Bone: Functions, Structure and Physiology

Joana da Costa Reis and Maria Teresa Oliveira

Abstract In this chapter, bone functions, regulation, morphological structure and physiology are revisited. Bone is a highly complex tissue, very sensitive and responsive to external and internal stimuli, and intimately intertwined with other organs. From embryogenesis to endocrine regulation and bone remodelling, a global assessment is presented. Considering the scope of this book, special emphasis is given to how cell structure and tissue organization modulate the response to mechanical stimuli.

1 Introduction

The deeply dynamic nature of bone may be missed by a less attentive eye. Bones are resilient and apparently quite rigid structures. They vary in shape, size and number and are divided in the axial and appendicular skeleton. Through life, they are subjected to loads and strains that temper their shape, with old matrices being replaced by newly formed ones, maintaining bone volume and strength. When trauma and fractures occur, bones are capable of healing if enough stability and proper alignment are guaranteed.

Osteogenesis, bone repair and remodelling are directed by the exchanges involving the environment, cell-to-cell interactions and cell–extracellular matrix.

Mechanical forces are crucial in early embryonic development. Morphogenesis is controlled through fluid flow mechanisms and by cellular contractility. Early embryo shaping depends on morula contraction determined by cohesivity. Multiple layers result in the development of endoderm, mesoderm and ectoderm in the

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blastula [1–4]. Mechanical forces, cell geometry, and oriented cell division together orchestrate normal airway tube morphogenesis [5] and may help determine the neocortical organization [6]. Cells generate tension through contraction of actin-myosin cytoskeleton filaments, which are transmitted through cadherin-mediated adhesion sites to surrounding structures, these being either cells or extracellular matrix. The cytoskeletal conformation and cell shape stress-dependent changes regulate cell phenotype; interactions with the extracellular matrix are of paramount importance for cell phenotype [2]. Organ lateralization and asymmetry depend on unidirectional fluid flow, generated by specialized motor protein complex dynein. The fluid flow induces differences in key molecules' expression (such as the TGF-family signalling molecules) [7–10]. Lateralization may also depend on fluid shear, in the embryo, by acting on a group of non-motile cilia, coupled to calcium channels; fluid flow generated shear may cause the intracellular calcium concentrations to increase and initiate the cascade of events responsible for lateralization [11].

The mechanical environment is also a determining factor for vasculogenesis, angiogenesis [12, 13] and neuronal development [14–17].

The embryo mesoderm is constituted by spindle- or star-shaped cells called mesenchymal stem cells (MSCs). MSCs are the utmost pluripotential cells in the organism, originating different tissues such as the connective tissue, muscle, cardiovascular tissue and the whole skeletal system. Bone, cartilage, tendons and ligaments develop through mechanisms of proliferation, migration and differentiation, but also programmed cell death/apoptosis [18].

We now begin to address how complex bone is in its functions, how its macroarchitecture, microarchitecture and arrangement at molecular level play together remarkably, ensuring its responsiveness to external and internal stimuli and close entwining with other organs.

2 The Complexity Behind Simplicity

2.1 Bone Functions

Osseous tissue is the most rigid and resilient tissue of the body. Bone is composed of dense connective tissue; it is the primary skeleton component, thus providing structure, support and protection to vital organs, like the brain (skull), the spinal cord (vertebrae) and the heart and lungs (ribs and sternum). Vertebrae also participate in the spine shock absorbance—providing adequate load cushioning for the fibrocartilaginous joints at the intervertebral discs. Long bones provide structure, stability and, along with the joints, enable body movement—providing levers for the muscles.

Moreover, bones act as the major source of blood, since haematopoiesis occurs in their medullary cavity. In infants, the bone marrow of all long bones is capable of blood synthesis. With ageing, part of the red marrow turns into yellow fatty marrow,

no longer capable of haematopoiesis. Functional red marrow in adults is limited to the vertebrae and the extremities of femur and tibia.

Bones also partake a vital role as:

- Mineral storage: mostly calcium, phosphate and magnesium; bone plays an important metabolic role, mediated by several hormones, regulating mineral homeostasis [19–21].
- Acid-base balance, as bone can buffer blood against extreme pH changes by absorbing or releasing alkaline salts, through bone cells' activity [22–24].
- Osteoblasts have been shown to produce growth factors, with production regulated by systemic hormones and local mechanical stress [25]. The bone matrix holds several growth factors such as insulin-like growth factors I and II, transforming growth factor beta, acidic and basic fibroblast growth factor, platelet-derived growth factor and bone morphogenetic proteins, released when resorption occurs [26].
- Adipose tissue storage (yellow bone marrow functions as a fatty acid/energy reserve) [27–29].
- Heavy metals and other foreign elements, after detoxification from the blood, are stored in bone and later excreted [30, 31].
- Bone functions as an endocrine organ, as it produces two known circulating hormones:

  a. Fibroblast Growth Factor 23 (FGF23): FGF23 was first described by Yamashita et al., and it is produced mainly by osteocytes [32, 33], but also by osteoblasts [34]. FG23 acts on the kidneys, inhibiting 1α-hydroxylation of vitamin D and promoting phosphorus excretion in urine [35–37]. FGF23 also decreases phosphorus absorption in the intestine, regulating inorganic phosphate metabolism and thus mineralization [35]. Serum calcium concentration regulates FGF23 production [38], thus making FGF23 into a calcium–phosphorus regulatory hormone [39]. Hence, FGF23 excess or deficiency results in anomalies of phosphate metabolism. Excess FGF23 hinders renal phosphate reabsorption and 1,25 dihydroxy vitamin D_3 [1,25$(OH)_2$D] production, causing hypophosphatemia and suppression of circulating 1,25$(OH)_2$D levels and, eventually, rachitic changes in bone [40]. These changes occur in autosomal dominant hypophosphatemic rickets and osteomalacia [41] and, in tumour-induced osteomalacia (TIO), a paraneoplastic syndrome [42]. In contrast, reductions in FGF23 cause tumoral calcinosis syndrome, characterized by hyperphosphatemia, increased 1,25$(OH)_2$D and soft tissue calcifications [40, 43]. An obligate FGF23 co-receptor was identified—Klotho [44]. Klotho is essential to activate FGF receptors and their signalling molecules. Secreted Klotho suppresses either by direct interaction or interference with receptors, the activity of several growth factors: insulin, insulin-like growth factor-1 (IGF-1) [45], Wnt [46] and TGF-β1 [47]. The FGF23-Klotho axis represents a specialized system responsible for the external and internal calcium and phosphorus balance in the bone, intestine and kidney. FGF23-Klotho axis works under parathormone regulation, with parathormone increasing serum FGF23 levels and directly

promoting FGF23 expression by osteocytes [48, 49]; FGF23 exerts negative feedback by inhibiting the parathyroid glands [50, 51]. FGF23 production in the osteocyte may be inhibited by osteopontin [52].

b. Osteocalcin is a protein produced by osteoblasts in bone, and it is a major regulator of insulin secretion by direct action over the pancreatic β-cell. Osteocalcin also increases insulin sensitivity of peripheral tissues, e.g. muscles and liver, up-regulating glucose uptake and energy expenditure, thus contributing to glycaemia regulation [53–56]; it also reduces fat deposition by inducing adiponectin secretion by adipocytes [57, 58]. Blood osteocalcin levels are significantly lower in diabetic patients when compared to non-diabetic controls, and osteocalcin levels are inversely related to fat mass and blood glucose [59, 60]. Lastly, osteocalcin influences male fertility, by enhancing testosterone production by Leydig cells in the testes [61].

2.2 Bone Structure and Mechanical Behaviour

Bone is a composite material; the inorganic portion of bone comprising 70% (of which 95% is hydroxyapatite and 5% are impurities impregnated in hydroxyapatite), whilst 22–25% are organic (of which 94–98% are mainly collagen type I and other non-collagen proteins and 2–5% are cells); 5–8% is water [62].

Bone mechanical properties depend on porosity, composition, mineralization degree and organization of solid matrix. Therefore, the mechanical behaviour of an entire bone is highly dependent on its properties at a microscale [63, 64].

Bone can be classified according to its structural features at a microscopic level in woven and lamellar bones.

Woven bone is immature or pathologic, primary bone, and it is present in growth, fracture healing and diseases such as Paget's disease. Cells and matrix are laid randomly. Woven bone is formed during intramembranous, endochondral or rapid appositional bone growth. In large animals (whether reptiles, birds or mammals), woven bone with large vascular canals is rapidly deposited in the subperiosteal region. Canals are lined with osteoblasts that gradually deposit lamellae until the canal has a reduced diameter; the resulting structure is a primary Haversian system or osteon. The random distribution of its components explains woven bone's isotropy.

Lamellar bone is organized, mature bone, and it is morphologically classified into two different types: cortical or compact bone and cancellous or trabecular bone. Cortical and cancellous bone types differ in both structure and functional properties, but both are highly anisotropic.

The typical structure of a long bone, such as the femur or the humerus, comprises the cylindric shaft, or diaphysis, and the extremities, or epiphyses (Fig. 1). The outer surface is covered by a layer of dense connective tissue called periosteum, except for the areas of mobile articulation, covered with hyaline cartilage. The periosteum is highly vascularized and responsible for appositional bone growth. The endosteum

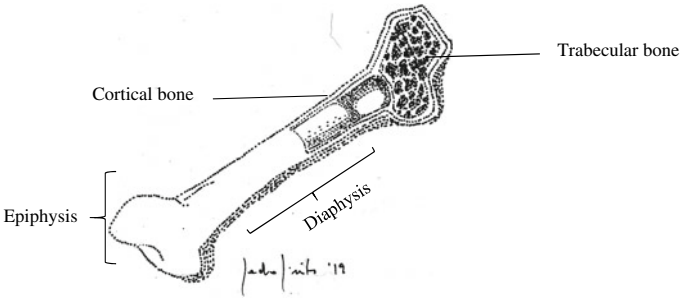

Fig. 1 Illustration of a long bone structure, showing the distribution of two different types of lamellar bone: cancellous and cortical compact bone

is a thin layer of connective tissue that lines the inner surface of the diaphysis, containing the medullary canal. The epiphysis consists of an outer layer of cortical bone surrounding the porous network formed by trabecular bone. Within the spaces between trabeculae lays red bone marrow [65]. Long bones, fundamental for load bearing and leverage, evolved as structures in which stiffness along the long axis was favoured.

Cortical bone (Fig. 2) accounts for approximately 80% of the skeletal mass. Cortical bone is vital to skeletal mechanical competence, both of long and flat bones. It is formed by tightly aligned collagen fibrils, making concentric lamellae. Each lamella is 2–3 μm thick and is arranged in distinct layers of parallel fibrils, each

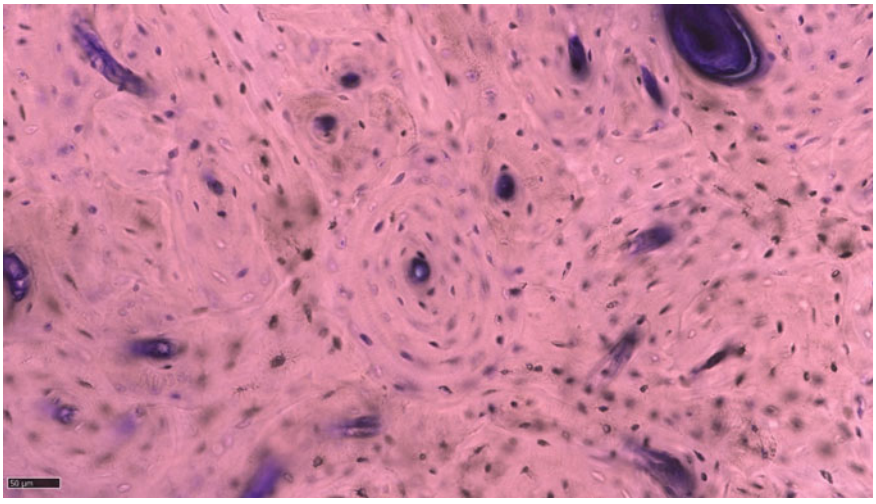

Fig. 2 Microphotograph of cortical bone in proximal tibia (undecalcified bone section of sheep tibia, Giemsa-Eosin, 40× magnification; slide digitalized using NanoZoomer SQ, Hamamatsu Photonics, Portugal). Haversian systems are evident, as are the concentric lamellae. Osteocytes are visible in their lacunae, in between lamellae

layer with a different fibril orientation [66]. Mineralization occurs by apatite crystals (mainly carbonated apatite) deposition within and around these fibrils. The lamellae form cylinders containing a hollow central canal where blood vessels and nerves run, composing the cortical bone microstructural unit, called Haversian system or osteon. From the centre of the osteon (Haversian canals), blood vessels form a three-dimensional network and penetrate the cortical bone layer perpendicularly (running within Volkman's channels) [67]. In between, the osteons are incomplete osteons, known as interstitial systems or interstitial bone.

Cancellous (or trabecular) bone is highly porous and adapted to compressive loads. The lamellae are organized in a parallel manner, forming trabeculae. These rod- and plate-shaped struts are organized into a flexible lattice with variable degrees of interconnectivity (Fig. 3).

The trabecular network is light and of utmost importance for load transfer in long bones, absorbing and distributing sudden stresses. In vertebrae, cancellous bone is the main load-bearing structure and essential for shock absorption. Trabeculae are approximately 200 μm thick and are orientated according to routine load-bearing direction [68]. This is evident in epiphyses and metaphyses of long bones, but also in the vertebrae and ribs. Trabeculae are covered by osteoblasts and bone-lining cells. Osteoblasts actively lay extracellular matrix (ECM), and bone-lining cells are in an inactive state. The metabolic rate of trabecular bone is higher than that of cortical bone and the remodelling phenomena more prominent. [18, 69].

Bone endures both compressive and tensile stresses. Bone is subjected to bending and torsion [62]. In humans, there is a large variation in strains, ranging from to 400 to 2000 μstrains or even as high as 4000 μstrains [62, 70, 71].

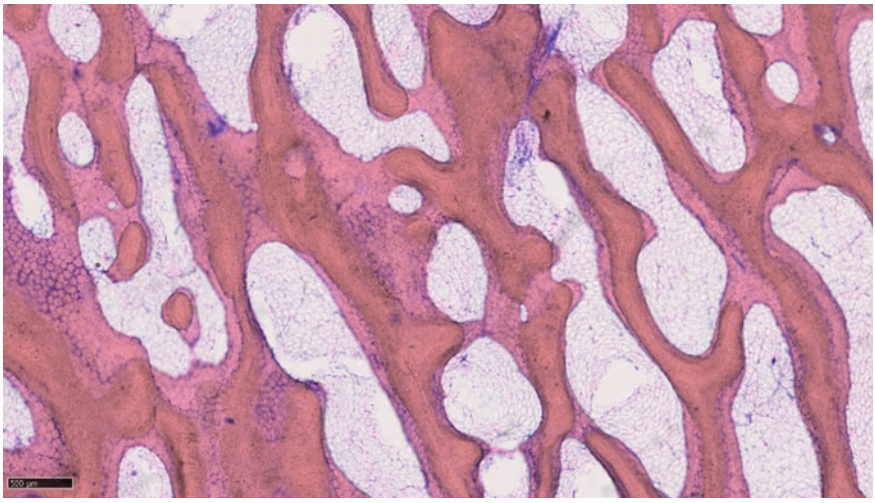

Fig. 3 Image of trabecular bone (undecalcified bone section of the proximal epiphysis of sheep's tibia, Giemsa-Eosin, 5× magnification; slide digitalized using NanoZoomer SQ, Hamamatsu Photonics, Portugal). The picture illustrates the sponge-like structure of cancellous bone

The bone exhibits a stress–strain response of sequential elastic and plastic responses. In its elastic region, no permanent damage is caused to the bone structure; if the stress increases, a gradual transition to a plastic response occurs. Post-yield deformations are permanent and cause trabecular fracture, cement lines and cracks. Crack formation and growth allow energy dissipation and are a powerful stimulus for bone remodelling in healthy bone.

