Understanding Mechanobiology: Physical Therapists as a Force in Mechanotherapy and Musculoskeletal Regenerative Rehabilitation

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Achieving functional restoration of diseased or injured tissues is the ultimate goal of both regenerative medicine approaches and physical therapy interventions. Proper integration and healing of the surrogate cells, tissues, or organs introduced using regenerative medicine techniques are often dependent on the co-introduction of therapeutic physical stimuli. Thus, regenerative rehabilitation represents a collaborative approach whereby rehabilitation specialists, basic scientists, physicians, and surgeons work closely to enhance tissue restoration by creating tailored rehabilitation treatments. One of the primary treatment regimens that physical therapists use to promote tissue healing is the introduction of mechanical forces, or mechanotherapies. These mechanotherapies in regenerative rehabilitation activate specific biological responses in musculoskeletal tissues to enhance the integration, healing, and restorative capacity of implanted cells, tissues, or synthetic scaffolds. To become future leaders in the field of regenerative rehabilitation, physical therapists must understand the principles of mechanobiology and how mechanotherapies augment tissue responses. This perspective article provides an overview of mechanotherapy and discusses how mechanical signals are transmitted at the tissue, cellular, and molecular levels. The synergistic effects of physical interventions and pharmacological agents also are discussed. The goals are to highlight the critical importance of mechanical signals on biological tissue healing and to emphasize the need for collaboration within the field of regenerative rehabilitation. As this field continues to emerge, physical therapists are poised to provide a critical contribution by integrating mechanotherapies with regenerative medicine to restore musculoskeletal function.

Regenerative medicine is an emerging field that combines advances in tissue engineering and molecular biology to replace or regenerate human cells, tissues, or organs with the goal of restoring or establishing normal function following loss due to injury, disease, or aging.[1] Regeneration in response to injury requires the recapitulation of specific events that occur during embryonic and fetal development, as well as a conducive cellular milieu, so that damaged regions are replaced with healthy tissue that has exactly the same composition, structure, and functional abilities as undamaged native tissue. Unfortunately, most musculoskeletal tissues in adults lack the ability to regenerate, with injury resulting in a repair response whereby fibrous connective tissue is laid down, forming a scar with inferior mechanical, physiologic, and functional properties.

Regenerative medicine has opened the possibility for full healing of injured or degenerated musculoskeletal tissues, thereby offering hope for people who have conditions that traditionally have had limited recovery potential. Examples of musculoskeletal conditions that may benefit from regenerative medicine approaches include: (1) injury-related conditions that use repair processes to heal, such as muscle strains, ligament sprains, tendon ruptures, and integument wounds; (2) injury-related conditions that exhibit compromised healing, such as osteochondral defects and non-united bone fractures; (3) injury-related conditions that have little prospect of healing, such as volumetric muscle loss and segmental bone defects; and (4) disease-related conditions, such as sarcopenia, osteoporosis, and osteoarthrosis. Examples of some regenerative therapies currently being used or developed for these conditions include the introduction of stem cells, progenitor cells, or biologically active molecules and the implantation of bioengineered scaffolds or ex vivo grown tissues. In this article, we provide a perspective of how mechanotherapies influence the development and healing of various tissues, with a particular emphasis on bone.

As the goal of regenerative medicine is to restore or establish normal function,
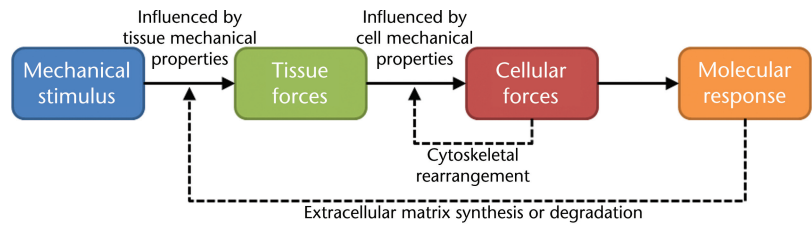
Figure 1.
Mechanical forces direct cellular activities to induce tissue adaptation. Extrinsically and intrinsically generated mechanical forces load musculoskeletal tissues, with the characteristics of the resultant tissue forces being dependent on the ability of the tissue to resist those forces. Tissue forces are transmitted to the micromechanical environment of resident cells, with cellular mechanical properties influencing the characteristics of the cellular forces. Cells can modify their micromechanical environment via cytoskeletal rearrangement, which feeds back to alter cellular sensitivity to incoming forces. When cellular forces are sufficient, the cell initiates a molecular response, which ultimately alters synthesis or degradation of the extracellular matrix. The latter alters tissue mechanical properties, which feeds back to influence tissue forces.

individuals who receive regenerative therapies will require rehabilitation to make best use of their restored anatomy and newly regained abilities. Physical therapists are specifically trained to assess and manage musculoskeletal pathologies and thus are well positioned to be important allies in musculoskeletal regenerative medicine. However, the role of physical therapists extends beyond the serial approach of simply reestablishing function at the organism level following tissue healing.[2,3] In particular, physical therapists have the potential to become the leaders in musculoskeletal regenerative rehabilitation.

