading of cells also modulates ECM turnover by regulating the expression of MMPs and their activity. In human vascular SMCs, a 5% static stretch for 24 h increased MMP-2 mRNA levels 50-fold compared to non-stretched controls, whereas 5% cyclic stretching at 1 Hz for 48 h decreased it (Asanuma et al. 2003). MMP-9 mRNA levels were increased after 48 h of static stretching compared with no stretching and cyclic stretching. This shows the selective response of different MMPs to various types of stretching. In rabbit tendon fibroblasts, cyclic biaxial stretching of 5% strain at 0.33 Hz for 6 h in the presence of IL-1 β increased the expression of MMP-1 and MMP-3 mRNA and induced a 20-fold increase in MMP-3 protein (Archambault et al. 2002). Also, both tensional and compressional forces increased MMP-2 mRNA in PDLFs (He et al. 2004). Furthermore, an equi-biaxial cyclic strain in bovine endothelial cells stimulated the production of MMP-2 mRNA expression and MMP-2 protein (von Offenbergh Sweeney et al. 2004). Similarly, in mouse vascular SMCs, a cyclic stretch of 15% at 0.5 Hz enhanced MMP-2 mRNA expression (Grote et al. 2003). A cyclic mechanical strain of 20% increased TNF- α production and MMP-2 and MMP-14 mRNA levels in human endothelial cells (Wang et al. 2003). However, in human vascular SMCs, cyclic mechanical strains of 1–4% at 1 Hz suppressed the PDGF and TNF- α -induced MMP-1 protein expression (Yang et al. 1998).

4 Interaction between mechanical loading and soluble factors

As noted above, mechanical loading promotes the release of soluble factors that can regulate ECM protein production in an autocrine or paracrine fashion, which modulates cell function. Several studies have explored the issue of interaction between mechanical loading and soluble factors. In rat cardiac fibroblasts, for example, a 20% mechanical stretching at 1.5 Hz for 48 h enhanced the cellular response to TGF- β 1 and IGF-I, which stimulated the procollagen mRNA expression by 4.3- and 3-fold, respectively, compared to the controls with growth factors alone (Butt and Bishop 1997). Furthermore, higher stretching magnitudes, either tension or compression, significantly increased the levels of TGF- β 1 in rat cardiac fibroblasts (Lee et al. 1999). Cyclic biaxial mechanical stretching (5%) of human tendon fibroblasts at 1 Hz also increased the secretion of TGF- β 1, bFGF, IL-6, TNF- α , and PDGF, which are known to stimulate cell proliferation, differentiation, and matrix formation (Skutek et al. 2001). Similar stretching studies in SMCs showed a time-dependent increase in PDGF and TGF- β 1 mRNA expression, which mediates the induction of cell proliferation and ECM proteins such as collagens I and IV and fibronectin (Li et al. 1998; Joki et al. 2000). Furthermore, cyclic biaxial mechanical stretching (20%, 1.5 Hz) increased the stimulatory effect of PDGF on procollagen production of pulmonary fibroblasts by 96% (Bishop et al. 1998). One possible mechanism by

which mechanical loads enhance the stimulatory effects of soluble factors such as PDGF may be that mechanical loads induce the over-expression of cell surface receptors as well as the augmentation of tyrosine phosphorylation of adhesive proteins (Tanabe et al. 2000). In order for cells to undergo a mitogenic response to mechanical loads, however, the presence of growth factors is necessary (Banes et al. 1995).