The mineral component contributes to compression strength, while collagen fibrils are fundamental for tensile strength. The mineral phase is highly related to stiffness, whilst collagen is determinant for toughness [72]. A higher Young's modulus corresponds to less ductility and higher brittleness [73].

Bone material properties reflect, therefore, high functional specialization and depend on architecture, composition and component spatial distribution.

2.2.1 The Bone Matrix

Structure and material properties of the bone depend on collagen. The collagen I molecule is composed of three long peptide sequences, arranged helicoidally. Collagen is produced by osteoblasts and goes through several enzymatic modifications whilst still within the cell [74]. After leaving the cell, collagen molecule undergoes further cross-linking within itself and with other collagen molecules. Collagen chain mutations lead to diseases such as osteogenesis imperfecta [74, 75]. The triple tropocollagen units are aligned in fibrils and display a permanent dipole moment. Consequently, collagen acts as a piezoelectric and pyroelectric material and as an electromechanical transducer [76, 77]. The native polarity and the piezoelectric properties of collagen are associated with the mineralization process. Under compression, negative charges on the collagen surface become uncovered and attract calcium cations, which are then tailed by phosphate anions [77, 78]. Collagen can actively control mineralization, functioning in synergy with other non-collagenous proteins, inhibitors of hydroxyapatite nucleation. The positive net charge close to the C-terminal end of the collagen molecules promotes the infiltration of the fibrils with amorphous calcium phosphate; at the gap and overlap regions of the collagen molecule, the clusters of charged amino acids form nucleation sites and the amorphous calcium phosphate are changed into parallel oriented apatite crystals [79].

Non-collagenous proteins such as osteopontin, fibronectin, osteonectin and bone sialoprotein are present in much smaller quantities but are, nonetheless, essential for normal bone function and properties.

Osteopontin (OPN) is a non-collagenous glycoprotein, present in the bone matrix, binding to the cell surface and hydroxyapatite. It is mostly produced by proliferating pre-osteoblasts, osteoblasts and osteocytes, but also by fibroblasts, osteoclasts and macrophages [80, 81]. OPN intervenes in cell migration, adhesion and survival in diverse cell types. OPN is a key player in bone remodelling processes. Its production is modulated by mechanical loading, being up-regulated both by loading and by loading deprivation [81–83]. OPN has been proved to inhibit mineralization, but

its deficiency significantly lessens bone fracture toughness and causes anomalous mineral distribution, leading to increased FGF23 production [52, 84–86].

Fibronectin mediates many cellular interactions with the ECM, playing an important part in cell adhesion, migration, growth and differentiation. It is determinant for vertebrate development and is mostly synthesized by osteoblast precursors and mature bone cells; it can also be produced at distant sites (such as the liver) and enters the systemic circulation. Some studies suggested that only circulating fibronectin exerts effects on the bone matrix [74, 87]. Fibronectin binds to collagen and may act as an extracellular scaffold, facilitating interactions of BMP1 with substrates [88]. Fibronectin may also be vital for the osteogenic differentiation of mesenchymal cells [89, 90].

Osteonectin or secreted protein acidic and rich in cysteine (SPARC) is secreted by osteoblasts during bone formation, and it is one of the most abundant non-collagenous proteins in the bone matrix. Osteonectin is a regulator of bone mineralization; its attachment to collagen can inhibit or promote mineral formation. It interacts also with apatite through its N-terminal domain, inhibiting crystal growth [91]. Osteonectin knockout mice suffer from osteopenia due to osteoblasts and osteoclasts defective function and low bone turnover. Changes in the osteonectin encoding gene have also been linked to idiopathic osteoporosis and osteogenesis imperfecta [92].

Thrombospondin-2 (TSP-2) is another matricellular protein that also exerts its effects on osteoblast proliferation and function, being involved in MSCs adhesion and migration; it has also influence on angiogenesis and tumour growth and metastization [93–96]. TSP-2 likely participates in bone remodelling, since it promotes osteoclastogenesis through the RANKL-dependent pathway [95, 96].

Bone sialoprotein (BSP) is a highly glycosylated and sulphated phosphoprotein that is found almost exclusively in mineralized connective tissues [97]. BSP knockout mice have higher trabecular bone mass and reduced amounts of cortical bone; they also present a very low turnover. BSP defective mice maintain unloading bone response, as opposite to OPN knockout mice [98]. The absence of BSP also leads to changes in the growth plates, decreased bone length and delayed ossification [99]. BSP and OPN are part of the small integrin-binding ligand N-linked glycoproteins (SIBLING) family, and the recent studies suggest the interplay in between these proteins is determinant in bone biology [100].

Proteoglycan (PG) encoding genes are expressed in skeletal and non-skeletal tissues but with stronger expression in bone, joints and liver. PGs are a large family of molecules and perform many biological functions. PGs help to structure bone by mediating collagen secretion and fibril organization; they also act as mineralization inhibitors. PGs also modulate cytokines and growth factors' biological activity in bone [101]. In bone, PrG4 gene expression is under the control of PTH [102].

From the reviewed above, it becomes clear that non-collagenous and collagen matrix proteins are fundamental for bone morphology and material properties, interacting with each other and with cells and responding to stimuli generated locally or systemically. It is also evident that matrix components have a multiplicity of functions. The role of a molecule is modulated by changes in its structure and by interactions with other substances.

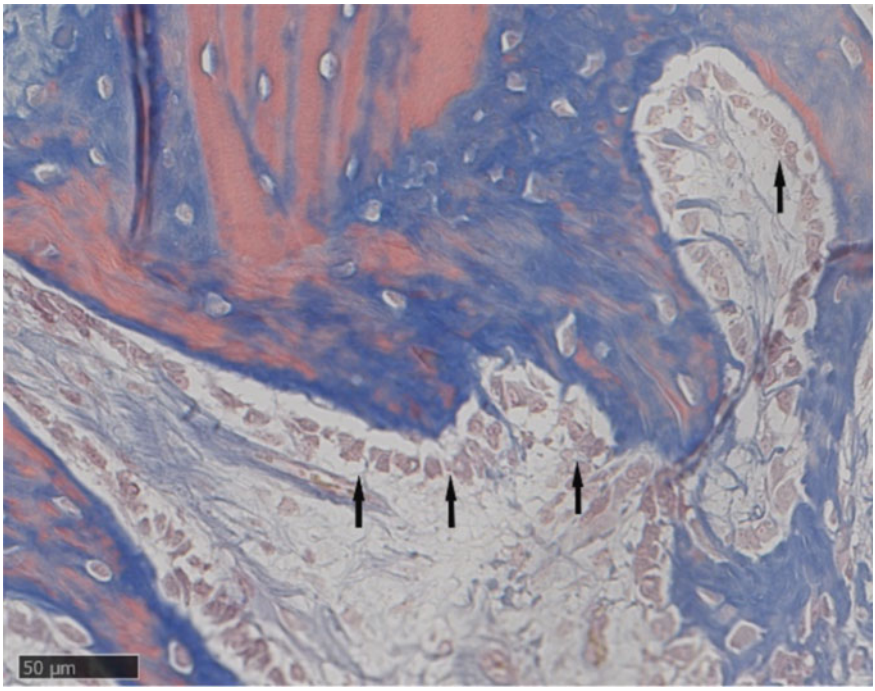

Fig. 4 Osteoblasts are cuboidal cells that when actively deposing matrix on bone surfaces (microphotograph, decalcified sheep bone section, Mason trichrome, magnification 40×; black arrows point line of active osteoblasts). When quiescent, osteoblasts appear as flat bone-lining cells

2.2.2 Bone Cell Population

Mature bone contains three core cell populations: osteoblasts, osteocytes and osteoclasts.

Osteoblasts

Osteoblasts arise from MSCs, sharing a common background with chondrocytes, myoblasts and fibroblasts. Osteoblasts differentiate under the influence of a variety of hormones, cytokines and the local mechanical environment [103]. These cells, when active, are cuboidal/round (Fig. 4), with specific features consistent with their secretory functions, such as prominent Golgi complexes and endoplasmic reticulum (with multiple vesicles and vacuoles); these are even more evident during matrix secretion and early stages of mineralization [104].

Osteoblasts can also remain on bone surfaces as flat bone-lining cells, in a quiescent state, with few apparent cell organelles. During the osteoblast maturation process, there are increased levels of expression of pro-collagen, osteopontin and

osteocalcin; bone sialoprotein seems to be more strongly expressed at intermediate phases of differentiation [105, 106]. Osteoblast differentiation is impaired when gap junctions are inhibited, suggesting communication to neighbouring cells is essential for differentiation [107]. Osteoblasts produce non-mineralized matrix—osteoid—that becomes gradually mineralized, wherein they become trapped and some differentiate into osteocytes. Runx2 induces the expression of major bone matrix protein genes in vitro. Runx2 expression is up-regulated in pre-osteoblasts, being maximal in immature osteoblasts and down-regulated in mature osteoblasts. Although Runx2 is weakly expressed in undifferentiated mesenchymal cells, it induces their osteogenic commitment [108]. Once Runx2 is activated, cells undergo the three stages of differentiation, with the synthetization of different molecules: in stage 1, the cells proliferate and express fibronectin, collagen, TGFβ receptor 1, and osteopontin; during stage, osteoblast will differentiate and act on the extracellular matrix through alkaline phosphatase and collagen; at stage 3, the osteoblast will assume its characteristic cuboidal shape and secrete significant amounts of osteocalcin. Osteocalcin will promote matrix mineralization [109]. Osteoblast differentiation is influenced by $1,25(OH)_2D_3$ and mechanical stimuli, amongst other factors [110].

Osteocytes

Osteocytes are the most abundant cells of bone, comprising more than 90% of the osteoblast lineage and contributing to bone formation and resorption [111, 112]. They are fully differentiated osteoblasts embedded in the mineralized matrix, inside the osteocytic lacunae. Lacunae are located between the lamellae and connected with surrounding lacunae by a canalicular system (Fig. 5). Osteocytes have long dendritic cell processes (50–60 by cell) that lay within the canaliculi. The extremities of the cell processes connect osteocytes amongst themselves and allow contact with osteoblasts and bone-lining cells [18, 113, 114]. The resulting functional syncytium shares a common environment [115].

Osteocytes have no matrix secretion functions; however, they are responsible for sensing changes in the bone structure and commanding bone remodelling.

Pre-osteoblasts and osteoblasts are less responsive to fluid shear stress than osteocytes. Mechanosensitivity seems to increase during differentiation. However, osteoblasts are able to modulate their response according to the mechanical stimuli intensity [62]. Osteocyte functions include mechanosensing and maintaining bone matrix [116, 117]. The sensation of electrical signals may be one of the functions of osteocytes, and electrical signals mediated by osteocytes may regulate the cell behaviour in bone tissue [118]. Flexoelectric fields are generated by fractures in the bone mineral and may be large enough to induce osteocyte apoptosis and initiate bone remodelling [119].

The same mechanical stimulus may cause a different response in osteocytes according to their cell body shape [120]. Recently, a study reports that osteocyte plasma membrane disruptions, caused by mechanical loading, act as triggering mechanosensing events, both in vitro and in vivo [121].

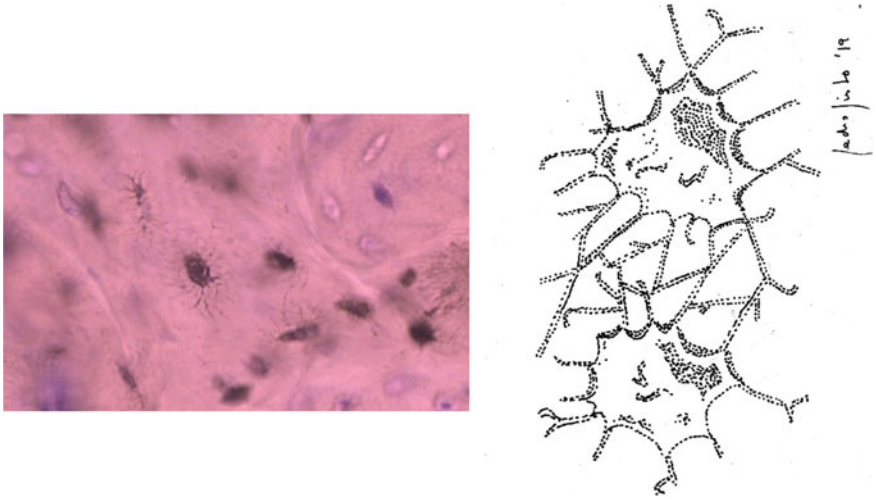

Fig. 5 Detail of microphotograph of an undecalcified bone section of sheep tibia, Giemsa-Eosin, on the left, showing osteocytes (Giemsa-Eosin, 40× magnification and 200% zoom; slide digitalized using NanoZoomer SQ, Hamamatsu Photonics, Portugal). The canaliculi where cell processes run are observable. The image on the right illustrates a simplified version of the resulting three-dimensional syncytium

Osteocytes' early response to mechanical loading results in vesicular ATP release by exocytosis, tuned according to the magnitude of the stimulus [122]. Mechanical stimulation of osteocytes also causes fluctuations in intracellular calcium levels; these are responsible for calcium-dependent actin contraction and release of extracellular vesicles containing bone regulatory proteins [123]. In fact, osteocytes respond to mechanical stimuli by producing various messenger molecules, such as nitric oxide and prostaglandins, namely prostaglandin E2 (PGE2) [117, 124, 125]. This response is dependent on the function of stretch-activated calcium channels [126], although reserves of intracellular calcium also contribute [123]. PGE2 has anabolic effects, stimulating osteoblast activity and new bone formation [127]. Nitric oxide inhibits bone resorption, by suppressing osteoclast formation and increasing the expression of osteoprotegerin [128, 129].

The lifespan of osteocytes is highly variable and likely associated with the rate of bone remodelling, depending on mechanical and environmental factors such as hormones; osteocytes apoptosis may be inhibited or induced by a variety of physiological and pathological conditions. Osteocyte apoptosis may be induced by biological effectors such as hormones, without being accompanied by increased osteoclastogenesis [130–134].