Musculoskeletal regenerative rehabilitation can be defined as the integration of principles and approaches from rehabilitation and regenerative medicine, with the ultimate goal of promoting the restoration of function through musculoskeletal tissue regeneration and repair.[4] This definition does not confine the role of physical therapists to restoring function *after* tissue regeneration or repair but also enables therapists to play an active role by facilitating regeneration and repair at the tissue level *during* healing. In addition, the definition encourages therapists to contribute to the conception and development of novel regenerative therapies by working collaboratively with other disciplines involved in regenerative medicine in a team-based approach to optimize functional outcomes.[2,3]

The success of therapies in regenerative medicine at repairing or regenerating musculoskeletal tissues ultimately depends on the therapies being accepted and incorporated into the native tissue (eg, in the case of ex vivo grown tissues or bioengineered scaffolds) and creating a musculoskeletal tissue with optimized mechanical characteristics (eg, in the case of biologic or pharmaceutical agents). One group of therapies that physical therapists have in their repertoire that have great potential of having additive, or even synergistic, effects when introduced in conjunction with regenerative medicine treatments is mechanotherapies.

Mechanotherapy

Musculoskeletal tissues are critical for load bearing but also generate, absorb, and transmit force, thereby enabling functional movement. Given their mechanical role, it follows teleologically that musculoskeletal tissues are capable of responding and adapting to their mechanical environment. Mechanical forces direct cellular activities influencing the tissue-level processes of growth, modeling, remodeling, and repair, with the ultimate outcomes being altered tissue mass, structure, and quality (Fig. 1). Nearly every physical therapy intervention in musculoskeletal rehabilitation introduces mechanical forces, regardless of whether the forces are generated extrinsically via therapist intervention (eg, during joint or tissue mobilization or
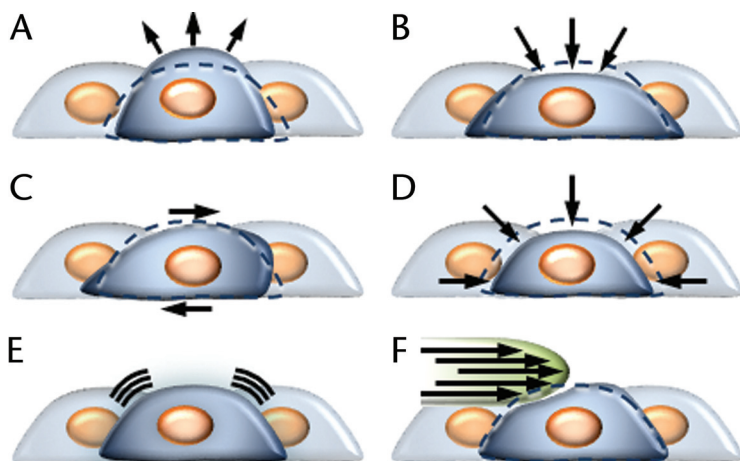
Figure 2.
Common micromechanical stimuli to which musculoskeletal cells are exposed: (A) tension—pulling force that increases cell dimensions in the direction of pull; (B) compression—pushing force that decreases cell dimensions in the direction of push; (C) shear—parallel forces pushing or pulling in opposite directions to distort the cell; (D) hydrostatic pressure—pressure exerted by surrounding fluid that changes cell volume; (E) vibration—oscillating, reciprocal back-and-forth shaking of a cell; and (F) fluid shear—force created by the flow of fluid parallel to a cell membrane.

via the introduction of external therapeutic modalities) or intrinsically within the individual themselves via the prescription of exercise therapy. As an exhaustive review of the numerous forms of mechanotherapies used by physical therapists is outside the scope of the current perspective article, readers are referred to the following reviews that detail various forms of mechanical interventions, including: joint mobilizations,[5] muscle or tendon stretching,[6] resistance exercises,[7] vibration platforms,[8] interventional ultrasound,[9] and massage.[10]

Remedy through mechanical intervention has been sought for thousands of years; however, physical medicine was not recognized as a medical specialty until the early-to-mid 19th century.[11] During that period, as medicine became more specialized, terms such as "physical medicine," "physical therapy," "physiotherapy," and others used to describe the use of exercise and physical manipulation were collectively known as "mechanotherapy." Forming organized groups of physicians and therapists helped establish mechanotherapy as a recognized medical intervention; however, the definition of mechanotherapy

remained ambiguous for decades, and limited empirical evidence brought broad interpretations and clinical implementation.

