

Mechanotransduction in bone: Intervening in health and disease

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Abstract

Mechanotransduction has been proven to be one of the most significant variables in bone remodeling and its alterations have been shown to result in a variety of bone diseases. Osteoporosis, Paget's disease, orthopedic disorders, osteopetrosis as well as hyperparathyroidism and hyperthyroidism all comprise conditions which have been linked with deregulated bone remodeling. Although the significance of mechanotransduction for bone health and disease is unquestionable, the mechanisms behind this important process have not been fully understood. This review will discuss the molecules that have been found to be implicated in mechanotransduction, as well as the mechanisms underlying bone health and disease, emphasizing on what is already known as well as new molecules potentially taking part in conveying mechanical signals from the cell surface towards the nucleus under physiological or pathologic conditions. It will also focus on the model systems currently used in mechanotransduction studies, like osteoblast-like cells as well as three-dimensional constructs and their applications among others. It will also examine the role of mechanostimulatory techniques in preventing and treating bone degenerative diseases and consider their

applications in osteoporosis, craniofacial development, skeletal deregulations, fracture treatment, neurologic injuries following stroke or spinal cord injury, dentistry, hearing problems and bone implant integration in the near future.

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Key words: Mechanotransduction; Bone remodeling; Bone disease; Bone health

Core tip: Mechanotransduction has been shown to be of major significance in modulating bone remodeling under physiological and pathological conditions. Therefore the study of the underlying mechanisms is of major importance and necessary step towards the better understanding of bone biology as well as the development of therapeutic strategies against conditions characterised by deregulated mechanotransduction. This review will consider the molecular mechanisms behind mechanotransduction as well as the scientific models currently used for its better understanding. It will also focus on mechanostimulatory techniques that could be used against a variety of deregulated mechanotransduction-related diseases.

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INTRODUCTION

Bone tissue biology

The importance of bones for a living organism is undeniable and goes far from just providing structural support for the body, protecting vital organs and exchanging minerals. Bones also comprise a multi-functional system that

interacts with other systems and abnormalities in bone tissues may result in mild or severe diseases.

Bone tissue is composed of the bone matrix and five different cell types. The bone matrix contains an inorganic (carbonated hydroxyapatite) and an organic phase (mainly type I collagen and several growth factors) whilst the cellular content of the bone tissue comprises of osteoprogenitors, osteoblasts, osteocytes, osteoclasts and lining cells^[1]. Osteoprogenitor cells comprise pluripotent cells of mesenchymal origin, localised on bone surfaces^[1] which have the ability, under the appropriate conditions, to commit and differentiate towards osteoblasts^[1]. On the same bone osteoblasts, the bone forming cells, are cited. They are responsible for the protein synthesis of the bone matrix as well as its calcification^[1]. The cavities of the calcified bone matrix bear osteocytes which comprise entrapped inactive osteoblasts forming a net of communicating cells inside the calcified matrix^[1]. Osteoclasts are large multinucleate cells of blood monocyte origin, settled inside bone resorption lacunae and they are responsible for bone resorption in bone remodeling areas^[1]. Lining cells comprise inactive osteoblasts with the ability to protect bone surfaces from bone resorption^[1].

Runt-related transcription factor 2 transcription factor in bone biology

Runt-related transcription factor 2 (Runx2) or core-binding factor subunit alpha-1 (Cbf α 1), the major osteo-specific transcription factor^[2] is responsible for the regulation of osteoblast differentiation as well as for hypertrophic cartilage synthesis^[2,3]. Its expression is necessary and sufficient for the commitment of mesenchymal cells towards the osteoblastic cell line^[4].

Abnormalities in Runx2 expression are indicative of its importance in bone biology. When Runx2 is expressed ectopically it has been shown to lead to increased expression of osteocalcin, alkaline phosphatase, collagenase-3, bone sialoprotein and collagen type I α 1^[5]. Osteoblast maturation in mice bearing a mutant *runx2* gene is inhibited and thus so are the procedures of intramembranous and endochondral ossification^[6,7]. Furthermore, it has been shown that differentiation of stem cells in adipocytes and chondrocytes in *runx2* knockout mice has not been impaired. In addition, heterozygous mice (*runx2*^{+/+}) developed characteristic skeletal abnormalities similar to human heritable skeletal disorder cleidocranial dysplasia (CCD) abnormalities^[8]. On the other hand, tissue-specific Runx2 over-expression in transgenic mice results in decreased bone density, bone fractures and osteopenia^[7,9,10].

Bone remodeling

Bone remodeling, the continuous bone reconstruction is of major importance for conserving bone structural integrity as well as for the bone to perform its metabolic role by modulating calcium and phosphorus levels in the body^[1].