In addition to these studies investigating the regulation of ECM synthesis through the release of soluble factors by mechanical loading, the interactive effect of growth factors/cytokines with mechanical loading on ECM synthesis has been investigated. IGF-I enhanced the synthesis of total protein and proteoglycan in bovine articular cartilage whereas static compression decreased the synthesis; however, the combination of both produced a decrease in synthesis after the initial 4 h, followed by a return to the initial levels at 24 h (Bonassar et al. 2000). One suggested mechanism by which static compression affects the action of IGF-I is that transport of IGF-I through the ECM is altered by compression. Kinetic studies indicated that biosynthetic responses to mechanical compression alone occurred more quickly than the response to IGF-I. In bovine cartilage explants, IGF-I stimulated the synthesis of protein and proteoglycan by 90 and 120%, respectively, whereas dynamic loading increased the synthesis by 40 and 90%, respectively. When applied together, the two stimuli enhanced protein synthesis and proteoglycan synthesis by 180 and 290%, respectively (Bonassar et al. 2001). Based on this study, two mechanisms have been proposed: first, dynamic compression may enhance the access of articular chondrocytes to IGF-I by enhancing IGF-I transport and second, dynamic compression may accelerate the biosynthetic response of the tissue to the growth factor. The combined effect of dynamic shear strain on articular cartilage and IGF-I also showed similar responses (Jin et al. 2003). However, dynamic shear strain itself did not affect IGF-I uptake or transport, suggesting a specific signal transduction mechanism sensitive to the deformation of chondrocytes by tissue shear stresses.

In addition to IGF-I, the role of IL-1 in the pathway linking mechanical forces and ECM synthesis has also been investigated. IL-1 is known to decrease ECM protein synthesis in cartilage. Similar to the kinetics of decreased biosynthesis caused by static compression, the rate of proteoglycan synthesis decreases after exposure to IL-1 α . Cartilage explants compressed in the presence of an IL-1 receptor antagonist increased proteoglycan synthesis (Murata et al. 2003); both IL-1 α and IL-1 β mRNA were detected in cartilage that was compressed 50% for 3 h. This study suggests that compression-induced proteoglycan synthesis is mediated via an IL-1 receptor.

5 Mechanical loading effects on tissue engineering constructs

Mechanical loads not only affect the ECM synthesis of native tissues, but also modulate the ECM structure, composition,

and mechanical properties of tissue engineering constructs. In SMC-seeded fibronectin-coated scaffolds fabricated from type I collagen and polyglycolide, cyclic mechanical stretching of a 7% strain at 1 Hz for 5–20 weeks stimulated the expression of SMC elastin and type I collagen mRNA (Kim et al. 1999). Furthermore, the application of cyclic stretch for 10 weeks induced cell alignment — a high percentage of the cells were aligned perpendicular to the stretching direction. Cyclic stretching for 20 weeks also increased the tensile strength and Young's moduli of the SMC engineered tissues whereas both decreased over time without mechanical stretching. The application of cyclic stretching at 10% for 4–8 days to SMC-seeded collagen gels increased the Young's modulus, changed the morphology of tissue constructs, with both collagen fibers and SMCs oriented circumferentially, and upregulated elastin mRNA while downregulating the collagen mRNA expression (Seliktar et al. 2000; Seliktar et al. 2001). MMP-2 protein levels were also significantly upregulated by this stretching protocol and its action on ECM remodeling may explain the altered levels of elastin and collagen (Seliktar et al. 2001).