The first formal definition of mechanotherapy was published in 1890 as "the employment of mechanical means for the cure of disease."[12(p181)] The term remained relatively unchanged until it was updated in 2009 to "the employment of mechanotransduction for the stimulation of tissue repair and remodeling."[13(p248)] The revised description highlighted the cellular basis of tissue responses and the distinction between healthy and injured tissues. More recently, the definition was again updated to reflect the influence of mechanotherapy on tissues outside of the musculoskeletal system.[14] In keeping with those revisions, we propose a definition of *mechanotherapy* as "any intervention that introduces mechanical forces with the goal of altering molecular pathways and inducing a cellular response that enhances tissue growth, modeling, remodeling, or repair." As such, we seek to highlight the multisystem hierarchy (molecules – cells – tissues), which is responsive to mechanical signals, and to recognize the influence of mechanother-

apy on the tissue-level processes responsible for the development, maintenance, healing, and regeneration of tissues. Additionally, although musculoskeletal tissues are the primary focus of the current article, it is important to acknowledge that essentially every cell type within the body is responsive to mechanical signals, extending the principles of mechanotherapy to nonmusculoskeletal tissues.

Mechanotransduction

In order for physical therapists to fully contribute to regenerative medicine and be viewed as the leaders in regenerative rehabilitation, there is a need to understand how mechanotherapies work at the cellular and molecular levels. Although the adaptive ability of tissues in response to mechanical stimuli has long been established, the precise mechanisms underlying the response at the cellular and molecular levels have only recently begun to be unraveled and remain to be fully elucidated. Nevertheless, it is accepted that the mechanism involves some form of mechanotransduction, which refers to the conversion of a biophysical force into a cellular and molecular response.

Mechanotransduction at the Cellular Level

Mechanotransduction requires a mechanical signal to be transmitted to the microenvironment of a cell and for the cell to possess machinery to sense the signal. Cells can be exposed to a variety of micromechanical stimuli, with the precise nature of the stimulus depending on the mechanical properties of the cells themselves and the interaction between the incoming mechanical signal and the extracellular matrix (ECM) (Fig. 1). Common stimuli include tension, compression, and shear; however, cells also can be exposed to other mechanical stimuli, such as hydrostatic pressure, vibration, and fluid shear (Fig. 2).

The tissue in which a cell resides and the location of the cell within that tissue influence the forces to which the cell is exposed; yet, the exact nature of the forces may not always be evident. For example, it may be assumed that mecha-

nosensitive cells in bone are predominantly exposed to compression, whereas those in tendon are exposed to tension due to the function of the tissues in which they reside. However, long bones (eg, tibia and femur) are curved and bend when axially loaded to generate compressive forces within the tissue on the side the bone is bending toward and tensile stresses within the contralateral side.[15] Thus, bone cells can be exposed to either compressive or tensile forces (although fluid shear appears to be the most likely signal involved in skeletal mechanotransduction [discussed later]).[16] Similarly, although tendons are exposed to large tensile forces in their role of transmitting muscle forces, the tensile loading of collagen can cause cell-occupying spaces to narrow, resulting in the generation of compressive forces, whereas differential elongation of adjacent collagen fibers can generate microscopic shearing forces.[17] Also, cells located in tendon near bony prominences (eg, within the supraspinatus tendon as it passes through the subacromial space or the Achilles tendon near its calcaneal insertion) can be exposed principally to compressive, rather than tensile, forces.[17]

By understanding the forces to which cells are exposed and respond, it may be possible to develop novel means of introducing those forces to induce a desired cellular response and resultant tissue adaptation. In particular, it may be possible to encourage the commitment of endogenous adult stem and progenitor cells to a particular lineage to enhance regenerative potential. There is a reciprocal relationship between cells and tissue during development wherein the tissue type influences the forces to which cells are exposed, while forces determine cellular differentiation and subsequently what tissue type is produced.[18] By introducing specific forces at specific times, resident regenerative cells can be encouraged to commit to a specific lineage and produce a particular tissue type.[19]

Although the nature and timing of the applied forces are critical for cellular responses, the biochemical and physical properties of the matrix to which the cells are attached are equally important. Recent work has shown that altering the stiffness of the ECM directs stem cell differentiation, where increased stiffness directs differentiation to more mechanically competent tissues, including cartilage and bone, but away from commitment to adipose and neuronal tissues.[19] Studies also have shown that controlling the area in which stem cells can adhere to the matrix regulates lineage fate. Stem cells forced to attach on small fibronectin island-like posts assumed a rounded shape, whereas cells attached to larger islands had an elongated morphology with increased Ras homolog gene family member A (RhoA) and Rho-associated protein kinase (ROCK) activity, resulting in enhanced osteogenic commitment.[20] As RhoA/ROCK signaling increases actin stress fiber formation, the spacing of the substrate to which the cells attach informs the physical structure of the cells, and thus their differentiation potential. This knowledge is critical when designing engineered substrates for tissue regeneration, which should not only incorporate bioactive anabolic molecules but also take into account the spatial and physical properties of the matrix to which they are attached. Work is continuing toward generating synthetic extracellular environments capable of directing stem cells to regenerate specific tissues.[21] Introducing appropriate physical stimuli to these engineered components may enhance their regenerative capacity.