Shortly, bone remodeling activation depends mostly on local factors and their effects on mesenchymal progenitor cells. Bone reconstruction initiates with osteo-

clasts performing bone resorption and forming cavities inside the bone. At the end of this phase, osteoclasts produce the appropriate signals for the initiation of bone synthesis^[1]. Osteoblasts quickly cover the cavity surfaces and synthesize new bone. Those two bone remodeling phases, bone formation and resorption are closely correlated and interconnected. This means that under normal conditions, the newly formed and the reabsorbed bone quantities are equal^[11]. Impaired bone remodeling may lead in pathophysiological bone conditions like osteoporosis, Paget's disease, orthopedic disorders and osteopetrosis among others^[1].

Research has shown that the GH-IGF-1 axis may also be of significance in the modulation of bone mass quantity and quality. More specifically, growth hormone (GH) is suggested to potentially play a role on bone remodeling^[12]. However, the exact mechanisms through which GH acts on osteoblast biology have not been elucidated^[12].

Role of RANK/RANKL/OPG pathway in bone remodeling

The receptor activator for nuclear factor κ B (RANK)/receptor activator for nuclear factor κ B ligand (RANKL)/osteoprotegerin (OPG) system comprises the main modulator of bone remodeling^[13]. More specifically, pre-osteoclasts express RANK in their surface. Its ligand, RANKL, is produced in osteoblasts, stromal cells as well as activated T cells^[14]. In osteoblasts and under steady-state conditions, vitamin D, parathyroid hormone and prostaglandins lead in induced RANKL expression. The binding of RANK and RANKL leads in osteoclast differentiation^[15,16]. More specifically, during normal bone remodeling, RANKL is produced by cells of the bone marrow- supporting tissue and osteoblasts. RANKL binds to RANK on pre- osteoclasts resulting in their maturation and activation. Nuclear-factor κ B (NF- κ B), which is of importance in inflammation response, also plays a central role in osteoclast activation. NF- κ B performs both aforementioned functions through regulation from interleukin-6 (IL-6). Pro-inflammatory cytokines play an important role in bone remodeling as indicated by the presence of interleukin-1 (IL-1), IL-6 and tumor necrosis factor- α (TNF- α) receptors on pre-and mature osteoclasts^[17]. OPG is produced by osteoblasts and has the ability to bind to RANKL and block its functions resulting in decreased bone resorption^[17,18].

MECHANOTRANSDUCTION

Bone remodeling and mechanotransduction

Bone remodeling is a strictly regulated process, largely modulated by the application of different mechanical stimuli or by metabolic stress on the bone^[3].

More specifically, local mechanical stress leads in bone resorption as an initial response^[19]. The nature of the mechanical stimulus is of importance in the regulation of bone remodeling since different types of mechanical stimuli result in different responses. For example, con-

stant repetitive application of mechanical force inducing high stress levels or unusual load distribution results in elevated bone synthesis and high bone mass. Furthermore, short pauses between long periods of mechanical loading have been shown to enhance bone strength and structure^[20]. However, static load, slow rates of pressure rotation as well as “predictable” pressure application, lead in decreased bone synthesis, enhanced bone resorption and thus low bone mass^[21,22].

Bone remodeling and mechanostimulation have been shown to roughly follow these rules: Bone synthesis is promoted by dynamic and not static loading application. Short-term load applications are sufficient for adaptive response initiation and lead in increased bone formation whereas long-term load applications result in decreased bone synthesis and enhanced resorption^[23,24]. In addition, the repetition of the same mechanical stimulus results in decreased response due to signaling prediction^[25]. The application of these rules is evident in the effects of space microgravity, osteoporosis or paralysis on bone tissues, where bone loss is observed^[20,26], as well as in the effects of tennis at a professional level on bone tissues, where bone growth is observed^[27].

Mechanisms in mechanotransduction

Signals of mechanical nature induce in osteoblasts and osteocytes the production and secretion of different types of molecules, which modulate osteoblast differentiation and proliferation^[3]. Such mechanical stimuli can include flow of fluids, strain of the substrate, membrane deformation or stimulation of integrins, vibration, altered gravity forces and compressive loading^[3]. Bone remodeling functions, after the application of different mechanical stimuli, are locally regulated by cytokines and growth factors among other molecules. More specifically, IL-1 β , TNF- α , prostaglandin E2 (PGE2)^[26,28], IL-6, IL-8, RANKL, OPG^[27,29-31], insulin-like growth factor (IGF), transforming growth factor β -1 (TGF β -1) and fibroblast growth factor (FGF)^[32,33] have been demonstrated to be induced after application of mechanical stimuli. Additionally, it has been shown that mechanical stimulation in osteoblasts results in increased mRNA levels of osteopontin, osteocalcin, platelet derived growth factor and collagen types I and III^[34,35].

