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SYSTEMATIC REVIEW

# Mechanotransduction in osteogenesis

**S. Stewart,**
**A. Darwood,**
**S. Masouros,**
**C. Higgins,**
**A. Ramasamy**

*The Royal British Legion Centre for Blast Injury Studies, Imperial College London, London, UK*

**Bone is one of the most highly adaptive tissues in the body, possessing the capability to alter its morphology and function in response to stimuli in its surrounding environment. The ability of bone to sense and convert external mechanical stimuli into a biochemical response, which ultimately alters the phenotype and function of the cell, is described as mechanotransduction. This review aims to describe the fundamental physiology and biomechanisms that occur to induce osteogenic adaptation of a cell following application of a physical stimulus. Considerable developments have been made in recent years in our understanding of how cells orchestrate this complex interplay of processes, and have become the focus of research in osteogenesis. We will discuss current areas of preclinical and clinical research exploring the harnessing of mechanotransductive properties of cells and applying them therapeutically, both in the context of fracture healing and de novo bone formation in situations such as nonunion.**

Cite this article: *Bone Joint Res* 2020;9(1):1–14.

**Keywords:** Mechanotransduction, Mechanoreceptor, Bone

## Article focus

- A comprehensive review of the fundamental concepts of mechanotransduction in osteogenesis.
- The biological mechanisms and pathways of mechanisms are reviewed, and how these pathways can be modulated in vitro and in vivo.
- Highlights current areas of preclinical and clinical research in the field of mechanotransduction.

## Key messages

- Mechanotransduction underpins the biomechanisms in which bone cells adapt their function and behaviour based on their physical environment and forces exerted on the cell.
- This represents an exciting area of research in orthopaedics, which has the potential to allow us to modulate the function of bone cells in vivo to create new bone.

## Strengths and limitations

- Summative assessment of the current areas of novel research in the field of mechanotransduction and osteogenesis.
- Highlights areas of potential therapeutic targets for utilizing the pathways involved in mechanotransduction in fracture healing and nonunion.
- This study has explored a number of promising preclinical and clinical areas of research. In many of the preclinical and clinical applications highlighted, however, these interventions may be subjected to experimental bias as assessments of the therapies are funded or carried out by key stakeholders in the technologies. It is clear that further independent research is required.

## Introduction

All living organisms are subject to external physical forces in their environment. The conversion of these physical forces into biochemical signals and integration of these signals into a functional response is termed mechanotransduction. On a cellular level, a mechanical stimulus generates a biochemical signal, which in turn can bring about a number of intracellular processes. These include activation of complex signalling pathways, upregulation or downregulation of gene expression, and alteration of protein synthesis, resulting in adjustment of the intracellular and extracellular environment in response to the initial mechanical stimulus. This mechanosensitive feedback modulates cellular

Correspondence should be sent to S. Stewart; email: s.stewart18@imperial.ac.uk

doi: 10.1302/2046-3758.91.BJR-2019-0043.R2

*Bone Joint Res* 2020;9(1):1–621.

functions as diverse as migration, proliferation, differentiation, and apoptosis, and is crucial for organ development and homeostasis.[1]

The embodiment of the importance of mechanotransduction on physiological function and processes can be observed by its effect on bone. Mechanotransduction is a critical determinant of new bone formation, repair and regeneration, and adaptation of the skeleton to its external environment. An understanding of these processes and how they can be engineered as therapeutic applications to heal bone has therefore been the subject of much recent orthopaedic research. In this review, the underlying mechanisms of mechanotransduction in osteogenesis are discussed, as well as current therapeutic applications and future research foci in this promising field of orthopaedic regenerative medicine.

**Cellular physiology of mechanotransduction in bone.** The process by which a mechanical force is converted into a biochemical signal that ultimately leads to the production of new bone is complex, and can be considered in four distinct phases: mechanocoupling, how mechanical loading causes deformation of bone cells; biochemical coupling, how bone cell deformation is converted into intracellular signalling pathways; transmission of signal, how the biochemical signal is transmitted from the sensor cell to the effector cell; and effector cell response, how the effector cell's response to the signal leads to new bone formation.[2]

**Mechanocoupling.** Wolff[3] first postulated that bone is a dynamic entity, the innate structure of which can be influenced by and adapt to its surrounding environment in order to meet varying physical demands. In bone, mechanical stimuli orchestrate the architecture, shape, strength, and quality that bone exhibits through the modulation of the three principal bone cells: osteoblasts, osteoclasts, and osteocytes.

Of the bone cell population, osteocytes are the most prolific, comprising 90% to 95% of the bony cellular blueprint.[4] Distributed uniformly through the cortical and cancellous bone and displaying multiple cytoplasmic appendages, they are well placed for detecting small shifts in movement and fluid, and generating an 'amplified' biochemical response intracellularly.[5,6] Intercellular signalling is also enhanced by their long cytoplasmic processes,[7] ensuring that a mechanotransductive signal is propagated throughout the local osteocytic network.

Osteocytes are located in the mineralized extracellular matrix (ECM) of bone in spaces termed lacunae (from the Latin *'lacus'*, meaning lake or pool). The dendritic processes of osteocytes connect with adjacent osteocyte processes through canaliculi (meaning 'small channel/pipe' in Latin). Together, this system is termed the lacunocanalicular (LC) network.

Within the LC network, osteocytes are enveloped by an interstitial fluid. This fluid can serve as a medium through which shear forces act on the osteocytes. Bone deformations secondary to a mechanical stimulus result in interstitial fluid being squeezed over the osteocytes. In turn, this interstitial fluid flow generates shear stress across the cell membrane (Figure 1). Paradoxically, physiological loading of bone induces a tissue-level strain of only up to 0.2%, whereas far higher strain levels of 0.5% are required to produce a cellular response in vitro.[8,9] When these higher levels of strain are applied in vitro they are sufficient to cause cellular damage. This paradox may be explained by the simplifications in the loading microenvironment that in vitro cell-culture studies induce, compared with the complex intercellular events occurring in vivo.[10]

However, other theories have been proposed as to how the cell amplifies the shear forces applied across it to generate a cellular response. The 'hoop strain' theory was originally put forward by You et al[11] who postulated that physiological forces applied to bone are amplified to induce a biochemical signal without concomitant damage to the cell (Figure 2). The model proposes that fluid flow through the pericellular matrix induces strains in the intracellular actin filaments of the osteocyte cytoskeleton that are twice the magnitude of those seen at a macroscopic whole-bone level. The term 'hoop' relates to the manner in which the osteocytic cytoskeleton is compressed to produce circumferential deformations (20 to 100 times greater than the deformation of the bone at tissue level), following application of shear loading.[12] The greater the magnitude of shear load, the greater the amplification of cytoskeletal strain.