Young osteocytes are polarized towards the mineralization front, just like osteoblasts are, with the nucleus remaining close to vessels [104]. As lamellar bone matures, the osteocytes tend to spread their processes perpendicular to the longitudinal axis of trabeculae and long bones and appear as flattened cells. In immature bone,

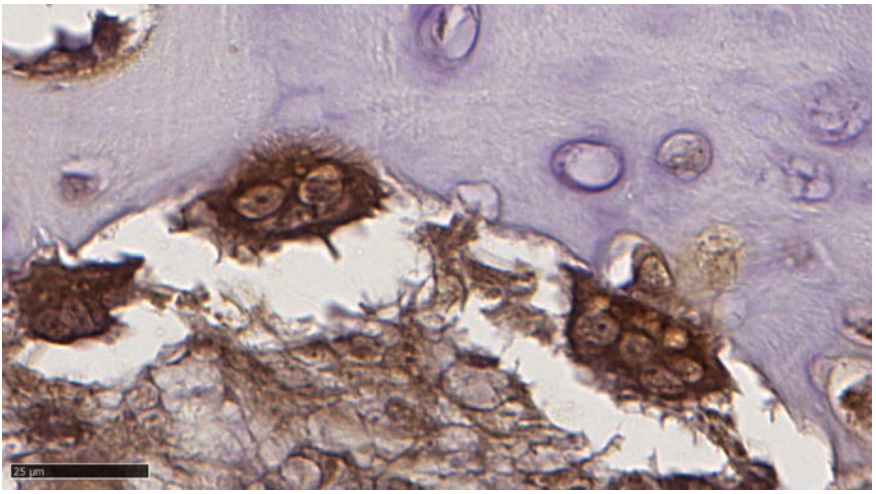

Fig. 6 A microphotograph of TRAP positive osteoclasts firmly attached to the bone surface. The ruffled border membrane is visible in direct contact with bone. This is the resorbing organelle; along its enlarged ruffled contact surface, proton pumps lower the local pH, dissolving hydroxyapatite

plump osteocytes with randomly distributed processes predominate [130]. Osteocyte density is closely related to bone architecture and thus to its mechanical behaviour [135].

Ageing has been correlated with smaller canaliculi, in lower numbers per lacuna, leading ultimately to reduced mechanosensitivity in the aged individual [136, 137].

Osteoclasts

Osteoclasts are multinucleated cells and belong to the same lineage as macrophages and monocytes (Fig. 6). Like macrophages, osteoclasts are able to merge and form multinucleated cells and to phagocytize [138]. The cell precursor may differentiate into either an osteoclast or a macrophage. The differentiation path depends on the progenitor cell being exposed to a receptor activator of several ligands (receptor activator of nuclear factor-κB ligand—RANKL, osteoprotegerin and osteoclast differentiation factor—ODF) or to colony-stimulating factors related to immune system [139–141].

The osteoclast presents distinctive functional features:

- osteoclasts can attach firmly to the bone surface, isolating the area under the cell membrane from its surroundings; the membrane domain responsible for the isolation of the resorption site is called sealing zone [142, 143];
- osteoclasts acidify the mineral matrix by the action of protons pumps at the ruffled border membrane, a resorbing organelle; the lowering of the pH causes the dissolution of the hydroxyapatite crystals [144–146];

- osteoclasts are capable of synthesizing and secreting enzymes such as tartrate-resistant acid phosphatase (TRAP) and cathepsins in a directional manner; the proteases secreted by osteoclasts cleave the organic matrix; through the combined action of lysosome enzymes, matrix metalloproteinases and the pH reduction, bone is resorbed [147, 148];
- osteoclasts can phagocytize the resultant organic debris and minerals, removing them from the resorption lacunae, through a transcytosis process [149, 150].

The bone resorption process begins with differentiation and recruitment of osteoclast precursors, which merge and originate matured multinucleated bone-resorbing osteoclasts. Bone resorption begins when the osteoclast attaches to the mineralized bone matrix through the interaction of integrins with matrix proteins, like osteopontin and bone sialoprotein, previously laid down by osteoblasts [143].

2.3 Regulation of Bone Metabolism (Modelling/Remodelling)

The bone cell populations are responsible for bone remodelling and repair. These processes are regulated systemically by hormones, neuropeptides and other mediators and locally by cytokines and growth factors [151, 152].

2.3.1 Parathormone (PTH), Vitamin D and Calcitonin

The bone mineral metabolism (calcium and phosphorus) is regulated by parathormone (PTH), calcitonin, FGF23 and vitamin D.

PTH is a peptide hormone produced by the parathyroid glands in response to low levels of extracellular ionized calcium, detected by specific cell-surface calcium-sensing receptors located in the parathyroid glandular tissue. High levels of PTH increase the number of osteoclasts and trigger resorption of bone matrix, with consequent release of calcium phosphate and increasing calcemia. This mechanism has developed as a protection against acute hypocalcemia. Inversely, low levels of PTH cause the elevation of osteoblast numbers. PTH also acts on osteoblasts' receptors, stimulating proliferation and differentiation and inhibiting apoptosis [153]. PTH also regulates kidney function by impairing phosphate reabsorption and promoting its excretion, by stimulating calcium reabsorption and up-regulating a hydroxylase enzyme (CYP27B1), thus promoting 1,25(OH)2 vitamin D3 synthesis [154].

Circulating hormonal metabolite, 1α,25-dihydroxy vitamin D3 (1,25(OH)2D3), enhances several physiological functions, including intestinal calcium and phosphate absorption, bone phosphate and calcium resorption, and renal calcium and phosphate reabsorption, which results in a rise in the blood calcium and phosphate, required for bone passive mineralization of unmineralized bone matrix to occur [155, 156]. Additionally, 1,25(OH)2D3 stimulates differentiation of osteoblasts and the expression of several bone proteins, like bone-specific alkaline phosphatase, osteocalcin,

osteonectin, osteoprotegerin and other cytokines, and influences the proliferation and apoptosis of other bone cells, including hypertrophic chondrocytes [157]. This may help explain why endogenous PTH levels can have anabolic and catabolic effects and are associated with differential skeletal effects on cortical and trabecular bones [158].

Calcitonin is produced by parafollicular cells of the thyroid, in direct response to extracellular calcium, through the same sensor that regulates the production of PTH. It inhibits matrix resorption, promotes calcium and phosphate excretion, thus reducing calcium and phosphate serum levels; calcitonin inhibits osteoclast mobility and the secretion of proteolytic enzymes [159, 160].

2.3.2 Growth Hormone (GH)

Growth hormone or somatotropin is secreted in pulses by the anterior pituitary gland, inducing bone longitudinal growth [161]. It also induces organs such as the liver and the skeleton to synthesize somatomedins that influence growth, such as insulin-like growth factor 1 (IGF-1) and 2 (IGF-2) [162]. The chondrocytes in the epiphyseal plate are stimulated not only by IGF1 and IGF2 but also directly by GH; proliferative and hypertrophic chondrocytes also secrete IGFs; IGF-1 acts inhibiting further GH secretion [163, 164].

According to Ohlsson et al. [162], GH action in bone remodelling follows a "biphasic model": initially, it increases bone resorption, causing bone loss, followed by a phase of increased bone formation. When bone formation is more stimulated than bone resorption (transition point), the bone mass increases. A net increase of bone mass will be seen after 12–18 months of GH treatment in GH-deficient adults [165]. GH increases bone growth, by increasing both periosteal and endocortical bone formation, bone mineral content (BMC) and bone mineral density (BMD). GH acts synergistically with PTH to increase bone growth and bone formation, bone density and mass and to decrease bone resorption [166].

2.3.3 Insulin and Insulin-like Growth Factors (IGF-1 and IGF-2)

IGF-1 stimulates chondrocyte proliferation in the growth plate, thus playing a crucial role in longitudinal bone growth [167]. It is also involved in the formation of trabecular bone [168]. Insulin and IGF-1 have anabolic effects over the osteoblast and promote bone development, mainly through the activation of Akt and ERK signalling pathways; also, IGF-1 is capable of inducing osteoblasts in vivo proliferation whilst inhibiting the gene expression of osteocalcin, a marker for differentiating osteoblasts; insulin enhances osteocalcin expression but has no effect on osteoblast proliferation [169]. Additionally, insulin indirectly enhances Runx2 expression, a regulator of osteoblast differentiation [55, 169]. A study with insulin-deficient type I diabetic mice showed that these mice presented a decreased expression of Runx2 and the Runx2-regulated genes, like osteocalcin and collagen type I, and a secondary

decrease in bone formation. Bone loss was restored after insulin treatment, which increased Runx2 expression and the expression of related genes [170].

Likewise, IGF-2 potentiates BMP-9-induced osteogenic differentiation and bone formation [171] through PI3K/AKT signalling. Moreover, a recent study in mice aortas showed that IGF-2 induces the expression of miR-30e, in a feedback loop. miR-30e is a major down-regulator of osteogenic differentiation of MSCs and smooth muscle cells [172].

2.3.4 Sex Steroids (Oestrogen and Testosterone)

Bone metabolism is strongly influenced by sex steroids. Oestrogen is an important regulator of skeletal development and homeostasis, both in men and women, exerting direct and indirect effects on the skeleton [173–175]. Indirectly, it influences, for example, the calcium intestinal absorption [176, 177] and secretion [178], and the calcium renal excretion; oestrogen also influences the secretion of PTH [179, 180]. Oestrogen maintains bone homeostasis by inhibiting osteoblast and osteocyte apoptosis [134, 181, 182], and it inhibits osteoclast formation and activity, inducing osteoclast apoptosis [183–187]. Oestrogen deficiency causes bone loss and osteoporosis [188].

Androgens are also important to bone homeostasis. However, their role is likely more important during growth and contributes, via the GH/IGF system, to bone formation at the periosteum [189]. Androgens contribute to the maintenance of cancellous bone mass and integrity, regardless of age or gender [190, 191]. Androgen-deprivation therapy has negative effects on bone mineral density; these effects can be partially delayed by exercise, in the lumbar vertebrae but not in the hip [192].

2.3.5 Thyroid Hormones

The skeleton is a target tissue for thyroid hormones, namely for thyroid hormone 3,5,3′-L-triiodothyronine (T3). Thyroid hormones influence bone growth during early development and adult bone turnover and maintenance. They act both directly, by stimulating bone resorption and formation, and indirectly, by enhancing the effects of growth hormone over tissues. Hypothyroidism causes impaired bone formation and growth delay; thyrotoxicosis is a recognized cause of secondary osteoporosis, and abnormal thyroid hormone signalling has been recognized as an osteoarthritis' risk factor [193]. T3 stimulates osteoblast proliferation and differentiation, with bone matrix secretion, modification and mineralization. Thus, bone turnover is increased by thyroid hormones, which is confirmed by increased biochemical markers of bone turnover, such as osteocalcin and bone-specific alkaline phosphatase [194–196], and therefore, bone loss can occur [160, 197]. Thyroid-stimulating hormone (TSH), produced by the hypophysis, has direct effects on bone turnover [198], and TSH receptors have been found on osteoblasts and osteoclasts, although available data does not allow conclusions on whether TSH inhibits, increases or does not affect osteoblast

differentiation and function [193]. Still, recombinant TSH showed antiresorptive effects in ovariectomized rats [198, 199] and lower TSH levels—with no apparent association with free T4 levels—have been related to hip fracture risk, supporting the idea that TSH effect on the skeleton may be independent of free T4, though its action on dedicated membrane receptors can be up-regulated by modulators [196, 200].

2.3.6 Leptin ("Satiety" Hormone)

Leptin is produced mainly in adipose tissue, and it is a regulator of food intake and energy expenditure through its effects on the central nervous system (CNS). Its influence in bone metabolism probably follows two pathways: a central pathway, activating the sympathetic nervous system that inhibits bone formation, and a peripheral pathway, promoting bone formation through leptin receptors on osteoblastic cells [201, 202]. Leptin inhibits osteoclast generation [203], promotes the decrease in cancellous bone and increases in cortical bone, thus enhancing bone enlargement [204, 205]; it also increases osteoblast number and activity, acting primarily through the peripheral pathways [206]. Another study showed that leptin increases bone mineral content and density, especially at the lumbar spine [207]. However, in the ovine foetus, leptin infusion caused increased femur porosity and connectivity density, and vertebral trabecular thickness whilst leptin receptor antagonist infusion decreased trabecular spacing and increased trabecular number, degree of anisotrophy and connectivity density in the lumbar vertebrae; effects differed in females and males [208]. Leptin also increases the expression of IGF-1 receptor and IGF-1 receptor messenger [209]. During infancy and childhood, leptin and IGF-1 were associated with body composition in preterm-born children. The same study also describes leptin association with bone parameters in early infancy, but not in childhood [210]. These results suggest leptin role on bone metabolism and architecture may vary with gender, age and interaction with other hormones and factors. Leptin is also a key up-regulator of FGF23 secretion [211], and it has been described as a direct enhancer of parathormone secretion [212].

2.3.7 Bone Morphogenetic Proteins (BMPs)

BMPs are a group of 15 growth factors also known by cytokines, which belong to the transforming growth factor β (TGF-β) superfamily, with the ability to induce the formation of bone [213] and cartilage [214]. BMPs play a major role in the regulation of osteoblast lineage-specific differentiation and later bone formation [215]. Alterations in BMPs activity are often associated with a great variety of clinical pathologies, like skeletal and extra-skeletal anomalies, autoimmune, cancer and cardiovascular diseases [216]. BMPs crosstalk with several other major signalling pathways, e.g. Wnt, Akt/mTOR, miRNA, among others, having Runx2 as a key integrator [216, 217]. Among all BMPs, BMP9 has stronger osteogenic inductive activity over MSCs [215,

218, 219]; BMP9 also acts synergistically with TGF-β and GH to enhance bone formation [216, 220, 221]. In addition to BMP9, other BMPs also have shown the ability to induce osteogenesis in vivo, such as BMP2, BMP6 and BMP7 [222–224], with recombinant human BMP2 and BMP7 already being commercialized with the purpose of enhancing bone healing [225]. However, recent studies indicate the existence of age-related differences in BMP2-mediated bone regeneration, including relative dose sensitivity [226]. Contrariwise, BMP3 is known to be a negative regulator of bone formation and BMP4 has been shown to decrease trabecular bone formation in a murine model [219, 227].

2.4 Bone Remodelling and Cell Interchange

Healthy bone, both cortical and trabecular, is continuously remodelling, a dynamic process with bone resorption and formation. Bone remodelling is modulated by mechanical loading, blood calcium levels and a wide range of paracrine and endocrine factors.

The bone remodelling process depends on the coordinate actions of osteoblasts, osteoclasts, osteocytes and osteoblast-derived bone-lining cells, along with other cells, such as macrophages and immune cells. The ensemble constitutes the "basic multicellular unit" (BMU) or "bone remodelling unit" (BRU). In the BMU, the amount of bone lysis achieved by osteoclasts is equal to the amount of bone produced by osteoblasts. The balance between osteoblastic and osteoclastic activity is known as coupling. Frost proposed that bone longitudinal growth, modelling and BMU-based remodelling activities were modulated by a "mechanostat", a mechanism modulating bone mass in the function of mechanical use, in which BMUs would play a central role, along with bone longitudinal growth and modelling (bone formation). Bone modelling was thus considered as an adaptative response to overloading and remodelling as a response to underloading, with given strain set points for each process [228].

Osteoclasts and osteoblasts within the BMU may function under the control of other cell types, since osteoblasts and osteoclasts may perform their functions in the absence of each other [229, 230]. Cells from the osteoblast lineage express receptors for cytokines and other local secreted factors that stimulate osteoclast formation [231]. The BMU can be inhibited by old age, drugs, endocrine, metabolic or inflammatory diseases.

Regardless of the triggering stimulus, osteoclast formation depends on RANKL. Osteoblasts express membrane-bond RANKL, and this regulatory molecule interacts with a receptor (receptor activator of nuclear factor-κB—RANK), expressed on the surface of osteoclast precursors. The RANK activation by RANKL is essential for the fusion of the osteoclast precursor cells and osteoclast formation [232].