Although some of the molecules taking part in mechanotransduction are known, the mechanisms behind it have not been fully elucidated.

The stage of osteoblast differentiation is shown to be of importance in osteoblast proliferation, apoptosis and translation of mechanical cues^[36]. Furthermore, it has been shown that undifferentiated mesenchymal stem cells seem to respond more successfully to load application than mesenchymal stem cells that have already started to differentiate^[37].

A diversity of molecules have been considered to play the role of mechano-sensors in differentiated osteoblasts: mechanical stimulation has been shown to lead in enhanced sensitivity and elevated open cation channels number^[38,39], increased communication through gap junc-

tions between osteoblasts as well as increased integrin production in osteoblasts^[39]. Actin cytoskeleton abnormalities have been shown to prevent mechanical signaling and therefore the integrin network has been considered as the main candidate for transduction of mechanical signals^[39]. On the other hand, a considerable number of research groups argue that cytoskeletal components involved in mechanotransduction differ depending on different types of stress or the response under study^[39].

Integrins comprise transmembrane receptors connecting the extracellular matrix to the cytoskeleton^[40]. Under mechanical signal application, integrins form complexes with molecules of the cytoskeleton with the help of the Rho family of Ras-related GTPases^[40]. Rho family members also induce multiple kinase cascades and particularly mitogen-activated protein kinase (MAPK) cascades^[40]. Rho and other Ras-related GTPases have been shown to play a role in osteoblast response after application of mechanical pressure^[41]. More specifically, it has been shown that the continuous application of mechanical forces leads in deregulation of Rab and Rho GTPases activity in osteoblast-like cells^[41].

Recently, another molecule, Polycystin-1 (PC1), was suggested to provide a link between environmental mechanical signals and their transformation towards biochemical signals. It has been shown that PC1 not only functions as a mechanosensor but that also conveys mechanical signals through the calcineurin/nuclear factor of activated T-cells (NFAT) signaling pathway and thereby regulates osteoblast-specific gene transcription as well as osteoblast differentiation^[42].

The primary cilium, a cellular sensory system, has also been demonstrated to be of importance in the transfer of mechanical signals as well as in mesenchymal stem cell differentiation. Additionally it was shown that the cilium modulates fluid flow mechanotransduction in human mesenchymal stem cells by maintaining fluid flow-induced osteogenic gene expression elevation and preventing fluid flow-induced increased proliferation^[43].

Following the reception of mechanical cues, the signal conveying the mechanical conditions of the extracellular environment is carried towards the nucleus through MAPK kinases and more importantly through extracellular signal-regulated kinases (ERKs) and c-Jun N-terminal kinases (JNKs)^[44,45]. ERKs, which in human osteoblasts seem to be induced by growth factors, estrogen and fluoride among others^[45], have been shown to play a significant role in osteoblast maturation and in osteoblast biology in general^[45-49]. Furthermore, duration and strength of JNK/ERK signaling is indicated to be significant in gene expression^[50].

Following ERK/JNK activation, the signal is transmitted to transcription factors that alter gene expression, like Jun and Fos family members^[51]. In their turn, c-Jun and Fos family members interact to form activator protein-1 (AP-1) transcription factor, which has been shown to be of major importance in osteoblast differentiation^[52] since it regulates the expression of collagen type I, osteocalcin, osteopontin and osteonectin^[52].

Application of continuous mechanical pressure in osteoblast-like cells as well as osteoblasts resulted in increased production of AP-1 components through activation of MAPK cascades^[41,53,54]. However, data on c-Jun expression after mechanical stimulation are inconclusive with some research groups arguing that human osteoblast-like cells after mechanical loading over-express c-Jun^[53] whereas others have opposing results^[55,56]. However, the above mentioned differences could be attributed to application of different stress type or usage of different cell system. Finally, different types of mechanical pressure applied on osteoblasts seem to result in different composition AP-1 and therefore regulate gene transcription accordingly depending on the extracellular signal applied^[57].

Application of short-term mechanical pressure activates both JNK2 and ERK2, with following activation of downstream molecules, like c-Jun, which alter the expression of osteoblastic genes^[54]. More specifically, it has been demonstrated that short-term continuous mechanical stimuli of physiological intensity in osteoblast-like cells activates JNK and ERK members resulting in enhanced AP-1 DNA binding activity on the human *L/B/K ALP* gene and thus osteoblast differentiation^[54]. This is further evidenced by the observation that osteoblast-like cells receiving mechanical stimuli synthesized increased quantities of type 1 collagen and osteocalcin, markers of early osteoblast differentiation^[58].