Although mechanocoupling physiology has been researched most extensively in osteocytes, other cells that are integral to bone formation also play a pivotal role in mechanocoupling. Whereas osteocytes have been shown to be most responsive to fluid flow in the mechanocoupling process,[13] deformation of osteoblasts leading to downstream mechanotransductive processes have been shown to occur in the presence of stretch. Application of a stretching force to osteoblasts generating strain increases their proliferation,[14] and increases twofold the production of the bone matrix protein collagen I.[15] Moreover, this was not reproduced in the presence of fluid flow, suggesting that osteoblasts are optimally responsive to physical deformation by strain. Mesenchymal stem cells (MSCs) are undifferentiated, multipotent cells that are found at high concentrations in the bone marrow. Application of a physical force can direct them towards an osteogenic lineage through similar mechanocoupling processes observed in osteocytes and osteoblasts. Similar to bone cells, MSCs also possess an actin cytoskeleton, which remodels in response to mechanical stretching. This remodelling instigates subsequent mechanotransductive pathways, ultimately leading to the differentiation of MSCs into bone-forming osteoblasts.[16,17] This ability of MSCs to be directed into an osteogenic lineage by physical forces provides further adaptive measures beyond those observed in

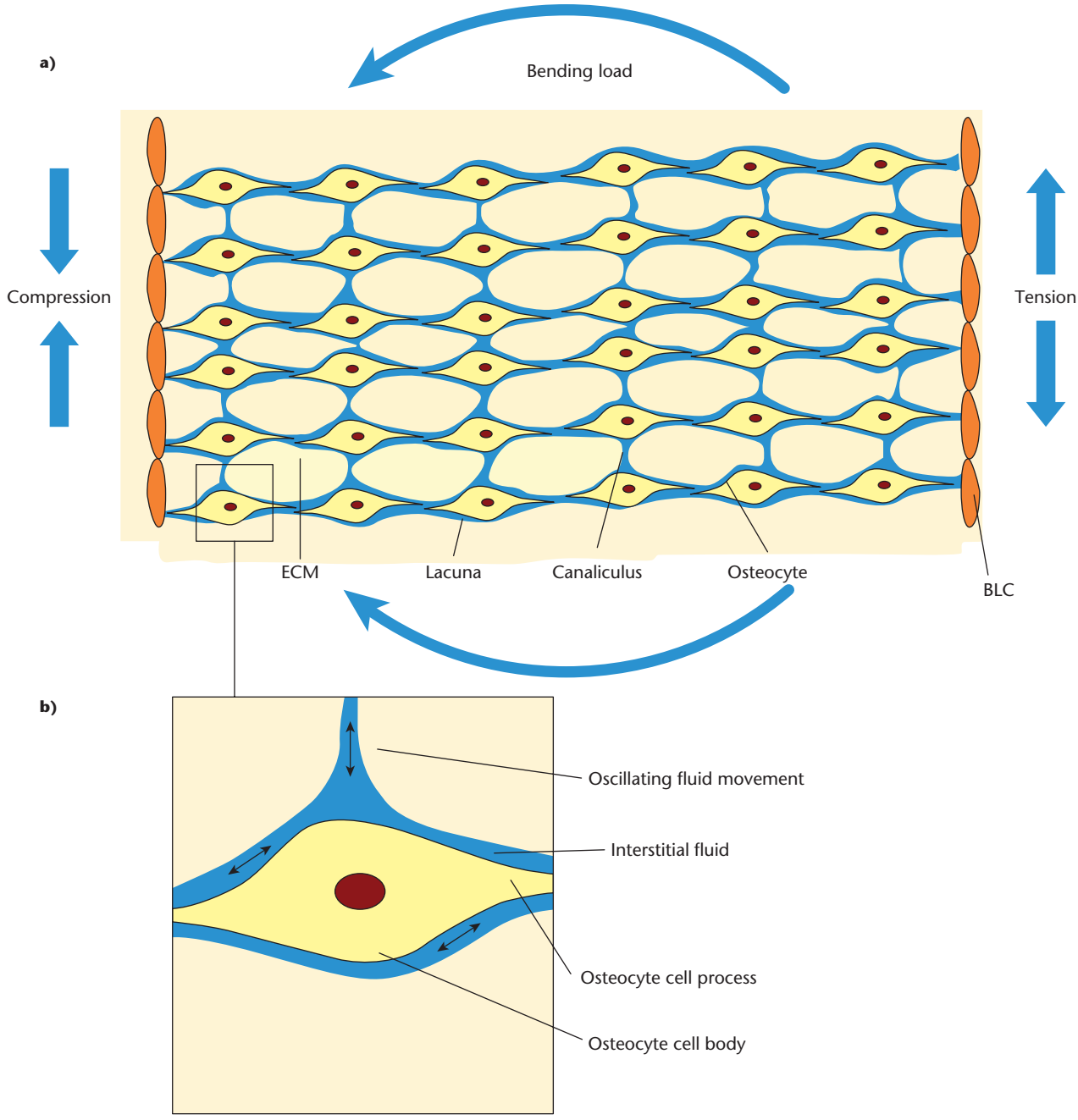


Fig. 1

Bone cellular architecture. a) Mechanical loading of bone causes tension and compression forces across the bone's lacuno-canalicular (LC) network. b) The tension/compression forces cause interstitial fluid shift within the LC network in an oscillatory manner across the cell membrane. BLC, bone lining cell; ECM, extracellular matrix. Adapted with permission from **Duncan RL, Turner CH.** Mechanotransduction and the functional response of bone to mechanical strain. *Calcif Tissue Int*. 1995;57(5):344-358.

the bone cell population, and through which bone can alter its architecture.

**Biochemical coupling.** The process through which a bone cell detects changes to its external environment and generates a biochemical signal involves numerous pathways, many of which are highly associated (Figure 3). These pathways include detection of mechanical forces by receptors ('mechanosensors') in the cell membrane, activation of intracellular signalling networks, and the generation of substances that act either within or outside the cell to exert an osteogenic effect.

**Mechanosensors.** Any receptors that can detect alterations in external and internal forces have been termed 'mechanosensors', and a comprehensive review of mechanosensors can be read elsewhere.[10,18,19] A number of mechanosensors have been shown to be integral in recognizing mechanical forces acting on a bone cell, and will be discussed here.

