The effect of mechanical loads in the differentiation of precursor cells into mature cells

Dalit Shav and Shmuel Einav

Department of Biomedical Engineering, Tel Aviv University, Tel Aviv, Israel; Stony Brook University, Stony Brook, New York, USA

Address for correspondence: Prof. Shmuel Einav, Ph.D., Berman Professor of Vascular Bioengineering, Tel Aviv University, Department of Biomedical Engineering, Tel Aviv 69978, Israel. Dalit Shav, MSC, Department of Biomedical Engineering, Tel Aviv University, Tel Aviv, Israel. dalitr@eng.tau.ac.il

Stem cell differentiation, both in vivo and in vitro, is regulated by a variety of signals. These signals can be of biochemical origin, such as those from growth factors and cytokines, or from different mechanical loads, such as fluid shear stress and matrix elasticity. The mechanisms by which the mechanical loads affect precursor cell differentiation are not entirely understood, but their role in regenerative medicine and cell therapy could be of vast importance. This paper reviews the role of mechanical loads on the differentiation of precursor cells.

Keywords: stem cells; differentiation; precursor cells; morphogenesis; mechanical loading; matrix stiffness; extra cellular matrix

Introduction

Cells are exposed in vivo to a complex of mechanical forces. The ability of cells to sense and respond to the mechanical environment is part of their mechanism for preserving the organism’s homeostasis. Mechanical stresses regulate a diverse array of physiological functions, ranging from sound sensation by the cells of the inner ear, to the detection of fluid shear stress imparted by blood flow across the endothelium. Mechanical cues modulate almost all aspects of cell function, including growth, differentiation, migration, gene expression, protein synthesis, and apoptosis. Mechanical forces participate in differentiation and morphogenesis from the level of individual cells to whole organism patterning. For example, fluid flow is an important factor in vasculogen-esis; and evidence suggests that fluid shear stress rather than fluid transport is essential to the re-modeling of the early vasculature. Contraction of the actin cytoskeleton through non-muscle myosins is a common mechanism for controlling differentiation in many developmental events, from gastrulation to cardiogenesis. Finally, novel experimental approaches suggest critical roles for the actin cytoskeleton and the mechanical environment in the differentiation of mesenchymal stem cells to different cell types.

One of the more visible signs of the effect of mechanical forces in morphogenesis is our fingerprints—the patterns of tiny ridges and valleys on the tip of each finger that take on definite shapes during the 13th week in the uterus. The fingertip skin ridges are shaped by the combined effects of cell proliferation and compressive stresses that develop in the dermal cell layer. They are not regularly spaced, and changes in the local environment cause small variations in them. In the case of identical twins, where even DNA analysis cannot differentiate between them, their fingerprints are not identical; while some characteristics such as pattern type and ridge count are usually similar, twins differ in the smaller details including where skin ridges meet, end, or bifurcate. This is caused by the small variations in the mechanical forces that each twin will experience in utero due to his/her different location. Thus, small environmental changes can induce different morphogenesis during the maturation process.
Mechanical forces affect morphogenesis in vivo

The role of mechanical forces is an important factor in many vital developmental events. A good example of this is seen in Kartagener’s syndrome, in which a mutation causes a loss in cilia function. The syndrome affects mainly the lungs and fertility. In addition, 50% of patients suffer from left–right reversal of the major visceral organs on account of an early embryonic event when the epithelium in the midline of the early embryo, which has cilia, should cause a flow to the left. This flow helps the embryo to determine right from left; the embryo senses the fluid flow and upregulates a signaling molecule on the left side. When there is a defect in the cilia motor proteins, there is a randomization of the left–right flow and consequently of the left–right patterning.

One of the most critical effects of fluid flow on differentiation events and morphogenesis is its control on vasculogenesis: the development of new blood vessels directly from precursor cells. In mouse development, vasculogenesis starts outside the embryo, where cells migrate to form “blood islands” in the yolk sac primary vascular plexus. When fetal circulation begins, these islands remodel to form a vascular tree with branching arteries and veins. Stopping circulation to the yolk sac prevents the remodeling, even though the blood islands continue to grow. Although thought to be caused by the lack of transport of nutrients, oxygen, or other growth factors, it was discovered that the real cause is the presence of fluid shear stress. By physically sequestering the erythroblasts, the hematocrit is greatly reduced and the pre-existing yolk sac vessels do not mature into an organized vascular tree. Artificially increasing the fluid viscosity by adding starch rescues blood vessel formation, demonstrating that it is shear stress and not transport that is required for the vascular remodeling during vasculogenesis.