PGE2 production has been shown to be induced in osteoblast-like cells after mechanical stimulation^[59] and in osteoblasts under the effect of physiological stress, growth factors, hormones, trauma or inflammatory cytokines and its production leads in cAMP-dependent IGF-1 induction in osteoblasts^[3]. IGF-1 and IGF-2, in turn, induce osterix (Osx) transcription factor expression in osteoblasts^[60], induce osteoblast function *in vitro* as well as lead in increased bone mass *in vivo*^[61]. PGE2 is also shown to lead in increased Runx2 expression *in vivo*^[62]. Downstream of PGE2, TGF- β expression, which leads in proliferation of osteoblasts and extracellular matrix synthesis^[63], has been found increased in human osteoblast-like cells under mechanical stimulation. Furthermore, TGF- β receptor 1 comprises a Runx2 target in osteoblasts^[64]. Those two observations combined explain why Runx2 knockout mice demonstrate characteristic abnormal extracellular matrix formation due to decreased number of mature osteoblasts^[65,66].

Nitric oxide (NO) production in osteoblasts is another response to mechanical stimulation. NO functions through the MEK/ERK cascade by binding to a regulatory site on Ras leading in cell proliferation and extracellular matrix production^[67]. Following, cyclooxygenase 1 (Cox1), Cox2, ERK1 and ERK2 are activated and result in bone matrix formation^[68].

Additionally, signals of mechanical nature have been shown to promote vascular endothelial growth factor-, bone morphogenetic protein 2 (BMP-2)- and BMP-4- dependent and PGE2- independent increased expression of IGF-1^[69]. BMPs result in bone synthesis in osteoblasts^[70]

and BMP-2 expression promotes Runx2, Osx and Dlx5 expression^[71].

Mechanical cues also promote the expression of genes that encode for c-Fos, early growth response factor 1 (Egr-1) and basic fibroblast growth factor (bFGF) which have been shown to promote cell growth in MC3T3-E1 osteoblasts^[22].

The nature the mechanical signal determines whether bone or cartilage formation will occur^[72]. More specifically, application of pressure of high frequency and low intensity in bone cells *in vitro*, results in elevated extracellular matrix (ECM) disposition and thus increased bone formation^[73]. On the contrary, mechanical loading of high intensity on osteoblasts leads in BMP extracellular antagonists expression and therefore results in inhibition of osteoblast development^[74]. In addition, the application of continuous mechanical forces on osteoblastic cells *in vitro* promotes inflammatory cytokines and their receptors expression^[75]. More specifically, IL-1b production is found elevated under such mechanical stimuli, and is accompanied by RANK-RANKL signaling pathway activation and thus bone resorption^[76]. Stimuli from short periods of fluid flow or cyclic substrate tension at physiological intensity levels promote osteoblast proliferation and survival^[77]. Mechanical signals of physiological intensity levels are associated with survival of human osteoblasts and several studies suggest that pro-survival proteins promote the production of survival factors like IGF-1 or IGF-2 and activate estrogen receptor^[78]. It has also been shown that gravitational force maintains osteoblast survival whereas when gravitational force is not taking place, osteoblasts are led to apoptosis through reduced DNA binding of an important for survival transcriptional factor^[18]. *In vivo*, the absence of mechanical signals promotes osteoblast apoptosis and thus osteoporosis^[72]. The application of excessive mechanical force *in vitro* leads in cell detachment from their adhering surface^[79] as well as in a form of programmed cell death called anoikis^[80].

Mechanical stimulation in osteocytes has also been under investigation since it may lead in better mechanotransduction understanding and may represent a potent therapeutic target against bone degenerative diseases. Recent studies have underlined the role of osteocytes in bone remodeling since their absence in mice led in fragile bones, microfractures, deregulated osteoblast functions, bone loss in the trabeculae as well as adipose tissue proliferation in the marrow indicating an aging skeleton. In addition, these mice could not experience bone loss due to unloading, an event that indicates osteocytes' importance in the procedure of mechanotransduction^[81] (Figure 1).

Runx2 in mechanotransduction

Runx2 which is known to play a significant role in osteoblast differentiation has been shown to be the recipient of mechanical signals in human osteoblast-like cells^[82]. As it has been demonstrated, continuous mechanical stimuli of low intensity in human osteoblast-like cells of the periodontal ligament (PDL) result in elevated Runx2