As implied above, the role of mechanical loading in the development of engineered cartilage has been gaining attention in recent years as a potential treatment for articular cartilage defects. Chondrocytes cultured in hydrogels can respond to mechanical loading after they have been given enough time to develop a functional ECM within the hydrogel. In order to create a mechanically competent tissue, chondrocyte-seeded agarose disks were subjected to dynamic compressive loading (Mauck et al. 2000). A custom-designed bioreactor was used to load cell-seeded agarose disks at a strain amplitude of 10% at 1 Hz for 5 days a week for 4 weeks. Results showed that mechanically loaded disks yielded a six-fold increase in the equilibrium aggregate modulus over free-swelling controls after 28 days of loading. At day 21, the sulfated GAG content and hydroxyproline content were also greater in dynamically loaded disks compared to the free-swelling controls. The initial cell seeding density also influenced the mechanical properties of the loaded disks (Mauck et al. 2002). For example, the application of dynamic compression of a 10% compressive strain at 1 Hz to constructs seeded at 20×10^6 cells/ml enhanced GAG and collagen content ($\sim 150\%$) and mechanical properties (\sim threefold increase in aggregate modulus) compared to the free-swelling controls. However, no difference in the mechanical properties was found in constructs seeded at 60×10^6 cells/ml compared to the free-swelling control. Further investigation revealed that increasing feed media volume and serum supplementation with increasing cell seeding densities are necessary along with dynamic compression in the development of functional articular cartilage (Mauck et al. 2003b). In addition, a recent study has shown that, applied in concert, dynamic deformational loading and either TGF- β 1 or IGF-I increased the aggregate modulus of engineered constructs greater than the sum of either stimulus applied alone (Mauck et al. 2003a). This result suggests further that there is a synergistic action of growth factors and dynamic loading.

In addition to studies concerning mechanical loads and ECM synthesis in chondrocytes in scaffolds, the modulation of chondrocyte differentiation by mechanical loading has been investigated (Takahashi et al. 1998; Elder et al. 2000). It was reported that chondrogenesis was differentially affected by 2 h of dynamic and compressive loading. It was also shown that the cyclic loading-induced stimulation of chondrogenesis depends on the loading frequency and duration (Elder et al. 2000, 2001).

The utility of mechanical loading in the preconditioning of engineered cartilage constructs has also been investigated. For instance, isolated articular cartilage cultured in collagen gels exposed to uniaxial static compression for 24 h inhibited collagens I and II and aggrecan mRNA expression, whereas dynamic compression showed no significant difference in collagen II and aggrecan mRNA levels (Hunter et al. 2002). In contrast, dynamic compression stimulated matrix synthesis, especially that of proteoglycans (Buschmann et al. 1995; Davisson et al. 2002; Chowdhury et al. 2003; Lee et al. 2003).

For the proper development of functional tissue engineered constructs, it is essential to include the mechanical loading factor because connective tissues like tendons bear mechanical loads *in vivo*. Therefore, bio-artificial tendons (BATs) using native tendon cells in a molded type I collagen matrix that can be subjected to mechanical loading have been developed (Garvin et al. 2003). After uniaxial stretching of BATs at 1% strain for 1 h/day at 1 Hz for 8 days, avian flexor tendon cells expressed collagen genes I, III, and XII as well as aggrecan and fibronectin. The expression levels of these ECM genes were consistent with the expression levels of cells grown on collagen-coated substrates and with those of native cells. The mechanical properties of loaded BATs also increased with time, the average elastic modulus for control BATs on day 7 was 0.49 MPa and that for the mechanically conditioned BATs was 1.8 MPa. In addition, mechanically stretched BATs had a 2.9-fold greater ultimate tensile strength compared with that of the non-stretched controls.

In tissue engineering constructs, preloading and stretching magnitudes are important issues that need to be considered. Contracted fibroblast-seeded collagen constructs were preloaded to either 2 or 10 mN and then subjected to a cyclic tensile strain of 10% at 1 Hz for 24 h (Berry et al. 2003). It was found that the cyclic stretching increased cell proliferation in the constructs preloaded to both 2 and 10 mN, whereas collagen synthesis was increased in the constructs preloaded at 2 mN only. Furthermore, the stiffness of the constructs was also enhanced only in the group preloaded at 2 mN. Taken together, these studies point to the utility of appropriate mechanical loading in the successful engineering of tissue constructs.

