

## MECHANOBIOLOGY OF ADULT AND STEM CELLS

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### Abstract

Mechanical forces, including gravity, tension, compression, hydrostatic pressure, and fluid shear stress, play a vital role in human physiology and pathology. They particularly influence extracellular matrix (ECM) gene expression, ECM protein synthesis, and production of inflammatory mediators of many load-sensitive adult cells such as fibroblasts, chondrocytes, smooth muscle cells, and endothelial cells. Furthermore, the mechanical forces generated by cells themselves, known as cell traction forces (CTFs), also influence many biological processes such as wound healing, angiogenesis, and metastasis. Thus, the quantitative characterization of CTFs by qualities such as magnitude and distribution is useful for understanding physiological and pathological events at the tissue and organ levels. Recently, the effects of mechanical

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loads on embryonic and adult stem cells in terms of self-renewal, differentiation, and matrix protein expression have been investigated. While it seems certain that mechanical loads applied to stem cells regulate their self-renewal and induce controlled cell lineage differentiation, the detailed molecular signaling mechanisms responsible for these mechano-effects remain to be elucidated. Challenges in the fields of both adult- and stem-cell mechanobiology include devising novel experimental and theoretical methodologies to examine mechano-responses more closely to various forms of mechanical forces and mechanotransduction mechanisms of these cells in a more physiologically accurate setting. Such novel methodologies will lead to better understanding of various pathological diseases, their management, and translational applications in the ever expanding field of tissue engineering.

**Key Words:** Fibroblasts, Chondrocytes, Smooth muscle cells, Endothelial cells, Stem cells, ECM, Mechanotransduction, Tissue engineering. © 2008 Elsevier Inc.

## LIST OF ABBREVIATIONS

2D, Two-dimensional; 3D, Three-dimensional; ACL, Anterior cruciate ligament; ALP, Alkaline phosphatase;  $\alpha$ -SMA,  $\alpha$ -smooth muscle actin; BMSC, Bone marrow stem cell; CBF, CCAAT-binding factor; Cbfa1, Cbfa1/Runx2 is a key transcription factor associated with osteoblast differentiation; CHP, Cyclic hydrostatic pressure; CNF-1, Cytotoxic necrotizing factor type 1; COX-2, Cyclooxygenase-2; cPLA<sub>2</sub>, calcium-dependent cytosolic phospholipase A<sub>2</sub>; CREB, cAMP-response element binding protein; CTF, Cell traction force; CTFM, Cell traction force microscopy; ECM, Extracellular matrix; EPCs, Endothelial progenitor cells; FAs, Focal adhesions; FAK, Focal adhesion kinase; FRET, Fluorescence resonance energy transfer; FPCGs, Fibroblast-populated collagen gels; GPCRs, G-protein-coupled receptors; ICAM1, Intercellular adhesion molecule-1; IGF-1, Insulin-like growth factor-1; IHP, Intermittent hydrostatic pressure; IL-1, Interleukin-1; MAPK, Mitogen-activated protein kinase; MCL, Medial collateral ligament; MCP1, Monocyte chemotactic protein-1; mDia1, A mammalian homolog of *Drosophila* diaphanous protein; MLCK, Myosin light chain kinase; MMPs, Matrix metalloproteinases; MSCs, Mesenchymal stem cells; NF- $\kappa$ B, Nuclear factor- $\kappa$ B; OCN, Osteocalcin; PDGF, Platelet-derived growth factor; PDGFRb, Platelet-derived growth factor receptor, beta polypeptide; PDL, Periodontal ligament; PECAM-1, Platelet endothelial cell adhesion molecule-1; PGA, Poly(glycolic acid); PGE<sub>2</sub>, Prostaglandin E<sub>2</sub>; ROCK, Rho-associated kinase; SMAD, Small mothers against decapentaplegic, which are a class of proteins that modulate the activity of TGF- $\beta$

ligands; SMCs, Smooth muscle cells; TGF- $\beta$ , Transforming growth factor- $\beta$ ; VCAM1, Vascular cell adhesion molecule-1; VEGF, Vascular endothelial growth factor; VEGFR2, Vascular endothelial growth factor receptor 2; VSMC, Vascular smooth muscle cell.

## 1. INTRODUCTION

Mechanical forces that originate externally from the environment influence many aspects of human health and disease (Banes *et al.*, 1990). Gravity, tension, compression, fluid shear stress, and hydrostatic pressure are just a few examples of the forces that constantly act on cells within organs and tissues (Davies *et al.*, 1995; Grodzinsky *et al.*, 2000; Kakisis *et al.*, 2004; Lehoux *et al.*, 2006; Silver *et al.*, 2003; Wang, 2006). Besides these external mechanical forces, cells also generate their own mechanical forces, known as cell traction forces (CTFs). Cells use CTFs to migrate, maintain their shape, and generate mechanical signals. As such, CTFs play a fundamental role in many biological processes such as wound healing, angiogenesis, and metastasis (Wang and Lin, 2007).

Different types of cells in the body are subjected to various levels of mechanical forces. Fibroblasts of the skin, lung, heart, tendons and ligaments, vascular smooth muscle cells (SMCs) and endothelial cells in blood vessels, and chondrocytes in cartilage are all types of cells that are subjected to large mechanical forces, or loads, and are referred to as mechano-responsive cells. Cellular responses to loads depend on loading conditions (e.g., the type, magnitude, duration, and frequency of loading); they also depend on cell type, cell source, developmental stage, and cell microenvironment, such as surrounding matrix proteins as well as soluble factors (Frangos, 1993; Grinnell, 2003; Vandeburgh, 1992). Cells use multiple sensing mechanisms to detect mechanical loads and transduce them into intracellular signals that lead to modulation of many vital cellular functions, such as proliferation, differentiation, migration, adhesion, apoptosis, and gene and protein expression (Bartling *et al.*, 2000; Chien *et al.*, 2005; Geiger and Bershadsky, 2002; Hsieh and Nguyen, 2005; Pradhan and Sumpio, 2004; Sarasa-Renedo and Chiquet, 2005; Wang *et al.*, 2007). The mechanotransduction by which cells transduce mechanical forces into biochemical responses have been under intensive investigation for the past two decades. Highly coordinated extensive cellular components including the cytoskeleton, adhesion complexes, and ion channels have been implicated as the predominant mediators of mechanotransduction (Burridge and Chrzanowska-Wodnicka, 1996; Ingber, 1991; Sadoshima and Izumo, 1997).

While it has been long established that mechanical forces are essential in the regulation of tissue homeostasis and remodeling, only recently has the concept that mechanical forces are also essential regulators in the development of successful tissue engineering constructs for tissue repair and replacement become evident (Akhyari *et al.*, 2002; Garvin *et al.*, 2003). A recent advance made in the tissue engineering field is the selective differentiation of mesenchymal stem cells (MSCs) into specific cell lineages by applying various mechanical loading conditions, along with providing the appropriate matrix environment and biochemical factors (Altman *et al.*, 2002; Huang *et al.*, 2004a; Park *et al.*, 2007). Although many types of cells respond to mechanical forces much like they respond to biochemical stimuli, cellular mechanotransduction mechanisms, especially for stem cells, are far less explored and understood.

## 2. APPLICATION OF EXTERNAL MECHANICAL FORCES TO CELLS

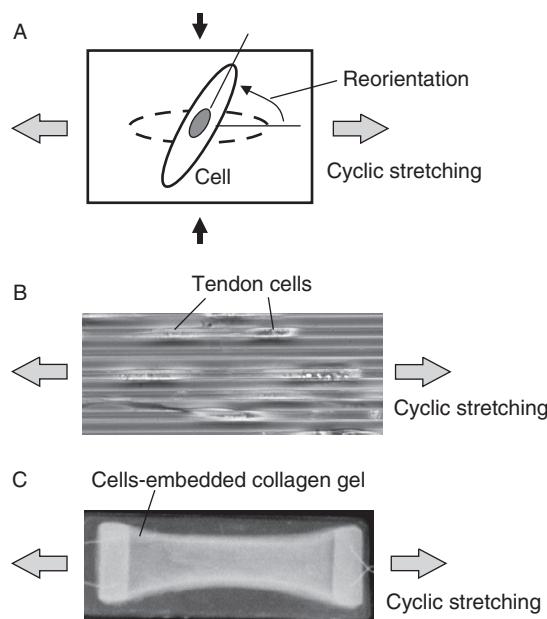
Over the years, various *in vitro* systems have been developed by applying mechanical forces to a population of cells to study cellular mechanobiological responses (Brown, 2000; Huang *et al.*, 2004b) as well as mechanics of single cells such as cellular deformation and viscoelasticity (Bao and Suresh, 2003; Zhu *et al.*, 2000). These systems take into account *in vivo* loading conditions of various types of cells. For fibroblasts, epithelial cells, and SMCs, mechanical stretching was applied to a population of cells using deformable elastic substrates (e.g., silicone membrane) that were coated with extracellular matrix (ECM) proteins for promoting cell attachment (Banes *et al.*, 1985; Brown, 2000; Leung *et al.*, 1977; Wang *et al.*, 1995). For endothelial cells lining the blood vessel wall, fluid shear stresses were applied using flow chambers (Brown, 2000; Chun *et al.*, 1997; Hermann *et al.*, 1997), as these cells are predominantly subjected to shear stress from blood flow *in vivo* (Davies, 1995). On the contrary, chondrocytes in cartilage are subjected to compression as well as hydrostatic pressure *in vivo*. Therefore, corresponding mechanical forces were applied to these cells using various types of *in vitro* systems (Brown, 2000; Huang *et al.*, 2004b). One advantage of these systems is that the loading parameters, such as loading magnitude and frequency, can be easily controlled. Another advantage of the systems is that the mechanical properties of the substrates (e.g., stiffness) and their surface chemistry can be readily modified.