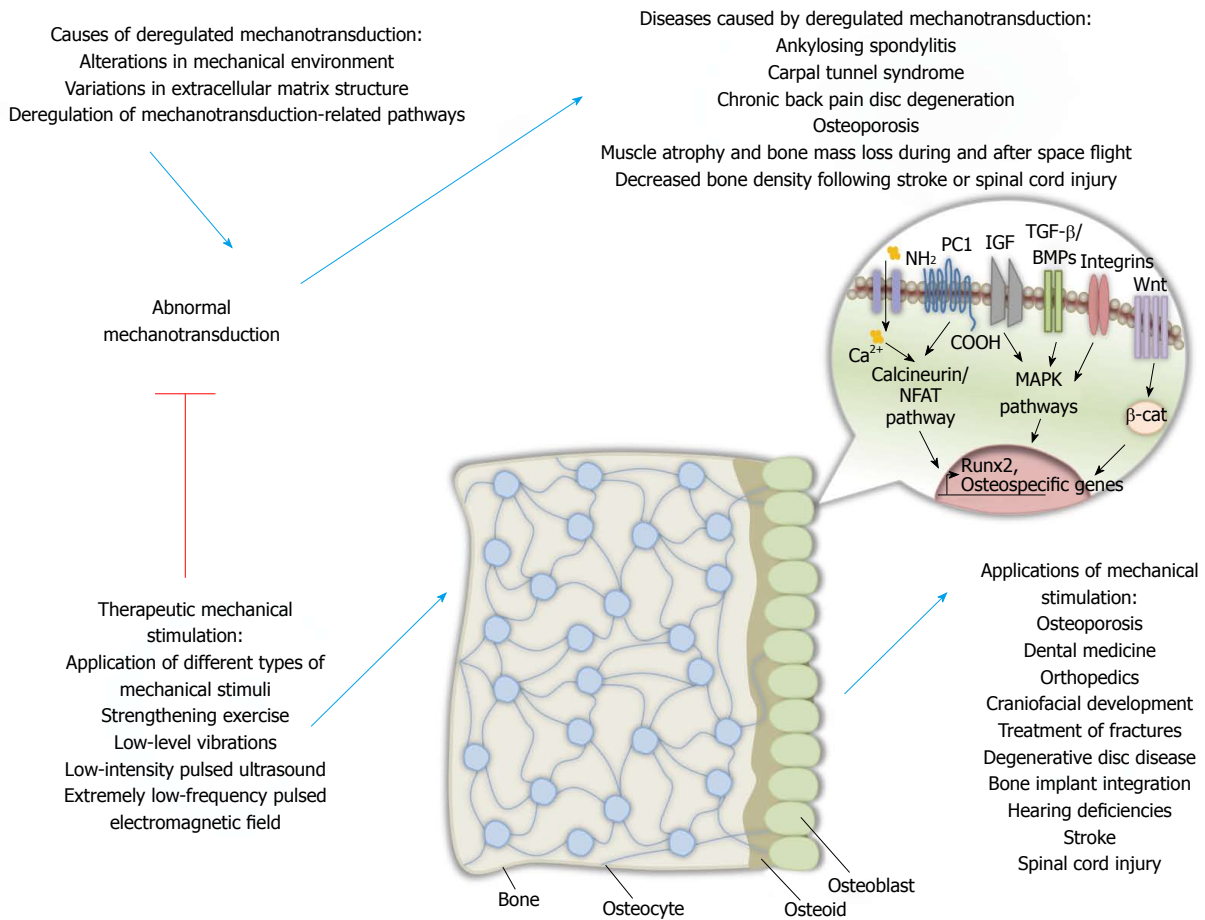


Figure 1 Mechanotransduction: Deregulation, associated disorders and therapeutic implications. Causes and effects of distorted mechanotransduction and the role of mechanical stimulation in the treatment of various pathophysiology. PC1: Polycystin-1; IGF: Insulin-like growth factor; TGF-β: Transforming growth factor β; BMP: Bone morphogenetic protein. NFAT: Nuclear factor of activated T-cells; MAPK: Mitogen-activated protein kinase; Runx2: Runt-related transcription factor 2.

expression and DNA-binding capacity. The mechanical signal, according to the researchers, initiates at the plasma membrane and more specifically from integrins and travels towards the nucleus through MAPK cascades. In the nucleus, the signal targets Runx2 and induces its expression^[82]. More specifically, Runx2 demonstrates increased expression at both mRNA and protein levels as well as elevated DNA binding activity. During this process, ERK1 and ERK2 are activated in a parallel manner with the Runx2 DNA-binding capacity elevation. After their activation, ERKs interact, phosphorylate and activate Runx2 *in vivo* causing osteoblast maturation^[7,82].

Runx2 expression depends on an autoregulatory mechanism^[83]. More specifically, activated by mechanical stimuli ERKs phosphorylate and activate already existing Runx2 molecules. Those activated Runx2 molecules bind to Runx2 promoter inducing Runx2 expression^[82]. In addition, a canonical AP-1 binding site has been found in Runx2 promoter which potentially plays a role in the regulation of Runx2 expression. AP-1 and Runx2 proteins have also been shown to interact and regulate collagenase-3 expression^[84].