6 Cellular mechanotransduction

Cells sense mechanical forces and then respond to regulate ECM synthesis and turnover. Although the exact mechanisms

by which these cells sense mechanical forces and convert them into biochemical signals are still unknown, some possible mechanisms have been proposed. The ECM–integrin–cytoskeleton pathway is one of the signaling pathways most studied. Cells attach to the ECM via integrins that are linked to the cytoskeleton. This provides a structural connection to transmit mechanical signals from the ECM to the cell (Juliana and Haskill 1993; Maniotis et al. 1997). The major cellular components involved in the mechanotransduction mechanisms are the integrins, cytoskeleton, G proteins, receptor tyrosine kinases (RTKs), mitogen-activated protein kinases (MAPKs), and stretch-activated ion channels (Fig. 1). It should be noted, however, that these components are related in a cell either physically, functionally, or both.

In this section, the role of these cellular components in the mechanotransduction mechanisms is briefly reviewed. Many reviews are available that focus on different types of cells, including dermal fibroblasts (Silver et al. 2003), cardiac fibroblasts (MacKenna et al. 2000), cardiac myocytes (Sadoshima and Izumo 1997), SMCs (Osol 1995), endothelial cells (Davies 1995), and bone cells (Duncan and Turner 1995). In addition, a review that focuses on the mechanotransduction at cell–matrix and cell–cell contacts is also available (Chen et al. 2004). These reviews are excellent references for those who are interested in a more in-depth understanding of cellular mechanotransduction mechanisms.

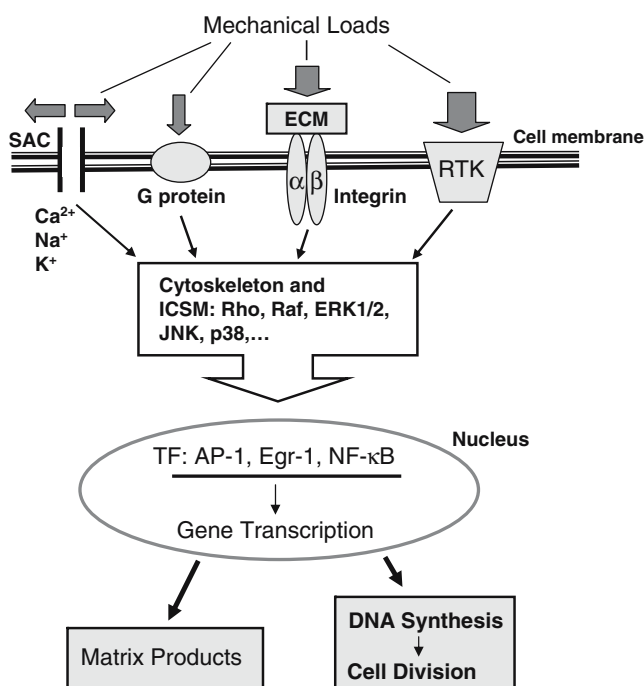


Fig. 1 A conceptual illustration of cellular mechanotransduction mechanisms. (ICSM: Intracellular signaling molecules; TF: Transcriptional factors)

6.1 Integrins

Integrins are the main mechanoreceptors that link the cytoskeleton to the ECM. They contain a large ECM domain responsible for binding to substrates, a single transmembrane domain, and a cytoplasmic domain (Hynes 1992). Serving as both adhesive receptors (Albelda and Buck 1990; Aplin et al. 1999) and mechanotransducers (Ingber 1991; Juliana and Haskill 1993; Giancotti and Ruoslahti 1999; Katsumi et al. 2004), integrin receptors transmit signals across the membrane after binding ECM ligands, thereby regulating various cellular functions, including cell attachment, proliferation, migration, and differentiation (Coppolino and Dedhar 2000). In addition, integrins bind paxillin, caveolin, and focal adhesion kinase and through these binding proteins, integrins are able to recruit kinases to activate pathways that lead ultimately to ERK1/2 and JNK phosphorylation (see below) (Iqbal and Zaidi 2005).