It is noteworthy that recent in vitro studies showed that fluid flow can cause differentiation of mesenchymal precursor cells into endothelial cells. This was demonstrated when mesenchymal progenitor cells plated on a collagen-based gel and exposed to fluid flow spontaneously formed tubular structures.

Fluid flow is an important mechanical stimulation, although other mechanical forces can participate in the differentiation of precursor cells. For example, gastrulation, the first major morphologic change in the embryo of the fruit fly D. melanogaster, is controlled by contraction of non-muscle myosin. Before gastrulation, the middle of the D. melanogaster embryo appears roughly as a cylindrical tube composed of a single layer of cells. Gastrulation begins when a furrow appears across the length of the tube, and the furrow deepens until it creates a new layer of cells by folding within itself. The primary regulator of this event is the contraction of the actin cytoskeleton by the type II non-muscle myosins (NNM II).

While muscle-specific type II myosin motors are known in the generation of muscle contractions, all cells contain related type II myosins, the “non-muscle myosins,” which bind to actin and control contraction of the cytoskeleton. Before gastrulation begins, myosin is located in a sharp ring around the inner circumference of the cells. Just as the initial furrow appears, myosin disappears from the inner surface and relocates to the outer surface precisely where the furrow is forming. The presumed mechanical effect is that myosin is causing the outer (apical) surface to contract, creating compression of the cells simultaneously with the loss of tension around the inner surface. This causes the cell layer to buckle inwards and deepens the furrow on the outside, leading to invagination.

Precursor cell differentiation in vitro

The differentiation of precursor cells in vitro offers another example of the role of mechanical forces. Stem cell differentiation is regulated by a variety of cues including growth factors, cytokines, and mechanical loading, among which extracellular matrix (ECM) stiffness has emerged as an important component. While growth factors are more likely to exert influence earlier in development compared to matrix, as the growth factors signal to cells to produce matrix, the ECM maintains the ability to stimulate similar cell responses in embryonic stem cells.

Matrix elasticity is one of the mechanical stimulations that cells feel and respond to, with numerous studies demonstrating that matrix mechanics modify the phenotype of a variety of cells including fibroblasts, normal or cancerous epithelial cells, neurons, myocytes, osteoblasts, and even stem
cells. Although individual cell types vary in their responses, increased matrix stiffness generally results in increases in protein phosphorylation, stress fiber assembly, and cellular stiffness. The elastic modulus $E$ of solid tissues quantifies the stress (force) required to strain (lengthen) a tissue, and ranges from about 0.1 to 1 kPa for especially soft tissues such as brain, fat, and liver, to about 10 kPa for static muscle, and around 20 to 100 kPa for stiff, non-calcified scars, tendons, and cartilage.

The composition, concentration, and elastic modulus of the matrix are important in controlling cell differentiation. For example, matrix elasticity of different tissues (e.g., soft brain tissue versus calcified bone) plays an important developmental role: muscle cells will need a compliant matrix that can deform during contractions, whereas bone cells need a stiffer matrix where they can mineralize. The role of the ECM in this process is not entirely understood.

It has been known since the 1970s that differentiation of precursor cells into chondrocytes and adipocytes in vitro is promoted by high cell density. Embryonic mesodermal cells isolated from chick limb buds will spontaneously undergo chondrogenesis when cultured at a very high density. Similarly, subsets of 3T3 fibroblastic cells will spontaneously accumulate lipid when kept in high-density confluent monolayers. Of interest, disrupting the actin cytoskeleton by treatment with cytochalasin B will stimulate the differentiation of precursor cells into chondrocytes and adipocytes.

Two mechanisms that control this differentiation in vitro have been suggested: The first is that differentiation depends on the cell’s normal three-dimensional round shape, which could affect spatial patterns of signaling through integrin or cadherin complexes on the cell surface. According to the second mechanism, both high cell density and round shape minimize the contact with the hard tissue culture surfaces, thereby reducing the area available for the formation of focal adhesions, stress fibers, and myosin contraction.

Naïve mesenchymal stem cells (MSCs) grown under identical media conditions express key markers of early neurogenic, myogenic, and osteogenic lineages on gels with a controlled elastic modulus that correspond, respectively, to tissue elasticities of brain (soft 1 kPa matrices), muscle (stiffer 11 kPa substrates), and pre-mineralized collagenous bone (comparatively rigid 34 kPa matrix). These differentiation responses are observed at all levels, from RNA to protein production to morphology and cell stiffness.

