Review

Mechanotherapy: revisiting physical therapy and recruiting mechanobiology for a new era in medicine

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It has long been thought that the effectiveness and efficiency of physical therapy would improve if our understanding of the cell biology/biochemistry that participates in mechanics could be improved. Traditional physical therapy focuses primarily on rehabilitation, but recent developments in mechanobiology that illuminated the effects of physical forces on cells and tissues have led to the realization that the old therapy model should be updated. To achieve this here, the term mechanotherapy is proposed and recent studies showing how mechanotherapies target particular cells, molecules, and tissues are reviewed. These studies show how mechanical force modulates integrin-mediated processes and other mechanosensors such as gap junctions, hemichannels, primary cilia, transient receptor potential channels (cell targeting), and intracellular mechanosignaling pathways (molecule targeting). The role of mechanical force in various therapies, including microdeformation, shockwave, tissue expansion, distraction osteogenesis, and surgical tension reduction (tissue targeting) therapies, is reviewed. This review aims to jumpstart research into this field, which promises to generate a new era of viable and novel pharmacological and engineering interventions that can overcome human diseases.

Introduction

History of mechanotherapy and the new definition

Our bodies are constantly subjected to mechanical forces that directly affect cellular functions. The effects of gravity on mineral deposition in bones and shear force on atherosclerotic plaque formation in blood vessels are just two examples. The spatial and temporal responses to mechanical forces are currently a growing field of medical research. Of particular interest is how these mechanical forces can be shaped to promote healing or tissue homeostasis or reverse pathogenic processes.

The term mechanotherapy was coined in the 19th century and, as indicated by the Oxford English Dictionary, was initially defined as 'the employment of mechanical means for the cure of disease'. In the 20th century, this term was frequently supplanted by massotherapy. Currently, the term is used to indicate physical therapies, namely, exercise therapeutics for injured tissues in the musculoskeletal system. The main aim of these adjuvant therapies is rehabilitation after the patient recovers from a trauma or surgery. These therapies are seldom used as therapeutic interventions in specific diseases. In 2009, the term was extended to denote 'the employment of mechanotransduction for the stimulation of tissue repair and remodeling' [1]. Typical examples of classical physical therapies are massage and orthopedic rehabilitation, which aim to promote symptom relief or functional recovery towards predisabled/presurgical levels with or without the help of specific equipment or devices.

However, the rapid advances in modern molecular biology, biomechanics, and tissue engineering suggest that physical therapy may also help in the healing or homeostasis of tissues outside the musculoskeletal system as well as in combating specific pathophysiologies and diseases. To promote this understanding, the term mechanotherapy should be updated. The present paper proposes a new definition of mechanotherapy, namely, 'therapeutic interventions that reduce and reverse injury to damaged tissues or promote the homeostasis of healthy tissues by mechanical means at the molecular, cellular, or tissue level' (Figure 1). Candidate target molecules of such mechano-interventions should be seen in the context of their dynamic, integrated, and homeostatic *in vivo* macro- and microenvironments. Thus, mechanotherapies are active mechano-interventions that aim to convert potentially destructive mechanical effects into constructive influences and target normal mechano-adaptation to promote recovery.

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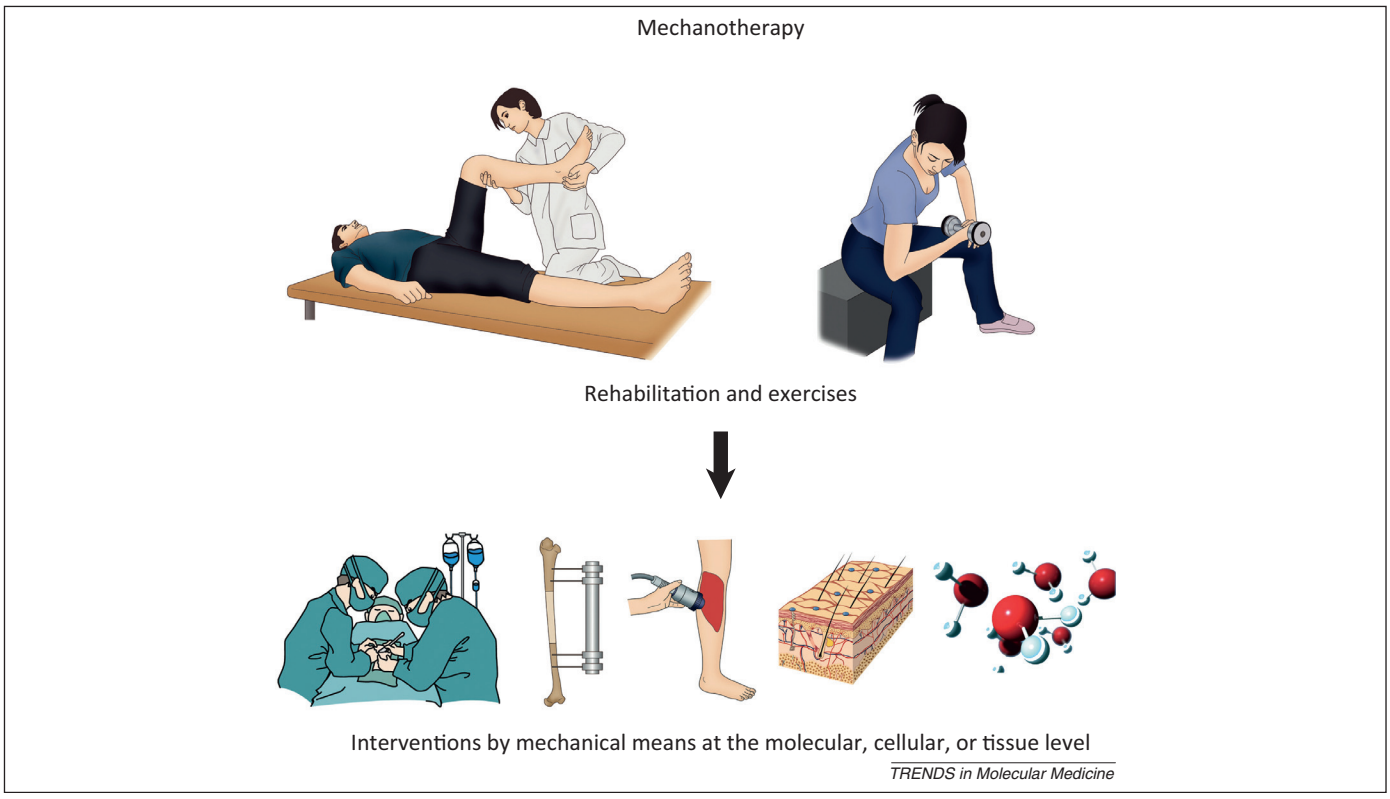