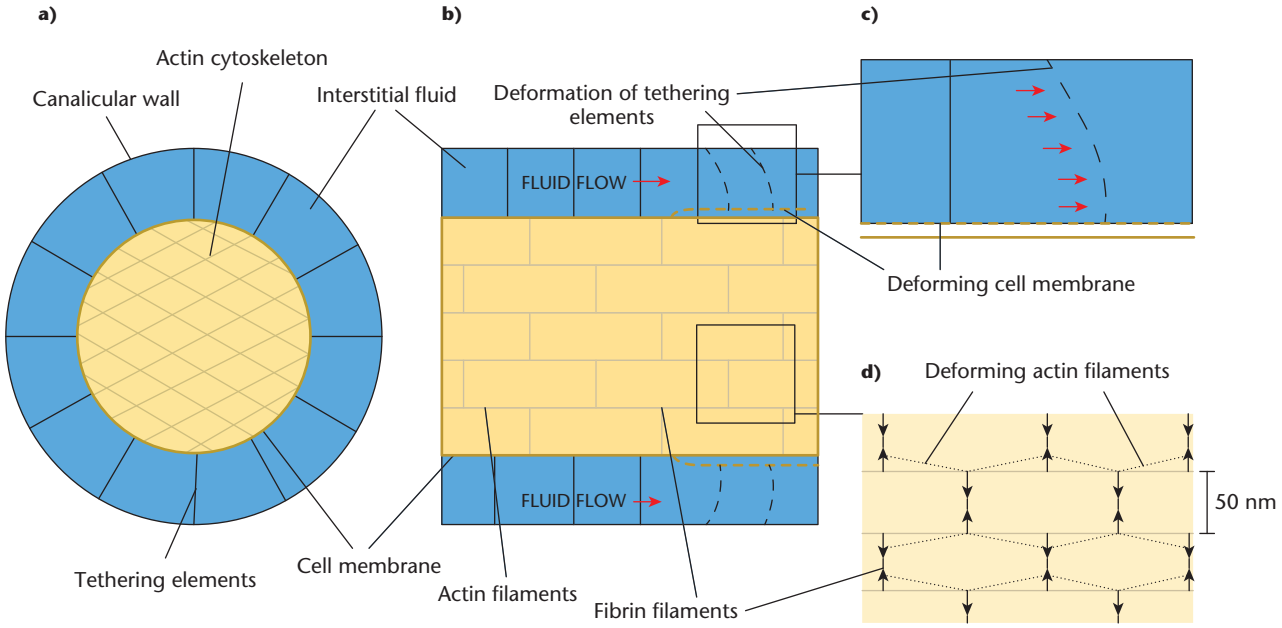


Fig. 2

Mechanocoupling. a) Axial section of osteocyte process with surrounding interstitial fluid in canaliculus. b) Longitudinal section of osteocyte process. Actin filaments span the process and are attached to the canalicular wall by tethering elements. Applied loading produces interstitial fluid movement producing a drag force on the tethering elements. c) Force balance on tethering elements: as the tethering elements deform, they pull the cell membrane outwards. d) Force balance on osteocyte skeleton: deformation of the cell membrane produces amplified strain on the actin cytoskeleton. The small vertical arrows indicate the direction of loading throughout the fibrin filaments. Adapted with permission from **You L, Cowin SC, Schaffler MB, Weinbaum S.** A model for strain amplification in the actin cytoskeleton of osteocytes due to fluid drag on pericellular matrix. *J Biomech*. 2001;34(11):1375-1386.

**Integrins.** Integrins are transmembrane proteins that have been identified as being part of a critical, two-way mechanotransductive pathway termed the 'ligand-integrin-cytoskeleton' linkage. The integrins act as a link between extracellular ligands, including ECM ligands and soluble ligands, transmitting forces into the intracellular actin cytoskeleton ('outside-in'), while also propagating signals from intracellular domains into the ECM to affect the binding affinity of extracellular molecules ('inside-out'). The integrin complexes are made up of $\alpha$ and $\beta$ subunits, with the $\beta 1$ subunit type acting as the predominant functional unit in osteoblasts.[20] Expression of a dominant-negative, osteoblast-specific $\beta 1$ subunit in transgenic mice resulted in offspring with reduced cortical bone formation,[21] while osteoblasts exposed to fluid flow shear stress upregulated $\beta 1$ integrin expression.[22]

Mechanotransduction via the 'outside-in' manner is predominantly controlled by integrins acting through focal adhesion complexes. Focal adhesions (FAs) are macromolecular protein complexes formed by the binding of integrins to intracellular 'linker proteins'. The linker proteins include talin, vinculin, paxillin, p130Cas, and focal adhesion kinase (FAK)[23-25]. Once adhered to the integrins, the FAs form a connection between the ECM and the cytoskeleton (Figure 3a). These FAs serve to transmit forces from 'outside in' to the actin cytoskeleton via their attachments to linker proteins, which subsequently activate mechanotransduction signalling cascades. Activation of these signalling cascades, the most well understood being kinase pathways (see 'Kinase pathways'), ultimately leads to activation of the master transcription factor of osteoblast differentiation, runt-related transcription factor 2 (RUNX2).

**Membrane-spanning proteins.** Ion channels have been shown to be key players in osteogenic mechanotransduction. Located in bone cell membranes, they have been found to be sensitive to strains from stimuli including stretching and fluid shearing via cellular ion fluxes.[26] A subset of these ion channels are connexins, hexagonal-shaped proteins that form intramembranous pores, and have been found on the appendages of osteocytes and between osteocytes and osteoblasts (Figure 3c).[27] These can align with other connexin-displaying cells, forming a functional connection known as a 'gap junction' through which small molecules including calcium, adenosine triphosphate (ATP), and cyclic adenosine monophosphate (cAMP) can be transported.[28]

The presence of gap junctions between adjacent osteocytes and osteoblasts suggests that these channels play a vital role in enhancing intercellular communication. Osteocytes are ideally placed within matrix to detect surrounding physical stimuli through mechanisms already described (see 'Mechanocoupling'). In order to transmit this information to osteoblasts on the bone surface, information needs to propagate through the cellular network. Yellowley et al[27] demonstrated the ability of osteocytes to communicate with osteoblasts