Blocking non-muscle myosin-II (NMM II) inhibits the elasticity differentiation in MSCs with no major effect on other aspects of cell function and shape. Inhibition of actin polymerization or the cytoskeletal contraction (by overexpression of RAC) will stimulate the differentiation of MSCs to chondrocytes and adipocytes. On the other hand, stimulating contraction of the actin cytoskeleton (by over-expression of either Rho or Rho-associated kinase [Rock]) will cause differentiation of MSCs into osteoblasts. Moreover, culturing MSCs on hard substrates will activate NMM II contraction and promote differentiation towards osteoblasts, whereas culturing MSCs on soft substrates, or minimizing the contact with hard surfaces, which minimizes the cytoskeletal contraction, promotes differentiation towards adipocytes and chondrocytes. This suggests that the differentiation of MSCs is controlled by the level of NMM II contraction inside the cell and the stiffness of its surroundings.

The common scheme of mechano-transduction is: (a) local displacement of sensors at the cell surface; (b) force transmission through the cytoskeleton; and (c) action of the transmitted forces on remote mechanotransduction sites causing morphologic and functional responses. In addition, sustained stimulation of mechanical forces causes adaptation to the stimuli, while different patterns of flow cause different responses.

In the case of substrate elasticity sensing, a large number of membrane-localized proteins and their interactions trigger the mechanisms behind the different responses of the cells. Ligand–receptor binding is clearly the first step of cell–substrate or cell–cell interactions. However, integrins not only bind to ECM ligands, but also establish intracellular connections with the cytoskeleton; when binding to the ECM, integrins form clusters and focal adhesion complexes with the cytoskeleton in a force-dependent manner. Integrins that are bound to the ECM induce further signaling, and cytoplasmic signaling proteins such as Rac and Rho activators are clearly involved in establishing focal complexes and maturing them to focal contacts.
Cell contractility is due to molecular motors which are also needed for adhesion maturation and maintenance of focal complexes and contacts. NMM II is important in the contractility of non-muscle cells and starts many mechano-sensing and signal transduction events. Blebbistatin, a selective NMM II inhibitor that blocks the motor activity of NMM II without affecting the actin-binding properties, completely shuts down the differential response of adult MSCs to matrix elasticity. This is done by preventing the active contraction within the cell, thereby rendering the cell unable to perceive the substrate elasticity. In the presence of this drug, the main bodies of cells on all substrates remain small and round, which shows that NMM II is crucial for cell spreading. How NMM II, the cytoskeleton, and other proteins regulate force-generation, mechano-sensing, signal transduction, and adhesion of cells remains to be elucidated.5

Inhibition of NMM II appears to prevent the response of the cells to the varying stiffness of different substances. On the other hand, initial data with MSCs and the use of mitomycin C, which inhibits cell proliferation and is used as an anti-cancer drug, suggests that these cells divide a bit more rapidly on soft gels in the presence of the drug versus the controls. This proliferation of MSCs on soft gels in the presence of the drug is similar to that on moderate-to-stiff gels. In contrast, adhesion on rigid glass substrates appears to be so strong that proliferation is inhibited with or without the drug. These results suggest that cells in the very soft parts of a solid tumor, which one might speculate include the necrotic core, will divide and grow slightly more upon delivery of an anti-cancer drug. Given the potential pathophysiologic relevance of matrix elasticity, such matrix-moderated effects on proliferation and on drug-induced effects on proliferation should be more seriously factored into therapeutic applications.5

Soluble growth factors are potent regulators of normal and pathologic processes. Mechanical factors are emerging as equally important, but there is no clear mechanism linking the different factors. Recent works have suggested that cell-generated mechanical tension results in the release of active transforming growth factor-β (TGF-β) from stiff ECM, providing a mechanism for differentiation and maintenance of myofibroblasts in processes like fibrosis.5