Figure 1. The definition of 'mechanotherapy'. The concept of 'mechanotherapy' should be expanded so that it not only includes physical therapy (e.g., massage and orthopedic rehabilitation) but also encompasses interventions by mechanical means at the molecular, cellular, or tissue level that reduce and reverse the injury to damaged tissues or that promote the homeostasis of healthy tissues.

To develop mechanotherapies, several disciplines have to be integrated. These disciplines include mechanotransduction, which elucidates the processes by which physical forces are sensed, transduced, and then transformed into intracellular biochemistry and gene expression [2]. They also include bioinformatics: studies have shown that by combining computer science and information technology, mechano-responses can be modeled accurately. Another important discipline is tissue engineering and regenerative medicine: advances in this field have produced mechanically viable and functionally active products that permit the reversal of pathophysiologies/diseases.

Classification of mechanotherapy

Given the many disciplines that are involved and the many ways they can be applied, mechanotherapy can be classified in various ways: on the basis of the mechanics involved, the tissues that are targeted, or the levels of the mechano-interventions (Table 1). Because the most successful mechanotherapies to date, namely, the microdeformation, shockwave, and tissue expansion therapies, center on soft tissues (discussed below), this review describes these therapies after first reviewing mechanotherapies that are at the cellular and molecular levels. Within this structure, the molecular mechanisms that underlie the effectiveness of these mechanical manipulations will be discussed. The aim of this review is to inspire further pharmacological and engineering exploration. Note that traditional physical therapies such as massage and electrotherapy will not be fully discussed.

Mechanotherapy at the cellular level

Our improving understanding of mechanics-driven disease-associated cellular dysfunction is gradually paving

Table 1. Classification of mechanotherapies

Classification	Type		Example
Mechanics-dependent	Constructive	Positive	Tissue expansion
		Negative	Negative pressure wound healing
	Destructive	Physiological	Tension-shielding therapy
		Pathological	Mechanics-unload protection
	Auxiliary		Hyperbaric oxygen
Target tissue-dependent	Soft tissue		Tissue expansion
	Hard tissue		Distractive osteogenesis
Purpose-dependent	Treatment		Shock wave therapy for wound healing
	Prevention		Surgical tension reduction
Intervention level-dependent	Tissue level		Vacuum-assisted closure
	Cellular level		Cell shape-guided migration or lineage switching
	Molecular level		Mechanotransduction signaling molecule

the way for the development of interventions that target these mechanisms. Of particular interest currently are the integrin-mediated mechanisms that alter various cellular functions, including cytoskeleton-related tensegrity, cell–matrix interactions, and cell shape-dependent functions. Other mechanosensors are also of considerable interest, including gap junctions (GJs), hemichannels, primary cilia, and transient receptor potential (TRP) channels.

Transmembrane integrins transfer mechanical forces from the extracellular matrix (ECM) to the cytoskeleton by inducing the formation of focal adhesion complexes. This reorients the signal transduction machinery of the cell (e.g., tyrosine kinases) [3], which in turn alters cytoskeletal functions and induces ECM remodeling [4]. The cytoskeleton can thus be seen as a 'global signal integrator' [5]. This function is mediated by the tensegrity architecture of the cytoskeleton, which is a self-assembling system driven by structural hierarchies and the tensile pre-stress of the cell that yields a dynamic balance between counteracting forces of compression and tension; this leads to a self-equilibrated mechanical stability. This architecture links macro-mechanic forces to molecular changes [4,6,7]. Tensile forces received by the cytoskeleton of a cell from the ECM not only change the cell that receives the signals but are also transferred to neighboring cells through cadherin-containing cell–cell adhesion complexes [8]. Thus, cells are connected to each other and to the ECM, thereby forming a dynamic system that can be manipulated by potential mechanotherapeutic interventions at the cellular level. Examples of promising targets are the epigenetically upregulated integrins in Hep3B cells: targeting these integrins could abrogate cellular migration, thereby preventing hepatocellular carcinoma migration [9].