In terms of cellular force–sensing machinery, most attention has focused on the ECM-integrin-cytoskeletal signaling axis. Cells of the musculoskeletal system possess transmembrane receptors called integrins, some of which connect extracellularly to ECM proteins and intracellularly to the cytoskeleton, which consists of actin filaments, nonmuscle myosin, and associated proteins (Fig. 3).[22] The cytoskeleton is prestressed and has a tensegrity architecture, a design achieving structural cohesion by creating a dynamic balance between the counteracting forces of compression and tension on the individual struts within the cell.[23] The self-equilibrated mechanical environment within a cell means that any change in
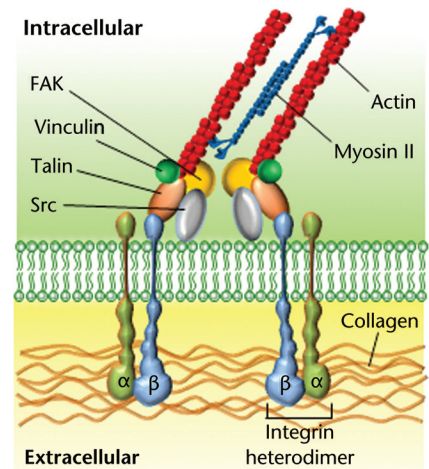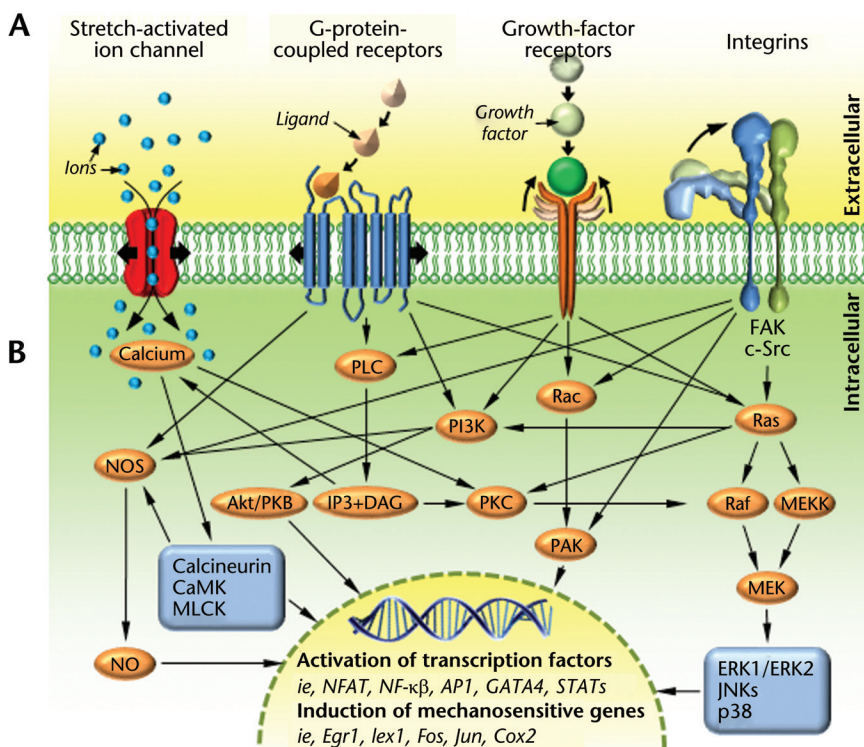

Figure 3.
Transducing mechanical signals into biochemical responses requires unique machinery. Forces are transmitted at the matrix/cell membrane interface where specialized complexes called focal adhesions form. Integrins span the plasma membrane, uniting the extracellular matrix with the internal actin cytoskeleton. Linker proteins, such as vinculin and talin, reinforce the structural integrity of the adhesion complex, and associated signaling effectors, including focal adhesion kinase (FAK) and Src, activate biochemical signaling pathways in response to force.