When stretching cells, the substrate underlying a population of cells can be stretched either uniaxially or biaxially. Under uniaxial stretching, the substrate is lengthened in its stretching direction whereas it is compressed in its perpendicular direction. This type of stretching is quite suitable for mechanical loading of cells from tendons and ligaments, such as the patellar

tendon and anterior cruciate ligament (ACL), as these cells are aligned with their long axis along the tendons or ligaments and are therefore subjected to mainly uniaxial stretching *in vivo*. On the contrary, biaxial stretching can be applied to the substrate by stretching it in two mutually perpendicular directions. The stretching, however, can be either an equibiaxial stretch, where substrate strains are the same in all directions, or nonequibiaxial stretch, where substrate strains vary with respect to stretching direction (Lee *et al.*, 1996). This type of mechanical stretching is most suitable for dermal fibroblasts, as they are randomly oriented in the ECM and are stretched in all directions *in vivo*.

Several biaxial stretching systems have been developed to provide a mechanical environment for cultured cells with a number of advanced features, including input force quantitation, loading parameter controls, and homogeneous deformation of a cell population (Lee *et al.*, 1996; Sotoudeh *et al.*, 1998; Waters *et al.*, 2001). However, there are a few limitations associated with these systems. The first is that the input strain is measured on the loading system (referred to as a “clamp-to-clamp” strain), or on the substrate (referred to as substrate strain), not actually on the cells. Similarly, the second limitation is that only a fraction of the input strain may actually be delivered to the cells. This limitation is partially due to the differential adherence of individual cells in the population of cells: some cells may adhere to the matrix more strongly than others, subjecting them to different strain levels. Consequently, cellular responses in these *in vitro* systems are heterogeneous, and gene and protein expressions measured only represent the average response of a population of cells to mechanical stretching.

An additional limitation associated with uniaxial stretching systems is that cells on the substrate assume a random orientation in static culture, but when being stretched, they orient away from the stretching direction and toward a direction that has minimal substrate deformation (Wang *et al.*, 1995, 2001b). Consequently, the strains acting on the cells that have reoriented are minimal. To overcome this cell reorientation problem, cell alignment was induced using microgrooved substrates (Mata *et al.*, 2002; Walboomers *et al.*, 1999, 2000; Wang and Grood, 2000). Fibroblasts on microgrooved substrates were shown to align with the microgrooves and maintain an elongated shape, mimicking the cell alignment and orientation *in vivo* (Wang *et al.*, 2003b) (Fig. 7.1). Moreover, application of cyclic uniaxial stretching to cells on the microgrooved surface did not change cell alignment regardless of initial cell orientation with respect to stretching direction (Loesberg *et al.*, 2005; Wang *et al.*, 2005b). The microgrooved substrate has also been used to show that the mechanobiological response of fibroblasts to mechanical stretching depends on their orientation with respect to stretching direction (Wang *et al.*, 2004b). An additional advantage of using the microgrooved substrate is that it can also control the organization of collagen matrix produced by cells in culture (Wang *et al.*, 2003a).



**Figure 7.1** The need to control cell alignment and organization in studying cellular mechanobiological response. When cells are grown on smooth culture surfaces and subjected to cyclic uniaxial stretching, they tend to reorient toward a direction with minimal substrate deformation (A). This cell reorientation during mechanical stretching makes it complicated, if not impossible, to properly interpret experimental results as substrate strains acting on cells during stretching keep changing. One approach to control such cell reorientation response is using microgrooved substrate (B). Regardless of stretching or not, cells remain aligned in microgrooves and are therefore subjected to relatively constant stretching. A more physiological experimental model is including matrix such as collagen gel (C) to surrounding cells as *in vivo*. In such a model, the cells deform the collagen gel matrix because of the traction forces they produce and at the same time are subjected to external mechanical stretching. One disadvantage of using collagen gel is its low mechanical strength for mechanical stretching. Therefore, many bioscaffolding materials with a higher mechanical strength are used to embed cells and apply mechanical loads to cells. This approach has been widely used in functional engineering of tissue constructs.

### 3. CELL-GENERATED MECHANICAL FORCES

#### 3.1. Cell traction force

Cells can respond to external mechanical forces, but just as importantly, cells also generate internal forces, or cellular contraction, resulting from actin–myosin interactions. Intracellular contraction is transmitted to the underlying substrate, and the forces on the substrate are called CTFs. CTFs are essential for cells to migrate, maintain shape, and generate mechanical

signals (Wang and Lin, 2007). As such, CTFs are implicated in many biological processes including wound healing, embryogenesis, angiogenesis, and inflammation (Li *et al.*, 2007). Detailed knowledge of how CTFs are regulated and transmitted to the ECM is thus important for understanding physiological and pathological events at the tissue and organ levels.

Several intracellular proteins, including Rho proteins, Rho-associated kinase (ROCK), and mammalian homolog of *Drosophila* diaphanous protein (mDia1) (Anderson *et al.*, 2004), are known to regulate the formation of stress fibers and focal adhesions (FAs), and thus CTF generation and transmission (Watanabe *et al.*, 1999). Moreover, mitogen-activated protein kinases (MAPKs) can phosphorylate myosin light chain kinase (MLCK) and increase MLC phosphorylation, which leads to actomyosin contraction and hence, CTF generation.

### 3.2. Cell traction force versus $\alpha$ -smooth muscle actin

During wound healing, large traction forces are generated at the wound site by myofibroblasts. Myofibroblasts contain a contractile apparatus—actin filaments and nonmuscle myosin II, which is similar to that of SMCs (Gabbiani, 2003; Tomasek *et al.*, 2002). Unlike fibroblasts, however, myofibroblasts express  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA), form  $\alpha$ -SMA-containing stress fibers (Grinnell, 1994), and generate greater contractile force than fibroblasts (Hinz *et al.*, 2001).  $\alpha$ -SMA is a prominent actin isoform in vascular SMCs and generally comprises 14–18% of total actin content (Arora and McCulloch, 1994). The actin isoform has been recognized as the underlying molecule that enhances traction forces of myofibroblasts (Herman, 1993; Serini and Gabbiani, 1999). Overexpression of  $\alpha$ -SMA upregulates the myofibroblast traction force (Hinz *et al.*, 2001). A recent study was able to show that while  $\alpha$ -SMA is not required for CTF generation, its expression upregulates CTF magnitude in a nearly linear fashion (Chen *et al.*, 2007). While appropriate traction forces of myofibroblasts are required for wound closure, ECM regeneration, and remodeling, the excessive traction force of myofibroblasts that persistently exist in the wound site is responsible for wound contracture, fibrosis, and other fibro-proliferative disorders during pathological conditions (Gabbiani, 2003).

The mechanisms by which  $\alpha$ -SMA protein expression regulates myofibroblast traction force are currently under investigation. They may be related to modification of stress fibers and FAs. In myofibroblasts, there is an abundant  $\alpha$ -SMA in stress fibers and at FA sites (Hinz *et al.*, 2001). The presence of  $\alpha$ -SMA may allow the stress fibers and FAs to sustain large traction forces (Goffin *et al.*, 2006; Wang *et al.*, 2006a). Also, the incorporation of  $\alpha$ -SMA into actin filaments may enhance force transmission between cortical actin filaments and FAs (Dugina *et al.*, 2001; Hinz and Gabbiani, 2003). The N-terminal sequence AcEEED of  $\alpha$ -SMA has been identified to

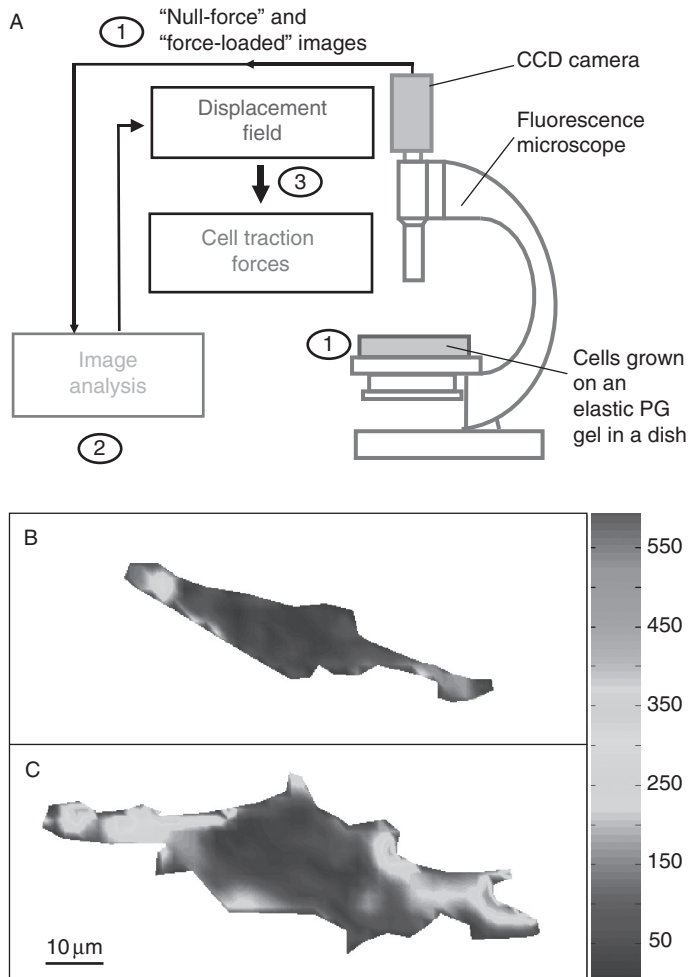
be crucial for incorporation of  $\alpha$ -SMA into stress fibers and for force generation (Chaponnier *et al.*, 1995; Clement *et al.*, 2005). Finally, high levels of  $\alpha$ -SMA expression may increase CTF by Rho-dependent activation in addition to enhancing stress fiber formation (Bogatkevich *et al.*, 2003; Skalli *et al.*, 1990).

It is well recognized that transforming growth factor- $\beta$  (TGF- $\beta$ ) promotes differentiation of fibroblastic cells into myofibroblasts by upregulation of  $\alpha$ -SMA and thus enhances CTF (Desmouliere *et al.*, 1993; Evans *et al.*, 2003; Kopp *et al.*, 2005). The TGF- $\beta$ -induced  $\alpha$ -SMA expression requires the induction of the extra type III domain A (ED-A) form of the matrix protein fibronectin and signaling molecules of small mothers against decapentaplegic (SMAD) family (Kobayashi *et al.*, 2006; Moustakas *et al.*, 2001; Serini *et al.*, 1998). Many soluble factors such as basic fibroblast growth factor (bFGF), prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), and  $\gamma$ -interferon inhibit TGF- $\beta$  1-upregulated  $\alpha$ -SMA expression and thus likely cause the downregulation of CTF (Burgess *et al.*, 2005; Hjelmeland *et al.*, 2004; Kawai-Kowase *et al.*, 2004; Kolodsick *et al.*, 2003; Yokozeki *et al.*, 1999).