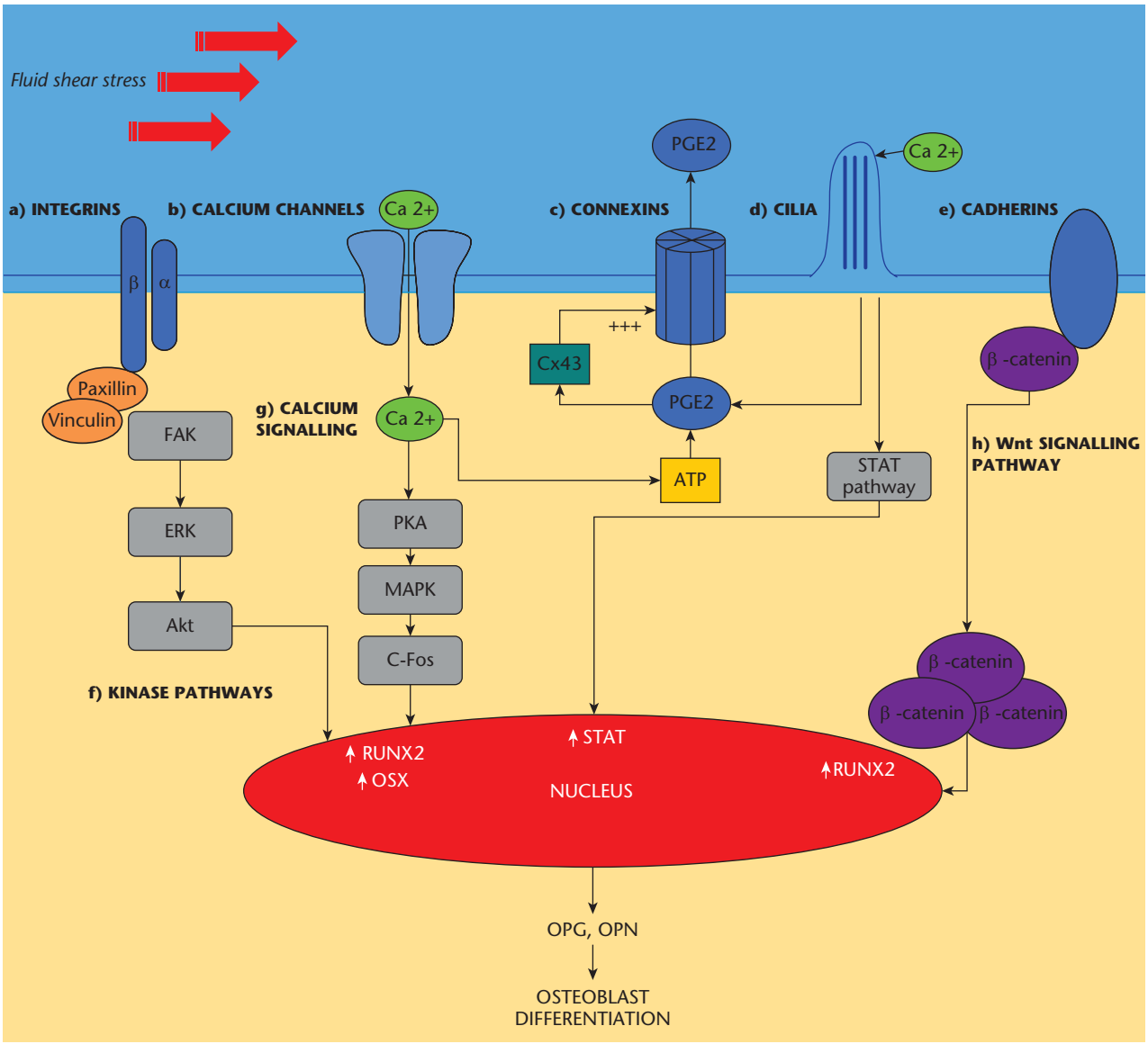


**Fig. 3**

Biochemical coupling. Mechanosensors: a) Transmembrane proteins termed 'integrins' form focal adhesions with linker proteins, stimulating kinase pathways. b) Voltage-sensitive calcium ($Ca^{2+}$) channels stimulate influx of calcium into the cell. c) Hexagonal connexins allow for efflux of newly synthesized prostaglandin 2 (PGE2), and their presence is upregulated by PGE2 via connexin 43 (Cx43). d) Primary cilia regulate bone cell function in response to fluid shear stress through calcium ion influx (activating signal transducer and activators of transcription (STAT) signalling pathways) and prostaglandin release. e) Cadherins span the cell membrane and dissociate with β-catenin in response to fluid shear stresses. Signal transduction pathways: f) Kinase pathways are activated by integrins and intracellular calcium release, resulting in upregulation of osteogenic transcription factors including runt-related transcription factor 2 (*Runx2*) and Osterix (*OSX*). g) An influx of calcium ions stimulates PGE2 synthesis via adenosine triphosphate (ATP), and activates kinase pathways. h) The wingless integrated (Wnt) signalling pathway is activated following dissociation of β-catenin from cadherin receptors. Accumulation and translocation of β-catenin to the cell nucleus stimulates upregulation of *RUNX2*. Akt, protein kinase B; ERK, extracellular signal-related kinase; FAK, focal adhesion kinase; MAPK, mitogen-activated protein kinase; OPG, osteoprotegerin; OPN, osteopontin; PKA, protein kinase A.

via changes in intracellular calcium, providing evidence that calcium and other small signalling molecules may use gap junctions to relay the mechanical signal detected by osteocytes deep in the matrix to the bone-producing osteoblasts on the bone surface. Calcium ion channels situated in bone cell membranes also facilitate the transmembranous passage of calcium ions in and out of the cell to modulate extracellular and intracellular signalling (Figure 3b).

**Primary cilia.** Primary cilia are immotile tubules that project from the cell surface of most human cell types, and sense physical forces in the extracellular environment (Figure 3d). The role of cilia in modulating osteogenic mechanotransductive pathways, particularly in MSCs, has been an area of interest in recent years. Deletion of a gene that codes for primary cilia formation is associated with a statistically significant decrease in bone formation in response to mechanical loading of MSCs.[29].

Hoey et al[30] demonstrated that oscillatory fluid flow enhanced the expression of osteogenic genes including cyclo-oxygenase-2 (COX-2) and bone morphogenetic protein 2 (BMP2) in MSCs, through a cilium mediated mechanism. Moreover, the proliferation rate of the MSCs increased twofold on exposure to a higher magnitude of oscillatory fluid flow. This ability of MSCs not only to increase expression of key genes linked with osteogenesis but also to increase in numbers, represents a possible mechanotransductive target through which MSC differentiation to cells of a bone lineage can be controlled. In recent years, researchers have employed the use of nanomechanical stimulation on MSCs to target their differentiation endpoint, and are described below in 'Applications' in greater detail.[31-34]

On a molecular level, a number of events must take place in order to induce mechanotransduction within the cell following cilia activation. Calcium ions are trafficked through polycystine channels located at the base of the cilia in response to bending deformations. Subsequent depolarization of the membrane acts to trigger the signal transducer and activators of transcription (STAT) pathway and wingless integrated (Wnt) signalling pathway (see 'Calcium signalling'), ultimately resulting in osteogenic gene transcription in the cell nucleus.[35] Evidence of the role of cilia in cell-to-cell communication, an important aspect of mechanotransduction for transmission of information in a cellular network, has been demonstrated by upregulation of osteogenic genes in MSCs exposed to mechanically stimulated osteocytes.[36] Upregulation of these genes such as osteopontin (OPN) and COX-2 is subsequently absent when primary cilia formation is inhibited on the osteocyte prior to stimulation.[36] It is therefore likely that cilia play a key role in both intracellular and extracellular biochemical signalling in mechanotransduction.

**Cadherins.** Cadherins are a subgroup of transmembranous cell membrane glycoproteins, and a wide range exists in the human osteoblast.[37] They have both extra- and intracellular functions and play a central role in the control of osteoblastic differentiation. Dominant negative cadherins inhibit osteoblast differentiation in vitro.[38] Intracellularly, cadherins associate with proteins including $\alpha$- and $\beta$-catenin (Figure 3e). Application of fluid-induced shear stress in osteoblasts has been shown to trigger a disassociation between the cadherin and $\beta$-catenin complex, causing a rise in free $\beta$-catenin in the cytoskeleton.[39,40] $\beta$-catenin has been shown to induce expression of RUNX2, which is a master transcription factor of osteogenesis,[41] and OPN, the glycoprotein secreted to the mineralizing ECM during bone development.[42] The ability of cadherins to modulate the level of $\beta$-catenin in response to an extracellular force, thereby determining the expression of two fundamental osteogenic genes, provides convincing evidence that cadherins may be key orchestrators in the process of bone formation as a consequence of mechanotransductive processes.

**Signal transduction pathways.** Following stimulation of mechanosensors in the cell membrane, a number of pathways are activated. Once activated, these pathways have two pivotal endpoints. The first endpoint is the generation of biochemical signalling molecules within the sensor-cell nucleus that correlate with osteogenesis, producing an autocrine biochemical response. The second endpoint is the generation of biochemical signalling molecules, which are subsequently released from the sensor cell to act on a target cell (the 'effector' cell).

**Kinase pathways.** Protein kinases are enzymes that modulate the action of proteins through phosphorylation, and the sequential, coordinated action of multiple intracellular kinases are termed 'kinase pathways'. Kinase pathways are pivotal in cellular mechanotransductive processes, being activated by extracellular forces in a number of cell types including MSCs, endothelial cells, and myocytes.[44-46] Thus, kinase pathways act as a vital 'linker' between the mechanotransductive message received by the mechanosensors in the cell membrane and the transfer of that message into the intracellular environment.