NF-κB transcription factor in mechanotransduction

NF-κB transcription factor which is implicated in inflammatory response signaling^[51] also plays a crucial

role in osteoclast formation and thus bone resorption^[85]. NF-κB, which is activated either through the RANK-RANKL system or potentially through integrins that transmit signals of mechanical nature to src-kinases^[86], besides its role in osteoclast maturation, may be implicated in osteoblast differentiation under mechanical stimulation. This is indicated by the fact that NF-κB is found to be activated and then translocated in the nucleus of osteoblasts that receive mechanical stimuli^[26,87] where it has been hypothesized to promote the transcription of osteoblast-specific genes.

MODEL SYSTEMS IN MECHANOTRANSDUCTION STUDY

The *in vitro* study of mechanostimulation in osteoblasts, has been made possible with the usage of osteoblast-like cells that are acquired either from healthy tissue (human PDL or mouse MC3T3-E1 calvaria cells) or from osteosarcomas (MG-63, SaOs cells). Different types of mechanical stimulation are applied on the aforementioned cell models, each causing a different response in osteoblast-like cells^[5]. Such types of mechanical stimulation include fluid flow, four-point bending and substrate

stretch, gravity force, vibration, magnetic bead twisting and atomic force or shockwaves among others^[88].

Periodontal ligament (PDL) cell system is a helpful model for the study of mechanotransduction signaling cascades in osteoblasts^[89]. More specifically, PDL cells are undifferentiated mesenchymal fibroblasts^[90] that bear all the characterized properties of osteoblasts. Furthermore, these cells are adapted to receive mechanical pressure, either because of physiological conditions or orthodontic treatments. Under specific conditions, PDL cells have the ability to differentiate towards more specialized cells capable of taking part in the regeneration and repair of the periodontal ligament as well as its surrounding hard tissue^[91].

Furthermore, three dimensional (3-D) constructs, like polydimethylsiloxane microdevices and human trabecular 3-D bone scaffolds, have been used to investigate the effects of mechanical stimulation on osteoblasts^[92].

Scientists are trying to develop an effective way to monitor the levels and characteristics of mechanical pressure applied as well as a way to measure the rates of tissue regeneration. In order to achieve the first part, scientists have made either fixation devices with different mechanical pressure characteristics and then monitor their effects *in vivo* or custom-made devices that accurately control the mechanical stimulation characteristics. With the first type of devices they are able to study bone tissue regeneration under more physiological conditions while with the second they assess the effects to a specific loading signal^[93]. In order to study the effect of mechanical signals on healing processes at organs, it is necessary to develop techniques to assess their mechanical environment *in vivo*. Today, we have found ways to determine loading applied on the affected limb^[94], load distribution between implant and bone^[95-97], and assess interfragmentary movements^[94,98] but the development of techniques to study the intermediate steps and not only the final outcome of loading are imperative.

MECHANOTRANSDUCTION IN BONE DISEASE

As mentioned before, deregulated bone remodeling is the main cause of a number of bone diseases. Bone remodeling abnormalities may be due to genetic alterations. For example, a mutant *runx2* gene can result in human heritable skeletal disorder CCD^[99,100]. A mutation in *runx2* gene may also lead in cancer metastasis to bone tissues since Runx2 is responsible for the expression of genes that are implicated in cancer development and more specifically, in cell metastasis in bone. Among those genes regulated by Runx2 are those encoding matrix metalloproteinases (MMPs) MMP-9 and MMP-13 as well as osteopontin and bone sialoprotein^[101]. Abnormal mechanotransduction due to lack of mechanical loading or other causes may result in bone remodeling deregulations like ankylosing spondylitis, carpal tunnel syndrome, chronic back pain disc degeneration and osteoporosis.

Recent studies have shown that annulus fibrosus (AF) cells that originate from non degenerative tissue respond to cyclic tensile strain through IL-1 and IL-4 dependent mechanisms, something that does not apply in AF cells coming from degenerative tissue^[102]. Furthermore, annulus fibrosus cells from degenerative discs have been found to have little capacity to successfully respond to application of mechanical stimuli and exhibit an intense response to inflammatory stimuli. The above observations may explain the different responses observed in patients with intervertebral disc degeneration after specific therapies^[103].