### 3.3. Cell traction force microscopy

To determine CTFs, several methods have been developed (Wang and Lin, 2007). These include the use of thin silicone membranes (Harris *et al.*, 1980), microfabricated cantilevers (Galbraith and Sheetz, 1997), micropost force sensor arrays (Li *et al.*, 2007; Tan *et al.*, 2003), and cell traction force microscopy (CTFM) (Butler *et al.*, 2002; Dembo and Wang, 1999; Yang *et al.*, 2006). A unique strength of CTFM is that it can quantify traction forces of both individual cells and a group of cells (Li *et al.*, 2008; Wang *et al.*, 2002). The current CTFM methods involve three major steps. The first step involves three parts: (a) plating cells on an elastic polyacrylamide gel (PG) embedded with fluorescent microbeads in a plastic dish; (b) taking digital images of individual cells on the PG substrate using a charge-coupled device (CCD) camera system on an inverted microscope, which yields “force-loaded” images; and (c) removing cells and then taking digital images at the same location, which yields a “null-force” image. The second step determines the substrate displacement field produced by CTFs by pairing “null-force” and “force-loaded” images using image analysis algorithms. The third and final step determines the CTFs from the substrate displacements by computation based on elasticity theory (Wang and Lin, 2007) (Fig. 7.2).

A limitation of using the CTFM method to determine CTFs is that, unlike *in vivo*, cells reside on a two-dimensional (2D) substrate without the surrounding ECM. An alternative approach to assess CTFs is the use of collagen gels to embed cells. Fibroblast-populated collagen gels (FPCGs) is such a commonly used experimental model. Fibroblasts within the gels



**Figure 7.2** (A). Schematic of cell traction force microscopy (CTFM). The CTFM technique involves three major steps (numbered 1, 2, and 3. See also the text for detailed description). Using CTFM, traction forces of individual cells can be determined; (B). the CTFs of a fibroblast; and (C). the CTFs of a myofibroblast. Adopted from Fig. 1 in Chen *et al.* (2007).

generate traction forces, which deform the gels (Cukierman *et al.*, 2002; Grinnell, 2003). The FPCG contraction may be estimated by measuring area changes in FPCG (Campbell *et al.*, 2004) or quantified using a culture force monitor (Campbell *et al.*, 2003). These two methods have been used to show that TGF- $\beta$ 1 induces a larger contraction than TGF- $\beta$ 3 (Campbell *et al.*, 2004), and that “healing ligament fibroblasts,” even after being

passed a few times, generate a larger contraction than their normal counterparts (Agarwal *et al.*, 2006), suggesting that cells after culturing retain their phenotype *in vivo* to certain degrees.

### 3.4. Roles of cell traction force in cell migration and tissue morphology

CTFs are essential for enabling cell migration. There are three sequential steps in each cell migration event: (a) the protrusion force generated at the cell's leading edge by one-directional actin polymerization drives the formation of new lamellipodia and filopodia and FAs at the front; (b) the CTFs pull the cell body forward; and (c) the cell detaches at the rear. The direction of the CTFs at the front and the rear of the cell always points inward (Galbraith and Sheetz, 1997). These centripetal CTFs work toward breaking cell's FAs (Cramer *et al.*, 1997). As the migrating cell forms stronger FAs at the front than at the rear (Schmidt *et al.*, 1993), the imbalance between the adhesion force and the traction force at the front edge and at the rear is the net driving force for cell migration. In normal fibroblasts, the lamellipodia provides nearly all the forces needed for protrusion of the front edge. The pattern changes of CTF often occur ahead of changes in the cell migration direction. This suggests a front locomotion mechanism of cell migration in which the dynamic traction force at the cell's front actively pulls the cell body forward. When fibroblasts are transformed with H-ras, spots of weak, transient CTFs are scattered among small pseudopods and arranged in random directions. These weak, randomized CTFs result in the abnormal migration behavior of H-ras-transformed fibroblasts (Munevar *et al.*, 2001).

Besides its role in cell migration, CTF also regulates tissue patterning and morphogenesis by modulating both ECM and cell growth. It has been noted that the traction force generated by a migrating cell normally ranges from tens to even hundreds of nano-Newtons (Burton *et al.*, 1999; Lee *et al.*, 1994; Tan *et al.*, 2003), a force that is far greater than the net force that pulls the cell forward (Lee *et al.*, 1994). The larger-than-needed CTFs have been postulated to be used by fibroblasts to help construct connective tissue during morphogenesis (Stopak *et al.*, 1985). When fibroblasts are embedded in collagen gel, the CTF makes the collagen fibers aligned, creating patterns which are similar to tissue or organ capsules (Harris *et al.*, 1981). Also, when fluorescently labeled collagen is injected into chicken embryos, CTFs are thought to be responsible for the rearrangement of the collagen gel into anatomical patterns (Stopak *et al.*, 1985). CTF regulates morphogenesis through modulation of Rho signaling. Epithelial branching is prohibited in embryonic mouse lung rudiments when cell tension is dissipated by treatment with ROCK inhibitor Y27632. On the contrary, lung branching is greatly enhanced when CTF is increased with Rho activator CNF-1 (Moore *et al.*, 2005).

## 4. MECHANOBIOLOGICAL RESPONSES OF CELLS

### 4.1. Adult cells

Mechanical forces are potent regulators of matrix gene and protein expression in various types of cells, particularly those from connective tissues subjected to large mechanical forces *in vivo*, such as skin, tendon, and ligaments (Chiquet, 1999; Shimizu *et al.*, 1998; Wang *et al.*, 2007). Collagen is the most abundant component of the ECM in these and many other tissues and therefore, many *in vivo* and *in vitro* investigations of mechanical loading effects have focused on the relationship between mechanical loads and collagen synthesis. While it is difficult to draw general conclusions from *in vitro* studies due to the array of factors affecting cellular mechanobiological response, many *in vitro* studies have consistently shown enhanced cellular collagen expression in response to mechanical loading, which is associated with interactions with exogenous growth factors in various types of cells and/or the production of autocrine growth factors by loaded cells (Kim *et al.*, 2002; Mouw *et al.*, 2007; Nakatani *et al.*, 2002; O'Callaghan and Williams, 2000). For example, when fetal rat cardiac fibroblasts were grown on an elastin substrate, cyclic biaxial stretching increased type I procollagen synthesis in the presence of serum or growth factors such as TGF- $\beta$ 1 and IGF-1 (Butt and Bishop, 1997). Adult rat cardiac fibroblasts on a collagen substrate responded to 10% static uniaxial stretching with elevated TGF- $\beta$ 1 activity as well as increased mRNA levels of collagen type III without affecting collagen type I mRNA levels (Lee *et al.*, 1999). A similar increase in collagen type III mRNA levels was observed in neonatal rat cardiac fibroblasts on a laminin substrate under biaxial stretching (Atance *et al.*, 2004). The speculation is that type III collagen mRNA is increased soon after the onset of mechanical loading while there is a delay in the stimulation of collagen type I mRNA. These events closely resemble those of *in vivo* animal models of pressure-overloaded hypertrophied myocardium, where an increase in type III collagen is often seen early on followed by a large sustained increase in type I collagen (Cleutjens *et al.*, 1995). This phenomenon may indicate the extent of tissue damage and wound healing, since early deposition of type III collagen followed by type I collagen is characteristic of tissue repair (Woo *et al.*, 1999).

This phenomenon is further emphasized by the differential responses of ACL fibroblasts and medial collateral ligament (MCL) fibroblasts to mechanical loading. It is known that an injured ACL does not heal, whereas an injured MCL heals well (Frank *et al.*, 1983a,b). In support of this clinical observation, *in vitro* experiments show that cyclic biaxial stretching of ACL fibroblasts increases only type I collagen mRNA expression whereas MCL fibroblasts under similar stretching conditions respond with an increase in

type III mRNA expression and a decrease in collagen type I mRNA expression (Hsieh *et al.*, 2000).

While it is known that cells respond to mechanical forces by changing their expression of genes such as collagen, the cellular mechanobiological response is generally cell type-dependent. For example, the application of uniaxial stretching of ACL fibroblasts increased both collagen type I and collagen type III mRNA levels (Kim *et al.*, 2002). However, tendon fibroblasts under uniaxial stretching increased collagen type I mRNA levels without a significant change in collagen type III mRNA, and this increase in collagen type I gene expression was stretching magnitude-dependent (Yang *et al.*, 2004). On the contrary, tendon fibroblasts in collagen gels subjected to cyclic uniaxial stretching expressed collagen genes I, III, and XII as well as fibronectin and tenascin (Garvin *et al.*, 2003), suggesting that the ECM influences cellular mechanobiological response. The type of loading condition also has differential effects on periodontal ligament (PDL) fibroblast response. Cyclic equibiaxial stretching increased the expression of collagen type I, whereas the same magnitude of cyclic compression decreased it (Howard *et al.*, 1998). This phenomenon may explain *in vivo* tooth remodeling, as PDL *in vivo* is subjected to tension on one side but compression on the other side, and the tension side is characterized by bone synthesis whereas the compression side is characterized by bone resorption.

Like fibroblasts, chondrocytes are also responsive to mechanical forces (Hall *et al.*, 1991; Lammi *et al.*, 1994). Compression, a major form of mechanical loading on cartilage, modulates cartilage-specific macromolecule biosynthesis and matrix deposition by chondrocytes (Guilak *et al.*, 1994). The matrix molecule expression of chondrocytes depends on loading magnitude, frequency, and duration (Gray *et al.*, 1989; Ragan *et al.*, 1999). Two major cartilage matrix components, aggrecan and type II collagen, are independently regulated by intermittent hydrostatic pressure (IHP). For example, expression of aggrecan mRNA levels increased in response to low magnitudes and short duration of IHP (Ikenoue *et al.*, 2003; Smith *et al.*, 2000). Type II collagen mRNA levels, on the contrary, were not affected by short duration or low magnitude and increased only at higher magnitudes applied at intervals and longer durations.