A number of different kinase pathways are found in osteoblasts and have been implicated in osteogenesis.[46-48] Focal adhesion kinases are attached to intramembranous integrin channels and FAs. Following mechanical stimulation of integrins by external fluid flow, their extensive networks with other kinases, including extracellular signal-related kinases (ERKs) and protein kinase B (Akt), bring about upregulation of the OPN gene in osteoblasts. Osteopontin is a glycoprotein found in the ECM and is enhanced during bone formation under mechanical stress.[49] Evidence of FAK mediating the increase in RUNX2 and Osterix (OSX), two transcription factors that are key regulators of osteoblastic differentiation and osteogenesis, further demonstrates the importance of this kinase pathway in altering cell responses to mechanical stimuli.[50]

**Calcium signalling.** Mechanical stimulation of osteocytes by fluid flow in the LC network activates voltage-sensitive calcium channels leading to a rapid increase in intracellular calcium levels.[51,52] Acting as a second messenger, calcium initiates the activation of a number of mechanically regulated signalling cascades, including ATP and nitric oxide (NO). Intracellular calcium mobilization additionally acts to stimulate kinase signalling pathways, bringing about the same biochemical outputs described above (Figure 3g).

A rise in intracellular calcium stimulates the release of prostaglandin 2 (PGE2), a key anabolic regulator of bone formation and one of the key paracrine signallers. Prostaglandin 2 is released from connexins in the cell membrane and communicates with other osteocytes through gap junctions as an intercellular messenger. In this capacity, PGE2 has been shown not only to increase

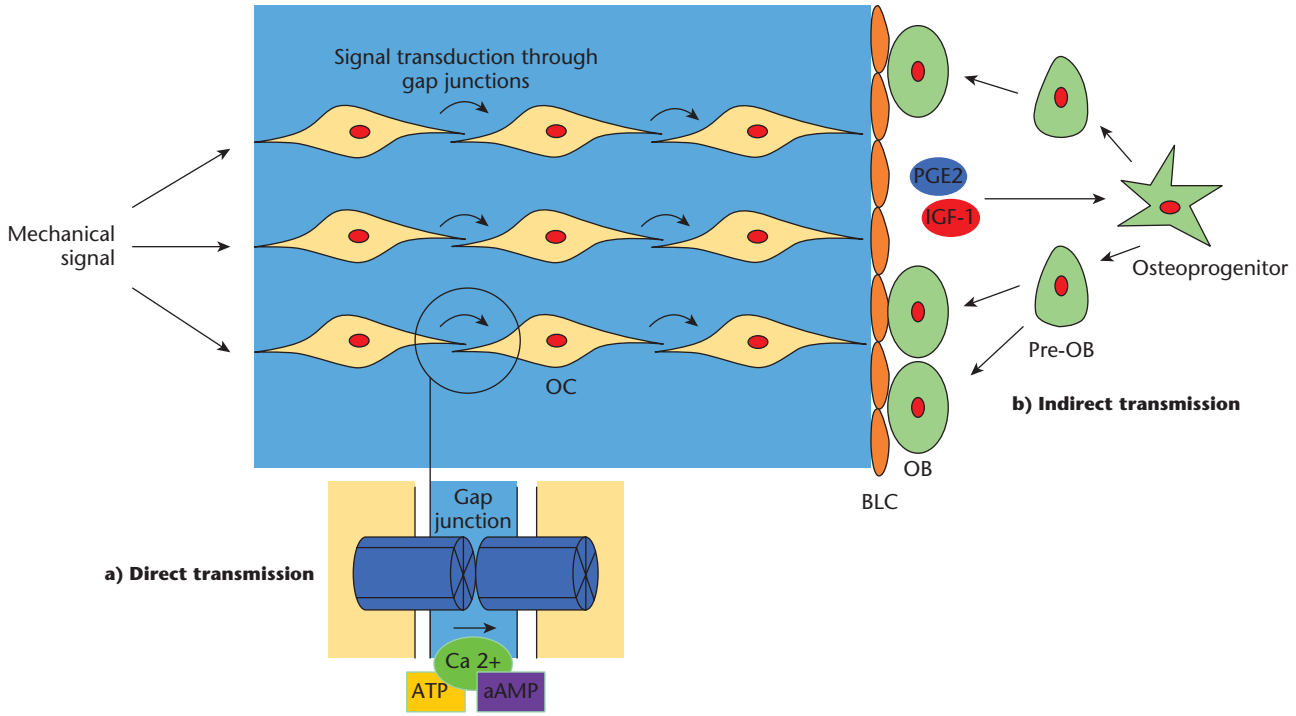


Fig. 4

Transmission of signal. a) Direct transmission: gap junctions form between adjacent connexins on cell membranes, allowing the passage of small molecules such as calcium, adenosine triphosphate (ATP) and cyclic adenosine monophosphate (cAMP). This propagates mechanotransductive signals through the network of osteocytes to the bone lining cells (BLCs). b) Indirect transmission: BLCs release paracrine factors including prostaglandin E2 (PGE2) and insulin-like growth factor 1 (IGF-1), stimulating osteoprogenitor cells to differentiate in preosteoblasts and subsequently osteoblasts. These new osteoblasts attach to the bone surface and produce new bone matrix. $Ca^{2+}$, calcium; OB, osteoblast; OC, osteoclast. Adapted with permission from **Duncan RL, Turner CH.** Mechanotransduction and the functional response of bone to mechanical strain. *Calcif Tissue Int*. 1995;57(5):344-358.

the sensitivity of bone to external loads acting in a positive feedback manner following its release, but also to recruit and stimulate the differentiation of precursor cells to an osteoblastic lineage.[26] Furthermore, generation of PGE2 modulates the presence of the connexins themselves, through increasing the amount of connexin 43 (Cx43) protein, which leads to upregulation of channel expression in the membrane, allowing for more PGE2 release from the cell.[53]

**Wnt/β-catenin signalling.** The Wnt signalling pathway is an evolutionary conserved signalling pathway that, upon activation, causes an accumulation of β-catenin in the cytoplasm and its eventual translocation into the nucleus to act as a transcription factor activator. Intracellularly, the cytoplasmic β-catenin protein is bound to the transmembrane cadherin mechanosensor (Figure 3h). Following mechanical stimulation of the cell, the cadherin dissociates with the β-catenin molecule, raising the intracellular concentration of β-catenin.[39] Translocation of the accumulated β-catenin into the cell nucleus activates the transcription of downstream genes strongly associated with osteomodulation such as RUNX2 gene expression.[54]

The Wnt pathway also plays a role in paracrine signalling. Following release of PGE2 in response to shear stress, it coordinates the Cx43 expression and gap junctions in the cell membrane described above, leading in turn to greater release of PGE2 from the cell and enhanced interosteocyte communication.[55]

**Transmission of signal.** The transmission of a biochemical signal between sensor and effector cell arises through a number of mechanisms and can be classified as direct or indirect. Direct mechanisms include membrane-to-membrane contact between cells. Indirect mechanisms relate to the diffusion of signalling factors from sensor cells, which can act on specific receptors on effector cells to evoke an osteogenic response (Figure 4).