6.2 Cytoskeleton

The fundamental structural unit of the cytoskeleton is a filamentous network of microfilaments, microtubules, and intermediate filaments (Ingber 1998). Microfilaments, comprised of actin monomers attached with small binding proteins (e.g., α -actinin, filamin A, talin, and vinculin), are semi-flexible. Microtubules are rod-like, stiff polymers, whereas intermediate filaments are very flexible, elongated polymers (Oddou et al. 2000). The elastic and flexible nature of the cytoskeletal components provides the mechanical properties required for resistance to deformations, and hence allows the cell to maintain its shape in the presence of mechanical stress. The mechanical properties of the cells also influence how they respond to mechanical stress because how the cell deforms under mechanical forces depends on their mechanical properties (Wang et al. 2002).

Furthermore, mechanical loads that are transferred across the integrins can be transduced into a chemical response through changes in the cytoskeletal structure at the site of receptor binding or at other locations inside the cell (Ingber 1997). It has been shown that mechanical stresses that act on magnetic beads coated with an integrin ligand are transmitted to the cytoskeleton (Wang et al. 1993). Results from many studies with various cell types and model systems have also shown that a mechanical stress applied to integrins can alter cytoskeletal structure and activate signal transduction and gene expression in a stress-dependent manner (Schmidt et al. 1993; Wang et al. 1993; Urbich et al. 2002). In vascular endothelial cells, for example, the connections between integrins and their specific ECM ligands are essential for relaying the signals induced by shear stress to intracellular pathways (Jalali et al. 2001). In rat vascular SMCs, the interactions between integrins and specific matrix proteins are found to be responsible for sensing mechanical strain as well (Wilson et al. 1995). Rho, a member of the Ras superfamily of small GTPases, is implicated in the integrin mediated signal transduction. In particular, Rho plays a major role in regulating

actin stress fiber formation and the focal adhesion assembly (Ridley and Hall 1992; Pavalko et al. 1998).

6.3 G proteins

G proteins are another family of membrane proteins that are involved in modulating the mechanotransduction pathways. G protein subunits are localized at the sites of focal adhesions, which are the sites of mechanotransduction (Hansen et al. 1994). Mechanical forces on a cell bring conformational changes to G proteins, which initiate signaling cascades, thus leading to cell growth. The activation of G proteins by shear stress and cyclic stretching has been demonstrated in cardiac fibroblasts (Gudi et al. 1996, 1998). Furthermore, activation of G proteins has been investigated in endothelial cells by subjecting them to uniaxial strain at various strain magnitudes, rates, and cycle numbers. The results showed a rapid activation of G proteins in a strain-magnitude and strain-rate dependent manner (Clark et al. 2002), confirming the participation of G proteins in the mechanoreception of mechanical strain.

6.4 Receptor tyrosine kinases

The activation of G proteins by mechanical stress triggers a cascade of downstream signaling events which causes a generation of second messengers. RTKs are a diverse group of transmembrane proteins involved in the signal transduction whose activation seems to play a major role in integrin-mediated signaling. Many growth factors, such as EGF and PDGF, bind to cell surface RTKs (Ullrich and Schlessinger 1990). The binding of the ligand causes receptor dimerization, which results in a cascade of complex signaling events (Cantley et al. 1991; Chao 1992; Karin 1992). RTKs also induce tyrosine phosphorylation and the activation of Raf-1 and ERKs or MAPKs (Boulton et al. 1991; Cobb et al. 1991a,b; Kyriakis et al. 1992).

6.5 Mitogen-activated protein kinases

MAPKs play an important role in cell signaling as well. Signals originating from mechanical forces can lead to gene expression and protein synthesis through the MAPK pathway. The phosphorylation of one of the MAPKs (ERK1 and 2), for instance, leads to the activation of regulatory proteins in the cytoplasm and nucleus. It has been shown that shear stress induced a transient activation of ERK in bovine aortic endothelial cells (Yamazaki et al. 1993; Jo et al. 1997). Mechanical stretching also rapidly activated ERKs in human pulmonary epithelial cells and in fetal lung fibroblasts (Hubmayr et al. 1996; Chess et al. 2000). In addition, p38 MAPK and JNK/SAPK (stress activated protein kinase) are activated by various cellular stresses. In the chondrocytes of articular cartilages, for instance, mechanical compression activates p38 MAPK, SAPK, and ERK1/2 phosphorylation (Fanning