In addition to the types of cells and mechanical loading, cell-ECM interactions are also known to affect collagen expression. For example, fetal lung fibroblasts responded to cyclic biaxial stretching by enhancing collagen type I mRNA expression when the cells were cultured on a laminin or elastin substrate but not on a fibronectin substrate (Breen, 2000). Cell response also varied depending on whether stretching was applied statically or dynamically. A 5% static stretching increased ratio of collagen type III to type I by 5% as compared to unstretched controls, whereas a 5% cyclic stretching induced a 70% increase (Carver *et al.*, 1991).

The load-induced collagen expression in many types of cells has been related to the response of growth factors to mechanical loading. TGF- $\beta$ 1 is one of the most potent inducers of procollagen  $\alpha$ 1(I) gene expression (Coker *et al.*, 1997). In two studies using tendon fibroblasts and ACL fibroblasts, cyclic uniaxial stretching increased collagen expression with a concomitant increase in expression of TGF- $\beta$ 1 mRNA and protein (Kim *et al.*, 2002; Yang *et al.*, 2004). In human dermal fibroblasts, a similar parallel increase in TGF- $\beta$ 1, procollagen  $\alpha$ 1(I) mRNA levels, and total collagen synthesis occurred when cells were subjected to cyclic biaxial stretching (Parsons *et al.*, 1999). A TGF- $\beta$ 1-mediated increase in gene expression of collagen type I, III, and V was observed in response to cyclic biaxial stretching in human ligament cells (Nakatani *et al.*, 2002). Vascular SMCs also responded to cyclic stretching by increasing collagen type I mRNA expression with a parallel increase in TGF- $\beta$  expression (Joki *et al.*, 2000; Li *et al.*, 1998). Endothelial cells increased TGF- $\beta$  mRNA expression and total collagen synthesis in response to cyclic stretching as well (O'Callaghan and Williams, 2000).

The presence of two potential strain response regions within the proximal promoter may be responsible for the stretching-induced collagen expression that have been identified (Lindahl *et al.*, 2002). One contains an inverted CCAAT-box, whose binding activity of CCAAT-binding factor, CBF/NF-Y, is enhanced by both mechanical stretching and TGF- $\beta$ 1 at this site (Lindahl *et al.*, 2002). CBF regulates human COL1A1 promoter activity in human dermal fibroblasts, and binding activity is higher in scleroderma fibroblasts, which produce excessive collagen (Saitta *et al.*, 2000). This observation suggests that transcription factor CBF/NF-Y may be involved in the upregulation of collagen gene expression. Furthermore, CBF has been shown to bind to SMAD proteins, the major components of TGF- $\beta$  signaling pathway, and Sp1, a human transcription factor (Bishop and Lindahl, 1999; Chen *et al.*, 1999). SMAD and Sp1 proteins cooperate to mediate TGF- $\beta$ 1-induced collagen expression (Poncelet and Schnaper, 2001). Also, mechanical compression of rabbit chondrocytes transfected with human COL2A1 gene increased the level of type II collagen mRNA expression by transcriptional activation, possibly through the Sp1-binding sites residing in the proximal region of COL2A1 gene promoter (Mouw *et al.*, 2007).

Besides inducing the expression of collagen and TGF- $\beta$ , mechanical stretching also elicits a cellular inflammatory response. In tendon cells, application of uniaxial mechanical stretching increased the expression of calcium-dependent cytosolic phospholipase A<sub>2</sub> (cPLA<sub>2</sub>) and cyclooxygenase-2 (COX-2), and the production of PGE<sub>2</sub> at stretching magnitudes of 8% and 12% (Wang *et al.*, 2003b, 2004a). Similarly, tendon explants subjected to cyclic compressive loading at 3 MPa and 12 MPa induced a loading magnitude and duration-dependent increase in the production of PGE<sub>2</sub>, a product of

the inducible enzyme COX-2 (Flick *et al.*, 2006). COX-2 is a mechano-sensitive enzyme as demonstrated in the dynamic compression of cartilage explants (Gosset *et al.*, 2006), where cartilage explants under intermittent compression and dynamic compression increased COX-2 expression and PGE<sub>2</sub> production (Fermor *et al.*, 2002; Gosset *et al.*, 2006). Mechanical stretching of tendon cells also caused a synergistic effect on production of matrix metalloproteinases (MMPs) with interleukin-1 $\beta$  (IL-1 $\beta$ ) (Archambault *et al.*, 2002; Yang *et al.*, 2005). IL-1 $\beta$  is a potent inflammatory cytokine, which induces expression of catabolic mediators MMPs, COX-2, and PGE<sub>2</sub> in tendon fibroblasts (Thampatty *et al.*, 2007; Yang *et al.*, 2005). In PDL fibroblasts, similar catabolic effects due to mechanical stretching were also noted (Shimizu *et al.*, 1998; Yamaguchi *et al.*, 1994). In chondrocytes, large cyclic tensile loading (15–18% equibiaxial strains) increased proinflammatory gene expression as evidenced by enhanced iNOS (inducible nitric oxide synthase) RNA and nitric oxide (NO) production (Agarwal *et al.*, 2004).

The inflammatory process of vascular endothelial cells and SMCs are also regulated by mechanical forces in the form of shear stress (Cunningham and Gotlieb, 2005; Harrison *et al.*, 2006). Shear stress increases intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin expression induced by TNF- $\alpha$  in endothelial cells (Chiu *et al.*, 2004; Walpola *et al.*, 1995). Nonlaminar flow can result in gene expression of proinflammatory transcription factors such as nuclear factor-kappaB (NF- $\kappa$ B) in the vascular wall (Brooks *et al.*, 2004; Nagel *et al.*, 1999). The activation of NF- $\kappa$ B generally leads to increased expression of proinflammatory genes including those encoding cytokines, VCAM-1, ICAM-1, and tissue factor monocyte chemotactic protein-1 (MCP-1) (Harrison *et al.*, 2006). NF- $\kappa$ B activation and ICAM-1 expression depend on the integrin activation pathway *in vitro*. Fluid shear stress triggers the conformational activation of integrins which mediate NF- $\kappa$ B activation. A pathway upstream of integrin activation identified is a mechanosensory complex that comprises platelet endothelial cell adhesion molecule-1 (PECAM-1), VE cadherin, and vascular endothelial growth factor receptor-2 (VEGFR-2). In PECAM-1-knockout mice, NF- $\kappa$ B and downstream inflammatory genes in the regions of disturbed flow are no longer activated (Tzima *et al.*, 2005).

While the *in vitro* studies described above provide useful insight into the role of mechanical forces in tissue homeostasis and pathophysiology, there are a number of factors that still need to be taken into account for proper interpretation of experimental data. For example, cell shape is usually not controlled in these studies *in vitro*, so cells are likely in different phenotypic states. Micropatterning technology has been demonstrated to be able to control for cell shape (Chen *et al.*, 1998), and this technology may be a useful tool in precisely defining cellular mechanobiological responses in future studies. Cell orientation is another factor that needs to be considered

in cell stretching experiments, as cells on smooth culture surfaces under cyclic uniaxial stretching are known to reorient toward a direction along which mechanical force or substrate deformation on the cells is minimal (Wang *et al.*, 2001b). Thus, the cell reorientation must be prevented so that individual cells are all subjected to the same substrate strains during cell stretching experiments. One solution to this problem would be to use equibiaxial stretching systems, which produce isotropic strains on smooth surfaces such that cells are subjected to the same surface strains regardless of their orientation. However, equibiaxial stretching may not be physiological for those cells such as tendon and ligament fibroblasts that are under uniaxial stretching *in vivo*. Another solution, as noted earlier, is the use of micro-grooved substrate to control cell orientation by taking advantage of cell contact guidance behavior (Wang and Grood, 2000).

There are other factors that also need to be carefully considered. Surface topography is one such important factor that can influence cell shape, orientation, and adhesion on the substrate (Chou *et al.*, 1995). The coating of a substrate with an ECM protein is another important factor that can modulate cell function (Breen, 2000; Reusch *et al.*, 1996). The ECM proteins typically used as substrate coating for cell attachment include collagen type I, fibronectin, elastin, and laminin. It is known that cell responses to mechanical loading depend on the type of matrix protein coated. For example, collagen type I is expressed by fibroblasts cultured on laminin and elastin but not on fibronectin under mechanical loading conditions (Breen, 2000). Similarly, SMCs increase myosin expression in response to mechanical loading when cultured on laminin or collagen matrix but not on a fibronectin matrix (Reusch *et al.*, 1996). Since heterodimeric integrin family constitutes major cellular receptors for ECM proteins, binding to different integrin types may elicit different signaling pathways. Therefore, the matrix protein selected for the coating of a substrate is crucial in determining specific cellular mechanobiological responses.

Finally, while the *in vitro* systems used in these studies offer convenience in investigating cellular mechanobiological responses, it is considered less physiological because cells on the 2D substrates (e.g., silicone membrane) often used in cell stretching experiments lack the ECM surrounding the cells. Therefore, three-dimensional (3D) experimental models have been developed to include collagen matrices, which allow the study of cellular mechano-responses in a system that is more representative of the *in vivo* environment (Grinnell, 1994, 2003). The ability of cells to generate tension within a collagen matrix is crucial in determining cell fate, as fibroblasts that can generate tension in attached collagen matrices proliferate, whereas cells that cannot in floating matrices become quiescent and apoptotic (Grinnell *et al.*, 1999; Rosenfeldt and Grinnell, 2000). The utility of collagen gels, however, is limited by their low mechanical strength to sustain repetitive mechanical loading, which is a significant drawback for the development of

tissue constructs in the tissue engineering field. Biomaterials with a large mechanical strength were therefore used as scaffolding materials (Wang *et al.*, 2006b). One example is the native silk fibroin fibers, which has been explored for engineering the ACL in cultures under dynamic mechanical loading (Vunjak-Novakovic *et al.*, 2004). Another example of biomaterials includes poly(glycolic acid) (PGA) (Kim *et al.*, 2000). Application of cyclic mechanical stretching to fibronectin-coated PGA scaffolds seeded with SMCs upregulated cellular expression of elastin and collagen and also increased the Young's modulus and ultimate strength of the tissues (Kim *et al.*, 1999). The findings of this study suggest that the appropriate combinations of mechanical loading and polymeric scaffolds can enhance the mechanical properties of the tissues.