**Direct transmission.** Gap junctions form channels between neighbouring cells through the docking of two hemichannels termed ‘connexons’. Small ions including calcium, ATP, and cAMP can pass through these adjacent junctions into adjacent cells.[28] This in turn can serve to activate signalling pathways in their neighbouring cells, forming an osteocytic network through which a mechanical stimulus can be propagated. Although bone has not traditionally been considered to have excitability, increasing evidence has shown that bone can act in a similar manner to a neural network through these gap junctions,[56] transmitting mechanical stimuli throughout neighbouring cells, generating an amplification of the biochemical response from the sensor cell.

**Indirect transmission.** Paracrine communication between bone cells through indirect transmission forms a pivotal pathway in the generation of new bone. As described above, PGE2 is released from connexon channels in response to a mechanical stimulus. Other paracrine mediators include prostacyclin and insulin-like growth factor-I (IGF-I). Lean et al[57] demonstrated how intercellular communication forms a critical step in mechanically transduced osteogenesis. Following mechanical stimulation, they observed increased expression of IGF-I in osteocytes, followed by increased expression of type 1 collagen and osteocalcin (OCN) on the bone surface 48 hours later. They hypothesized that IGF-I stimulates osteoprogenitor cells to divide and differentiate into osteoblasts, which attach to the bone surface and produce new bone matrix.

Although paracrine signalling appears to be a critical element to osteogenesis following mechanotransduction, autocrine signalling is arguably as important a pathway. Autocrine signalling refers to the sensor cell acting simultaneously as an effector cell, bringing about osteogenic changes in its own genomic footprint following mechanical stimulation. Experimental studies have observed the autocrine manner by which osteoblast-like cells increase expression and production of matrix proteins in response to stretching.[58,59] PGE2 is an example of a substance that has a role in both an autocrine and paracrine signalling capacity. Intracellularly, PGE2 has been shown to increase levels of calcium and cAMP.[60] Furthermore, PGE2 upregulation is associated with the induction of prostaglandin G/H synthase messenger RNA (mRNA), a major enzyme involved in the conversion of arachidonic acid to prostaglandin.[61] Both examples illustrate how PGE2 may act in an autocrine manner to further upregulate its own production within a cell.

Extracellularly, PGE2 can act on osteoblasts to stimulate alkaline phosphatase and collagen synthesis, while also increasing preosteoblast proliferation and attachment to the bone surface.[62-65] PGE2 therefore has the ability to stimulate not only existing osteoblast function to lay down new bone, but also to increase the production of osteoblasts through the modulation of progenitor cells.

**Effector cell response.** Following the action of osteogenic autocrine or paracrine factors on cells, the cellular response eventually manifests as a tissue response. De novo bone formation is approximately correlated to the rate of change of the applied load, and typically takes three to five days following a mechanical stimulus before bone formation is observed.[2] This lag period likely reflects the time required for the cascade of mechanotransduction events described above to take place.

The relevance of the rate of change of the applied load is also important. Static loading of cells sufficient to induce strain does not induce osteogenic changes,[66] emphasizing the importance of a dynamic process of mechanical loading in osteogenesis. Application of dynamic mechanical loading in excess of 1,000 μstrain will lead to bony remodelling, resulting in increased bone mass, whereas dynamic application of strain levels of exactly 1,000 μstrain is only sufficient to maintain bone mass.[67] Moreover, at lower levels of strain there will be inadequate stimulus, resulting in failure of activation of the mechanotransduction cascade and a consequent reduction in bone mass. This explains why loading of bone through weight-bearing exercise brings about an increase in bone mass density, whereas disuse from a physical disability or hypogravity environments results in rapid bone loss. Bone can therefore finetune its density requirements in an autonomous, homeostatic manner on a microscopic scale in order to meet the functional, macroscopic requirements of the skeleton in its environment.

**Mechanotransduction mechanisms in fracture healing.** This review has so far focused on the mechanotransduction mechanisms in osteogenesis, one of many factors required for successful fracture healing. Certainly, de novo bone is formed during fracture repair through mechanotransductive events already described. However, there are a number of other events that take place in fracture healing through mechanisms of mechanotransduction that can serve as targets for modulation and manipulation.

Angiogenesis, the formation of new blood vessels, is an integral part of bone remodelling following fracture. Micromovement at the fracture site generates fluid flow and shear stress on the cell membrane, which can induce mechanocoupling and subsequent activation of the pathways already described that lead to upregulation of both osteoblast formation and osteoblast activity. Fluid flow-induced shear stress applied to osteoblasts in vitro have also been shown to modulate the expression of genes associated with angiogenesis. A study by Thi et al[68] applied pulsatile flow to osteoblasts in vitro and examined all genes that were upregulated following this physical stimulus. The most markedly upregulated gene clusters were related to angiogenesis, blood vessel morphogenesis, blood coagulation, regulation of osteoblast differentiation, and prostaglandin biosynthesis. Furthermore, they demonstrated upregulation of at least seven genes that code for angiogenesis, with vascular endothelial growth factor (VEGF)-A being upregulated most strongly at 5.7-fold.

The mechanotransduction pathways associated with osteogenesis and those associated with angiogenesis are likely to be tightly coordinated. We have already described how PGE2 has both autocrine and paracrine effects in relation to osteogenesis. Its autocrine trait has

**Table I.** Preclinical and clinical applications of osteogenic mechanotransductive techniques

| Factor | Vibrations and nanovibrations | Low-intensity pulsed ultrasound | Extracorporeal shockwave treatment | Electrical stimulation |
|---|---|---|---|---|
| Mechanism | Whole body vibration: 0.1% strain, 10 to 90 Hz<br>Nanovibrations: nanoscale displacements of 1kHz | Ultrasound waves<br>Intensity of 0.5 mW/cm$^2$ to 50 mW/cm$^2$<br>Application with probe over fracture or nonunion site | Pressure wave rapidly increasing to > 10 MPa over nanosecond timescale<br>Applied externally or internally with a probe | Oscillatory electromagnetic fields generated around fracture site<br>Multiple generation modalities in clinical use |
| Physiological action | Improvement in angiogenesis at the fracture site<br>Some enhancement to bone stiffness and strength | Micromechanical stress at target site | Cascade of osteogenesis-inducing steps<br>Generation of oxygen free radicals<br>Hyperpolarization of cell membrane inducing osteogenic growth factor TGF-β1 | Simulate an enhanced loading environment on bone via piezoelectric effect |
| Clinical relevance | Improving bone density<br>No current use in fracture healing | Fracture-healing adjuvant therapy | Fracture-healing adjuvant therapy | Fracture-healing adjuvant therapy |
| Evidence base | Review of 19 studies of effect on fracture healing[72]<br>No clear effect to make clinical recommendations | Review of 17 sham controlled trials failed to categorically confirm clinical utility[73] | Systematic review of level 4 studies reported overall union rate of 76%[74] | Recent Cochrane review inconclusive and unable to support widespread clinical use[75] |

TGF-β1, transforming growth factor beta-1.

also been shown to govern the control of angiogenesis. PGE2 causes rapid induction of VEGF mRNA and VEGF production in osteoblasts,[69] which acts not only to promote angiogenesis but also to mediate osteoblast proliferation and differentiation.[70,71]

The innate ability of bone cells to fundamentally alter their function following a mechanical stimulus has proven to be a therapeutic target in recent years of orthopaedic research. Given the need for micromovement at the site of fracture to generate healing, it follows that iatrogenically generated forces applied to bone will bring about improved fracture healing. Those modalities that have demonstrated both preclinical and clinical evidence of being able to modulate osteogenesis through mechanotransductive pathways are described in Table I.