force within the ECM to which a cell is attached results in disruption of cellular mechanical homeostasis. The resultant conformational changes within the cytoskeleton directly alter chromatin structure and thus modulate gene transcriptional activity via direct connections between cytoskeletal elements and the DNA itself,[24] or by activating intermediate molecular signals by interactions between integrins and intracellular signaling molecules (eg, focal adhesion kinase [FAK] and Src tyrosine kinase).[25] Also, as cells are attached to one another via cadherin-containing adhesion complexes, disruption of cellular mechanical homeostasis of one cell may be mechanically transferred to neighboring cells.[26]

Although it is possible that the ECM-integrin-cytoskeleton axis principally acts to alter the mechanosensitivity of a cell by changing the cell's internal stiffness and how much it pulls on the surrounding ECM,[27] it also is possible that the actual conversion of a mechanical stimulus into a molecular response (ie,

Figure 4.
A variety of extracellular receptors activate an overlapping network of mechanosensitive pathways. (A) Musculoskeletal cells can sense incoming mechanical signals using a diverse group of transmembrane mechanosensitive proteins (mechanosensors), including stretch-activated ion channels, cell-membrane spanning G-protein-coupled receptors, growth-factor receptors, and integrins. The mechanical stimulation of these proteins can lead to changes in their affinity to binding partners or ion conductivity. (B) Mechanical stimulation of the mechanosensors and alteration in their binding capacity or ion conductivity converts the mechanical signal into a biochemical signal (biochemical coupling) triggering intracellular signaling cascades. Many of the pathways overlap sharing signaling molecules. The convergence of the pathways results in the activation of select transcription factors, including nuclear factor of activated T cells (NFAT), nuclear factor-$\kappa\beta$ (NF-$\kappa\beta$), activator protein 1 (AP1), GATA4 (a member of the transcription factor family characterized by the ability to bind the DNA sequence "GATA"), and signal transducer and activator of transcription factors (STATs). The transcription factors translocate to the nucleus and modulate the expression of a panel of mechanosensitive genes, including early growth response 1 (Egr1), lex1, Fos, Jun, and cyclo-oxygenase-2 (Cox2). Ultimately, the net sum of gene-expression reprogramming determines the functional response of the cell to a mechanical stimulus. Akt/PKB=protein kinase B; CaMK=calcium/calmodulin-dependent kinase; DAG=diacyl-glycerol; ERK=extracellular signal-regulated kinase; FAK=focal adhesion kinase; IP3=inositol triphosphate; JNKs=c-Jun N-terminal kinases; MEK=mitogen-activated protein kinase; MEKK=mitogen-activated protein kinase; MLCK=myosin light-chain kinase; NO=nitric oxide; NOS=nitric oxide synthase; PAK=p21-activated kinase; PI3K=phosphoinositide 3-kinase; PKC=protein kinase C; PLC=phospholipase C; Raf=rapidly accelerated fibrosarcoma kinase; Ras=rat sarcoma small GTPase.

Mechanotransduction at the Molecular Level

Once a cell has detected a local mechanical stimulus, the signal needs to be converted into a biochemical response. This process is commonly referred to as *biochemical coupling*. As there are multiple potential mechanosensory mechanisms, there also are multiple signaling pathways that a cell may use to create a biochemical response. Although a detailed description of all of the constantly evolving biochemical pathways underlying mechanotransduction within the musculoskeletal system is beyond the scope of the current article, several common pathways are detailed in Figure 4.

To highlight a few example pathways, integrin-mediated transmission of membrane strain induces activation of several kinases, including focal adhesion kinase (FAK) and Fyn at focal adhesions (contact points where the cell attaches to the matrix),[25] as indicated in Figure 4. These signals activate Akt, resulting in downstream activation of both β-catenin and RhoA, resulting in repression of adipogenic genes. Thus, signals emanating from focal adhesions diverge into 2 pathways, resulting in β-catenin nuclear translocation, which alters transcriptional control,[29] and activation of RhoA, which increases cell stiffness.[25] Here, mechanical transmission through integrins results in reduced formation of fat from mesenchymal stem cell (MSC) precursors. Another example is the ability of force to regulate intracellular calcium entry (Fig. 4). Pharmacological inhibition of mechanosensitive calcium channels results in reduced anabolic responses in bone.[30] Recent work also has implicated an auxiliary voltage sensitive calcium channel subunit, which is partially anchored in the cell membrane, capable of attaching to the ECM, in the mechanical activation of osteocytes.[31] Additionally, these channels are important in cartilage, where inhibition reduces load-induced osteoarthritis in mice.[32]

For a more in-depth discussion of the numerous molecular pathways responsive to mechanical signals, we direct readers to recent reviews.[17,28,33] It is important to point out that some pathways are complementary, whereas oth-

mechanotransduction) is primarily mediated by conformational changes in transmembrane mechanosensitive proteins. These proteins include stretch-activated ion channels, cell membrane spanning G-protein-coupled receptors, and growth factor receptors, to name a few (Fig. 4A).[28] Any changes in normal intra-cellular force transmission through changes in intracellular or ECM structure and organization may lead to altered forces acting on these proteins, resulting in a change in their affinity to binding partners or ion conductivity and the initiation of downstream molecular signaling pathways.