The ECM is a dynamic, mobile, and multifunctional regulator of cellular behavior, and thus is not just a scaffold for cells and a storehouse for cytokines. The cells also use the elasticity/rigidity of the ECM microenvironment to actively exert traction force on the ECM, which in turn alters the ECM [10]. Thus, there is a dynamic mechanical equilibrium between cellular traction forces and ECM resistance sites that links the cells and the ECM in a state of mechano-reciprocal isometric tension [11]. Several lines of evidence suggest that manipulating this mechanical equilibrium could promote tissue regeneration. First, different matrix elasticities direct the human mesenchymal stem cell (MSC) lineage differentiation in a highly specific manner: in identical serum conditions, soft, stiffer, and rigid matrices induce neurogenic, myogenic, and osteogenic differentiation, respectively [12]. Second, matrix rigidity is the foundation of durotaxis, which is where gradients in substrate rigidity guide cell migration. Fibroblasts have a preference for stiff substrates and, thus, when they are placed on flexible polyacrylamide sheets, they will migrate from the soft to the stiff side [13]. Moreover, the migration speed of human glioma cells *in vitro* is affected by the ECM geometry: for a given ECM stiffness, these cells move more quickly if they are confined to narrow channels than if they are located in wide channels or on unconstrained 2D surfaces [14]. This is attributed to an increased polarization of cell–ECM traction forces. Third, the cell stiffness of metastatic cancer cells is >70% lower than that of normal cells in the same sample [15]. This low stiffness may be caused by a loss of actin filament and/or microtubules and the subsequent lower density of scaffold [16].

These observations suggest that the cytoskeleton is the axiomatic target of mechano-interventions. By targeting the cytoskeleton, thereby distorting the cell or changing the cellular geometry, one may be able to control the fate and behaviors of that cell. Indeed, new studies show that altering the cell shape changes cellular function, probably because altering the cytoskeleton generates organizational guidance cues for the cell [17]. Several lines of evidence support this hypothesis. First, tension-dependent changes in cell shape and cytoskeletal structure not only influence cell cycle progression but also control cell proliferation [18]. Second, cell shape change and/or compromised structural integrity of the cytoskeleton can be an apoptosis signal [19]. Third, cell shape distortion can govern stem cell lineage switching: human MSCs will differentiate into bone or adipose tissue when their spreading is promoted or restricted, respectively [20]. Fourth, during migration, constraining the cell shape controls the direction in which the cell extends its leading edge. [21]. Moreover, by completely preventing cell spreading, the cellular phenotype can be switched from differentiation to apoptosis, even when cells remain anchored to the ECM [22]. Fifth, vascular smooth muscle cells (VSMCs) adopt a contractile phenotype when a spindle-like cell shape is forced, even in the absence of concurrent changes in contractile markers [17].

The discoveries showing that integrin acts as a mechanosensor and could participate in mechanotherapy have heightened interest in the relationship between integrins and several other cellular mechanosensors, namely, GJs, hemichannels, primary cilia, and TRP channels. GJs are membrane-spanning channels through which small molecules (<1 kDa) are passed. They are composed of two juxtaposed hemichannels formed by connexins (Cxs) [23]. The shear-induced opening of Cx43 hemichannels, which allows the passage of bone anabolic factors such as prostaglandin E_2, requires that integrin $\alpha_5\beta_1$ interacts directly with Cx43. This interaction could be a target for preventing bone loss due to mechanical immobilization [24]. Primary cilia, which are the microtubule-based organelles that project from the cell surface, can serve as mechanosensory organelles in chondrocytes or endothelial cells (ECs) in response to compression [25] or fluid shear [26], respectively. Indeed, the removal of these cilia abolishes the ability of the cells to sense such stimuli [27]. The primary cilia of VSMCs express α_3- and β_1-integrins and ciliary integrin–ECM interactions direct, at least in part, VSMC migration in a wound scratch model [28]. The TRP channels are a group of ion channels that are mostly located on plasma membranes. It has been suggested that these channels are important in relaying mechanical stimuli, although they may participate in mechanosensitivity indirectly rather than via their direct activation [29,30]. These observations suggest that these channels could be a target of mechanotherapies. The link between integrin and TRP channels is shown by the fact that TRPV4 is activated in mechanical hyperalgesia in rats and that this is associated with a direct interaction with integrin α_2 and Src tyrosine kinase [31]. Notably,

treatment of rat, mouse, and guinea pig models with capsazepine, a TRPV1 antagonist, reverses mechanical hyperalgesia in inflammatory and neuropathic pain [32].

Mechanotherapy at the molecular level

Progress in mechanotransduction research has led to the identification of target signaling pathway molecules that can be used to promote soft tissue and bone cell health or to treat diseased cells in these tissues.