## Preclinical applications

**Vibrations and nanovibrations.** Reproducing the micromovement that fractures experience through secondary healing has been a field of prolific research in recent years[76-78]. Vibration is the mechanical stimulus produced through oscillatory movement, and the permutation of factors including frequency, amplitude, and acceleration result in different intensities and effects of the vibration. This systemic application of vibrations has been coined 'whole body vibration' (WBV). Although its use in improving bone density has been translated into clinical research,[79,80] its application to improve fracture healing and union rates in a clinical setting remains unexplored.

A recent preclinical systematic review examined the efficacy of WBV on fracture healing.[71] The review examined 19 animal studies where WBV was applied to either a closed or open acute fracture model. There was notable heterogeneity between the vibration regimen and delivery methods, although all were high-frequency vibrations (20 Hz to 90 Hz). The majority of studies reported an improvement in fracture healing, although a number reported inconclusive evidence and one study even showed that application of the vibration disrupted the fracture site.[81] In three of the four studies where the effect of vibration on angiogenesis was investigated, WBV enhanced angiogenesis at the fracture site and the surrounding muscles,[82-84] suggesting that vibration improves blood supply in order to enhance bone repair. However, the authors concluded that further studies with more concordant methodologies are needed to fully elucidate the osteogenic power of WBV before it is translated into clinical trials.[81]

A particular area of interest in the preclinical field of mechanotransduction and regenerative medicine has been the use of nanoscale mechanical stimulation, also known as nanovibration.[32,33,85,86] Ito et al[85] were the first to demonstrate genomic changes in cells following application of high-frequency piezo vibrations, where 'piezo' refers to the generation of vibrations through electricity. Their work demonstrated alteration in gene expression coding for cytoskeleton in embryonic fibroblasts, paving the way for further work examining the osteogenic effect of mechanotransduction through nanovibration. Application of nanoscale vibrational displacements of 1,000 Hz frequency on MSCs promoted osteoblastic differentiation through the statistically significant upregulation of OPN, OSX, OCN,

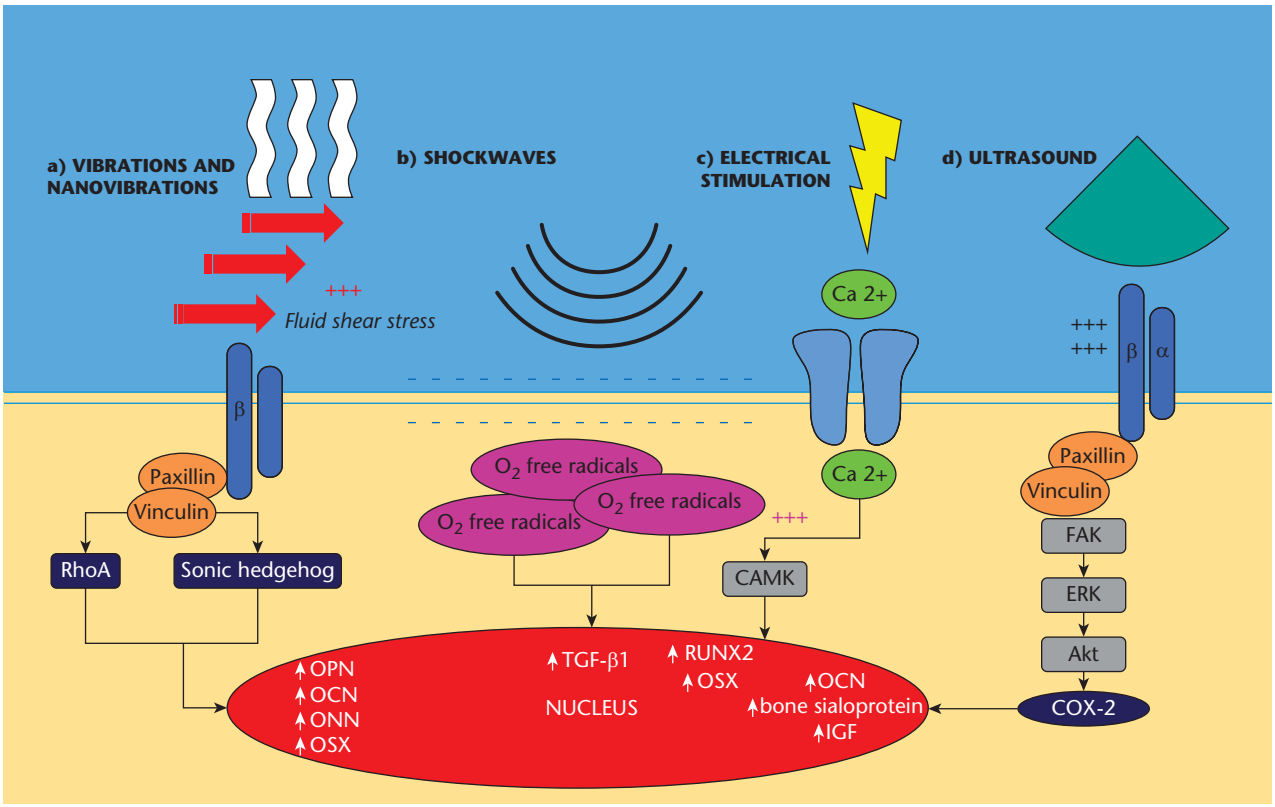


Fig. 5

Applications of mechanotransduction. a) Shockwaves induce hyperpolarization of the cell membrane with subsequent induction of the osteogenic growth factor, transforming growth factor beta-1 (TGF-β1), as well as synthesizing oxygen free radicals. b) Vibrations and nanovibrations enhance the oscillatory fluid flow in the interstitial fluid of the lacuno-canalicular (LC) network, increasing the fluid shear forces that stimulate integrin receptors. Upregulation of integrin activity stimulates Sonic Hedgehog pathways and Rho A pathways leading to upregulation of osteopontin (OPN), osteocalcin (OCN), osteonectin (ONN), and Osterix (OSX) proteins. c) Electrical stimulation can increase the influx of calcium through voltage-gated ion channels, modulating calmodulin kinase (CAMK) pathways leading to increased expression of the osteogenic gene *Runx2* and OSX. d) Ultrasound waves enhance integrin receptor activity, stimulating cyclo-oxygenase-2 (COX-2) pathway synthesis via protein kinase B (AKT), focal adhesion kinase (FAK), and extracellular-signal related kinase (ERK) pathways. This leads to the upregulation of osteocalcin, bone sialoprotein and insulin growth factor. RhoA, Ras homolog family member A.

and osteonectin (ONN), and, to a lesser degree, *Runx2* and BMP2 (Figure 5a).[32-34]

## Clinical applications

**Shockwaves.** A shockwave is a sonic pulse with defined physical characteristics. Initially, there is a very rapid positive rise in pressure over nanoseconds to approximately 100 MPa, followed by a longer period of negative pressure lasting several microseconds.[87] The shockwave generates two effects: the primary effect is the direct mechanical forces that result in the maximal kinetic energy concentration, while the secondary effect is the indirect mechanical forces generated by cavitation.[87,88] As the wave propagates through a medium, there is alternating compression and relaxation of the surrounding medium as the wave reflects off structures of different impedance. These changes in local density generate cavitation bubbles that collapse asymmetrically, producing local shear forces by high-velocity 'jet streams'.[89]