ers are redundant, making it a challenge to decipher the precise contribution and timing of activation of individual pathways. Also, there are likely many other pathways, intermediary molecular players, and pathway interactions that are yet to be identified. Once the mechanical stimulus is transduced into a biochemical signal, it is either transmitted to the nucleus, where it induces expression of mechanosensitive genes, or propagated intercellularly to effector cells. The latter is facilitated by small channels, called connexins, positioned in the cell membrane, which allow small peptides to be passed to neighboring cells or into the extracellular space.[28]

Decoding the biochemical pathways and players involved in mechanotransduction extends beyond scientific curiosity. Altered or reduced mechanotransduction is considered to contribute to a number of musculoskeletal disorders, ranging from osteoporosis and osteoarthritis to muscular dystrophies and sarcopenia.[34] Identifying molecules involved in mechanotransduction may reveal novel targets for therapeutic intervention that not only aid in the management of mechanotransduction-related disorders but also aid in stimulating musculoskeletal tissue regeneration. Manipulation of a target to induce a biochemical signal may independently induce a cellular response or have additive effects when combined with a mechanotherapy. More interestingly, use of pharmacological agents to "target" specific molecular pathways involved in mechanosensitive responses may result in a greater response when superimposed with an appropriate mechanotherapy, resulting in an overall enhanced anabolic stimulus than with pharmacological intervention or the mechanotherapy alone.

Integrating Knowledge of Mechanotransduction Into Regenerative Rehabilitation

Acquiring knowledge in mechanotransduction is a critical component for physical therapists to become leaders in regenerative rehabilitation. Mechanotransduction forms the foundation of mechanotherapies, with mechanotherapies forming one of the largest groups of interventions prescribed in physical ther-

apy. By understanding the mechanical stimuli to which musculoskeletal cells best respond and the mechanisms these cells use to convert mechanical signals into molecular responses, physical therapists may augment the response of musculoskeletal cells to mechanical stimuli. The net result can be the additive or synergistic facilitation of tissue regeneration and restoration of function in individuals receiving regenerative therapies.

Regenerative Rehabilitation: Mechanotransduction at the Cellular Level

Physical therapists typically use extrinsically or intrinsically generated mechanical stimuli to create a tissue force with the goal of evoking a cellular and molecular response (Fig. 1). However, individuals requiring regenerative therapy often have limited or restricted load-bearing capacity, as the introduction of such loads may be potentially detrimental. By understanding the microscopic forces to which cells are exposed and respond, it may be possible to develop novel means of introducing mechanical forces without generating excessive forces at the tissue level.

An example of where the microscopic force that cells are exposed to has been partly deconstructed to develop potential novel mechanotherapies is in bone. There is general consensus that osteocytes embedded throughout the bone matrix are the mechanosensors within the skeletal system.[35] Physical deformation (strain) of the bone matrix is not sufficient to deform the osteocyte cell membrane and initiate a response; however, axial compression and bending increase intramedullary pressure, inducing the flow of interstitial fluid from areas of high pressure (compression) to low pressure (tension) within the lacunocanalicular network housing osteocytes and their dendritic processes.[16] Although extravascular pressure drives a baseline flow of interstitial fluid, flow is heightened by the superimposition of intermittent mechanical loading and exposes osteocytes to fluid flow shear forces.[36] Thus, a small level of tissue strain induces enhanced shear at the cell membrane, enhancing the mechanical stimulus engendered to the cells.

Based on the purported mechanical milieu that osteocytes are exposed to during tissue loading (ie, fluid flow shear forces) and the observation that pressurization of the intramedullary cavity causes an outward pressure gradient that induces interstitial fluid flow,[37,38] investigators have begun exploring how to exogenously enhance intramedullary pressure in the absence of significant tissue loading. Example interventions currently in preclinical development include oscillatory muscle stimulation, dynamic flow stimulation, and dynamic joint loading.[38-43] Each of these modalities increases intramedullary pressure in preclinical animal models to induce bone adaptation, with very negligible tissue loading.[38-40,42-44] Importantly, as the intramedullary cavity contains both hematopoietic cells and MSCs, which are responsive to hydrostatic pressure and fluid flow shear forces, induction of altered intramedullary pressure and interstitial fluid flow via exogenous means has the potential to contribute to regenerative processes.[45] Ultimately, the intramedullary pressure and interstitial fluid flow modalities need to be scaled up to humans before their clinical utility can be realized, but they provide an example of how the microscopic force to which cells are exposed and respond can be developed into potential novel mechanotherapies.