Both down-regulation and up-regulation of RANKL expression by osteoblasts under similar mechanical stimulation have been described [233, 234]. Osteoblasts

subjected to different mechanical stimuli respond by an increase in RANKL-bound and a decrease in soluble RANKL secretion [235].

The RANKL/RANK coordinated effects can only be understood by adding osteoprotegerin to the axis. Cells from osteoblastic lineage produce osteoprotegerin (OPG). OPG is soluble and blocks the interaction between RANKL and RANK, acting as a decoy receptor for RANKL. OPG thus inhibits osteoclast formation and induces osteoclast apoptosis [236]. Osteoblasts, in addition, secrete macrophage colony-stimulating factor-1 (M-CSF-1); M-CSF-1 promotes osteoclast precursor proliferation and RANK expression [237, 238]. Osteoblast-like cells' cultures mechanically stimulated may respond by a decrease in the production of OPG, without a change in the RANKL production, with a consequent increase in the ratio of RANKL/OPG. This could translate into increased bone remodelling. However, subjecting osteoclast-like cells to the same mechanical stimuli regimen, decreased TRAP and a period of stimulation of one minute at 0.3 Hz frequency, a decrease in cell fusion and resorption activity was observed [239].

RANKL expression by osteoblast lineage cells is enhanced when microdamage within the bone matrix occurs. Microdamage may occur under physiological bone loading and in pathological conditions. The presence of microcracks is sensed by osteocytes and may induce osteocyte apoptosis; osteocyte apoptosis may also be induced by disuse and is closely correlated with higher bone remodelling levels [131, 240–245].

Pulsating fluid flow (PFF)-treated osteocyte cultures conditioned the culture medium, inhibiting osteoclast formation and decreasing in vitro bone resorption. These effects have not been detected in the medium from PFF-treated fibroblast cultures [246]. In osteocytes subjected to PFF, nitric oxide is involved in the up- and down-regulation of at least two apoptosis-related genes (Bcl-2 and caspase-3, with antiapoptotic protective and pro-apoptotic functions, respectively) [247]. Nitric oxide (NO) is a second messenger molecule produced in response to mechanical stimulation of osteoblasts and osteocytes, and other cell types such as endothelial cells, with a large variety of biological functions [248–251].

Osteocytes, thus, regulate osteoclastogenesis and osteoclast activity through soluble factors and messenger molecules.

Other pathways are relevant for osteoblasts, osteocytes and osteoclasts interweaved regulation, such as the Notch signalling pathway. In osteocytes, the Notch receptor's activation induces OPG and Wnt signalling, decreasing cancellous bone remodelling and inducing cortical bone formation [252]. Wnt/Lrp5 signalling in osteocytes has been considered as a key pathway for bone response to loading [253].

2.5 Bone Mechanotransduction

Bone mechanotransduction, essential in health and disease states, is not yet fully understood. The elements involved in transduction include the ECM, cell–cell

adhesions, cell–ECM adhesions, cell membrane components, specialized surface processes, nuclear structures and cytoskeleton.

2.5.1 The Cell Membrane Elements, Cell–Cell and ECM–Cell Adhesions

Cell membrane-associated mechanotransduction mechanisms depend on the integrity of the phospholipid bilayer. Mechanotransduction pathways are disrupted if membrane cholesterol is depleted, inhibiting the response to hydrostatic and fluid shear stress [254, 255]. Cytoskeleton actin polymerization and assembly are influenced by membrane cholesterol levels [256, 257]. However, it has been proposed that actin polymerization during synaptic vesicle recycling is influenced by vesicular cholesterol, but not plasma membrane cholesterol, as suggested by a study wherein the inhibition of actin polymerization by the extraction of vesicular cholesterol resulted in the dispersal of synaptic vesicle proteins [258]. But even with a functional cell membrane, if integrin binding is impaired, actin cytoskeleton will not re-organize in response to shear stress [259]. However, nanometre- to micron-sized tears, reparable defects in the cell plasma membrane promote particle flux across the cell membrane, namely Ca^{2+} influx [121].

Integrins are cell adhesion receptors, heterodimers of non-covalently associated 18α and 8β subunits, in mammals, that can combine to generate 24 different receptors with different binding properties and different tissue distributions [260, 261]. These subunits possess an extracellular portion with several domains, able to bind to large multi-adhesive ECM molecules, which in turn bind to other ECM molecules, growth factors, cytokines and matrix-degrading proteases [260]. Integrins were first acknowledged as bridging the ECM and the cell cytoskeleton, including the actin cytoskeleton but also the intermediate filament network, essentially vimentin and laminin [262]. Cells use multiple mechanisms to sense and respond to mechanical stress applied to integrins [263]. Recruitment of vimentin has been shown to depend on integrin β3 subunits, underpinning the relationship between the various cytoskeletal elements and integrins [264]. The cytoplasmatic portions of integrin β subunit bind to talin, which can also directly bind to vinculin and actin filaments [265]. On the other hand, integrin α4 subunit binds to paxillin [266], a protein that integrates sites of cell adhesion to the ECM.

Integrins allow communication between structures in the interior and outside of the cell, in a bidirectional way. The inside-out signalling turns the integrin extracellular domains into the active conformation. In the outside-in pathway, when an integrin binds to the extracellular ligand, it clusters with other bound integrins, forming focal adhesions, highly organized intracellular complexes; these are connected to the cytoskeleton. The focal adhesions integrate a range of different molecules, including the cytoplasmic portions of the clustered integrins, proteins of the cytoskeleton and signalling molecules [265, 267]. Initial adhesions to substrates are characterized by punctuating areas at the limits of lamellipodia, usually known as focal complexes. Focal adhesions are the mature form of cell–matrix adhesion, with an elongated

shape, and are associated with bundles of actin and myosin (stress fibres). There is a specialized form of focal contact, in which integrin binds to fibronectin fibrils and tensin but with low levels of tyrosine kinases [268, 269]. Most focal adhesions also contain several types of signalling molecules like tyrosine phosphatases and tyrosine kinases and adaptor proteins [267, 270–272].

Matrix proteins may also modulate cell adhesion; connective tissue growth factor (CTGF), which is a matrix protein, enhances osteoblast adhesion (via $\alpha V\beta_1$ integrin) and cell proliferation, by inducing cytoskeletal reorganization and Rac1 activation [273]. Another matrix protein—osteoactivin—also modulates osteoblast adhesion, differentiation and function, stimulating alkaline phosphatase (ALP) activity, osteocalcin production, nodule formation, and matrix mineralization [274]. $\alpha 5\beta 1$ integrin interacts with its high-affinity ligand CRRETAWAC, enhancing the Wnt/β-catenin signalling mechanism to promote osteoblast differentiation independently of cell adhesion [275].

Cell adhesion and mechanical stimulation depend on integrin mediation [276]. Forces applied to integrin receptors cause local adhesion proteins to be recruited, and the cell adapts by making the integrin–cytoskeleton linkages more rigid; myosin II contraction makes the cell apply tension to the substrate [277]. Different signalling pathways are triggered by sensed stress through integrin receptors. Sequential expression of integrin ligands (osteopontin, fibronectin and bone sialoprotein) in response to mechanical stimulation of osteoblasts has been described [278]. Bonds between integrin and ligands become stronger in the presence of cell tension [279].

Osteocytes are highly specialized in their interaction with ECM; osteocyte cell bodies express β1 integrins while cell processes express β3 integrins, the latter in a punctuate distribution [280–283]. Thi et al. [284] identified the cell processes as the mechanosensory organs in osteocytes. It has been demonstrated that integrin $\alpha V\beta 3$ is essential for the maintenance of osteocyte cell processes and also for mechanosensation and mechanotransduction by osteocytes, by ATP release that triggers calcium signalling [285, 286]. β1 integrins have been shown to regulate specific aspects of mechanotransduction, namely the cortical osteocyte response to disuse [283]. In osteoblasts, a mechanical load applied to β1 integrin subunit results in calcium influx [287], independently from gap junctions [288]. Another study showed that ERK1/2 activation by strain prevented osteocyte apoptosis but required the integrin/cytoskeleton/Src/ERK signalling pathway activation [133].

Apart from integrin, other membrane proteins are responsible for the conduction of mechanical stimuli. Cadherins, which connect to the cytoskeleton, also mediate force-induced calcium influx [289, 290] and participate in the Wnt/β-catenin pathway [291]. In osteoblasts, it has been suggested that GPI-anchored proteins may play an important role in mechanosensing, by demonstrating that the overexpression of GPI-PLD, an enzyme that can specifically cleave GPI-anchored proteins from cell membranes, inhibits flow-induced intracellular calcium mobilization and ERK1/2 activation in MC3T3-E1 cells [255]. Ephrins (ligands) and Ephs (receptors) contribute to cell–cell interactions between osteoclasts and osteoblasts, helping to regulate bone resorption and formation, and appear to be necessary for hMSC differentiation [292, 293]. Lastly, another family of proteins—galectins—is also involved

in regulating osteogenesis; for example, Gal-3, which is expressed both by osteocytes and osteoblasts, plays a significant role as a modulator of major signalling pathways, such as Wnt signalling, MAPK and PI3K/AKT pathways [294]; Gal-8 induces RANKL expression by osteoblasts and osteocytes, osteoclastogenesis and bone mass reduction in mice [295]; and Gal-9 induces osteoblast differentiation through the CD44/Smad signalling pathway in the absence of bone morphogenetic proteins (BMPs) [296].

Gap junctions are transmembrane channels that connect the cytoplasm of adjacent cells. Only small metabolites, ions and signalling molecules like calcium and cAMP pass through these channels since the molecular weight must be lower than 1 kDa [297, 298]. Gap junctions are essential for bone mechanosensation since in osteoblastic cells, the PGE2 production induced by fluid flow is dependent on intact gap junctions; if these are disturbed, PGE2 production does not occur [288, 299]. Mice lacking Cx43 gap junctions in osteoblasts and/or osteocytes exhibit increased osteocyte apoptosis, endocortical resorption and periosteal bone formation [300].

2.5.2 Primary Cilia

In different cell types, different structures ensure recognition of mechanical stimuli; kidney epithelial cells possess a single microvillar projection on their apical surface (primary cilia). A similar structure was described in osteoblasts and osteoblast-like cells [301, 302]. Primary cilia originate in the centrosome and project from the surface of bone cells; its deflection during flow indicates that they have the potential to sense fluid flow. These cilia deflect upon application of 0.03 Pa steady fluid flow and recoil after cessation of flow [303, 304]. In bone, primary cilia translate fluid flow into cellular responses, independently of Ca^{2+} flux and stretch-activated ion channels [303]. It has been demonstrated in vitro that, apart from mediating the up-regulation of specific osteogenic genes, primary cilia are also chief mediators of oscillatory fluid flow-induced extracellular calcium deposition, thereby playing an essential role in load-induced mineral matrix deposition [301]. A study using knockout mice of Kif3a, which results in defective primary cilia, showed that primary cilia are essential for the ability of pre-osteoblasts to sense strain-related mechanical stimuli at a healing bone–implant interface, inducing osteoblast further differentiation [305]; using the same animal model, another study shown primary cilia were paramount for MSCs to sense mechanical signals and enhance osteogenic lineage commitment in vivo [306]. Primary cilia must also be present in osteocytes for pulsed electromagnetic fields to inhibit osteocyte-mediated osteoclastogenesis and inhibit osteocyte apoptosis, modulate cytoskeletal distribution and decrease RANKL/OPG expression [95, 96].

Concerning osteocytes, there is still conflicting information regarding in vivo expression of cilia. Their role as mechanosensors depends on the type and number of cells with cilia and on the local mechanical environment. The incidence of primary cilia in osteocytes has been described as of 4%; this may indicate that cilia function

as mechanosensors on a selected number of cells or that cilia function in concert with other mechanosensing mechanisms [307].

2.5.3 The Cytoskeleton

The cell cytoskeleton network is coupled to the ECM through specific transmembrane receptors. Integrins connect to the cytoskeleton through focal adhesions that gather actin-associated proteins such as talin, vinculin, paxillin and zyxin. Both paxillin and zyxin belong to a group of LIM domain structural proteins, which have been suggested as mechanoresponders responsible for regulating stress fibres assembly, repair and remodelling in response to changing forces [308]. Focused stresses applied to the surface of the cellular membrane are transferred across the network of cell adhesions, microfilaments and microtubules and affect distant cellular sites such as the mitochondria and nucleus or the cell membrane on the opposite side. The transmission of strain towards the ECM stimulates structural changes at a higher organization level, making it stronger [309, 310].

The cell deformation in consequence of applied stress does not correspond to the predicted behaviour of an isotropic viscoelastic material; the interior of the cell, the cytoskeleton, is anisotropic. The intricate network of microtubules and microfilaments, how it spreads and is connected to the point of applied force, may result in structures away from the load application point to be further displaced than closer ones; displacements towards the origin of the compressive stimulus are also possible. Behaving in an anisotropic way, cells can respond to an external force according to its magnitude and direction [311–313]. An intact cytoskeleton is necessary for the rendering of applied forces into mitochondria movements. Since mitochondria are semi-autonomous organelles, highly dynamic, the distress caused by mechanical stimuli exerts biological effects on their function [313], both in health and disease [314].

It is, therefore, logical that mechanical properties of the ECM affect the behaviour of cells from osteoblastic lineage, with mature focal adhesions and a more organized actin cytoskeleton associated with more rigid substrates, suggesting that controlling substrate compliance enables control over differentiation [315] and that this influence on differentiation is independent of protein tethering and substrate porosity [316].

Other factors are determinant for cell fate. A recent in vitro study showed similar patterns in cell growth, differentiation and gene expression in human osteoblasts and endothelial cells when implanted in two different ceramic scaffolds—β-tricalciumphosphate and calcium-deficient hydroxyapatite. These scaffolds had different chemical and physical characteristics, with results suggesting that the interaction between different cell types and scaffold materials is crucial for growth, differentiation and long-term outcomes of tissue-engineered constructs [317]. It has also been highlighted the importance of surface roughness of the biomaterials in

osteogenic differentiation and the contribution of specific integrin subunits in mediating cell response to different materials [318]; additionally, the application of synthetic integrin-binding peptidomimetic ligands (αVβ3- or α5β1-selective) to a titanium graft enhanced cell adhesion, proliferation, differentiation and ALP expression in vitro osteoblast-like cells, resulting in a higher mineralization on the surfaces coated with the ligands [319].

The biochemical nature of the substrate, its rigidity and spatial organization is recognized by cells through signalling from molecular complexes that are integrin-based.

In most anchorage-dependent cells, cell spreading on ECM is required for cell progression and growth; increasing cytoskeletal tension results in cell flattening, a rise in actin bundling and bucking of microtubules. Spread cells can transfer most of the load to the ECM.

The cell shape is influenced by how the cytoskeleton organizes its elements and it is determinant for cell function. For example, osteocyte morphology and alignment differ in two types of bone, fibula and calvaria, probably due to different mechanical loading patterns, which influence the cytoskeletal structure and thus cell shape [320]. Also, osteocyte and lacunae morphology may vary in pathological bone conditions, and these morphological variations may be an adaptation to the differences in matrix properties and, thus, different bone strain levels under similar stimuli [321]. Osteocyte morphology is characterized by long dendritic-like processes, cell shape also assumed by osteoblast MC3T3 cells cultured in 3D; however, differences in cytoskeleton elements in the processes of these two cell types may indicate differences in function; microtubules are predominant on osteoblasts' processes while actin ensures integrity of osteocytes' cytoplasmatic projections [322]. Osteocyte sensitivity to mechanical load applied to the microparticles varies between those attached to the cell bodies and the ones attached to the cell processes: a much smaller displacement of the second ones is needed to cause an intracellular calcium influx that rapidly propagates to the cell body; if local stimulus is applied to the cell body, the reaction is slower and a higher displacement is needed to cause the calcium transient [323].