TGF-β is a matrix-associating, protein growth factor with important roles in embryogenesis, malignancy, and fibrosis. TGF-β is secreted from cells as part of a latent complex that adheres to matrix proteins such as fibrillin, proteoglycans, and fibronectin. This complex interacts directly with integrins, providing a cellular grip on extracellular stores of TGF-β. The important finding is that the cell exerts tension on the latent complex through the integrin grip, causing conformational changes probably like those occurring in other structural proteins under force, and releasing TGF-β in an active form to diffuse and bind cell surface receptors; the latter sets off a molecular cascade that increases the synthesis of TGF-β and α-smooth muscle actinin (α-SMA). A soft matrix would deform under the stresses, leaving the latent complex intact and TGF-β sequestered. A stiff matrix would resist the deformation, resulting in deformation of the latent complex and the release of active TGF-β. Matrix stiffness, cell tension, and TGF-β release are therefore all required to increase the abundance of cytoskeletal α-SMA. The feed-forward, load-limiting mechanism promotes and maintains myofibroblast differentiation (and therefore continued fibrogenesis) in the continuous remodeling that occurs as soft wounded tissue is pulled together in healing, scar formation, and even in pathologic fibrosis. Finally, it is appealing to speculate that mechano driven mechanisms might also be responsible for the release of other growth factors sequestered in the matrix. Thus, matrix stiffness could regulate the equilibrium between storage and release of matrix-bound growth factors.5

Biomaterials and mechanical approaches to the differentiation of stem cells

Stem cells play increasingly prominent roles in tissue engineering and regenerative medicine. Undifferentiated cells that remain pluripotent give rise to tumors known as teratomas. Hence, it is critical for any therapeutic strategy employing a stem cell-based approach to ensure complete and irreversible differentiation of stem cells into the desired progenitors or terminal target cell type. This may be accomplished by supplementing the appropriate trophic factors in the culture medium, or by delivering them from a scaffold in a controlled manner. The mechanical properties of a scaffold or culture
surface can also exert a significant effect on the differentiation of the seeded stem cell.8

Current approaches for the derivation of stem cell–based implantable grafts can be classified into four strategies. The first step in all strategies is to increase their number. To achieve this, cells (embryonic stem cells or somatic stem cells isolated from pediatric or adult patients) are expanded in culture on biomaterial-based bioreactors. Tissue scaffolds are then tailored according to the intended therapeutic purpose.8 (1) In the most common strategy, stem cells are differentiated into the target cell type before being seeded into scaffolds to constitute the grafts. In cases where instructive signals are incorporated into the scaffolds, differentiation can take place in situ in the scaffolds. (2) In the second strategy, stem cells differentiate directly in the scaffold before implantation. This strategy is probably more

![Figure 1](image)

**Figure 1.** Immunostaining for myogenic proteins sarcomeric myosin and Myo D of primary myoblasts from skeletal and heart muscles obtained from 1- to 2-day-old neonatal rats.

![Figure 2](image)

**Figure 2.** Comparison of the molecular profile of cardiac muscle (CM) cells, skeletal muscle (SM) cells, and mesenchymal stromal cells (MSCs). The different cell populations displayed shared or distinct gene patterns that specified their lineal diversification. (A) Localization of the primers used for PCR. (B) Expression of analyzed genes on cells at different stages of differentiation.
suited to adult stem cells. (3) In the third strategy, stem cells are partially differentiated into progenitor cells before or after seeding into scaffolds to give rise to proto-tissues. When implanted, these constructs transiently release progenitors that migrate into surrounding regions, where they undergo terminal differentiation, integrate, and contribute to regeneration of the lesioned areas. Prolonged release of stem/progenitor cells may be achieved when a suitable scaffold is used to maintain them in a partially differentiated state. (4) Injectable grafts, composed of naïve or stimulated stem cells encapsulated in biodegradable hydrogels, constitute the fourth strategy. This strategy is attractive for soft tissue repair or treatment of solid tissues with critical size defects that are too fragile for surgical intervention.8

Another important aspect of investigating the effects of mechanical forces on cultured cells is how to stimulate the cell culture with a mechanical load. Over the years a wide variety of laboratory apparatuses have been devised for mechanical stimulation of cell and tissue cultures, including cells cultured on different scaffolds. These devices can be categorized according to their primary loading modality: compression (hydrostatic pressure or direct platen contact on a tissue explant or cells seeded in matrix), longitudinal stretch, bending, axi-symmetric substrate bulge, in-plane substrate distention, fluid shear stress, or combined substrate distention and fluid shear. All these devices act on cell culture systems with controlled delivery of a quantified mechanical input. Laboratory apparatuses devised for that purpose span a considerable range of complexity and sophistication and feature mechanical input signals of varied levels of precision and homogeneity.9