Mechanotransduction signaling pathways contain numerous potential targets for interventions. Intracellular calcium ion signaling, prostaglandin and nitric oxide (NO) signaling, and Wingless-type (Wnt)/β-catenin signaling are involved in bone repair and regeneration [33], whereas to prevent or treat benign or malignant fibroproliferative disorders, integrin signaling, transforming growth factor-β (TGF-β)/Smad, mitogen-activated protein kinase (MAPK) signaling, Rho-associated protein kinase (ROCK) signaling, tumor necrosis factor-α (TNF-α)/nuclear factor kappa light-chain-enhancer of activated B cells (NF-κB) signaling, and Wnt/β-catenin pathways would be good candidates (Figure 2) [34]. Given the mechanosensitivity and mechanoresponsiveness of a particular target cell (e.g., osteocytes and fibroblasts), it may be possible to manipulate the behaviors of these cells by inhibiting specific mechanosignaling molecules, thereby preventing or treating the corresponding diseases (e.g., osteoporosis and fibrosis, respectively). Such inhibition could be generated by small interfering RNA (siRNA), neutralizing antibodies, or competitive inhibitor proteins. Naturally, because many molecules play multiple roles in various pathways and the different pathways crosstalk, such molecular therapies will have to be sufficiently specific to avoid uncontrollable and unwanted effects [35]. Nevertheless, the field of mechanotransduction-oriented molecular therapy has enormous potential.

Mechanotherapies that target mechanotransduction signaling pathways can mainly aim to modulate one of their four phases [36]: (i) the mechanocoupling phase, where the external mechanical signal is converted into a mechanical signal in the vicinity of the cell; (ii) biochemical coupling, where the local mechanical signal is transduced into a biochemical signal, resulting ultimately in genetic or protein changes; (iii) signal transmission, where the biochemical signal is then passed from the sensor cells to the effector cells; and (iv) the effector cell response (Figure 2). A recent study has shown that controlling the coupling of the physical forces with the chemical signaling networks by spatiomechanical regulation effectively controls cellular behavior: the simple mechanical restriction of surface molecule movement can alter downstream cellular activities, as observed by changes in the cytoskeleton morphology of human breast cancer cells and their recruitment of a disintegrin and metalloprotease 10, as the cellular response to ephrin-A1, when their erythropoietin-producing hepatocellular receptor A2 (EphA2) is interfered with