During space flight, astronauts are exposed to microgravity and thus altered mechanical stimuli are applied on their skeletons. As a result, their muscles atrophy and their bones experience bone mass loss. Short exposure to microgravity has been shown to result in increased bone resorption evidenced by the urinary calcium excretion observed^[104]. Under long periods of microgravity, the structural alterations occurring in bones have even more crucial effects on bone strength than was previously thought while counteracting measurements like exercise seem to have little or no effects^[104]. The mechanism behind bone loss is not yet clarified but probably is a result of decreased hydrostatic pressures and thus decreased intramedullary pressure which may lead in reduced fluid flow shear stresses on osteocytes and thus enhanced bone loss. Since exercise does not seem to prevent bone loss, it has been suggested that the decreased hydrostatic pressure may result in impaired mechanosensitivity in the bone tissue. Furthermore, other physiologic alterations on the body under reduced gravity conditions may contribute to the observed bone loss in co-operation with the reduced hydrostatic pressures like low vitamin D levels, oxidative stress, radiation exposure and acidosis^[105-109].

Neurologic injury results in bone loss in the affected paretic limb whereas the other limb is characterized either by reduced or increased bone mass. Those effects are probably due to alterations in muscle mass and strength and load pressure applied. More specifically, strokes result in decreased bone density mostly in the paretic limb and its effects are more intense in the upper extremities. The pattern of bone loss observed in stroke patients is generally limited to the paretic side and is more evident in the upper extremities than in the lower extremities. The pathogenesis of the observed bone loss after stroke probably depends between others on immobilization, duration of paresis, loss of muscle activity, endocrine disorders, nutritional deficiencies as well as medications^[110].

Following spinal cord injury, bone loss is observed in pelvis and lower extremities of paraplegics and in the upper and lower extremities of tetraplegics after spinal cord injury^[111]. Those effects are predominantly observed in trabecular bone. Recent data indicate the presence of endocortical resorption without periosteal synthesis^[112]. Absence of mechanical stimulation, muscle contraction, neuroendocrine alterations as well as neural innervation alteration are probably responsible for the observed bone

loss after those types of injuries^[113,114] (Figure 1).

MECHANOSTIMULATION IN THERAPY OF BONE DISEASE

Pharmaceutical treatments like anabolic treatments or treatments with anti-resorptive agents have been the norm in order to achieve increased bone density until now^[3]. Nowadays, mechanical stimulation is considered to be of great importance in designing new therapies for bone diseases, avoiding this way the unwanted side effects of pharmaceutical products.

A number of studies demonstrate the role of mechanostimulation in acquiring a higher bone mass quantity and thus its role in treatment of bone diseases. For example, it has been shown that low intensity mechanical signals result in bone remodeling activation and increased bone mass and that following a period of time confer regenerative abilities to bone tissues^[115]. It has also been observed that mechanical signal application on PDL and osteoblast cell lines leads in enhanced OPG expression^[116,117] and therefore in RANK-RANKL signaling interruption which results in decreased osteoclastogenesis. Furthermore, mechanical stimulation has been shown to activate Cox enzymes and prostaglandins which reduce RANKL production and thus block bone resorption *in vitro*^[77,118]. Mechanical stimuli have also been demonstrated to activate the Wnt-b-catenin pathway on osteoblasts resulting in enhanced osteoblast differentiation and bone synthesis^[119]. Studies on three dimensional models have showed that osteoblasts receiving dynamic application of mechanical pressure, expressed elevated ALP, Runx2 and osteocalcin levels^[120,121]. Additionally, application of mechanical pressure resulted in increased mineralized matrix production in 3-D, partially demineralized bone scaffold-cultured human bone marrow stromal cells^[122].

Considering the aforementioned and other results, researchers have turned to mechanical stimulation in order to design treatments against bone diseases which will avoid the undesirable effects of pharmacological treatments^[115]. Application of mechanostimulation has already a variety of applications in dentistry, orthopedics, the craniofacial development and treatment of fractures.

More specifically, strengthening exercises in osteoporotic patients has been shown to result in increased bone mineral content^[123] and physical exercise has been observed to prevent post-menopausal and age-related ECM bone mineral decrease^[124]. Moreover, other types of mechanical stimulation like low-level vibrations at intensity safe for the bone integrity may play a protective role in osteoporosis^[125]. A functional mechanical environment seems to be of importance in the treatment of degenerative disc disease as well as other skeletal deregulations^[126]. Mechanical signals of specific ratio^[127], form^[128] and intensity in osteoblasts have also been shown to be beneficial in bone fracture treatment^[128]. Additionally, low-intensity pulsed ultrasound has been indicated to promote osteoblast differentiation and bone formation in bone frac-

tures^[129]. Extremely low-frequency pulsed electromagnetic field has been demonstrated to result in osteoblast proliferation and maturation^[130].

In addition, mechanostimulation was found to have positive effects in bone implant integration by modulating osteoblast differentiation through regulation of Cbfa1 as well as osteocalcin levels. Cbfa1 and osteocalcin levels were shown to be frequency-, magnitude-, and duration of mechanical application- dependent. Furthermore, osteoblast cells under strain in the implant seem to produce factors that have the ability to activate DNA synthesis and thus cell proliferation in a larger scale than non-strained cells^[131].