There are two mechanotransductive mechanisms to convert the force from the shockwave into a biological signal. The first mechanism is through hyperpolarization of the cell membrane (Figure 5b). Wang et al[90,91] demonstrated application of a shockwave-induced cell membrane hyperpolarization in bone marrow stromal cells, with subsequent induction of the osteogenic growth factor, transforming growth factor beta-1 (TGF-β1). The second mechanism is through the generation of oxygen free radicals. In a different study, Wang et al[92] observed the transformation of human mesenchymal progenitor cells into cells of osteogenic lineage following shockwave therapy, with a concomitant rise in oxygen free radicals or 'superoxide'. They asserted that these two mechanisms of mechanotransduction are not mutually exclusive, and the hyperpolarization of a cell membrane sets in motion a cascade of intracellular osteogenesis-inducing steps, including superoxide induction and TGF-β1 synthesis. Together, these can act to convert multipotent progenitor cells into cells of an osteogenic lineage.

Delius et al[93] were among the first authors to assess the osteogenic effects of shockwaves in vivo. Shockwave application to a rabbit femur stimulated microscopic

changes including fracture of cancellous trabeculae and leakage of bone marrow. They postulated that compression of marrow within the medullary canal, leading to marrow hypoxia and subperiosteal haemorrhages, stimulated osteogenesis at the site of the bony defect leading to new bone growth and cortical thickening. Subsequent clinical trials have looked at the use of extra-corporeal shockwave therapy (ESWT) in both the acute setting for fracture healing and also in the nonacute setting of stimulating bone healing in a nonunion. A recent review article summarized the findings of 11 studies utilizing ESWT in the treatment of acute long bone fractures and delayed unions/nonunions.[94] Only one of the studies was a randomized controlled trial, with the remainder being case series illustrating level 4 evidence, thus being of insufficient quality to be able to make a recommendation. Furthermore, lack of a standardized definition of nonunion and variable treatment regimens render quantitative analysis difficult. However, an overall mean union rate of 76% provides encouraging scope for more stringent clinical trials.

**Electrical stimulation.** Electrical stimulation (ES) products are commonly available and relatively economical adjuvants in modern fracture care, with several different technologies that currently exist.[95] Current approaches can be grouped into three main device classes: direct current (DC) products comprising a cathode at the fracture site and an anode in remote subcutaneous tissues; inductive coupling (IC) utilizing coils carrying electromagnetic currents; and capacitive coupling (CC) technologies using two capacitor plates at either end of the fracture site.[95] An external power source induces an electrical field between the two capacitors. Griffin and Bayat[92] reviewed 140 in vitro and clinical studies evaluating ES for bone healing. The summary of evidence suggested that all three types of ES enhance growth factors, in turn increasing cell proliferation and enhancing callus formation (Figure 5c).

While the osteogenic effect of ES appears promising in in vitro work, the clinical efficacy of the technology is controversial. A recent Cochrane review of randomized controlled trials of ES technologies in the treatment of nonunion adult long bone fractures, which assessed four trials involving 125 patients, was inconclusive and insufficient to inform clinical practice.[97] A 2016 systematic review of 15 sham controlled randomized trials found moderate quality evidence for ES reducing patient-reported pain and radiological nonunion with no difference in functional outcomes.[98] These findings were independent of the ES technology modality used. Mollon et al[99] conducted a meta-analysis of 11 trials relating to delayed or ununited long bone fractures, and did not find a statistically significant impact of ES technologies on this subset of fractures.

**Low-intensity pulsed ultrasound.** Low-intensity pulsed ultrasound (LIPUS) has been commercially available since its 1994 Food and Drug Administration (FDA) approval as an adjuvant therapy in the healing of primary fractures.[100] It exerts a micromechanical stress over its target site and has been shown in vitro to increase the incorporation of calcium ions in cultures of cartilage and bone cells while stimulating gene expression implicated in the healing process.[101] In vivo work by Naruse et al[102] implicates COX-2 as the central protagonist for mediating LIPUS-induced osteogenesis. Application of LIPUS on bone marrow stromal cells elevated levels of IGF-I, OCN, and bone sialoprotein mRNA, which were in turn eliminated by application of a COX-2 inhibitor (Figure 5d).[102] These findings have been substantiated by Tang et al[103] who demonstrated increased expression of COX-2 in osteoblasts via activation of integrins and subsequently kinase pathways following application of ultrasound.

The technology is now widely used in clinical practice, despite the exact biological mechanism of function remaining unknown. Low-intensity pulsed ultrasound was prescribed by 21% of Canadian trauma surgeons in the management of acute tibial fractures in 2008, and LIPUS technologies are available to be prescribed on the NHS in the UK.[104]

A number of preclinical and clinical studies have assessed the effects of this technology on fracture healing[105-108] however, its use remains controversial.[109] A 2017 systematic review of 17 sham controlled high-quality randomized trials assessed several outcomes such as functional recovery, number of subsequent operations, and time to radiological healing.[110] This review also assessed each included study for risk of bias and reliability. The review concluded that high-quality randomized trials showed no effect on pain reduction, time to full weight bearing, or adverse effects related to the device. Furthermore, when higher credence was given to trials at low risk of bias, there was moderate- to high-quality evidence that LIPUS failed to accelerate radiological healing.[110]

In conclusion, LIPUS promises a relatively low-cost, non-invasive technology to assist in fracture healing. Unfortunately, despite the presence of many trials in the field, a definitive answer in support of its use remains elusive.

It is evident from this review of the literature that the application of molecular-level mechanical forces has a clear osteogenic effect on both in vitro and in vivo cells. Macroscopically, the clinical evidence is less convincing. Current mechanotransductive technologies have failed to prove their utility despite tantalizing in vitro evidence and plausible biochemical mechanisms, leaving patients with the deleterious effects of fracture nonunion. A global consensus regarding the optimal way to treat nonunion

remains undecided, but stimulating de novo bone formation is likely to be pivotal in the development of an effective therapy.

Given how far the understanding of the physiological mechanisms underpinning mechanotransduction has progressed, together with the osteogenic properties that numerous in vitro and in vivo mechanotransduction studies have demonstrated, it is hopeful that future research will identify effective novel targets for de novo bone formation utilizing mechanotransduction.

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**Authors information**

- S. Stewart, MBChB, BMedSci (Hons), Centre for Blast Injury Studies,
- A. Darwood, BMBS, BMedSci, Centre for Blast Injury Studies,
- S. Masouros, PhD, Centre for Blast Injury Studies,
- C. Higgins, PhD, Centre for Blast Injury Studies,
- A. Ramasamy, MA, PhD, Centre for Blast Injury Studies, Imperial College London, London, UK.



**Funding statement**

- No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

**Conflict of interest statement**

- S. Masouros reports an institutional grant (paid to the Centre for Blast Injury Studies) from the Royal British Legion, related to this study. C. Higgins reports an institutional grant (paid to the Centre for Blast Injury Studies) from the Medical Research Council, related to this study.