et al. 2003). In adult cardiac fibroblasts, both ERK and JNK are activated by a cyclic mechanical load (MacKenna et al. 1998). Both MAPK and JNK are activated in response to static stretching in cardiac fibroblasts (Komuro et al. 1996). Phosphorylation of these second messengers leads to the activation of downstream transcriptional factors such as AP-1, Egr-1, and NF- κ B, which subsequently induce the expression of other signaling proteins (Hughes-Fulford 2004; Kakisis et al. 2004).

6.6 Stretch-activated channels

Besides the protein kinase signaling molecules, the activation of mechano-sensitive ion channels has also been proposed as a transduction mechanism (Hamill and Martinac 2001). Stretch-activated ion channels allow the movement of ions like Na⁺, K⁺, and Ca²⁺ in and out of cells (Sachs 1992; Ruknudin et al. 1993). In particular, changes in intracellular Ca²⁺ levels regulate a wide range of cellular processes, including cell growth, cell motility, contraction, apoptosis, and differentiation.

Mechanical stimulation elevates Ca²⁺ in many types of cells, including SMCs, fibroblasts, osteoblasts, and vascular endothelial cells (Shen et al. 1992; Sigurdson et al. 1992; Mow 1994; Pommerenke et al. 1996; Kirber et al. 2000). The stretching of cultured cardiac endothelial cells increased Ca²⁺ levels via the activation of stretch-activated channels (SACs), since this increase was blocked by SAC blockers such as gadolinium (Naruse and Sokabe 1993; Kohler et al. 1998). ERK1/2 activation by mechanical stretching also requires the activation of the Ca²⁺-sensitive EGF receptor, mainly via stretch-activated ion channels, leading to vascular smooth muscle growth (Iwasaki et al. 2000). Also, human disc cells respond to fluid shear stresses by increasing intracellular Ca²⁺ concentration (Elder et al. 2001). Therefore, regardless of the cell type, the integrins, cytoskeleton, RTKs, G proteins, MAP kinases, and Ca²⁺ are essential in enabling cellular mechanotransduction.

7 Summary

In this review, we attempted to clarify that various mechanical forces acting on load-sensitive cells *in vivo*, such as chondrocytes and tendon fibroblasts, regulate cellular functions, including gene expression, protein synthesis, cell growth, and differentiation. These forces balance the cellular synthesis and degradation of various matrix components, thus maintaining tissue homeostasis. However, excessive/abnormal mechanical loads may tilt the equilibrium from cellular anabolism to catabolism, consequently leading to tissue pathophysiological conditions, such as osteoarthritis, tendinopathy, and fibrosis in cartilage, tendon, lung, and skin. In an effort to better understand the causes of tissue disorders and to develop effective protocols for their treatment and prevention, it is necessary to study the effects of various mechani-

cal loading conditions on cells (e.g., loading magnitude, frequency, and duration). Also, the interaction between mechanical forces and soluble factors, including growth factors and cytokines (MacKenna et al. 2000), should be investigated to discern how changes in mechanical forces are associated with the development and remodeling of tissues as well as their disorders. Fundamentally, future research in the area of cell mechanobiology will require novel experimental and theoretical methodologies to determine whether particular cells respond directly to stress, strain, strain-rate, strain-energy, or other mechanical quantities (Humphrey 2001). Future research should also be directed at determining the type and magnitude of forces experienced at the cellular and sub-cellular levels and at identifying those “force sensors/receptors” (Janmey and Weitz 2004) that are responsible for initiating a cascade of molecular events, which results in altered cellular functions in response to mechanical loads.

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