**Therapeutic aspects**

Bone is a dynamic tissue with a high turnover rate. A number of stimuli affect bone turnover, including hormones, cytokines, and mechanical stimuli. The extent and quality of the resulting tissue depends upon all of these factors. Mechanical loading is a particularly potent stimulus for bone cells; it improves bone strength and inhibits bone loss with age. Bone can accumulate damage from this loading, but, unlike engineering materials, bone is capable of self-repair. The molecular mechanisms by which bone adapts to loading and repairs damage are becoming clear. Many of these processes have implications for bone health, disease, and the feasibility of living in weightless environments.4

In vivo, the skeleton is loaded in a dynamic fashion, which helps in the physiologic remodeling of bone density, and suggests that the bone cells can sense this loading. During adaptation to a new functional demand, remodeling of the skeleton involves recruitment of osteoclasts from the pluripotent hematopoietic cells of the marrow. Normal dynamic loading prevents bone resorption, but how biophysical factors reduce osteoclast activity is not understood. Rubin and colleagues showed that mechanical strain (2% at 10 cycles per minute) applied to murine marrow cultures and to primary stromal cells reduced stimulated osteoclast formation by 50%; this was accompanied by a decreased expression of mRNA levels of osteoclast differentiation factor (ODF), with no significant effect on the total cell count, thymidine uptake, or alkaline phosphatase activity. Soluble ODF (2 ng/mL) was able to reverse the effect of strain and return the osteoclast numbers to control. This indicates that osteoclast formation depends upon ODF expression, and that strain-induced reductions in this factor may contribute to the accompanying reduction in osteoclastogenesis.10

Mechanical loading of a rat’s tibia in vivo stimulates new bone formation and increases endocortical bone formation. The proposed mechanism behind this is increased bone formation by differentiation of osteoprogenitor cells from the marrow stroma into osteoblasts at the cortical bone surfaces. Using a specific dye to determine the origin of the endocortical osteoblasts, Turner and colleagues found that although the endocortical osteoblasts surface was significantly increased 48 h after loading, the percentage of labeled osteoblasts did not increase, suggesting that the newly differentiated osteoblasts on the endocortical surface did not originate from proliferating cells. However, 96 h after loading, 30–40% of the endocortical osteoblasts were labeled. Thus, the mechanical loading causes two osteoblastic responses: an immediate response within 48 h in which osteoblasts are recruited from non-dividing preosteoblasts and/or bone-lining cells, and a delayed response involving proliferation and differentiation of preosteoblasts that requires more than 3 days.11
Adult cardiac muscle lacks the ability to regenerate after myocardial infarction, resulting in necrosis of the infarcted ventricular tissues, which are replaced by scar tissue, leading ultimately to heart failure. Using fetal cardiomyocytes and cultured cardiomyocytes for tissue engineering limited the scar expansion and prevented the postinfarction heart failure. This is unfeasible in the clinical setting, however, since it is impossible to obtain donor fetal heart cells, making the development of new technologies to culture cardiomyocytes of prime importance.

Two cell types have been proposed for this purpose: satellite cells, which are present in the adult skeletal muscle and are thought to be its progenitors, but are not good candidates as their number and ability to proliferate and reconstruct muscle tissue decrease with age; and adult multi-potent mesenchymal stromal cells (MSCs), which are capable of creating some types of tissues in vitro under proper conditions. The conditions and molecular mechanisms of the proliferation/differentiation switch of MSCs into the required lineage are poorly understood.

We have compared myoblasts from cardiac and skeletal muscle for their gene expression to analyze the similarities and differences between these cell types, focusing on the transcriptional profiles of the different cells (Figs. 1 and 2). The profile of these cells allows highlighting the molecular pathways that correlate with the regulation of muscle cells from proliferation to differentiation and thus contributes to our understanding of the regulation of lineage fate decision and the mechanism by which mechanical loading can change the differentiation path the cells choose.

Conclusion

The amount of data regarding the contribution of mechanical loading in shaping the fate of precursor cells is constantly increasing. The effect of matrix elasticity, fluid shear stress, and other strains is becoming evident in vivo and in vitro. Knowledge of the mechanism and molecular pathways by which mechanical loading affects the differentiation of various precursor cells and the resulting cell lineage is of great significance both for understanding the biological events involved and ultimately for devising effective clinical treatments.

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Conflicts of interest

The authors declare no conflicts of interest.

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