Osteocytes are uniquely positioned to sense mechanical forces; however, MSCs in the bone marrow also perceive force, particularly direct membrane strain, as opposed to the fluid shear stress experienced predominantly by osteocytes.[46] As bone marrow MSCs can differentiate into a variety of tissue types, including fat, cartilage, tendon, and bone, understanding the optimal loading parameters to direct lineage commitment is critical for the incorporation of physical stimuli into engineered tissue components. An ex vivo study has shown that direct membrane strain restricts MSC adipogenesis, providing a larger pool of precursor cells available for differentiation toward cartilage, bone, or tendon.[47] These concepts have been carried over to the development of engineered cartilage grafts, where dynamic compression may

enhance formation and mechanical competence of cartilaginous grafts.[48]

Low-intensity vibration (LIV) also has been developed as a means of mechanically stimulating musculoskeletal cells in the absence of appreciable tissue deformation forces. Low-intensity vibration evolved from the observation that skeletal muscle not only imparts force on bone during locomotion to engender high strain magnitudes but also generates low-magnitude (<100 microstrain [$\mu\varepsilon$]), high-frequency (30- to 90-Hz) stimuli. It induces neither strain[49] nor fluid shear[50] and thus requires a distinct mechanism for the vibration signals to mediate cellular effects. It has been proposed that LIV induces acceleration of the cell nucleus, which may activate mechanosensitive signaling pathways, as the nucleus is tethered by the internal actin cytoskeleton. In support of this proposed mechanism, it was recently shown that LIV-induced activation of mechanosensitive pathways is reduced by physically disconnecting the nucleus from the supporting actin cytoskeleton.[51]

Rubin and colleagues have championed LIV as an exogenous stimulator of bone adaptation. Initially, they showed that adult sheep exposed to LIV stimuli with a magnitude of $<0.3g$ (where g equals the earth's gravitational field) and frequency of 30 Hz for 20 minutes per day over 1 year exhibited a 34% increase in proximal femur trabecular bone density compared with controls.[52] In subsequent clinical trials, Rubin and others[53,54] provided evidence suggestive of a beneficial skeletal effect of exogenously introduced LIV as an inhibitor of bone loss in: (1) a subset of women who were postmenopausal, (2) young women with low bone density, and (3) children with neurologically derived disabling conditions. Although each of these clinical studies possessed important limitations (eg, a relatively small sample size, nonblinding of participants, absence of group differences when using an intention-to-treat analysis) and a more recent study showed no effect of LIV in older adults,[55] the data provide the impetus to further explore LIV as an exogenous mechanical intervention for bone. In addition, the identification of LIV effects on bone heal-ing,[56] other tissues,[57] and stem cell fate[58] requires further consideration. For a more discussion on the clinical applications of vibration therapy, we direct the reader to a recent comprehensive review.[8]

A final example of a novel exogenously introduced mechanical stimulus that has the potential to be safely coupled with other regenerative therapies is low-intensity pulsed ultrasound (LIPUS). Although standard clinical ultrasound therapy has fallen out of favor, LIPUS is an established modality for the management of bone injuries. This modality refers to pulsed-wave ultrasound with a spatial-averaged, temporal-averaged intensity (I_{SATA}) of ≤100 mW/cm^2.[9] This intensity is much lower than that produced by conventional ultrasound units utilized in physical therapy and is introduced daily for 20 minutes. This modality appears to work through a number of different mechanotransductive pathways and has stimulatory effects on MSCs and both chondrogenesis and osteogenesis.[59] Clinical trials have shown LIPUS to reduce the time to both clinical and radiological healing of tibial, radial, and scaphoid fractures by 30% to 38% and stimulate union in 86% of individuals with a nonunited fracture.[60] Of note with regard to regenerative medicine, LIPUS has been safely coupled with other regenerative therapies to improve bone healing in studies involving stem cell therapies[61] and to promote allograft incorporation.[62]

Regenerative Rehabilitation: Mechanotransduction at the Molecular Level

Understanding the biochemical pathways through which mechanical signals are transduced enables potential molecular targets to be identified. Manipulation of a target to induce a biochemical signal may independently induce a cellular response or have additive effects when combined with a mechanotherapy. More interestingly, molecule targeting also may sensitize a specific mechanotransductive pathway such that the superimposition of mechanical loading results in a greater response than with mechanical loading or molecule targeting alone. In order to induce such synergistic effects, it may be necessary to carefully coordinate the timing of mechanical load introduction with peak sensitization of the mechanotransductive pathway.