Osteoblasts, osteoid–osteocytes and mature osteocytes have different mechanical properties. The elastic modulus is higher in the cell periphery than in the perinuclear region; the elastic modulus in both regions decreases as bone cells mature. These differences in elastic modulus probably depend on the number of actin filaments, as it has been shown in other cell types. Furthermore, focal adhesion area is smaller in mature osteocytes, when comparing to osteoblasts. If peptides containing RGD sequence are added to culture medium, both the focal adhesion area and the elastic modulus of osteoblasts decreases whilst remaining unaffected in osteocytes [324].

2.6 *Mechanotransduction Mechanisms*

The multitude of cellular structures, messenger substances, environmental factors and levels of organization of the organs involved in the mechanotransduction mechanisms in distinct cells and tissues makes it extremely complex to understand, predict and replicate how responses are composed at cellular, organ and living organism levels.

2.6.1 Strain, Frequency and Loading Duration

Bone remodelling is influenced by strain magnitude, frequency and loading duration. Wolff developed mathematical equations for trabeculae orientation and thickness prediction according to load [325]. Later, Turner enunciated three essential rules critical for bone remodelling [326]:

1. Remodelling is determined by dynamic loading, not by static loading;
2. Short periods of loading quickly trigger a response; prolonging loading times any further diminishes the magnitude of bone cell response;
3. Bone cells have memory and accommodate to routine loading, diminishing the amplitude of the response triggered by a same repeated stimulus.

Increasing loading frequency increased strain-related bone deposition in vivo, whilst decreasing the threshold for osteogenesis and bone formation [327]. Human osteoblasts subjected to strains varying from 0.8 to 3.2% respond to higher strain with increased expression of osteocalcin, type I collagen and Cbfa1/Runx2, and to lower strain magnitudes with an increase of alkaline phosphatase activity [328].

Bone formation depends on strain magnitude [329], along with the number of loading cycles at low frequencies [330]. Frost theorized that a minimum effective strain level was necessary to trigger bone formation, above 3000 micro-strain [228]. Strain distribution is also paramount for skeletal adaptation. Unusual strain distribution will rapidly trigger an osteogenic response, as suggested by the extensive periosteal and endosteal bone proliferation described by Rubin and Lanyon [331] in a study conducted in poultry. Rest periods between loading cycles also intensify osteogenic response [332] and maximize cell response [333]. During active exercise, peak strains in long bones may be high, but strains as low as 0.15% are enough to ensure osteoblast recruitment in vivo [331]. Human bone marrow stem cells show variable early osteogenic differentiation and gene expression accordingly with load and frequency regimen of cyclic hydrostatic pressure; osteogenic differentiation on the long term occurred under mechanical stimulation, independently of load magnitude and frequency, within the tested physiological ranges [334].

The adaptation of cortical bone is correlated with frequency, although not linearly; the changes in geometry are more significant with higher frequency, with a plateau for frequencies past 10 Hz [335].

Other mechanisms apart from direct deformation of cells are involved in bone mechanical stimulation. Bone's canalicular system is filled with fluid. Simulation

of osteogenic load levels has produced higher shear stresses due to fluid displacement in the canaliculi. The fluid flows within the canalicular system, wherein the osteocytes extend their cell processes, reinforcing osteocyte processes as the main mechanosensing organ in mature bone cells [336]. Multiple canaliculi intersect at points (canalicular joints); these occur with a density similar to that of lacunae and represent areas of enlarged space, with consequences on fluid flow variables such as fluid mass and velocity [337]. Microstructural changes associated with osteoporosis reduce interstitial fluid flow around osteocytes in the lacunar–canalicular system of cortical bone, impairing mechanosensation [338].

As reviewed previously, shear stresses resulting from fluid flow cause calcium influx through mechanosensitive channels [339, 340]. Calcium influx occurs is osteoblasts in response to oscillatory fluid flow [288].

Fluid also carries electrically charged particles. The resulting fluid flow phenomena are common to other biological tissues but not limited to living structures. The fact that fluid flow changes interfacial chemistry has been recognized; the flow of fresh water along the surfaces disturbs the equilibrium of dissolved ions, changing the surface charge and the molecular orientation of the water at the interface [341]. Likewise, when bone is deformed, a thin sheet of fluid with particles with charge opposite to that of the matrix and bone cells is formed [342]; when a non-uniform mechanical load is applied to the bone structure, the ions in the fluid move away from the matrix. Therefore, the displacement of the electrically charged fluid creates an electrical field aligned with the fluid flow. This causes an electrical potential, and the phenomenon is known as strain-generated bone streaming potential and has been described in bone [342–345]. The density of matrix fixed charges influences the magnitude of the generated streaming potential [346], so the mechanosensory ability along bone may vary and, ultimately, influence dynamic stiffness.

2.6.2 Bone Piezoelectricity and Flexoeletricity

Fukada and Yasuda first described bone piezoelectrical properties in 1957. In dry bone samples submitted to a compressive load, an electrical potential was generated, an occurrence explained by the direct piezoelectric effect [347]. In connective tissues, such as bone, skin, tendon and dentine, the dipole moments are related to the collagen fibres, composed by strongly polar protein molecules aligned [76, 348, 349].

Recently, it has been suggested that hydroxyapatite flexoelectricity is the main source of bending-induced polarization in cortical bone [119].

The architecture of bone itself, with its aligned lamellae, contributes to the existence of potentials through the bone structure [348].

Bone piezoelectric constants, i.e. the polarization generated per unit of mechanical stress, change with moisture content, maturation state (immature bone has lower piezoelectric constants when comparing to mature bone) and architectural organization (altered areas, such as bone neoplasia osteosarcoma, show lower values) [350]. In dentin, piezoelectric constants are higher when moisture contents increase, also behaving anisotropically; tubule orientation determined piezoelectricity, stronger

parallel to the tubules [351]. Wet bone also behaves as a piezoelectric material [347, 350, 352].

Bone piezoelectrical properties have risen interest, in the context of bone physiology and electromechanics. It has been related to bone remodelling mechanisms and to streaming potential mechanisms [353, 354]. Using a piezoelectric substrate and the piezoelectric converse effect were tested in vitro and in vivo with promising results, mechanically stimulating osteoblastic cells and bone, suggesting the potential for clinical application [355, 356]. The development of new synthetic scaffolds is a new emergent field for the bone tissue engineering industry. Hydroxyapatite/barium titanate [357] or polycaprolactone/barium titanate composites [358] with piezoelectric coefficients dependent on distribution and density of barium titanate particles aim to improve cell adhesion and differentiation. A wide range of biomaterials with piezoelectric properties, with potential application for bone regeneration, is available [359]. Mesenchymal cell differentiation in 3Dpiezoelectric scaffolds can be modulated by the voltage output (or streaming potential); lower voltage output scaffolds promoted chondrogenic differentiation while high voltage output promoted osteogenic differentiation [360]. Electromechanical stimulation also promoted improved differentiation when compared to mechanical load alone [360].

Due to the potential impact on therapeutic approaches to bone remodelling and healing, more and more research is being conducted on bioinspired approaches that consider piezoelectric bone properties.

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References

1. de Vries WN, Evsikov AV, Haak BE et al (2004) Maternal β-catenin and E-cadherin in mouse development. Development 131:4435–4445
2. Ingber DE (2006) Mechanical control of tissue morphogenesis during embryological development. Dev Biol 50:255–266
3. Oster GF, Murray JD, Harris AK (1983) Mechanical aspects of mesenchymal morphogenesis. J Embryol Exp Morphol 78:83–125
4. Takeichi M (1988) The cadherins: cell-cell adhesion molecules controlling animal morphogenesis. Development 102:639–655
5. Tang Z, Hu Y, Wang Z et al (2018) Mechanical forces program the orientation of cell division during airway tube morphogenesis. Dev Cell 44:313–325
6. Foubet O, Trejo M, Toro R (2018) Mechanical morphogenesis and the development of neocortical organisation. Cortex
7. Cartwright JHE, Piro O, Tuval I (2004) Fluid-dynamical basis of the embryonic development of left-right asymmetry in vertebrates. Proc Natl Acad Sci USA 101:7234–7239

8. Collignon J, Varlet I, Robertson EJ (1996) Relationship between asymmetric nodal expression and the direction of embryonic turning. Nature 381:155–158
9. Nakamura T, Mine N, Nakaguchi E et al (2006) Generation of robust left-right asymmetry in the mouse embryo requires a self-enhancement and lateral-inhibition system. Dev Cell 11:495–504
10. Okada Y, Nonaka S, Tanaka Y et al (1999) Abnormal nodal flow precedes situs inversus in iv and inv mice. Mol Cell 4:459–468
11. McGrath J, Somlo S, Makova S et al (2003) Two populations of node monocilia initiate left-right asymmetry in the mouse. Cell 114:61–73
12. Patwari P, Lee RT (2008) Mechanical control of tissue morphogenesis. Circ Res 103:234–243
13. Schmidt A, Brixius K, Bloch W (2007) Endothelial precursor cell migration during vasculogenesis. Circ Res 101:125–136
14. Anava S, Greenbaum A, Ben Jacob E et al (2009) The regulative role of neurite mechanical tension in network development. Biophys J 96:1661–1670
15. Bray D (1979) Mechanical tension produced by nerve cells in tissue culture. J Cell Sci 37:391–410
16. Dennerll TJ, Lamoureux P, Buxbaum RE, Heidemann SR (1989) The cytomechanics of axonal elongation and retraction. J Cell Biol 109:3073–3083
17. le Noble F, Klein C, Tintu A et al (2008) Neural guidance molecules, tip cells, and mechanical factors in vascular development. Cardiovasc Res 78:232–241
18. Carter DR, Beaupré GS (2001) Skeletal tissue histomorphology and mechanics. Skeletal function and form. Cambridge University Press, Cambridge, pp 31–52
19. Belanger LF (1969) Osteocytic osteolysis. Calcif Tissue Res 4:1–12
20. Teti A, Zallone A (2009) Do osteocytes contribute to bone mineral homeostasis? Osteocytic osteolysis revisited. Bone 44:11–16
21. Zallone A, Teti A, Primavera MV, Pace G (1983) Mature osteocytes behaviour in a repletion period: the occurrence of osteoplastic activity. Basic Appl Histochem 27:191–204
22. Green J, Kleeman CR (1991) The role of bone in the regulation of systemic acid-base balance. Kidney Int 39:9–26
23. Arnett T (2003) Regulation of bone cell function by acid-base balance. Proc Nutr Soc 62:511–520
24. Bushinsky DA, Krieger NS (2015) Acid-base balance and bone health. In: Holick MF, JNieves NW (eds) Nutrition and bone health. Humana Press Springer, New York, pp 335–357
25. Baylink DJ, Finkelman RD, Mohan S (1993) Growth factors to stimulate bone formation. J Bone Miner Res 8:S565–S572
26. Linkhart TA, Mohan S, Baylink DJ (1996) Growth factors for bone growth and repair: IGF, TGFβ and BMP. Bone 19:S1–S12
27. Krings A, Rahman S, Huang S et al (2012) Bone marrow fat has brown adipose tissue characteristics, which are attenuated with aging and diabetes. Bone 50:546–552
28. Rosen CJ, Ackert-Bicknell C, Rodriguez JP, Pino AM (2009) Marrow fat and the bone microenvironment: developmental, functional, and pathological implications. Crit Rev Eukaryot Gene Expr 19:109–124
29. Suchacki KJ, Cawthorn WP, Rosen CJ (2016) Bone marrow adipose tissue: formation, function and regulation. Curr Opin Pharmacol 28:50–56
30. Roelofs-Iverson RA, Mulder DW, Elveback LR et al (1984) ALS and heavy metals: a pilot case-control study. Neurology 34:393
31. Sharma B, Singh S, Siddiqi NJ (2014) Biomedical implications of heavy metals induced imbalances in redox systems. BioMed Research Int 2014:640754
32. Rhee Y, Bivi N, Farrow E et al (2011) Parathyroid hormone receptor signaling in osteocytes increases the expression of fibroblast growth factor-23 in vitro and in vivo. Bone 49:636–643
33. Yamashita T, Yoshioka M, Itoh N (2000) Identification of a novel fibroblast growth factor, FGF-23, preferentially expressed in the ventrolateral thalamic nucleus of the brain. Biochem Biophys Res Commun 277:494–498

34. Masuyama R, Stockmans I, Torrekens S et al (2006) Vitamin D receptor in chondrocytes promotes osteoclastogenesis and regulates FGF23 production in osteoblasts. J Clin Invest 116:3150–3159
35. Fukumoto S, Martin TJ (2009) Bone as an endocrine organ. Trends Endocrinol Metab 20:230–236
36. Haussler MR, Whitfield GK, Kaneko I et al (2012) The role of vitamin D in the FGF23, klotho, and phosphate bone-kidney endocrine axis. Rev Endocr Metab Disord 13:57–69
37. Shimada T, Hasegawa H, Yamazaki Y et al (2004) FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. J Bone Miner Res 19:429–435
38. David V, Dai B, Martin A et al (2013) Calcium regulates FGF-23 expression in bone. Endocrinology 154:4469–4482
39. Rodriguez-Ortiz ME, Lopez I, Muñoz-Castañeda JR et al (2012) Calcium deficiency reduces circulating levels of FGF23. J Am Soc Nephrol 23:1190–1197
40. Fukumoto S, Yamashita T (2007) FGF23 is a hormone-regulating phosphate metabolism-unique biological characteristics of FGF23. Bone 40:1190–1195
41. ADHR Consortium (2000) Autosomal dominant hypophosphataemic rickets is associated with mutations in FGF23. Nat Genet 26:345–348
42. Shimada T, Mizutani S, Muto T et al (2001) Cloning and characterization of FGF23 as a causative factor of tumor-induced osteomalacia. Proc Natl Acad Sci USA 98:6500–6505
43. Lyles KW, Halsey DL, Friedman NE, Lobaugh B (1988) Correlations of serum concentrations of 1,25-dihydroxyvitamin D, phosphorus, and parathyroid hormone in tumoral calcinosis. J Clin Endocrinol Metab 67:88–92
44. Urakawa I, Yamazaki Y, Shimada T et al (2006) Klotho converts canonical FGF receptor into a specific receptor for FGF23. Nature 444:770–774
45. Kurosu H, Yamamoto M, Clark JD et al (2005) Suppression of aging in mice by the hormone Klotho. Science 309:1829–1833
46. Liu H, Fergusson MM, Castilho RM et al (2007) Augmented Wnt signaling in a mammalian model of accelerated aging. Science 317:803–806
47. Doi S, Zou Y, Togao O et al (2011) Klotho inhibits transforming growth factor-β1 (TGF-β1) signaling and suppresses renal fibrosis and cancer metastasis in mice. J Biol Chem 286:8655–8665
48. López I, Rodríguez-Ortiz ME, Almadén Y et al (2011) Direct and indirect effects of parathyroid hormone on circulating levels of fibroblast growth factor 23 in vivo. Kidney Int 80:475–482
49. Quarles LD (2012) Role of FGF23 in vitamin D and phosphate metabolism: implications in chronic kidney disease. Exp Cell Res 318:1040–1048
50. Ben-Dov IZ, Galitzer H, Lavi-Moshayoff V et al (2007) The parathyroid is a target organ for FGF23 in rats. J Clin Invest 117:4003–4008
51. Krajisnik T, Bjorklund P, Marsell R et al (2007) Fibroblast growth factor-23 regulates parathyroid hormone and 1alpha-hydroxylase expression in cultured bovine parathyroid cells. J Endocrinol 195:125–131
52. Paloian NJ, Leaf EM, Giachelli CM (2016) Osteopontin protects against high phosphate-induced nephrocalcinosis and vascular calcification. Kidney Int 89:1027–1036
53. Ferron M, Hinoi E, Karsenty G, Ducy P (2008) Osteocalcin differentially regulates β cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice. Proc Natl Acad Sci USA 105:5266–5270
54. Ferron M, Wei J, Yoshizawa T et al (2010) Insulin signaling in osteoblasts integrates bone remodelling and energy metabolism. Cell 142:296–308
55. Fulzele K, Riddle RC, DiGirolamo DJ et al (2010) Insulin receptor signaling in osteoblasts regulates postnatal bone acquisition and body composition. Cell 142:309–319
56. Lee NK, Karsenty G (2008) Reciprocal regulation of bone and energy metabolism. Trends Endocrinol Metab 19:161–166
57. Reid IR, Ames R, Evans MC et al (1992) Determinants of total body and regional bone mineral density in normal postmenopausal women—a key role for fat mass. J Clin Endocrinol Metab 75:45–51