Mechanical stimulation has also its applications in the treatment of hearing problems. For example, SPAHA, which comprises a novel bone conduction hearing device, whose effects are accomplished through elastic bending of the bone and not the application of a point force which results in cochlea vibration as previous devices used to do^[132].

Exercise has not been shown to meliorate bone loss in space flights until now^[104]. Furthermore, there is no indication that osteoporosis drug therapies would be successful during or following space flight. Exercise seems to be helpful in increasing bone density after stroke or spinal cord injury according to a recent study^[133,134]. Bisphosphonates have been shown to be able to prevent bone loss after a stroke^[134]. Mechanical stimulation may have some positive effects on preventing bone loss after spinal cord injury, with early application demonstrated to bear better results^[135,136]. Furthermore, bisphosphonate early administration after spinal cord injury may be able to prevent bone loss^[137].

Researchers have investigated whether sympathetic nervous system inhibition could be beneficial against bone loss in osteopenia induced by absence of mechanical signals. They found that its inhibition led in blockade of neurectomy-induced bone resorption but further studies need to be conducted^[138].

Although mechanical loading is thought to be an anabolic beneficial procedure against osteoporosis, abnormal mechanotransduction in conjunction with age seem to counteract its beneficial effects in elderly people. Recently, a research group presented an agent-based model of real-time Ca²⁺/NFAT signaling in bone cells that successfully described periosteal bone synthesis induced by different types of mechanical stimulation in young and aged animals. The model demonstrated age-related pathway changes being responsible for the decrease in bone synthesis during senescence. This way the group managed to identify important pathway alterations that comprise potent therapeutic targets. In accordance, the researchers applied an *in vivo* intervention and showed that application of mechanical stimuli along with Cyclosporin A can prohibit the decrease in bone synthesis in the bones of elderly people. This study not only provided a potent inexpensive treatment for osteoporosis in the elderly but also demonstrated the significance of real-time cellular

signaling and *in silico* techniques in studying, intervening and treating bone diseases like osteoporosis^[139].

The primary cilium was shown to modulate fluid flow mechanotransduction in human mesenchymal stem cells by maintaining fluid flow-induced osteogenic gene expression elevation and preventing fluid flow-induced increased proliferation^[43]. Therefore, fluid flow systems may be effective in designing techniques to develop bone-like tissues for bone regenerative purposes. Furthermore, the role of cilium in developing techniques that imitate loading in order to treat bone loss in bone diseases needs to be investigated. Last but not least, studying the events taking place during acute proliferation of mesenchymal stem cells with not functional cilia receiving mechanical cues could help in understanding the mechanisms behind ciliopathies and cystic diseases^[43] (Figure 1).

CONCLUSION

Bone remodeling is of major importance for the proper structure and metabolic functions of the bone. Deregulations in bone remodeling can result in a variety of bone diseases like osteoporosis, hyperparathyroidism, hyperthyroidism, Paget's and osteopetrosis among others. Therefore, the investigation of mechanisms and pathways behind bone remodeling and mechanotransduction, which comprises of the most important variables of bone remodeling, is of great significance.

There is a lot that we don't know about bone biology and bone diseases as well as the implication of mechanical signals in the aforementioned procedures. The better understanding of the underlying mechanisms will potentially result in designing a successful strategy for treating bone diseases, avoiding the unpleasant side effects of conventional treatments like the administration of pharmaceutical substances. Furthermore, it will help us design techniques to successfully predict and prevent bone diseases when possible.

Undeniable is the necessity of innovative new ways to monitor bone density, to identify hormonal or metabolic risk factors for bone loss, to develop effective ways to apply mechanical stimulation with successful results against reduced bone density, to assess the effect of newly developed anabolic drugs against osteoporosis and their effects on bone loss characterizing bone diseases due to absence of mechanical stimuli, as well as to develop trials investigating the improvement of bone health under the afore mentioned conditions. In addition, the study on the effects of mechanostimulation on bone tissue and organ healing is of great significance for future interventions. In order for this to be achieved, we need to develop an effective way to monitor the levels and characteristics of mechanical pressure applied on bone tissue, a way to measure the rates of tissue regeneration as well as techniques to assess mechanical environment of organs *in vivo*^[106].

Currently, researchers have started using mechanostimulation with encouraging results for certain bone conditions but further study is required. Mechanostimula-

tion is considered to comprise the future in treating bone diseases that have their origin in absence of mechanical cues. Further investigation of the molecular players and pathways involved in mechanotransduction and bone remodeling will amplify our knowledge and understanding of these processes and help us build successful prevention, prediction and treatment strategies for a variety of bone diseases.

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