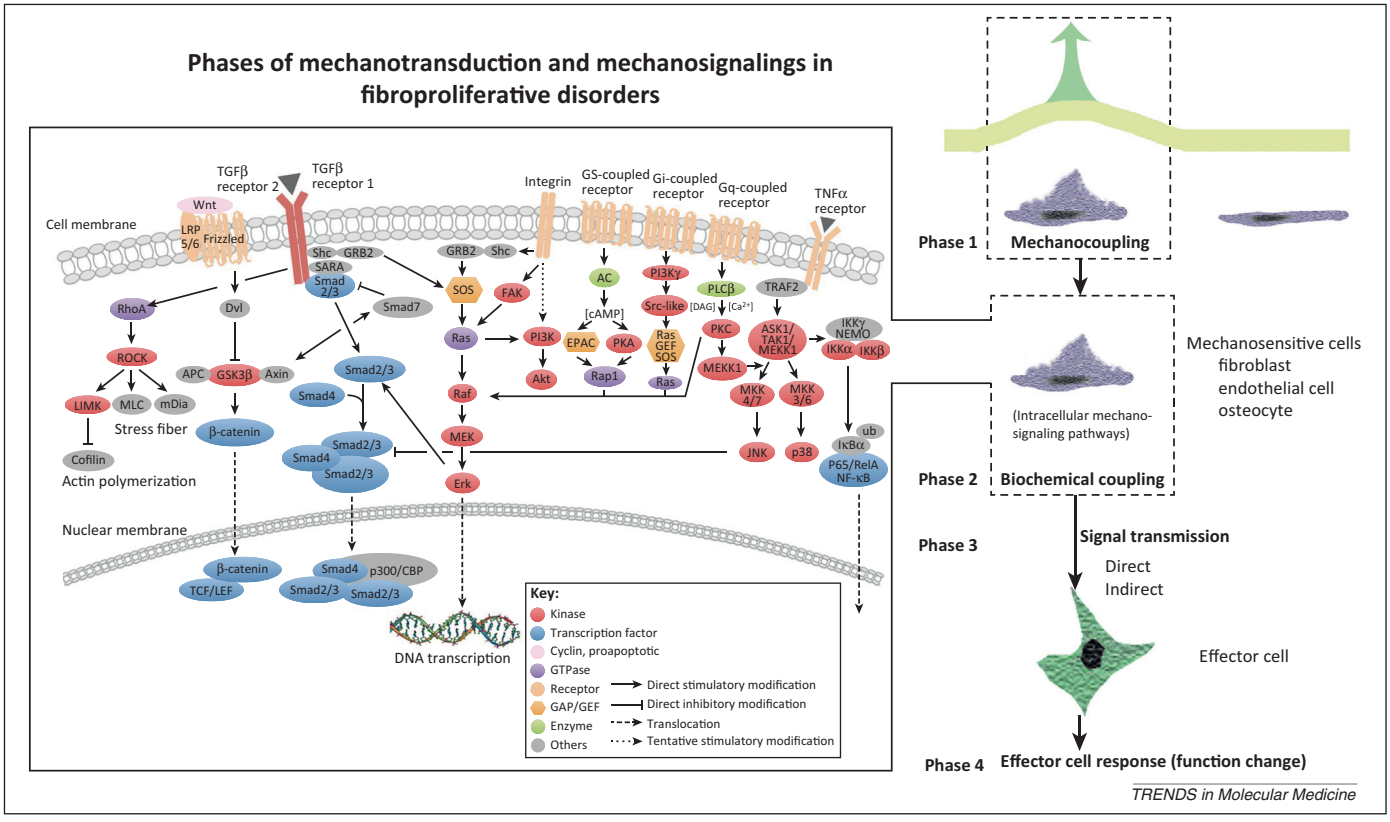

Figure 2. Mechanotransduction phases and the intracellular pathway cascades in soft tissue. Mechanotherapies that target mechanotransduction signaling pathways can mainly aim to modulate one of their four phases. Phase 1: the mechanocoupling phase, where the external mechanical signal is converted into a mechanical signal in the vicinity of the cell; phase 2: biochemical coupling, where the local mechanical signal is transduced into a biochemical signal, resulting ultimately in genetic or protein changes; phase 3: signal transmission, where the biochemical signal is then passed from the sensor cells to the effector cells; and phase 4: the effector cell response. Mechanosignaling varies in different diseases. For fibroproliferative disorders, the integrin, TGF-β/Smad, MAPK, RhoA/ROCK, TNF-α/NF-κB, and Wnt/β-catenin signaling pathways are good candidates for mechanotherapeutic interventions. Abbreviations: TGF-β, transforming growth factor-β; MAPK, mitogen-activated protein kinase; ROCK, Rho-associated protein kinase; TNF-α, tumor necrosis factor-α; NF-κB, nuclear factor kappa light-chain-enhancer of activated B cells; Wnt, Wingless-type.

transporting radially inwards in response to membrane-bound ligand stimulation by physical barriers nanofabricated on the underlying substrate [37]. This approach may yield promising therapeutic interventions in cancer [38]. Interventions can also target other signal transmission phases. For example, long-term treatment with TGF-β1 impairs stress-induced autoregulation of vascular tone by inhibiting mechanotransduction (but not mechanoreception). This effect involves RhoA membrane translocation, and the ultimate effect of the treatment is to reduce the basal levels of membrane-bound RhoA [39]. In addition, small molecule inhibition of focal adhesion kinase (FAK) effectively uncouples mechanical forces from pathological scarring [40]. Moreover, siRNA-induced FAK knockdown impairs the proliferation, differentiation, and collagen synthesis of cardiac fibroblasts in response to cyclic stretch, which indicates that it has the potential to reduce myocardial fibrosis [41]. However, because the field of molecular mechanotherapy is in its infancy, it is still too early to determine the efficiency, sensitivity, and specificity of mechano-interventions that target molecules in the cross-talking mechanotransduction cascades that operate in the dynamic temporal and spatial microenvironment of the cell.

Mechanotherapy at the tissue level

Tissue level mechanotherapies mainly focus on improving wound healing and include microdeformational wound therapy (MDWT), shockwave therapy, soft tissue expansion, distraction osteogenesis, and surgical tension reduction. Other procedures that shape mechanobiology at the tissue level include hyperbaric oxygen (HBO_2), lung-protective and cardiac offloading strategies.

MDWT

MDWT, also called vacuum-assisted closure (VAC) or negative pressure wound therapy (NPWT), involves the application of a vacuum to a wound surface by packing the wound with a porous polyurethane sponge and then sealing the wound with occlusive dressing connected to the vacuum [42] (Figure 3). It is effective for treating acute wounds as well as chronic, open, large, and contaminated wounds [43]. Its effects are largely the result of removing extracellular fluid, stabilizing the wound environment, generating contracture of the wound or macrodeformation, and inducing microdeformation at the foam–wound interface [44]. It also regulates neovascularization, inflammation, and cellular energy levels. The biomechanically driven neovascularization caused by MDWT over time is promoted via angiogenesis [45]. It may also be explained by the nonangiogenic expansion of pre-existing vessels [46]. The neovascularization is related to increased levels of vascular endothelial growth factor (VEGF) proteins and potentially involves the hypoxia-inducible factor-1α–VEGF pathway [47]. MDWT also removes infiltrating leukocytes *in vivo* meanwhile increasing gene expression of leucocyte chemoattractants such as interleukin-8 (IL-8) and CXCL5 [48]. In addition, the MDWT effects are mediated by early and continuous activation of mast cells, as shown by MDWT-induced mast cell-dependent collagen maturation [49]. Moreover, MDWT increases the immunoreactivity of