Studies of the combined skeletal effects of parathyroid hormone (PTH) and mechanical loading provide an example of where the coordinated introduction of a molecule and mechanotherapy can induce synergistic effects. Parathyroid hormone is an anabolic skeletal agent when introduced intermittently and stimulates osteocytes and osteoblasts, in part, through PTH type 1 receptor (PTH1R) activation. The PTH1R in these cells also plays a key role in the bone anabolic response to mechanical stimuli. As PTH and mechanical loading effects are colocalized through the PTH1R, simultaneous introduction of these agents may allow one modality to enhance the cellular response to the other. Indeed, a number of preclinical studies have demonstrated synergistic bone adaptive responses when PTH and mechanical loading were introduced in combination, with PTH appearing to sensitize the cells by enhancing the mobilization of intracellular calcium.[63] In order to translate these preclinical observations to the clinical setting, it is important to note that PTH has a short half-life (75 minutes) and reaches maximal serum concentrations within 15 to 45 minutes following subcutaneous injection.[64] Thus, coupling of a mechanotherapy with PTH administration should be performed in the period immediately following PTH administration in order to optimize any synergistic effects between the modalities.

The above example highlights the potential for synergistic effects when a mechanical stimulus is appropriately timed with the introduction of an agent that sensitizes a mechanotransductive pathway in bone. With the progressive development of new biologically active compounds and molecules that target mechanosensitive pathways, there is a need to explore their combined effects with mechanotherapies. For instance, monoclonal antibody therapies targeting myostatin are progressing toward clinical availability, with phase I and II trials being complete.[65] Myostatin is ex-

pressed in skeletal muscle throughout embryogenesis and is a negative regulator of adult skeletal muscle mass. It binds to activin receptor IIb to inhibit muscle protein synthesis and myoblast proliferation and differentiation.[66] Myostatin is involved in skeletal muscle mechanotransduction, with both physical activity and mechanical loading reducing myostatin signaling by provoking the release of the myostatin inhibitor, follistatin.[67] Inhibition of myostatin signaling through the delivery of propeptides, neutralizing antibodies, and other means stimulates muscle protein synthesis, resulting in gains in muscle mass and function.[65,68] Combining pharmacological inhibition of myostatin with mechanotherapies may lead to greater improvements in functional recovery, with initial work revealing myostatin inhibition to enhance the effects of exercise on performance in aged mice.[69] One further note with regard to regenerative therapies is that myostatin inhibits the activation of satellite cells and promotes transformation of myoblasts into scar-forming myofibroblasts as opposed to myofiber-forming myocytes.[70] Thus, inhibition of myostatin using a biologically active compound may potentiate skeletal muscle regeneration as opposed to repair, with the former potentially being further enhanced via the cointroduction of an appropriate mechanotherapy.

Physical therapists should explore not only the beneficial interactions induced by combining mechanotherapies and biologically active compounds but also unfavorable interactions. One such interaction is a pathway activated in a range of musculoskeletal cells involving the rapid increase in intracellular calcium concentration, induction of cyclo-oxygenase-2 expression, and release of prostaglandin E_2. Interference of this early signaling cascade by introduction of calcium channel blockers, nonsteroidal anti-inflammatory drugs (NSAIDs), or other compounds prior to the delivery of a mechanotherapy may negatively affect adaptive responses.[71] For instance, animal studies have demonstrated that bone formation is substantially blunted when NSAIDs are administered prior to the introduction of a mechanical stimulus.[71,72] Similarly, a recent randomized controlled trial suggested that individuals taking NSAIDs prior to exercise exhibited an impaired skeletal adaptive response.[73] These bone-related findings are supported by studies in muscle and tendon showing that NSAID administration prior to exercise may reduce adaptive responses.[74]

Conclusions

In the last 60 years, the fields of both rehabilitation science and regenerative medicine have expanded exponentially. What were once considered mutually exclusive disciplines are now beginning to recognize their synergistic contributions, resulting in even greater advancements in tissue engineering and rehabilitation outcomes. Regenerative medicine seeks to repair and replace damaged tissues. Likewise, the goal of rehabilitation is to expedite tissue healing to improve physical function. As nearly every rehabilitation intervention introduces force to the affected tissues, therapists must have an appreciation of how those mechanical responses influence biological signals to result in tissue healing. Physical mobility was not always recognized as a crucial component of the healing process, with bed rest once being the modality of choice. Although we now know that early physical intervention promotes healing and integration of implanted prostheses and engineered tissues, we must move beyond a precursory appreciation of the influence of mechanical force on human health. Effective integration of basic science discoveries is needed to guide development of disease- and patient-specific rehabilitation programs. Therapists are already working side-by-side with scientists, physicians, and surgeons to maximize the benefit of regenerative medicine interventions. By continuing to integrate and expand the understanding of tissue-, cellular-, and molecular-level mechanics into practice, physical therapists will become leaders in the discipline of regenerative rehabilitation, ultimately resulting in improved patient outcomes.

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