58. Ribot C, Tremollieres F, Pouilles JM et al (1987) Obesity and postmenopausal bone loss: the influence of obesity on vertebral density and bone turnover in postmenopausal women. Bone 8:327–331
59. Kindblom JM, Ohlsson C, Ljunggren Ö et al (2009) Plasma osteocalcin is inversely related to fat mass and plasma glucose in elderly Swedish men. J Bone Miner Res 24:785–791
60. Pittas AG, Harris SS, Eliades M et al (2009) Association between serum osteocalcin and markers of metabolic phenotype. J Clin Endocrinol Metab 94:827–832
61. Oury F, Sumara G, Sumara O et al (2011) Endocrine regulation of male fertility by the skeleton. Cell 144:796–809
62. Sommerfeldt D, Rubin C (2001) Biology of bone and how it orchestrates the form and function of the skeleton. Eur Spine J 10:S86–S95
63. Augat P, Schorlemmer S (2006) The role of cortical bone and its microstructure in bone strength. Age Ageing 35(suppl_2):ii27–ii31
64. Rho J-Y, Kuhn-Spearing L, Zioupos P (1998) Mechanical properties and the hierarchical structure of bone. Med Eng Phys 20:92–102
65. Van De Graaff K (2001) Skeletal system: introduction and the axial skeleton. In: Lange M, Tibbetts K, Queck K (eds) Human Anatomy, 6th edn. McGraw-Hill College, Boston, pp 131–171
66. Weiner S, Traub W, Wagner HD (1999) Lamellar bone: structure-function relations. J Struct Biol 126:241–255
67. Meyer U, Wiesmann HP (2006) Bone and cartilage. In: Schröder G (ed) Bone and cartilage engineering, 1st edn. Springer, Berlin, pp. 7–46
68. Oftadeh R, Perez-Viloria M, Villa-Camacho JC et al (2015) Biomechanics and mechanobiology of trabecular bone: a review. J Biomech Eng 137:010802
69. Currey JD (2003) The many adaptations of bone. J Biomech 36:1487–1495
70. Burr DB, Milgrom C, Fyhrie D et al (1996) In vivo measurement of human tibial strains during vigorous activity. Bone 18:405–410
71. Duncan RL, Turner CH (1995) Mechanotransduction and the functional response of bone to mechanical strain. Calcif Tissue Int 57:344–358
72. Zioupos P, Currey JD, Hamer AJ (1999) The role of collagen in the declining mechanical properties of aging human cortical bone. J Biomed Mater Res A 45:108–116
73. Turner CH (2006) Bone strength: current concepts. Ann N Y Acad Sci 1068:429–446
74. Young MF (2003) Bone matrix proteins: their function, regulation, and relationship to osteoporosis. Osteoporos Int 14:35–42
75. Bodian DL, Chan T-F, Poon A et al (2009) Mutation and polymorphism spectrum in osteogenesis imperfecta type II: implications for genotype-phenotype relationships. Hum Mol Gen 18:463–471
76. Fukada E, Yasuda I (1964) Piezoelectric effects in collagen. Jpn J Appl Phys 3:117–121
77. Noris-Suárez K, Lira-Olivares J, Ferreira AM et al (2007) In vitro deposition of hydroxyapatite on cortical bone collagen stimulated by deformation-induced piezoelectricity. Biomacromol 8:941–948
78. Ferreira AM, González G, González-Paz RJ et al (2009) Bone collagen role in piezoelectric mediated remineralization. Acta Microsc 18:278–286
79. Nudelman F, Pieterse K, George A et al (2010) The role of collagen in bone apatite formation in the presence of hydroxyapatite nucleation inhibitors. Nat Mater 9:1004–1009
80. Ashizawa N, Graf K, Do YS et al (1996) Osteopontin is produced by rat cardiac fibroblasts and mediates A (II)-induced DNA synthesis and collagen gel contraction. J Clin Invest 98:2218–2227
81. Perrien DS, Brown EC, Aronson J et al (2002) Immunohistochemical study of osteopontin expression during distraction osteogenesis in the rat. J Histochem Cytochem 50:567–574
82. Gross TS, King KA, Rabaia NA et al (2005) Upregulation of osteopontin by osteocytes deprived of mechanical loading or oxygen. J Bone Miner Res 20:250–256
83. Harter LV, Hruska KA, Duncan RL (1995) Human osteoblast-like cells respond to mechanical strain with increased bone matrix protein production independent of hormonal regulation. Endocrinology 136:528–535

84. Fisher LW, Torchia DA, Fohr B et al (2001) Flexible structures of SIBLING proteins, bone sialoprotein, and osteopontin. Biochem Biophys Res Commun 280:460–465
85. Jahnen-Dechent W, Schäfer C, Ketteler M et al (2008) Mineral chaperones: a role for fetuin-A and osteopontin in the inhibition and regression of pathologic calcification. J Mol Med 86:379–389
86. Thurner PJ, Chen CG, Ionova-Martin S et al (2010) Osteopontin deficiency increases bone fragility but preserves bone mass. Bone 46:1564–1573
87. Bentmann A, Kawelke N, Moss D et al (2010) Circulating fibronectin affects bone matrix, whereas osteoblast fibronectin modulates osteoblast function. J Bone Miner Res 25:706–715
88. Huang G, Zhang Y, Kim B et al (2009) Fibronectin binds and enhances the activity of bone morphogenetic protein 1. J Biol Chem 284:25879–25888
89. Kang Y, Georgiou AI, MacFarlane RJ et al (2017) Fibronectin stimulates the osteogenic differentiation of murine embryonic stem cells. J Tissue Eng Regen Med 11:1929–1940
90. Linsley C, Wu B, Tawil B (2013) The effect of fibrinogen, collagen type I, and fibronectin on mesenchymal stem cell growth and differentiation into osteoblasts. Tissue Eng Part A 19:1416–1423
91. Matlahov I, Iline-Vul T, Abayev M et al (2015) Interfacial mineral–peptide properties of a mineral binding peptide from osteonectin and bone-like apatite. Chem Mater 27:5562–5569
92. Rosset EM, Bradshaw AD (2016) SPARC/osteonectin in mineralized tissue. Matrix Biol 52:78–87
93. Delany AM, Hankenson KD (2009) Thrombospondin-2 and SPARC/osteonectin are critical regulators of bone remodelling. J Cell Commun Signal 3:227–238
94. Delany AM, Amling M, Priemel M et al (2000) Osteopenia and decreased bone formation in osteonectin-deficient mice. J Clin Invest 105:915–923
95. Wang M, Chao CC, Chen PC et al (2019) Thrombospondin enhances RANKL-dependent osteoclastogenesis and facilitates lung cancer bone metastasis. Biochem Pharmacol 166:23–32
96. Wang P, Tang C, Wu J et al (2019) Pulsed electromagnetic fields regulate osteocyte apoptosis, RANKL/OPG expression, and its control of osteoclastogenesis depending on the presence of primary cilia. J Cell Physiol 234:10588–10601
97. Ganss B, Kim RH, Sodek J (1999) Bone sialoprotein. Crit Rev Oral Biol Med 10:79–98
98. Malaval L, Wade-Guéye NM, Boudiffa M et al (2008) Bone sialoprotein plays a functional role in bone formation and osteoclastogenesis. J Exp Med 205:1145–1153
99. Holm E, Aubin JE, Hunter GK et al (2015) Loss of bone sialoprotein leads to impaired endochondral bone development and mineralization. Bone 71:145–154
100. Bouleftour W, Juignet L, Verdière L et al (2019) Deletion of OPN in BSP knockout mice does not correct bone hypomineralization but results in high bone turnover. Bone 120:411–422
101. Lamoureux F, Baud'huin M, Duplomb L et al (2007) Proteoglycans: key partners in bone cell biology. BioEssays 29:758–771
102. Novince CM, Michalski MN, Koh AJ et al (2012) Proteoglycan 4: a dynamic regulator of skeletogenesis and parathyroid hormone skeletal anabolism. J Bone Miner Res 27:11–25
103. Nakamura H (2007) Morphology, function, and differentiation of bone cells. J Hard Tissue Biol 16:15–22
104. Palumbo C (1986) A three-dimensional ultrastructural study of osteoid-osteocytes in the tibia of chick embryos. Cell Tissue Res 246:125–131
105. Bellows CG, Reimers SM, Heersche JNM (1999) Expression of mRNAs for type-I collagen, bone sialoprotein, osteocalcin, and osteopontin at different stages of osteoblastic differentiation and their regulation by 1,25 dihydroxyvitamin D_3. Cell Tissue Res 297:249–259
106. Bellows CG, Heersche JNM (2001) The frequency of common progenitors for adipocytes and osteoblasts and of committed and restricted adipocyte and osteoblast progenitors in fetal rat calvaria cell populations. J Bone Miner Res 16:1983–1993
107. Schiller PC, D'Ippolito G, Balkan W et al (2001) Gap-junctional communication is required for the maturation process of osteoblastic cells in culture. Bone 28:362–369

108. Komori T (2019) Regulation of Proliferation, Differentiation and Functions of Osteoblasts by Runx2. Int J Mol Sci 20:1694–1705
109. Rutkovskiy A, Stensløkken KO, Vaage IJ (2016) Osteoblast differentiation at a glance. Med Sci Mon Basic Res 22:95–106
110. van der Meijden K, Bakker AD, van Essen HW et al (2016) Mechanical loading and the synthesis of 1, 25 (OH) 2 D in primary human osteoblasts. J Steroid Biochem Mol Biol 156:32–39
111. van Bezooijen RL, Roelen BA, Visser A et al (2004) Sclerostin is an osteocyte-expressed negative regulator of bone formation, but not a classical BMP antagonist. J Exp Med 199:805–814
112. Bonewald LF (2007) Osteocyte messages from a bony tomb. Cell Metab 5:410–411
113. Jiang JX, Siller-Jackson AJ, Burra S (2007) Roles of gap junctions and hemichannels in bone cell functions and in signal transmission of mechanical stress. Front Biosci 12:1450–1462
114. Tate ML Knothe, Adamson JR, Tami AE, Bauer TW (2004) The osteocyte. Int J Biochem Cell Biol 36:1–8
115. Tate ML Knothe (2003) Whither flows the fluid in bone? An osteocyte's perspective. J Biomech 36:1409–1424
116. Burger EH, Klein-Nulend J (1999) Mechanotransduction in bone—role of the lacuno-canalicular network. FASEB J 13:101–112
117. Mullender M, El Haj AJ, Yang Y et al (2004) Mechanotransduction of bone cells in vitro: mechanobiology of bone tissue. Med Biol Eng Comput 42:14–21
118. Huang CP, Chen XM, Chen ZQ (2008) Osteocyte: the impresario in the electrical stimulation for bone fracture healing. Med Hypotheses 70:287–290
119. Vasquez-Sancho F, Abdollahi A, Damjanovic D et al (2018) Flexoelectricity in bones. Adv Mater 30:1705316
120. van Oers RF, Wang H, Bacabac RG (2015) Osteocyte shape and mechanical loading. Curr Osteoporos Rep 13:61–66
121. Yu K, Sellman DP, Bahraini A et al (2018) Mechanical loading disrupts osteocyte plasma membranes which initiates mechanosensation events in bone. J Orthop Res 36:653–662
122. Kringelbach TM, Aslan D, Novak I et al (2015) Fine-tuned ATP signals are acute mediators in osteocyte mechanotransduction. Cell Signal 27:2401–2409
123. Morrell AE, Brown GN, Robinson ST et al (2018) Mechanically induced Ca 2+ oscillations in osteocytes release extracellular vesicles and enhance bone formation. Bone Res 6:6
124. Cherian PP, Cheng B, Gu S et al (2003) Effects of mechanical strain on the function of gap junctions in osteocytes are mediated through the prostaglandin EP2 receptor. J Biol Chem 278:43146–43156
125. Klein-Nulend J, Helfrich MH, Sterck JGH et al (1998) Nitric oxide response to shear stress by human bone cell cultures is endothelial nitric oxide synthase dependent. Biochem Biophys Res Commun 250:108–114
126. Rawlinson SCF, Pitsillides AA, Lanyon LE (1996) Involvement of different ion channels in osteoblasts' and osteocytes' early responses to mechanical strain. Bone 19:609–614
127. Jee WSS, Mori S, Li XJ, Chan S (1990) Prostaglandin E2 enhances cortical bone mass and activates intracortical bone remodelling in intact and ovariectomized female rats. Bone 11:253–266
128. Fan X, Roy E, Zhu L et al (2004) Nitric oxide regulates receptor activator of nuclear factor κB ligand and osteoprotegerin expression in bone marrow stromal cells. Endocrinology 145:751–759
129. Kasten TP, Collin-Osdoby P, Patel N et al (1994) Potentiation of osteoclast bone-resorption activity by inhibition of nitric oxide synthase. Proc Natl Acad Sci USA 91:3569–3573
130. Hirose S, Li M, Kojima T et al (2007) A histological assessment on the distribution of the osteocytic lacunar canalicular system using silver staining. J Bone Miner Metab 25:374–382
131. Jilka RL, Noble B, Weinstein RS (2013) Osteocyte apoptosis. Bone 54:264–271
132. Lee KC, Jessop H, Suswillo R et al (2004) The adaptive response of bone to mechanical loading in female transgenic mice is deficient in the absence of oestrogen receptor-alpha and -beta. Endocrinology 182:193–201