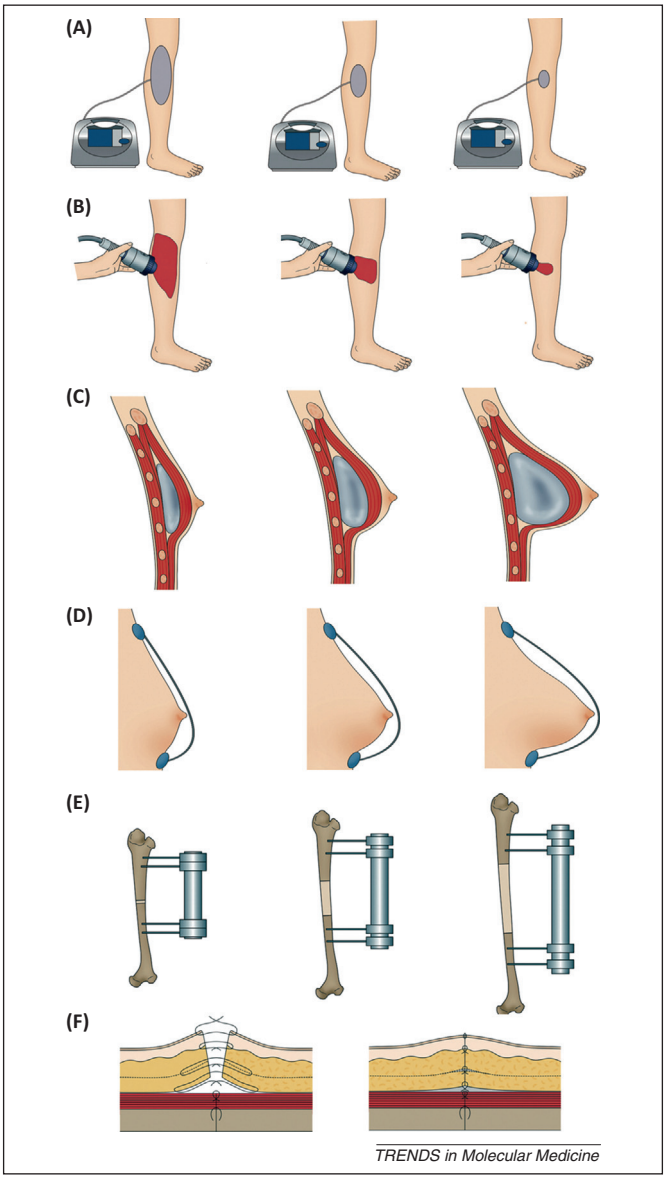

Figure 3. Mechanotherapies at the tissue level. **(A)** Microdeformational wound therapy: aberrant wound healing can be improved by microdeformational wound therapy (also known as vacuum-assisted closure) because the device provides mechanical stimulation that accelerates wound healing. **(B)** Shockwave therapy: although mechanical stimuli from outside the body can be harmful for wound healing, their careful application in extracorporeal shockwave therapy (ESWT) may improve wound healing. **(C)** Internal soft tissue expansion: the soft tissue expansion technique is a widely applied procedure that systematically overstretches the skin in a controlled mechanical manner to generate additional skin endogenously in a 3D manner. **(D)** External soft tissue expansion: the external volume expansion (EVE) devices apply force on tissues to expand their volume. They are now mainly used in the clinic for breast augmentation. **(E)** Distraction osteogenesis: also called bone expansion, induces osteogenesis and is used to correct limb and craniofacial complex defects. Direct membranous ossification across the distraction gap is induced by turning the nuts on the rods. This eventually results in the bridging of the large bony defect. **(F)** Surgical tension control: surgery involves the control of tissue tension. Surgical tension-reducing techniques, such as subcutaneous/fascial sutures, can reduce the risk of the development of pathological or ugly scars.

neuropeptides (substance P and calcitonin gene-related peptide) and neurotrophin (nerve growth factor) [50], which suggests that this therapy also upregulates neurogenic inflammation to some extent. In terms of cellular energy levels, there is *in vitro* evidence showing that MDWT of human dermal fibroblasts in a provisional wound matrix elevates their energy charge, ATP/ADP and cytochrome

c oxidase levels, and increases their protein concentrations of TGF-β, platelet-derived growth factor-α (PDGF-α), and PDGF-β [51]. Similarly, mouse dermal fibroblasts treated by a suction/foam/perfusion bioreactor show upregulation of basic fibroblast growth factor (bFGF), TGF-β1, Type I collagen α_1, and smooth muscle actin α_2 mRNA expression [52]. However, the mechanotransduction pathways involved in these effects remain to be clarified.

Shockwave therapy

The shockwaves that are commonly used in extracorporeal shockwave therapy (ESWT) are biphasic high-energy acoustic waves that can be generated by electrohydraulic, electromagnetic, or piezoelectric technologies. ESWT is a well-known lithotripsy method whose potential in promoting wound healing is currently being investigated vigorously (Figure 3). It has been shown to be effective in both diabetic [53] and surgical wounds [54]. There is evidence that it reduces wound size [55], accelerates re-epithelialization [56], improves blood perfusion [57], decreases pain [58], and reduces necrosis [59]. Histological analyses show that these clinical outcomes are induced by increased neovascularization [60], revascularization [61], collagen synthesis [62], and proliferation [57], and by reducing apoptosis [63]. At the molecular level, it has been shown that shock wave therapy upregulates TGF-β1 expression in fibroblasts [64] and suppresses the production of the proinflammatory cytokines IL-1β, IL-6, and TNF-α [65]. It also triggers anti-inflammatory activity by, for example, increasing neuronal NO synthase (nNOS) activity and NO production (in the C6 rat glioma cell line) while concomitantly downregulating NF-κB and TNF-α gene expression [66]. That shockwave therapy probably induces mechanotransduction and immunomodulation in wound healing shapes its future development [67].

Soft tissue expansion therapies

The soft tissue expansion technique is a widely applied procedure that systematically overstretches the skin in a controlled mechanical manner to generate additional skin endogenously. It can be performed in an invasive or noninvasive manner. An invasive form of this technique involves the surgical, subcutaneous insertion of an inflatable soft tissue expander, and gradual injection of saline solution into the expander in the following weeks. This causes the skin to become overstretched (Figure 3) and to generate new skin to accommodate the expander. The newly generated skin can then be used in plastic surgery to cover skin defects or reconstruct organs (e.g., ear and nose). At the biomechanical level, this technique exploits the viscoelastic properties of the skin, namely, tissue creep [68] (where a constant load extends the skin over time) and stress relaxation (where the force required to maintain a stretch at a fixed length decreases over time) [69]. Tissue creep is generated by mechanical creep, where the skin is incrementally stretched in an acute manner, followed by biological creep, where the persistent chronic stretching force generates new tissue partly by increasing mitotic activity and neovascularization [70]. At the molecular level, soft tissue expansion therapy involves strain-induced changes in growth factors [e.g., epidermal growth factor (EGF)], protein kinases (e.g., PKC), and second messengers (e.g., cAMP) [71].