## 4.2. Stem cells

Stem cells are cells characterized by the abilities to perpetuate themselves through self-renewal and to generate mature cells of a particular tissue through differentiation. Stem cells are rare in most tissues, and it appears that each tissue arises from tissue-specific stem cells (Reya *et al.*, 2001). Currently, most studies focus on stem cell biology, including isolation, identification, and characterization; fewer studies have investigated the effects of mechanical loading on stem cells (Table 7.1), which is vital for gaining a better understanding of the biology and pathology of load-bearing tissues.

Mechanical loading is essential for the development, function, and repair of the major components of the musculoskeletal system such as bones, tendons, ligaments, and cartilage. Much work concerning the mechano-biological responses of stem cells arise from the desire to create functional tissue engineering constructs. For example, concurrent application of tensile and rotational loading to human and bovine bone marrow stem cells (BMSCs) in collagen gels resulted in several features characteristic of ligament cells, including expression of collagen types I and III, and tenascin-C, increased cell alignment and density, and the formation of oriented collagen fibers (Altman *et al.*, 2002). The findings of this study show that mechanical loading alone can direct BMSC differentiation into a preferential ligament cell lineage without specific inducers of ligament cell differentiation. In another study, tissue engineering constructs were created for tendon repair using rabbit MSCs in type I collagen sponges (Juncosa-Melvin *et al.*, 2006). Compared to nonloading constructs, cyclic mechanical stretching of the tissue constructs resulted in three- and fourfold gene expressions of collagen type I and III, respectively, and 2.5 times greater stiffness of the tissue construct (Juncosa-Melvin *et al.*, 2006, 2007).

With appropriate mechanical loading, human MSCs (hMSCs) also can differentiate along the osteogenic pathway without the need for osteogenic supplements. For example, when subjected to uniaxial cyclic stretching,

**Table 7.1** Mechanobiological responses of stem cells

Type of cells	Type of loading	Loading conditions	Response of stem cells	Cell lineage commitment	References
Human and bovine BMSCs	Stretching and torsion	Translational 10%, rotational 25% in collagen gels	Increased expression of collagen types I and III, tenascin-C, increased cell alignment, density, formation of oriented collagen fibers	Ligaments	Altman <i>et al.</i> (2002)
Rabbit MSC	Cyclic stretching	4% in collagen gels, 8 h/day for 2 weeks	Increase in gene expression of collagen types I and III	Tendon	Juncosa-Melvin <i>et al.</i> (2006, 2007)
Human MSCs	Cyclic uniaxial stretching	10% and 12%, 4 h/day for 7 and 14 days in collagen matrices	Increase in BMP-2 mRNA levels	Osteogenic	Sumanasinghe <i>et al.</i> (2006)
Human MSCs	Cyclic equibiaxial stretching	3%, 8 h  8%, 48 h	Increase in gene expression of cBfa1, OCN, and ALP  Increase in gene expression of collagen types	Osteoblasts at low magnitude (3%), short duration (8 h)  Tendon/ligament at high magnitude (8%),	Chen <i>et al.</i> (2008)

(continued)

**Table 7.1** (continued)

Type of cells	Type of loading	Loading conditions	Response of stem cells	Cell lineage commitment	References
Human BMSCs	Cyclic uniaxial stretching	8%, 3 days	I, III, and tenascin-C for high magnitude Increase in the gene expression of Bfa1, ALP, OCN, collagen types I and III	long duration (48 h) Osteoblasts	Jagodzinski <i>et al.</i> (2004)
Rat bone marrow MSCs	Uniaxial cyclic stretching	2000 microstrains, 40 min	Increase in gene expression of cBfa1, Ets-1, and ALP, and cell proliferation	Osteoblasts	Qi <i>et al.</i> (2008)
Mouse bone marrow stromal cell line, ST-2	Equibiaxial stretching	0.8% and 5%, 6 h	Increase in gene expression of cBfa1, and increase in ALP activity	Osteoblastic	Koike <i>et al.</i> (2005)
Human BMSCs	Cyclic compression	5%, 10%, and 15%, 48 h 7994 Pa, 0.33 Hz, 7 days	Downregulation of ALP activity Increase in the gene expression of collagen type II and aggrecan	Chondrogenic	Angele <i>et al.</i> (2004)
Rabbit bone marrow MSCs	Cyclic compression	10%, 14 days	Increase in gene expression of collagen type II and aggrecan	Chondrogenic	Huang <i>et al.</i> (2004a)

Bovine BMSCs	Dynamic compression	10%, 16 days	Increase in gene expression of collagen type II and aggrecan	Chondrogenic	Mouw <i>et al.</i> (2007)
Rabbit BMSCs	Cyclic compressive loading	15%, 1 Hz, 4 h/day, 2 days	Increase in gene and protein expressions of Sox-9, c-Jun, and TGF- $\beta$	Chondrogenic	Huang <i>et al.</i> (2005)
Human MSCs	Intermittent hydrostatic pressure	0.1 MPa, 14 days	Increase in gene expression of Sox-9 and aggrecan	Chondrogenic	Miyanishi <i>et al.</i> (2006a,b)
Human MSCs	Cyclic compression	10 MPa, 14 days	Increase in collagen type II gene expression	Chondrogenic	Pelaez <i>et al.</i> (2008)
Human MSCs	Cyclic hydrostatic pressure	Steady at 7.5 MPa for 14 days, 4 h/day at 1 Hz in agarose gels	Increase in aggrecan gene expression at all frequencies; increase in collagen type II at 1 Hz only	Beginning stage of chondrogenesis	Finger <i>et al.</i> (2007)

(continued)

**Table 7.1** (continued)

Type of cells	Type of loading	Loading conditions	Response of stem cells	Cell lineage commitment	References
		Ramped at 1 MPa at day 1, 0.5 MPa increase in subsequent days, 7.5 MPa at day 14, 4 h/day at 1 Hz	No change in collagen type II, and aggrecan gene expression		
Human MSCs	Intermittent hydrostatic pressure	1 MPa, 1 Hz 4 h/day, 10 days in collagen sponges	Increase in gene expression of collagen type II, aggrecan, Sox-9, and collagen type I; increased accumulation of proteoglycan; no change in R unx2 mRNA levels	Chondrogenic	Wagner <i>et al.</i> (2008)
Human BMSCs	Cyclic uniaxial and equibiaxial stretching	10%, 1 Hz for 1–3 days on collagen or elastin membranes	Transient increase in $\alpha$ -SMA, SM-22 $\alpha$ and collagen type I gene expression by uniaxial stretching Downregulation of SM $\alpha$ -actin and SM-22 $\alpha$ by equibiaxial strain	Smooth muscle	Park <i>et al.</i> (2004)

Murine embryonic progenitor cell line	Cyclic equibiaxial stretching	10%, 1 Hz for 6 days	Increase in gene and protein expressions of $\alpha$ -SMA and SM-MHC; synergistic effect with TGF- $\beta$ 1	Smooth muscle	Riha <i>et al.</i> (2007)
Murine embryonic progenitor cell line	Steady fluid shear stress	15 dynes/cm <sup>2</sup> , 6 and 12 h	Increase in gene and protein expressions of CD31, VE-cadherin, vWF; increase in gene expression of angiogenic growth factors, VEGF, VEGFR; decrease in expression of growth factors (TGF- $\beta$ , PDGF- $\beta$ , and PDGFR) associated with SMC	Endothelial	Wang <i>et al.</i> (2005a)
Mouse embryonic stem cells	Fluid shear stress	1.5–10 dynes/cm <sup>2</sup> , 1–3 days	Increase in gene and protein expression of Flk-1, Flt-1, VE-cadherin, and PECAM-1	Endothelial	Yamamoto <i>et al.</i> (2005)

(continued)

**Table 7.1** (continued)

Type of cells	Type of loading	Loading conditions	Response of stem cells	Cell lineage commitment	References
Mouse embryonic stem cells	Cyclic uniaxial stretching	2, 4, 8, and 12%, 1 Hz, 24 h	Increase in gene and protein expression of VSMC markers, $\alpha$ -SMA, and SM-MHC	Smooth muscle	Shimizu <i>et al.</i> (2008)
Mouse embryonic stem cells	Fluid shear stress	10 dynes/cm <sup>2</sup> , 24 h	Increase in gene expression of $\alpha$ -SMA, SMA 22- $\alpha$ , PECAM-1, VEGFR-2	Cardiovascular	Illi <i>et al.</i> (2005)
Human MSCs	Cyclic uniaxial stretching	5%, 1 Hz, 2–4 days on collagen I coated micropatterned silicone	Increase in gene expression of SMC marker calponin 1; decrease in gene expression of cartilage matrix markers (biglycan, COMP, collagen type X1a, and collagen type XI a1	Smooth muscle	Kurpinski <i>et al.</i> (2006)

BMSC, bone marrow stem cell; MSC, mesenchymal stem cell; BMP-2, bone morphogenetic protein-2;  $\alpha$ -SMA,  $\alpha$ -smooth muscle actin; OCN, osteocalcin; PDGFR, platelet-derived growth factor receptor; ALP, alkaline phosphatase; SMC, smooth muscle cell; TGF- $\beta$ , transforming growth factor- $\beta$ ; PECAM-1, platelet endothelial cell adhesion molecule-1; VEGFR-2, vascular endothelial growth factor receptor-2; VSMC, vascular smooth muscle cell.

hMSCs in 3D collagen type I matrices significantly upregulated bone morphogenetic protein-2 (BMP-2) mRNA levels (Sumanasinghe *et al.*, 2006). Therefore, application of mechanical loads to tissue constructs may be a useful means of inducing desired stem cell differentiation and hence facilitating the fabrication of functional tissue constructs.