133. Plotkin LI, Mathov I, Aguirre JI et al (2005) Mechanical stimulation prevents osteocyte apoptosis: requirement of integrins, Src kinases, and ERKs. Am J Physiol 289:633–643
134. Tomkinson A, Reeve J, Shaw RW, Noble BS (1997) The death of osteocytes via apoptosis accompanies estrogen withdrawal in human bone. J Clin Endocrinol Metab 82:3128–3135
135. Metz LN, Martin RB, Turner AS (2003) Histomorphometric analysis of the effects of osteocyte density on osteonal morphology and remodelling. Bone 33:753–759
136. Milovanovic P, Zimmermann EA, Hahn M et al (2013) Osteocytic canalicular networks: morphological implications for altered mechanosensitivity. ACS Nano 7:7542–7551
137. Okada S, Yoshida S, Ashrafi S, Schraufnagel D (2002) The Canalicular Structure of Compact Bone in the Rat at Different Ages. Microsc Microanal 8:104–115
138. Rubin J, Greenfield EM (2005) Osteoclast: origin and differentiation. In: Farach-Carson MC, Bronner F, Rubin J (eds) Bone resorption. Springer, London, pp 1–23
139. Asagiri M, Takayanagi H (2007) The molecular understanding of osteoclast differentiation. Bone 40:251–264
140. Nakagawa N, Kinosaki M, Yamaguchi K et al (1998) RANK is the essential signaling receptor for osteoclast differentiation factor in osteoclastogenesis. Biochem Biophys Res Commun 253:395–400
141. Takayanagi H (2008) Regulation of osteoclastogenesis and osteoimmunology. Bone 42:S40
142. Marchisio PC, Cirillo D, Naldini L et al (1984) Cell-substratum interaction of cultured avian osteoclasts is mediated by specific adhesion structures. J Cell Biol 99:1696–1705
143. Väänänen HK, Horton M (1995) The osteoclast clear zone is a specialized cell-extracellular matrix adhesion structure. J Cell Sci 108:2729–2732
144. Baron R, Neff L, Louvard D, Courtoy PJ (1985) Cell-mediated extracellular acidification and bone resorption: evidence for a low pH in resorbing lacunae and localization of a 100-kD lysosomal membrane protein at the osteoclast ruffled border. J Cell Biol 101:2210–2222
145. Blair HC, Teitelbaum SL, Ghiselli R, Gluck S (1989) Osteoclastic bone resorption by a polarized vacuolar proton pump. Science 245:855–857
146. Rousselle AV, Heymann D (2002) Osteoclastic acidification pathways during bone resorption. Bone 30:533–540
147. Littlewood-Evans A, Kokubo T, Ishibashi O et al (1997) Localization of cathepsin K in human osteoclasts by in situ hybridization and immunohistochemistry. Bone 20:81–86
148. Vääräniemi J, Halleen JM, Kaarlonen K et al (2004) Intracellular machinery for matrix degradation in bone-resorbing osteoclasts. J Bone Miner Res 19:1432–1440
149. Salo J, Lehenkari P, Mulari M et al (1997) Removal of osteoclast bone resorption products by transcytosis. Science 276:270–273
150. Yamaki M, Nakamura H, Takahashi N et al (2005) Transcytosis of calcium from bone by osteoclast-like cells evidenced by direct visualization of calcium in cells. Arch Biochem Biophys 440:10–17
151. Harada SI, Rodan GA (2003) Control of osteoblast function and regulation of bone mass. Nature 423:349–355
152. Karsenty G, Kronenberg HM, Settembre C (2009) Genetic control of bone formation. Annu Rev Cell Dev Biol 25:629–648
153. Siddiqui JA, Partridge NC (2016) Physiological bone remodeling: systemic regulation and growth factor involvement. Physiology (Bethesda) 31:233–245
154. Murayama A, Takeyama K, Kitanaka S et al (1998) The promoter of the human 25-hydroxyvitamin D3 1 alpha-hydroxylase gene confers positive and negative responsiveness to PTH, calcitonin, and $1\alpha,25(OH)_2D_3$. Biochem Biophys Res Commun 249:11–16
155. Haussler MR, Whitfield GK, Haussler CA et al (1998) The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. J Bone Miner Res 13:325–349
156. Saini RK, Kaneko I, Jurutka PW et al (2013) 1, 25-dihydroxyvitamin d3 regulation of fibroblast growth factor-23 expression in bone cells: evidence for primary and secondary mechanisms modulated by leptin and interleukin-6. Calcif Tissue Int 92:339–353
157. Clarke B (2008) Normal bone anatomy and physiology. Clin J Am Soc Nephrol 3:S131–S139

158. Hong AR, Lee JH, Kim JH et al (2019) Effect of endogenous parathyroid hormone on bone geometry and skeletal microarchitecture. Calcif Tissue Int 104:382–389
159. Boissy P, Saltel F, Bouniol C et al (2002) Transcriptional activity of nuclei in multinucleated osteoclasts and its modulation by calcitonin. Endocrinology 143:1913–1921
160. Hadjidakis DJ, Androulakis II (2006) Bone remodelling. Ann NY Acad Sci 1092:385–396
161. Isaksson OG, Jansson JO, Gause IA (1982) Growth hormone stimulates longitudinal bone growth directly. Science 216:1237–1239
162. Ohlsson C, Bengtsson BA, Isaksson OG et al (1998) Growth hormone and bone. Endocrine Rev 19:55–79
163. Ranke MB, Wit JM (2018) Growth hormone—past, present and future. Nat Rev Endocrinol 14:285–300
164. Wu S, Yang W, De Luca F (2015) Insulin-like growth factor-independent effects of growth hormone on growth plate chondrogenesis and longitudinal bone growth. Endocrinology 156:2541–2551
165. Kuzma M, Kuzmova Z, Zelinkova Z et al (2014) Impact of the growth hormone replacement on bone status in growth hormone deficient adults. Growth Horm IGF Res 24:22–28
166. Guevarra MS, Yeh JK, Castro Magana M, Aloia JF (2010) Synergistic effect of parathyroid hormone and growth hormone on trabecular and cortical bone formation in hypophysectomized rats. Hormone Res Paediatr 73:248–257
167. Lupu F, Terwilliger JD, Lee K et al (2001) Roles of growth hormone and insulin-like growth factor 1 in mouse postnatal growth. Dev Biol 229:141–162
168. Zhang M, Xuan S, Bouxsein ML et al (2002) Osteoblast-specific knockout of the insulin-like growth factor (IGF) receptor gene reveals an essential role of IGF signaling in bone matrix mineralization. J Biol Chem 277:44005–44012
169. Zhang W, Shen X, Wan C et al (2012) Effects of insulin and insulin-like growth factor 1 on osteoblast proliferation and differentiation: differential signalling via Akt and ERK. Cell Biochem Funct 30:297–302
170. Fowlkes JL, Bunn RC, Liu L et al (2008) Runt-related transcription factor 2 (RUNX2) and RUNX2-related osteogenic genes are down-regulated throughout osteogenesis in type 1 diabetes mellitus. Endocrinology 149:1697–1704
171. Chen JH, Liu C, You L, Simmons CA (2010) Boning up on Wolff's Law: mechanical regulation of the cells that make and maintain bone. J Biomech 43:108–118
172. Ding W, Li J, Singh J et al (2015) miR-30e targets IGF2-regulated osteogenesis in bone marrow-derived mesenchymal stem cells, aortic smooth muscle cells, and ApoE −/− mice. Cardiovasc Res 106:131–142
173. Turner RT, Riggs BL, Spelsberg TC (1994) Skeletal effects of estrogen. Endocr Rev 15:275–300
174. Khosla S, Monroe DG (2018) Regulation of bone metabolism by sex steroids. Cold Spring Harb Perspect Med 8:a031211
175. Prince RL (1994) Counterpoint: estrogen effects on calcitropic hormones and calcium homeostasis. Endocr Rev 15:301–309
176. Liel Y, Shany S, Smirnoff P, Schwartz B (1999) Estrogen increases 1, 25-dihydroxyvitamin D receptors expression and bioresponse in the rat duodenal mucosa 1. Endocrinology 140:280–285
177. ten Bolscher M, Netelenbos JC, Barto R, van Buuren LM (1999) Estrogen regulation of intestinal calcium absorption in the intact and ovariectomized adult rat. J Bone Miner Res 14:1197–1202
178. Draper CR, Edel MJ, Dick IM et al (1997) Phytoegens reduce bone loss and bone resorption in oophorectomized rats. J Nut 127:1795–1799
179. Robinson LJ, Yaroslavskiy BB, Griswold RD et al (2009) Estrogen inhibits RANKL-stimulated osteoclastic differentiation of human monocytes through estrogen and RANKL-regulated interaction of estrogen receptor-α with BCAR1 and Traf6. Exp Cell Res 315:1287–1301

180. Väänänen HK (2005) Mechanism of osteoclast mediated bone resorption-rationale for the design of new therapeutics. Adv Drug Deliv Rev 57:959–971
181. Emerton KB, Hu B, Woo AA et al (2010) Osteocyte apoptosis and control of bone resorption following ovariectomy in mice. Bone 46:577–583
182. Kousteni S, Chen JR, Bellido T et al (2002) Reversal of bone loss in mice by nongenotropic signaling of sex steroids. Science 298:843–846
183. Faloni APDS, Sasso-Cerri E, Rocha FRG et al (2012) Structural and functional changes in the alveolar bone osteoclasts of estrogen-treated rats. J Anat 220:77–85
184. Faloni APS, Sasso-Cerri E, Katchburian E, Cerri PS (2007) Decrease in the number and apoptosis of alveolar bone osteoclasts in estrogen-treated rats. J Periodont Res 42:193–201
185. Hughes DE, Dai A, Tiffee JC et al (1996) Estrogen promotes apoptosis of murine osteoclasts mediated by TGF-β. Nature Med 2:1132–1136
186. Khosla S, Oursler MJ, Monroe DG (2012) Estrogen and the skeleton. Trends Endocrinol Metab 23:576–581
187. Rodan GA, Martin TJ (2000) Therapeutic approaches to bone diseases. Science 289:1508–1514
188. Riggs BL, Khosla S, Melton LJ (1998) A unitary model for involutional osteoporosis: estrogen deficiency causes both type I and type II osteoporosis in postmenopausal women and contributes to bone loss in aging men. J Bone Miner Res 13:763–773
189. Almeida M, Laurent MR, Dubois V et al (2016) Estrogens and androgens in skeletal physiology and pathophysiology. Physiol Rev 97:135–187
190. Compston JE (2001) Sex steroids and bone. Physiol Rev 81:419–447
191. Vanderschueren D, Vandenput L, Boonen S et al (2004) Androgens and bone. Endocr Rev 25:389–425
192. Taaffe DR, Galvão DA, Spry N et al (2019) Immediate versus delayed exercise in men initiating androgen deprivation: effects on bone density and soft tissue composition. BJU Int 123:261–269
193. Bassett JD, Williams GR (2016) Role of thyroid hormones in skeletal development and bone maintenance. Endocr Rev 37:135–187
194. El Hadidy M, Ghonaim M, El Gawad S, El Atta MA (2011) Impact of severity, duration, and etiology of hyperthyroidism on bone turnover markers and bone mineral density in men. BMC Endocr Disord 11:15
195. Harvey RD, McHardy KC, Reid IW et al (1991) Measurement of bone collagen degradation in hyperthyroidism and during thyroxine replacement therapy using pyridinium cross-links as specific urinary markers. J Clin Endocrinol Metab 72:1189–1194
196. Waring AC, Harrison S, Fink HA et al (2013) A prospective study of thyroid function, bone loss, and fractures in older men: The MrOS study. J Bone Miner Res 28:472–479
197. Britto JM, Fenton AJ, Holloway WR et al (1994) Osteoblasts mediate thyroid hormone stimulation of osteoclastic bone resorption. Endocrinology 134:169–176
198. Abe E, Marians RC, Yu W et al (2003) TSH is a negative regulator of skeletal remodelling. Cell 115:151–162
199. Sun L, Vukicevic S, Baliram R et al (2008) Intermittent recombinant TSH injections prevent ovariectomy-induced bone loss. Proc Natl Acad Sci USA 105:4289–4294
200. Neumann S, Eliseeva E, Boutin A et al (2018) Discovery of a positive allosteric modulator of the thyrotropin receptor: potentiation of thyrotropin-mediated preosteoblast differentiation in vitro. J Pharmacol Exp Ther 364:38–45
201. Chen XX, Yang T (2015) Roles of leptin in bone metabolism and bone diseases. J Bone Miner Metab 33:474–485
202. Shi Y, Yadav VK, Suda N et al (2008) Dissociation of the neuronal regulation of bone mass and energy metabolism by leptin in vivo. Proc Natl Acad Sci USA 105:20529–20533
203. Holloway WR, Collier FM, Aitken CJ et al (2002) Leptin inhibits osteoclast generation. J Bone Miner Res 17:200–209
204. Elefteriou F, Takeda S, Ebihara K et al (2004) Serum leptin level is a regulator of bone mass. Proc Natl Acad Sci USA 101:3258–3263

205. Hamrick MW, Ferrari SL (2008) Leptin and the sympathetic connection of fat to bone. Osteoporos Int J Establ Result Coop Eur Found Osteoporos Natl Osteoporos Found USA 19:905–912
206. Turner RT, Kalra SP, Wong CP et al (2013) Peripheral leptin regulates bone formation. J Bone Miner Res 28:22–34
207. Mantzoros CS, Magkos F, Brinkoetter M et al (2011) Leptin in human physiology and pathophysiology. Am J Physiol Endocrinol Metab 301:E567–E584
208. DeBlasio MJ, Lanham SA, Blache D et al (2018) Sex-and bone-specific responses in bone structure to exogenous leptin and leptin receptor antagonism in the ovine fetus. Am J Physiol Regul Integr Comp Physiol 314:R781–R790
209. Maor G, Rochwerger M, Segev Y, Phillip M (2002) Leptin acts as a growth factor on the chondrocytes of skeletal growth centres. J Bone Miner Res 17:1034–1043
210. Ruys CA, van de Lagemaat M, Lafeber HN et al (2018) Leptin and IGF-1 in relation to body composition and bone mineralization of preterm-born children from infancy to 8 years. Clin Endocrinol 89:76–84
211. Tsuji K, Maeda T, Kawane T et al (2010) Leptin stimulates fibroblast growth factor 23 expression in bone and suppresses renal 1α25-dihydroxyvitamin D_3 synthesis in leptin-deficient ob/ob mice. J Bone Miner Res 25:1711–1723
212. López I, Pineda C, Raya AI et al (2016) Leptin directly stimulates parathyroid hormone secretion. Endocrine Abstracts 41:GP144
213. Urist MR (1965) Bone: formation by autoinduction. Science 150:893–899
214. Kobayashi T, Lyons KM, McMahon AP, Kronenberg HM (2005) BMP signaling stimulates cellular differentiation at multiple steps during cartilage development. Proc Natl Acad Sci USA 102:18023–18027
215. Beederman M, Lamplot JD, Nan G et al (2013) BMP signaling in mesenchymal stem cell differentiation and bone formation. J Biomed Sci Eng 6:32–52
216. Rahman MS, Akhtar N, Jamil HM et al (2015) TGF-/BMP signaling and other molecular events: regulation of osteoblastogenesis and bone formation. Bone Res 3:15005
217. Lin GL, Hankenson KD (2011) Integration of BMP, Wnt, and notch signaling pathways in osteoblast differentiation. J Cell Biochem 112:3491–3501
218. Kang Q, Song WX, Luo Q et al (2008) A comprehensive analysis of the dual roles of BMPs in regulating adipogenic and osteogenic differentiation of mesenchymal progenitor cells. Stem Cells Dev 18:545–559
219. Kang Q, Sun MH, Cheng H et al (2004) Characterization of the distinct orthotopic bone-forming activity of 14 BMPs using recombinant adenovirus-mediated gene delivery. Gene Ther 11:1312–1320
220. Huang E, Zhu G, Jiang W et al (2012) Growth hormone synergizes with BMP9 in osteogenic differentiation by activating the JAK/STAT/IGF1 pathway in murine multilineage cells. J Bone Miner Res 27:1566–1575
221. Li RD, Deng ZL, Hu N et al (2012) Biphasic effects of TGFβ1 on BMP9-induced osteogenic differentiation of mesenchymal stem cells. BMB Rep 45:509–514
222. Cheng H, Jiang W, Phillips FM et al (2003) Osteogenic activity of the fourteen types of human bone morphogenetic proteins (BMPs). J Bone Joint Surg Am 85:1544–1552
223. Franceschi RT, Wang D, Krebsbach PH, Rutherford RB (2000) Gene therapy for bone formation: in vitro and in vivo osteogenic activity of adenovirus expressing BMP-7. Ann Arbor 1001:48109–1078
224. Jane JA Jr, Dunford BA, Kron A et al (2002) Ectopic osteogenesis using adenoviral bone morphogenetic protein (BMP)-4 and BMP-6 gene transfer. Mol Ther 6:464–470
225. Carreira AC, Lojudice FH, Halcsik E et al (2014) Bone morphogenetic proteins facts, challenges, and future perspectives. J Dent Res 93:335–345
226. Cheng A, Krishnan L, Tran L et al (2019) The effects of age and dose on gene expression and segmental bone defect repair after BMP-2 Delivery. JBMR Plus 3:e100681-11
227. Holien T, Westhrin M, Moen SH et al (2018) BMP4 gene therapy inhibits myeloma tumor growth, but has a negative impact on bone. Blood 132:1928