A noninvasive form of soft tissue expansion therapy is the traction-assisted dermatogenesis technique. Here, serial intermittent strips of skin tape are applied to the normal skin around defects. The aim is to stretch this normal skin as much as possible so that the resulting lax skin can later be used to cover the defects. It causes 2D skin expansion that is suitable for combination with other techniques such as MDWT that enable the reconstruction of complex dermal wounds by promoting both mechanical and biological creep [72].

The noninvasive external volume expansion (EVE) devices also apply force to enhance cell and tissue engraftment and to directly augment tissue volume, mainly for breast augmentation (Figure 3). The typical BRAVA device consists of a rigid plastic dome connected to a negative pressure pump and it serves to pre-expand the breast recipient site for fat grafting. It is useful for fat grafting because it produces bigger parenchymal spaces, reduces interstitial hypertension, augments contour irregularities, avoids variables such as centrifugation during grafting, and stimulates angiogenesis [73]. When this external, low-level and sustained mechanical distraction of around 20 mmHg vacuum pressure is applied through a brassiere-like system for over 10 weeks, it results in a stable long-term increase in breast size of 55% on average [74]. Furthermore, use of an EVE device on mouse integument (by using a soft silicone dome connected to a vacuum source) revealed that continuous 25 mmHg suction for 28 days led to a 2-fold increase in subcutaneous fat layer thickness and a 2.2-fold increase in adipocyte number [75].

Distraction osteogenesis therapies

Distraction osteogenesis, also called bone expansion, induces osteogenesis and is used to correct limb and craniofacial complex defects (Figure 3). A widely applied distraction osteogenesis method is the Ilizarov technique, which creates a dynamic mechanical environment that induces customized bone regeneration. It can effectively correct limb deformities caused by congenital defects, mal- or non-union open fractures, osteomyelitis, and tumor resection. This method involves the use of the Ilizarov external fixator, which is composed of metal rings, threaded rods, and Kirschner wires. Direct membranous ossification across the distraction gap is induced by turning the nuts on the rods. This eventually results in the bridging of the large bony defect [76]. In a mouse model, distraction osteogenesis activated bone morphogenetic protein (BMP) signaling molecules: the expression of the osteoinductive BMP2, 4, and 6 molecules was upregulated along with the expression of their activin receptors type 1 and type 2b, and the downstream transcription factors such as Smad1, 4, and 8. The expression of the BMP antagonists (noggin and chordin) and receptor antagonists (inhibin and BMP3) was also upregulated [77].

Distraction osteogenesis is also effective in craniofacial complex reconstruction. Versatile devices that induce external unidirectional or bidirectional distraction, multiplanar distraction, or internal distraction have been developed to induce mandibular distraction and widening,

ridge augmentation, and midface distraction [78]. It was found that high strain caused the formation of fibrous or cartilaginous tissue, whereas low strain induced bone formation [79]. Analysis of the molecular mechanisms involved in midpalatal suture expansion in mice revealed that one of the mechanosensors involved is polycystin-1 [the protein product of the polycystic kidney disease 1 (*Pkd1*) gene], and *Pkd1* is required for the survival, proliferation, and differentiation of periosteal osteochondro-progenitor cells in response to mechanical stimulation of the suture tissue [80]. Similarly, in a mandibular distraction osteogenesis model in rats, c-Src and BMP 2/4 expression colocalizes. This indicates the involvement of the integrin-mediated mechanotransduction pathway, of which c-Src is a key component [81].

Surgical tension reduction/tension shielding

Mechanotherapies are highly useful for preventing and treating pathological scars and reducing their recurrence. The association of pathological scars with skin tension is indicated by their site-specific distribution: most of these scars occur in areas of the body that are subjected to frequent mobility and/or high stretching tension, such as the chest wall [82]. Significantly, mechanical stress applied to a healing wound successfully produces hypertrophic scars in mice [83]. These destructive local mechanical forces on the wound can be alleviated by employing refined surgical tension-reducing techniques, such as a small-wave incision design [84], local flaps to cover the wound, silicon sheeting [85], and subcutaneous/fascial sutures (Figure 3) [86]. Recently, a stress-shielding method based on a dynamic polymer device was found to reduce the histological scar area of incisions in swine by 9-fold compared with incisions in a stressed state; a subsequent Phase I clinical study showed that the stress-shielding device decreased hypertrophic scar formation in humans with high-tension abdominoplasty incisional wounds that are prone to excess scarring [87].

Other biophysical therapies

Apart from these well-established clinical therapeutic approaches, all of which aim to employ mechanobiology, there are a number of other mechanics-related treatments that combat specific pathophysiologies/diseases but were found only incidentally to involve mechanobiology during analyses of the relevant disease etiology or the mechanisms by which these therapies work. These therapies include HBO_2 treatment and mechanics-unloading prevention therapies. HBO_2 treatment involves placing the patient in a chamber containing 100% oxygen under a pressure that is higher than the atmosphere. Its beneficial effects appear to relate to both the elevated pressure and the hyperoxia [88]. TGF-β and VEGF are upregulated by the treatment [89]. However, whether mechanobiology plays a key role in the positive effect of HBO_2 treatment remains to be determined.