Recently, several experimental approaches using various types of mechanical loading conditions have been developed for selective differentiation of stem cells into specialized cells. For instance, the influence of mechanical stretching on the gene expression of tendon-related and osteoblast-specific markers in hMSCs has been investigated recently (Chen *et al.*, 2008). hMSCs subjected to cyclic equibiaxial stretching with low magnitude (3% strain) and short duration (8 h) promoted expression of genes typical of osteoblasts, or cBfa1/Runx2, a key transcription factor associated with osteoblast differentiation, osteocalcin (OCN), and alkaline phosphatase (ALP), while cyclic stretching with high magnitude (8% strain) and long duration (48 h) upregulated genes (collagen type I and III, and tenascin-C) typical of tendon/ligament cells (Chen *et al.*, 2008). In contrast, cyclic uniaxial stretching (8% strain) of BMSC for 4 and 7 days preferentially upregulated differentiation markers typical of osteoblasts (ALP, OCN, cBfa1/Runx2) (Jagodzinski *et al.*, 2004). In rat bone marrow MSCs, a brief bout of cyclic uniaxial stretching induced cell proliferation, increased ALP activity, and upregulated expression of two osteogenic transcription factors (cBfa1/Runx2, Ets-1) as well as ALP (Qi *et al.*, 2008). However, the increase in transcription returned to control levels within 12 h after mechanical loading ceased, suggesting that the effect is transient. Stretching the cells for longer time periods may drive sustained osteoblastic differentiation. The effect of mechanical strain in mouse bone marrow stromal cell line ST-2 also showed that low equibiaxial strains for a short duration (0.8% and 5%, 6 h) stimulated osteoblastic differentiation (Koike *et al.*, 2005). In addition, application of equibiaxial strain induced changes in cell morphology, proliferation, and differentiation. At all strain magnitudes (5%, 10%, and 15%) applied, the proliferation of ST-2 cells increased in a stretching magnitude-dependent manner. The level of mRNA for Cbfa1/Runx2, a key regulator of cell growth and differentiation of mesenchymal bone cell progenitors, was upregulated at 0.8% and 5% strains at 6 h and but downregulated at 5%, 10%, and 15% at 24 and 48 h. ALP activity significantly increased at 0.8% and 5% at 24 h; however, the activity decreased at 10% and 15% at 48 h (Koike *et al.*, 2005). The difference in type of stretching and duration thus affect the differential response of MSCs in terms of morphology, proliferation, and differentiation.

TGF- $\beta$ 1-induced chondrogenesis of human BMSCs has been established previously (Barry *et al.*, 2001; Johnstone *et al.*, 1998). However, recent studies show that the application of compressive loading can direct controlled differentiation of BMSCs into mature chondrocytes equally as

effectively as chondrogenic mediums (Schumann *et al.*, 2006). Both cyclic mechanical compression and cyclic hydrostatic pressure (CHP) enhanced the chondrogenic phenotype of BMSCs with increased collagen type II, aggrecan, and proteoglycan contents (Angele *et al.*, 2003, 2004). Cyclic compression of rabbit BMSCs also increased expression of chondrogenic markers, aggrecan and collagen II, as effectively as TGF- $\beta$ 1 (Huang *et al.*, 2004a). In addition, combining cyclic compressive loading with TGF- $\beta$ 1 treatment promoted collagen type II expression more effectively than TGF- $\beta$ 1 alone. Significant stimulation of chondrogenic markers, collagen type II and aggrecan, was also reported in bovine BMSCs that were subjected to dynamic compression in the presence of TGF- $\beta$  and dexamethasone (Mouw *et al.*, 2007). Moreover, dynamic compression upregulated SMAD2/3 phosphorylation in samples with and without TGF- $\beta$ 1. The interaction between mechanical loading and TGF- $\beta$ 1 signaling could be due to a wide range of potential mechanisms. TGF- $\beta$ 1 signaling may lead to pSMAD activation of mechano-sensitive proteins, such as focal adhesion kinase (FAK) and paxillin, which might directly increase mechanosensitivity of cells. Alternatively, TGF- $\beta$  signaling may indirectly amplify the effects of mechanotransduction by increasing the transcription of downstream targets (Sox-9, collagen type II, aggrecan) of mechanical stimulation in chondrocytes. On the contrary, mechanical stimulation may modulate TGF- $\beta$  signaling by enhancing the production of TGF- $\beta$  or expression of its receptors through upregulation of mRNA levels, efficiency in translation, or both. For example, it was shown that cyclic compressive loading promoted gene expressions of Sox-9, c-Jun, and TGF- $\beta$  receptors and production of their corresponding proteins in rabbit BMSCs in 3D agarose culture (Huang *et al.*, 2005).

Combining IHP and TGF- $\beta$ 3 also had similar effects, showing increased gene expression of cartilage matrix proteins, collagen type II and aggrecan, and increased Sox-9 expression in hMSCs as pellet cultures (Miyanishi *et al.*, 2006a,b). When compared to unloaded controls, IHP at 0.1 MPa increased Sox-9 and aggrecan gene expression, while collagen type II increased only at IHP of 10MPa at 14 days (Miyanishi *et al.*, 2006a,b). The data show that the regulation of two major matrix proteins, collagen type II and aggrecan, are pressure magnitude-dependent. Similar pressure magnitude-dependent expression of collagen type II and aggrecan is reported in hMSCs in fibrin gels subjected to compression at various frequencies. Aggrecan gene expression was upregulated at all frequencies (0.1, 0.5, and 1 Hz), while only 1Hz frequency stimulated collagen type II gene expression when compared to control cells (Pelaez *et al.*, 2008). Exposure to IHP altered the morphological organization of the ECM surrounding the hMSCs into more uniform and compact structure compared to the sponge-like, dispersed matrix in cultures in absence of IHP (Miyanishi *et al.*, 2006b). Also, chondrogenic mRNA levels were increased with just exposure to IHP alone without

presence of TGF- $\beta$ 3. Although the exact mechanisms for chondrogenic differentiation in response to growth factors and mechanical loading are unknown, selective cell adhesion, integrin expression, or macromolecule assembly in response to loading may underlie this observation.

A recent study investigated the differential effects of ramped and steady applications of CHP on chondrogenic differentiation of hMSCs in absence of TGF- $\beta$  (Finger *et al.*, 2007). The findings indicate that hydrostatic pressure may induce chondrogenesis in hMSC-seeded agarose constructs without the need for TGF- $\beta$  stimulation. In addition, their data also show that hMSCs are capable of withstanding high initial pressure (7.5 MPa) that may initiate chondrogenesis faster than lower pressure (1 MPa). Specifically, steady and ramped application of CHP initially increased (day 4) and then decreased (days 9 and 14) collagen I gene expression and transiently increased Sox-9 gene expression. However, aggrecan and collagen type II were not detected within the initial 14 days of loading. Although previous studies observed increased collagen type II and aggrecan gene expression at 14 days, these studies applied a combination of chondrogenic media and hydrostatic pressure to hMSCs or rabbit bone marrow MSCs (Angele *et al.*, 2003, 2004; Huang *et al.*, 2005) or utilized different culture methods for cells as pellet cultures (Miyanishi *et al.*, 2006a), where close proximity of the cells may have accelerated the differentiation compared to those cultured in 3D gels. On the contrary, chondrogenic differentiation was initiated in both pellet and 3D alginate cultures of hMSCs in presence of TGF- $\beta$ 3 under intermittent dynamic compression (Campbell *et al.*, 2006). With the onset of chondrogenesis, the gene expression of collagen types II and X and aggrecan all increased over the 10-day culture period, with a transient expression of Sox-9 by day 8 followed by a decline. The accumulation of collagen X would indicate differentiation toward chondrocyte hypertrophy; however, many studies reported the expression of this gene at early stages of chondrogenesis under high cell density conditions, which is the case in this study. Transient gene expression of Sox-9 in early chondrogenesis also has been reported previously. Moreover, Sox-9 expression may be sensitive to factors such as cell density fluctuations, ECM environment, and gel encapsulation causing modification in cell morphology and nutrient delivery.

IHP applied to hMSCs for 10 days in collagen type I sponges in a mixed osteochondrogenic medium supplemented with TGF- $\beta$ 1 increased the gene expression of chondrogenic markers Sox-9, aggrecan, and collagen type-II (Wagner *et al.*, 2008). The upregulation of these genes supports the view that chondrogenesis is regulated in part by mechanical signals. Additionally, there were noticeable increases in proteoglycan accumulation and collagen type I gene expression. The increase in collagen type I expression observed in this study contradicts with another similar study (Huang *et al.*, 2004a), which may be likely due to a combination of scaffold, mechanical

loading conditions, and culture conditions. There was no difference in expression of Runx2 mRNA between the constructs exposed to hydrostatic pressure and unloaded controls, which suggests that neither osteoblast differentiation nor chondrocyte maturation was modulated by the hydrostatic pressure under these conditions. Compared to the constructs that were loaded without cells or unloaded constructs, cell/scaffold constructs that were exposed to HP had a much more compact structure. This observation suggests that MSCs subjected to HP actively contracted the soft matrices. The mechanism for contraction of fibroblasts and endothelial cells has been shown to depend on actin cytoskeleton and the RhoA/ROCK pathway, which regulates tension in the actin cytoskeleton (Kolodney and Wysolmerski, 1992). The disruption of the RhoA/ROCK pathway leads to decrease in contraction. Recent studies have shown that inhibition of the RhoA/ROCK pathway in mesenchymal limb bud cells transforms cell shape to a spherical chondrocyte morphology and decreases the downstream mRNA expression of chondrogenic genes collagen type II and aggrecan (Woods and Beier, 2006; Woods *et al.*, 2005). These observations suggest that cell shape, cytoskeletal tension, and chondrogenesis are interrelated.

Despite the variability in pressure application, culture conditions, and scaffolds in stem cell commitment to chondrogenesis *in vitro*, the overall findings of previous studies suggest that hydrostatic/compressive pressure may play critical role in cartilage development and regeneration *in vivo*.

The determination of how hemodynamic forces influence vascular cell differentiation represents another exciting area of mechanobiology research. Recent studies attempted to harness cyclic stretching to differentiate BMSCs to SMC lineage (Hamilton *et al.*, 2004; Park *et al.*, 2004). Uniaxial cyclic stretching induced differentiation of human BMSCs into SMCs, as they increased expression of SM  $\alpha$ -actin (or  $\alpha$ -SMA) and SM-22 $\alpha$ , which are marker proteins for contractile SMCs (Park *et al.*, 2004). However, cyclic equibiaxial stretching downregulated expression of SM  $\alpha$ -actin and SM-22 $\alpha$  (Park *et al.*, 2004). Therefore, the study showed that different types of mechanical stretching (i.e., uniaxial vs biaxial) produce differential effects on BMSC differentiation. In addition, DNA microarray analysis revealed that uniaxial stretching for 24 h increased SM contractile markers, SM-22 $\alpha$  and calponin, but did not significantly change the expression level of marker genes for other cell types such as osteoblasts, chondrocytes, ligaments, and endothelial cells. In contrast, 3% equibiaxial stretching promoted MSC differentiation into osteoblastic cells in osteogenic media (Simmons *et al.*, 2003). The type of stretching seems to have great influence on stem cell differentiation. The different strain distributions from uniaxial and biaxial stretching may regulate the conformation and/or localization of mechanotransduction signaling molecules and either turn on different signaling pathways or have opposite effect on the same pathway, leading to different cellular responses.

One potential problem with the studies, however, is that the cell populations were derived from bone marrow aspirates and hence may include various types of cells, which may complicate the interpretation of mechanical stretching effects. Therefore, the use of a homogeneous murine embryonic mesenchymal progenitor cell line (CH3/10T1/2), as used in another study, is advantageous for furthering the understanding of the effects of mechanical forces on vascular cell differentiation. After applying cyclic equibiaxial stretching to CH3/10T1/2 cells, the cells adopted an elongated, spindle-shaped morphology with parallel arrangement, suggesting a change toward a mature SMC phenotype. In addition, cyclic stretching increased the mRNA and protein levels of  $\alpha$ -SMA and smooth muscle myosin heavy chain (Riha *et al.*, 2007). Furthermore, when 10T1/2 cells were exposed to fluid shear stresses, the cells increased expression of endothelial specific markers such as CD31 and cadherin in addition to changes in cell morphology and alignment, suggesting that these cells differentiated into mature endothelial cells (Wang *et al.*, 2005a). Both studies show the induction of two different phenotypes (SMCs vs endothelial cells) from one mesenchymal cell line with two different types of hemodynamic forces (vascular stretching vs fluid shear stress).

Alteration of cell function by a mechanical stimulus such as shear stress can be used as a novel technique to induce stem cell differentiation. Fluid shear stress has been previously shown to affect the differentiation of endothelial progenitor cells (EPCs) (Yamamoto *et al.*, 2005). When EPCs were subjected to fluid shear stress, the cells elongated and oriented their long axes in the direction of flow. In addition, shear stress induced proliferation and expression of VEGF receptors, ICAM and VCAM, both at mRNA and protein levels when compared to static controls. Shear-stressed EPCs formed tube-like structures and developed an extensive tubular network faster than their static controls. Therefore, the utilization of mechanical force to manipulate EPCs may be useful in the development of efficient tissue engineered constructs or the maturation of EPC cultures *in vitro* for cell therapy. Fluid shear stress also has been shown to selectively differentiate Flk-1-positive embryonic stem (ES) cells into vascular endothelial cells. When Flk-1-positive ES cells were subjected to shear stress, their cell density markedly increased, and a larger percentage of cells were in S and G2(M) phase than they were in static controls (Yamamoto *et al.*, 2005). Shear stress significantly enhanced the expression of vascular endothelial cell-specific markers Flk-1, Flt-1, VE cadherin, and PECAM-1 at both mRNA and protein levels. Shear stress, however, did not have any effect on markers of epithelial or smooth muscle, namely, keratin, or  $\alpha$ -SMA. This study also reported that shear stress activates Flk-1 in a ligand-independent manner and that activation of Flk-1 plays a critical role in endothelial differentiation of Flk-1-positive ES cells. However, when the same cells were subjected to cyclic uniaxial stretching, the results were quite different. For example, cyclic uniaxial stretching (2%, 4%, 8%, or

12%, 1Hz) of Flk-1-positive ES cells for 24 h markedly increased VSMC markers  $\alpha$ -SMA and smooth muscle-myosin heavy chain (SM-MHC) in a stretching magnitude-dependent manner (Shimizu *et al.*, 2008). However, cyclic stretching (8%, 1 Hz, 24 h) significantly decreased the expression of EC marker Flk-1 but had no effect on the expression of other EC markers (Flt-1, VE cadherin, and PECAM-1), the blood cell marker CD3, or epithelial marker keratin. In addition, cell proliferation was increased, and cells oriented perpendicular to the stretching direction in response to cyclic stretching, which suggests that the cells' response to mechanical stretching may change during stretching. Finally, the PDGFRb kinase inhibitor completely blocked cyclic stretching-induced cell proliferation and VSMC marker expression. The results suggest that activation of PDGFRb plays an important role in VSMC differentiation from ES cells. Taken together, the results of the two studies using fluid shear stress and cyclic stretching suggest that both stimuli may act by a common mechanism in which growth factor receptors are activated by mechanical forces without ligand binding.

Directing stem cells into cardiovascular lineage utilizing mechanical stretching has been attempted recently. Mouse ES cells exposed to fluid shear stress (10 dynes/cm<sup>2</sup>, 24 h) induced several cardiovascular markers including  $\alpha$ -SMA, SMA 22- $\alpha$ , PECAM-1, and VEGF receptor-2 (Illi *et al.*, 2005). In addition, stress activated transcription from VEGFR-2 promoter. The study shows how laminar flow effects could be successfully utilized for cardiovascular differentiation. Besides fluid shear stress, mechanical stretching can also be used to direct ES cell differentiation into cardiovascular lineage. Equibiaxial strain (10%, 2 h) of ES cells induced HIF-1 $\alpha$  and VEGF mRNA and protein, which are involved in cardiovascular development (Schmelter *et al.*, 2006). Reactive oxygen species have been shown to function as transducers of mechanical strain-induced cardiovascular differentiation of ES cells (Schmelter *et al.*, 2006).

Cyclic uniaxial stretching applied to cells on elastic substrates causes cells to align perpendicular to the stretching direction, which is different from that in the vascular wall that is anisotropic and mainly in the circumferential direction. To simulate the vascular cell alignment and investigate the anisotropic mechanical sensing by MSCs, elastomeric membranes with parallel microgrooves were developed using soft lithography (Kurpinski *et al.*, 2006). This topographic pattern kept the cells aligned parallel to the stretching direction. MSCs in such microgrooves were subjected to cyclic uniaxial stretching (5%, 1Hz) for 2–4 days, and DNA microarray analysis revealed global gene expression changes, including an increase in smooth muscle marker calponin 1 and a decrease in cartilage matrix markers (biglycan, collagen type X  $\alpha$ 1, collagen type XI  $\alpha$ 1, and cartilage oligomeric matrix protein (COMP)) and MMP-1. Cyclic uniaxial stretching increased the contractile marker calponin but not SMC mature marker MHC, suggesting that additional factors, possibly soluble factors may be required for

the terminal differentiation of MSCs. Also, the increase in contractile markers and the decrease in cartilage markers by cyclic uniaxial stretching suggest that a more tension-bearing rather than compression-bearing tissue phenotype is promoted.

Taken together, these studies show that mechanical forces can induce or enhance the differentiation of stem cells into a specific cell lineage. However, cellular responses by mechanical forces must always be evaluated in the context of other microenvironmental factors such as culture media and ECM. A recent study has demonstrated that cyclic stretching inhibits human embryonic stem cell (hESC) differentiation in cells cultured on plates coated with Matrigel (Saha *et al.*, 2006). Cyclic biaxial stretching (10%) applied to the deformable substrate was found to inhibit hESC cell differentiation and to promote self-renewal, as measured by an increase in Oct4 and SSEA-4 expression. Mechanical stretching significantly repressed differentiation of hESCs cultured in mouse embryonic fibroblast-conditioned medium but had no effect on the differentiation of hESCs cultured in unconditioned medium (Saha *et al.*, 2006). Therefore, it appears that chemical signals act synergistically with mechanical loads to regulate the differentiation of hESCs. The differential effect of different mechanical loading conditions and the cross talk between mechanical loading and chemical factors in stem cell differentiation open up important avenues for further research.

In summary, various types of mechanical loading can be effectively applied to direct stem cell differentiation. The upregulation of specific differentiation markers greatly depends on magnitude, duration, and type of mechanical loading. For example, low magnitude, short duration biaxial stretching promotes markers of osteoblast-type gene expression, while high magnitude, long duration promotes markers typical of tendon/ligament type. In addition, uniaxial versus biaxial stretch has differential effects on stem cell differentiation, and the same is true with compressive loading and shear stress, which specifically promote chondrogenic phenotype or endothelial phenotype, respectively. Furthermore, several other mediators such as growth factors and matrix environment (ECM substrates/scaffolds) act in concert with mechanical loading to direct stem cell differentiation. Mechanical regulation of stem cells provides a rational basis for tissue engineering and regeneration.



## 5. MECHANOTRANSDUCTION MECHANISMS

Mechanical forces have a myriad of effects on cells, and the whole field of mechanotransduction is devoted to investigating how various forms of mechanical forces are transduced into biochemical cascades and cellular events

(Alenghat and Ingber, 2002; Davies, 1995; Ingber, 1997; Wang *et al.*, 2001c). Years of intensive research have concluded that the basic mechanotransduction components include integrins, FAs, cytoskeleton, ion channels, cell surface receptors, and various secondary signaling molecules, irrespective of cell type. Many excellent reviews on the mechanotransduction mechanisms are available (e.g., Davies, 1995; Duncan and Turner, 1995; Huang *et al.*, 2004b; Osol, 1995; Silver and Siperko, 2003), and interested readers should consult them for a more in-depth understanding of the topic.