Whereas the mechanical force in HBO_2 may serve as an 'accelerator', the reverse approach is used in lung-protective and cardiac offloading strategies: here, a 'brake' is applied to attenuate or reverse potential mechanics-related injury. Lung-protection strategies include the restriction of tidal volume and the use of relatively high levels of positive end-expiratory pressure; these approaches protect the lung from mechanical ventilation harm (e.g., barotraumas and oxygen toxicity) [90]. Studies on the effects of these strategies at the molecular level reveal that phosphoinositide 3-kinase (PI3K)-kinase/Akt/endothelial NOS (eNOS) signaling is protective in ventilation-associated lung injury in mice [91]. Moreover, incubation of fetal rat alveolar type II epithelial cells with IL-10 protect them from overstretch injury, thereby promoting their resistance to the negative effects of mechanical ventilation [92].

Cardiac offloading is a therapy for heart failure that works in a similar manner to the lung-protective strategy. Cardiac offloading with left ventricle offloading devices has been shown to improve oxygen supply and blood perfusion, and reduce the afterload of the myocardium [93,94]. In a rodent model of heart failure, mechanical unloading normalizes local Ca^{2+}-induced Ca^{2+} release and reverses pathological tubule remodeling, whereas t-tubules are disrupted in mechanical overload and heart failure [95].

Opportunities and challenges in the development of new mechanotherapies

The accumulating understanding and techniques in mechanobiology open the door to many potential novel mechanotherapies. This is particularly true in tissue engineering that aims to generate tissue *in vitro* for implantation. Given the daunting complexity of the *in vivo* biomechanical microenvironment, such *in vitro* tissue engineering is essential. However, to be successful, the *in vitro* system must not only use the three traditional tissue engineering components (namely, the stem cells, the scaffold, and the growth factor), it must also simulate the *in vivo* mechanical environment adequately. The appropriate application of mechanical stimuli will help to optimize the stem cell niche and enhance nutrient transport and waste removal (which will improve cell viability), customize scaffold compliance and substrate stiffness, and facilitate the synergistic effects of biochemical and mechanical factors. Physical interventions can also be used to directly regulate cell fates and functions into the desired direction. An example of this is cartilage tissue engineering: high osmotic pressure upregulates the catabolic function of chondrocytes [96], whereas cyclic hydrostatic pressure (HP) enhances the chondrogenic differentiation of adipose-derived stem cells in a 3D collagen scaffold [97]. These observations shed light on how to construct this avascular tissue, which has poor innate repair ability. Similarly, in bone engineering, cyclic HP via an *in vitro* bioreactor improves the osteogenic differentiation and maturation of human bone marrow-derived MSCs (although this is achieved to some extent at the expense of their proliferation and self-renewal) [98]. Notably, an *in vivo* bioreactor has been created in rabbits by injecting calcium-alginate gel between the tibia and the periosteum: this induces osteogenesis and allows bone that is biomechanically identical to native bone to be grown without having to use cell transplantation or growth factor administration [99]. This engineered bone shows complete integration after transplantation into contralateral tibial defects.

Although mechanotherapy is a highly promising field, developments in this field are confronted with hugely challenging questions, particularly with regard to specificity, selectivity, and timeliness. In terms of specificity, mechanical stimuli should be applied in a cell-type specific manner. Although all cells in the *in vivo* liquid microenvironment are subjected to some common mechanical forces such as gravity or HP, different types of cells [e.g., osteocytes, ECs, or fibroblasts] can vary in terms of their mechanosensitivities. In other words, their thresholds with regard to particular mechanical stimuli may differ. Such type, amplitude, duration, and frequency preferences could be utilized to make a therapy more cell-specific. In terms of selectivity, it is necessary to identify the key molecules whose targeting by a therapeutic intervention would have a highly selective effect. Signaling molecules often crosstalk and it can be challenging to identify and distinguish specific target 'signals' from 'noise', as well as to avoid side effects that arise from their interactions. Finally, the timeliness of mechanotherapy *in vivo* should be considered seriously. Mechanical forces of a therapy should be confined to a certain period of time, or applied in a dynamic and finely tuned manner. Indeed, it may be as therapeutic to halt an ongoing mechanical stimulus at a given time as it would be to actively initiate it.

The field of mechanobiology-based mechanotherapy is still young. However, it promises many viable and novel medical therapies. Ongoing and future research will no doubt gradually bring mechanobiology down from the ivory molecular tower, thereby making mechanotherapies clinically accessible on a larger scale.

Concluding remarks

In this article, the term mechanotherapy was redefined to better reflect the enormous promise of the mechanobiology field and our present understanding of the effects of mechanotherapy at the tissue, cellular, and molecular levels. We showed that potentially destructive mechanical effects can be converted into constructive influences and that normal mechano-adaptive responses can be actively targeted by mechano-interventions to combat specific pathophysiologies/diseases and promote healing or homeostasis. We emphasized the fact that candidate target molecules of mechano-interventions should be understood in the context of their dynamic and homeostatic *in vivo* macro- and microenvironments, as this will allow us to predict and improve therapeutic efficacy and reduce side effects. The coining of the term mechanotherapy aims to jumpstart research in this promising field that will promote a new era of medical, biological, and engineering interventions that can overcome human diseases.

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