One of the major components involved in mechanotransduction is integrins, which act as mechanosensors by themselves or in concert with cytoskeletal proteins (Ingber, 2002; Katsumi *et al.*, 2004; Schwartz *et al.*, 1995). Integrins in adherent cells, such as fibroblasts, are direct mechanosensors by physically connecting the ECM to the cytoskeleton (Stupack, 2007). Binding to ECM proteins, such as collagen and fibronectin, integrins also function as signaling receptors. ECM-attached integrins also physically link FAs to the actin cytoskeleton (Geiger and Bershadsky, 2002). Mechanical forces promote the assembly of FAs (Sawada and Sheetz, 2002; Wang *et al.*, 2001a) and trigger integrin-dependent signaling and activation of MAPKs. FA molecules may sense mechanical forces due to an altering of the relative positions of specific FA components (Geiger and Bershadsky, 2002). Another possible mechanism by which FAs sense mechanical forces involves the conformational changes of specific FA molecules. Interestingly, several FA proteins such as vinculin and fibronectin exist in active and inactive conformations. The transition from inactive to active conformation may occur in response to mechanical forces, which may then trigger the cascade of signaling events (Balaban *et al.*, 2001; Geiger *et al.*, 2001).

The actin cytoskeleton, on the contrary, is responsible for the transmission of tension to the nucleus. Tensional force within the actomyosin contractile system is regulated in part by the degree of phosphorylation of the myosin light chain (Kamm and Stull, 2001; Pfitzer, 2001). The activation site of RhoA, a member of a family of GTP-binding proteins, controls the development of FAs and stress fibers in adherent fibroblasts (Chrzanowska-Wodnicka and Burridge, 1996). The activities of RhoA on the actin cytoskeleton are mediated primarily through its downstream effector ROCK, which inactivates myosin phosphatase to induce the stabilization of filamentous actin and formation of stress fibers (Bershadsky *et al.*, 2006). Mechanical stretching causes a remodeling of the microfilament and microtubule networks, and prevention of this cytoskeletal remodeling mitigates stretch-induced increases in gene transfer and expression (Geiger *et al.*, 2006). Fluid shear stress also induces remodeling of the actin cytoskeleton with the formation of stress fibers through an array of signaling molecules including RhoA and Rac GTPase (McCue *et al.*, 2004; Noria *et al.*, 2004; Tzima *et al.*, 2002).

Besides the changes in the actin cytoskeleton in response to applied mechanical forces, an early cellular response is the influx of  $\text{Ca}^{2+}$  through stretch-activated channels (Wu *et al.*, 1999). The  $\text{Ca}^{2+}$  influx may then lead to intracellular activation of many molecules, including NF- $\kappa$ B, cAMP-response element binding protein (CREB), and membrane kinases, which specifically phosphorylate other signaling molecules, such as EGFR, which activates downstream MAPK signaling pathways (Iqbal and Zaidi, 2005; Rosen and Greenberg, 1996; Sadoshima and Izumo, 1997). Stretching-induced  $\text{Ca}^{2+}$  influx upregulates COX-2 expression via activation and translocation of NF- $\kappa$ B into the nucleus (Inoh *et al.*, 2002). However, it is not clear whether ion channels are indispensable components of the mechanosensor itself because integrin-mediated FA mechanosensor can still respond to applied forces in detergent-treated cells (Sawada and Sheetz, 2002).

There are similarities and differences between adhesion-dependent and ion channel-based mechanosensors (Geiger and Bershadsky, 2002). In both cases, a mechano-responsive element is linked via a motor protein (e.g., myosin II), to the cytoskeleton at the cell's interior and to an extracellular anchor, which is usually the ECM. It is speculated that channel-based mechanosensors induce global cellular signaling events, whereas FA-based mechanosensors elicit more localized events.

Another mechanotransduction mechanism is thought to originate at the cell membrane and involve G-protein-coupled receptors (GPCRs) (Sarasa-Renedo and Chiquet, 2005; White and Frangos, 2007). Using time-resolved fluorescence microscopy and GPCR conformation-sensitive fluorescence resonance energy transfer (FRET), fluid shear stress on endothelial cells was shown to increase the activity of bradykinin B2 GPCR (Chachisvilis *et al.*, 2006), which is known to be involved in fluid flow-dependent responses in endothelial cells (Groves *et al.*, 1995; Leeb-Lundberg *et al.*, 2005). In addition, single-molecule studies have identified certain structural motifs of proteins such as fibronectin that can be mechanically switched between conformations, thereby exposing potential cryptic sites, which may transduce mechanical force into biochemical signals by changing the relative distance of the two binding sites of the receptor or by changing the geometry of the binding site (Smith *et al.*, 2007; Vogel, 2006; Vogel and Sheetz, 2006).

A novel mechanotransduction mechanism that has been proposed recently considers a coupling between an external mechanical force and a protein redox (reduction/oxidation reaction) equilibrium by which the triggering of a biochemical signal is controlled (Grandi *et al.*, 2006; Sandal *et al.*, 2006). The disulfide bonds present in many ECM proteins act as redox switches for protein functions. Angiostatin, which contains disulfide bonds and is located in the basement membrane where mechanical forces act, has been shown to be an ideal model system for addressing the issues of the

possible triggering of biochemical signals by the combination of mechanical forces and redox equilibrium (Grandi *et al.*, 2006). Mechanical forces can partially unfold the structure of angiotatin, which can activate new binding capabilities and trigger new biochemical signals (Grandi *et al.*, 2006).

As noted above, many years of research has greatly increased our understanding of cellular mechanotransduction mechanisms—the centerpiece of cell mechanobiology. However, the precise details of how mechanical forces transduce into signaling events and how a cell decides its response from cross talks of many mechanotransduction signals still remain elusive. Furthermore, whether adult and stem cells use the same mechanotransduction mechanisms remains to be worked out. Thus, more research with novel experimental and theoretical methodologies is required.

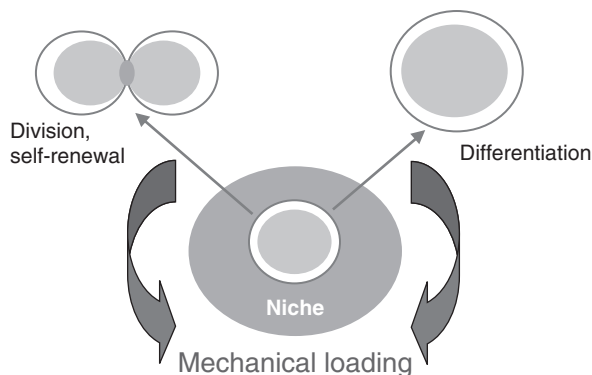
## 6. CONCLUDING REMARKS

Mechanical forces acting on cells within tissues and organs (e.g., tension, compression, hydrostatic pressure, and fluid shear stress) play a vital role in human health and disease. Years of intensive research have also established that mechanical forces stimulate ECM (e.g., collagen) gene and protein expression and induce cellular inflammatory response (e.g., expression of COX-2 and production of PGE<sub>2</sub>). Mechanical forces can also interact with soluble factors (e.g., TGF- $\beta$  and IL-1 $\beta$ ) in generating anabolic and catabolic cellular responses. However, while *in vitro* studies highlight the critical role of mechanical forces in cell biology and consequently tissue physiology and pathophysiology, the proper interpretation of experimental data requires consideration of many factors such as cell source, type, morphology, and organization; the manner of applying mechanical forces (e.g., uniaxial vs biaxial stretching); and the type of matrix protein coating, as these factors can all influence cellular response to mechanical forces.

Besides these external mechanical forces on cells, cell-generated forces, known as CTFs, play an important role in many biological processes, including wound healing and angiogenesis. Therefore, measurement methods such as CTFM have been developed to quantify CTFs. Current CTFM methods are limited in that they use a 2D substrate. To have much broader application, CTFM methods should be extended to more physiologically accurate 3D matrices.

The critical role of mechanical force in the development of functional tissue engineering constructs for repair, replacement, and regeneration of injured and diseased tissues has also been recognized. Much effort has been focused on the effects of mechanical loading on adult stem cells, such as BMSCs, in an attempt to obtain functional tissue engineering constructs.

Additionally, the application of mechanical forces in the form of tension, compression, and torsion induces controlled differentiation of MSCs into different cell lineages such as ligament and SMCs. A few studies have also examined how mechanical loading regulates ES cells in terms of self-renewal and differentiation. However, the molecular mechanisms by which mechanical loads activate or suppress signaling pathways that lead to the self-renewal and/or differentiation of ES cells and adult stem cells are not clear. Furthermore, the detailed mechanisms regarding both the interaction of different signaling pathways in modulating expression of mechano-responsive genes and also the cross talk between mechanical and chemical signaling pathways in ES cells and adult stem cells remain to be worked out. Mechanical forces acting on stem cells may trigger cell surface receptors and adhesion sites that activate signaling cascades responsible for the synthesis and secretion of key ECM components (Sebastine and Williams, 2006). Soluble mediators, along with nonprotein metabolic products, such as calcium, may also affect stem cell differentiation. Although the well-described stem cell niches, including neighboring cells and surrounding matrix, are known to regulate the balance of self-renewal and differentiation of adult stem cells (Moore and Lemischka, 2006; Scadden, 2006), the mechanical loading environment around individual stem cells may also be an important “niche factor” that may have an influence on the division, self-renewal, and differentiation (or the lineage commitment) of adult stem cells, especially those in load-bearing tissues such as bone, tendons, and ligaments (Fig. 7.3). Manipulation of the stem cell niche by targeting the mechanical environment surrounding stem cells may



**Figure 7.3** Schematic of the concept of mechanical loading as a niche factor of stem cells. Stem cell niche generally refers to local microenvironment that maintains stem cell identity and regulates stem cell function. It is suggested that the niche may also include mechanical loading (curved arrows), which, together with other intrinsic factors (e.g., growth factors), regulates stem cell division, self-renewal, and differentiation.

constitute an effective therapeutic treatment of musculoskeletal tissue diseases, in which mechanical forces are known to play a critical role in tissue pathophysiology. Finally, it should be noted that compared to numerous studies on adult cell mechanobiology, there are far fewer studies on stem cell mechanobiology; therefore, it is an emerging field to be explored fruitfully in future research.

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