228. Frost HM (1987) Bone “mass” and the “mechanostat”: a proposal. Anat Rec 219:1–9
229. Corral DA, Amling M, Priemel M et al (1998) Dissociation between bone resorption and bone formation in osteopenic transgenic mice. Proc Natl Acad Sci USA 95:13835–13840
230. Kong Y-Y, Feige U, Sarosi I et al (1999) Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 402:304–309
231. Suda T, Takahashi N, Udagawa N et al (1999) Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocr Rev 20:345–357
232. Miyamoto T, Suda T (2003) Differentiation and function of osteoclasts. Keio J Med 52:1–7
233. Fan X, Rahnert JA, Murphy TC et al (2006) Response to mechanical strain in an immortalized pre-osteoblast cell is dependent on ERK1/2. J Cell Physiol 207:454–460
234. Kreja L, Liedert A, Hasni S, Claes L, Ignatius A (2008) Intermittent mechanical strain increases RANKL expression in human osteoblasts. J Biomech 41:S462
235. Kim DW, Lee HJ, Karmin JA et al (2006) Mechanical loading differentially regulates membrane-bound and soluble RANKL availability in MC3T3-E1 cells. Ann NY Acad Sci 1068:568–572
236. Liu W, Xu C, Zhao H et al (2015) Osteoprotegerin induces apoptosis of osteoclasts and osteoclast precursor cells via the fas/fas ligand pathway. PLoS ONE 10:e0142519
237. Arai F, Miyamoto T, Ohneda O et al (1999) Commitment and differentiation of osteoclast precursor cells by the sequential expression of c-Fms and receptor activator of nuclear factor κb (RANK) receptors. J Exp Med 190:1741–1754
238. Romas E, Sims NA, Hards DK et al (2002) Osteoprotegerin reduces osteoclast numbers and prevents bone erosion in collagen-induced arthritis. Am J Pathol 161:1419–1427
239. Kadow-Romacker A, Hoffmann JE, Duda G et al (2009) Effect of mechanical stimulation on osteoblast- and osteoclast-like cells in vitro. Cells Tissues Organs 190:61–68
240. Bentolila V, Boyce TM, Fyhrie DP et al (1998) Intracortical remodelling in adult rat long bones after fatigue loading. Bone 23:275–281
241. Mann V, Huber C, Kogianni G et al (2006) The influence of mechanical stimulation on osteocyte apoptosis and bone viability in human trabecular bone. J Musculoskelet Neuronal Interact 6:408–417
242. Martin RB (2007) Targeted bone remodelling involves BMU steering as well as activation. Bone 40:1574–1580
243. Mori S, Burr DB (1993) Increased intracortical remodelling following fatigue damage. Bone 14:103–109
244. Noble BS, Peet N, Stevens HY et al (2003) Mechanical loading: biphasic osteocyte survival and targeting of osteoclasts for bone destruction in rat cortical bone. Am J Physiol 284:934–943
245. Verborgt O, Tatton NA, Majeska RJ, Schaffler MB (2002) Spatial distribution of Bax and Bcl-2 in osteocytes after bone fatigue: complementary roles in bone remodelling regulation? J Bone Miner Res 17:907–914
246. Tan SD, de Vries TJ, Kuijpers-Jagtman AM et al (2007) Osteocytes subjected to fluid flow inhibit osteoclast formation and bone resorption. Bone 41:745–751
247. Tan SD, Bakker AD, Semeins CM et al (2008) Inhibition of osteocyte apoptosis by fluid flow is mediated by nitric oxide. Biochem Biophys Res Commun 369:1150–1154
248. Rössig L, Haendeler J, Hermann C et al (2000) Nitric oxide down-regulates MKP-3 mRNA levels. J Biol Chem 275:25502–25507
249. Smalt R, Mitchell FT, Howard RL, Chambers TJ (1997) Induction of NO and prostaglandin E2 in osteoblasts by wall-shear stress but not mechanical strain. Am J Physiol 273:751–758
250. Zaman G, Pitsillides AA, Rawlinson SCF et al (1999) Mechanical strain stimulates nitric oxide production by rapid activation of endothelial nitric oxide synthase in osteocytes. J Bone Miner Res 14:1123–1131
251. van’T Hof RJ, Ralston SH (2001) Nitric oxide and bone. Immunology 103:255–261
252. Canalis E, Adams DJ, Boskey A et al (2013) Notch signaling in osteocytes differentially regulates cancellous and cortical bone remodelling. J Biol Chem 288:25614–25625

253. Bullock WA, Pavalko FM, Robling AG (2019) Osteocytes and mechanical loading: the Wnt connection. Orthod Craniofac Res 22:175–179
254. Ferraro JT, Daneshmand M, Bizios R, Rizzo V (2004) Depletion of plasma membrane cholesterol dampens hydrostatic pressure and shear stress-induced mechanotransduction pathways in osteoblast cultures. Am J Physiol Cell Physiol 286:831–839
255. Xing Y, Gu Y, Xu LC et al (2011) Effects of membrane cholesterol depletion and GPI-anchored protein reduction on osteoblastic mechanotransduction. J Cell Physiol 226:2350–2359
256. Klausen TK, Hougaard C, Hoffmann EK, Pedersen SF (2006) Cholesterol modulates the volume-regulated anion current in Ehrlich-Lettre ascites cells via effects on Rho and F-actin. Am J Physiol Cell Physiol 291:757–771
257. Qi M, Liu Y, Freeman MR, Solomon KR (2009) Cholesterol-regulated stress fibre formation. J Cell Biochem 106:1031–1040
258. Dason JS, Smith AJ, Marin L, Charlton MP (2014) Cholesterol and F-actin are required for clustering of recycling synaptic vesicle proteins in the presynaptic plasma membrane. J Physiol 592:621–633
259. Radel C, Rizzo V (2005) Integrin mechanotransduction stimulates caveolin-1 phosphorylation and recruitment of Csk to mediate actin reorganization. Am J Physiol 288:936–945
260. Barczyk M, Carracedo S, Gullberg D (2010) Integrins. Cell Tissue Res 339:269–280
261. Hynes RO (2002) Integrins: bidirectional, allosteric signaling machines. Cell 110:673–687
262. Nievers MG, Schaapveld RQJ, Sonnenberg A (1999) Biology and function of hemidesmosomes. Matrix Biol 18:5–17
263. Matthews BD, Overby DR, Mannix R, Ingber DE (2006) Cellular adaptation to mechanical stress: role of integrins, Rho, cytoskeletal tension and mechanosensitive ion channels. Journal Cell Sci 119:508–518
264. Bhattacharya R, Gonzalez AM, DeBiase PJ et al (2009) Recruitment of vimentin to the cell surface by β3 integrin and plectin mediates adhesion strength. J Cell Sci 122:1390–1400
265. Cram EJ, Schwarzbauer JE (2004) The talin wags the dog: new insights into integrin activation. Trends Cell Biol 14:55–57
266. Brown MC, Perrotta JA, Turner CE (1996) Identification of LIM3 as the principal determinant of paxillin focal adhesion localization and characterization of a novel motif on paxillin directing vinculin and focal adhesion kinase binding. J Cell Biol 135:1109–1123
267. Geiger B, Spatz JP, Bershadsky AD (2009) Environmental sensing through focal adhesions. Nature Rev Mol Cell Biol 10:21–33
268. El-Hoss J, Arabian A, Dedhar S, St-Arnaud R (2014) Inactivation of the integrin-linked kinase (ILK) in osteoblasts increases mineralization. Gene 533:246–252
269. Katz B-Z, Zamir E, Bershadsky A et al (2000) Physical state of the extracellular matrix regulates the structure and molecular composition of cell-matrix adhesions. Mol Biol Cell 11:1047–1060
270. Parsons JT (1996) Integrin-mediated signalling: regulation by protein tyrosine kinases and small GTP-binding proteins. Curr Opin Cell Biol 8:146–152
271. Teo BKK, Wong ST, Lim CK et al (2013) Nanotopography modulates mechanotransduction of stem cells and induces differentiation through focal adhesion kinase. ACS Nano 7:4785–4798
272. Yamada KM, Geiger B (1997) Molecular interactions in cell adhesion complexes. Curr Opin Cell Biol 9:76–85
273. Hendesi H, Barbe MF, Safadi FF et al (2015) Integrin mediated adhesion of osteoblasts to connective tissue growth factor (CTGF/CCN2) induces cytoskeleton reorganization and cell differentiation. PLoS ONE 10(2):e0115325
274. Moussa FM, Hisijara IA, Sondag GR et al (2014) Osteoactivin promotes osteoblast adhesion through HSPG and αvβ1 integrin. J Cell Biochem 115:1243–1253
275. Saidak Z, Le Henaff C, Azzi S et al (2015) Wnt/β-catenin signaling mediates osteoblast differentiation triggered by peptide-induced α5β1 integrin priming in mesenchymal skeletal cells. J Biol Chem 290:6903–6912
276. Carvalho RS, Schaffer JL, Gerstenfeld LC (1998) Osteoblasts induce osteopontin expression in response to attachment on fibronectin: demonstration of a common role for integrin

receptors in the signal transduction processes of cell attachment and mechanical stimulation. J Cell Biochem 70:376–390
277. Riveline D, Zamir E, Balaban NQ et al (2001) Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism. J Cell Biol 153:1175–1185
278. Carvalho RS, Bumann A, Schaffer JL, Gerstenfeld LC (2002) Predominant integrin ligands expressed by osteoblasts show preferential regulation in response to both cell adhesion and mechanical perturbation. J Cell Biochem 84:497–508
279. Friedland JC, Lee MH, Boettiger D (2009) Mechanically activated integrin switch controls α5β1 function. Science 323:642–644
280. Litzenberger JB, Kim JB, Tummala P, Jacobs CR (2010) β1 integrins mediate mechanosensitive signaling pathways in osteocytes. Calcif Tissue Int 86:325–332
281. Litzenberger JB, Tang WJ, Castillo AB, Jacobs CR (2009) Deletion of β1 integrins from cortical osteocytes reduces load-induced bone formation. Cell Mol Bioeng 2:416–424
282. McNamara LM, Majeska RJ, Weinbaum S et al (2009) Attachment of osteocyte cell processes to the bone matrix. Anat Rec 292:355–363
283. Phillips JA, Almeida EA, Hill EL et al (2008) Role for β1 integrins in cortical osteocytes during acute musculoskeletal disuse. Matrix Biol 27:609–618
284. Thi MM, Suadicani SO, Schaffler MB et al (2013) Mechanosensory responses of osteocytes to physiological forces occur along processes and not cell body and require αVβ3 integrin. Proc Natl Acad Sci USA 110:21012–21017
285. Haugh MG, Vaughan TJ, McNamara LM (2015) The role of integrin α V β 3 in osteocyte mechanotransduction. J Mech Behav Biomed Mater 42:67–75
286. Cabahug-Zuckerman P, Stout RF Jr et al (2018) Potential role for a specialized β3 integrin-based structure on osteocyte processes in bone mechanosensation. J Orthop Res 36:642–652
287. Pommerenke H, Schmidt C, Durr F et al (2002) The mode of mechanical integrin stressing controls intracellular signaling in osteoblasts. J Bone Miner Res 17:603–611
288. Saunders MM, You J, Trosko JE et al (2001) Gap junctions and fluid flow response in MC3T3-E1 cells. Am J Physiol—Cell Physiol 281:1917–1925
289. Gillespie PG, Walker RG (2001) Molecular basis of mechanosensory transduction. Nature 413:194–202
290. Kazmierczak P, Sakaguchi H, Tokita J et al (2007) Cadherin 23 and protocadherin 15 interact to form tip-link filaments in sensory hair cells. Nature 449:87–91
291. Marie PJ, Hay E (2013) Cadherins and Wnt signalling: a functional link controlling bone formation. BoneKEy Rep 2:4
292. Matsuo K, Otaki N (2012) Bone cell interactions through Eph/ephrin: bone modeling, remodelling and associated diseases. Cell Adh Migr 6:148–156
293. Tamma R, Zallone A (2012) Osteoblast and osteoclast crosstalks: from OAF to Ephrin. Inflamm Allergy-Drug Targets 11:196–200
294. Nakajima K, Kho DH, Yanagawa T et al (2016) Galectin-3 in bone tumor microenvironment: a beacon for individual skeletal metastasis management. Cancer Metastasis Rev 35:333–346
295. Vinik Y, Shatz-Azoulay H, Vivanti A et al (2015) The mammalian lectin galectin-8 induces RANKL expression, osteoclastogenesis, and bone mass reduction in mice. Elife 4:e05914
296. Tanikawa R, Tanikawa T, Hirashima M et al (2010) Galectin-9 induces osteoblast differentiation through the CD44/Smad signaling pathway. Biochem Biophys Res Commun 394:317–322
297. Flagg-Newton J, Simpson I, Loewenstein WR (1979) Permeability of the cell-to-cell membrane channels in mammalian cell junctions. Science 205:404–407
298. Steinberg TH, Civitelli R, Geist ST et al (1994) Connexin43 and connexin45 form gap junctions with different molecular permeabilities in osteoblastic cells. EMBO J 13:744–750
299. Saunders MM, You J, Zhou Z et al (2003) Fluid flow-induced prostaglandin E2 response of osteoblastic ROS 17/2.8 cells is gap junction-mediated and independent of cytosolic calcium. Bone 32:350–356

300. Bivi N, Condon KW, Allen MR (2012) Cell autonomous requirement of connexin 43 for osteocyte survival: consequences for endocortical resorption and periosteal bone formation. J Bone Miner Res 27:374–389
301. Delaine-Smith RM, Sittichokechaiwut A, Reilly GC (2014) Primary cilia respond to fluid shear stress and mediate flow-induced calcium deposition in osteoblasts. FASEB J 28:430–439
302. Myers KA, Rattner JB, Shrive NG, Hart DA (2007) Osteoblast-like cells and fluid flow: cytoskeleton-dependent shear sensitivity. Biochem Biophys Res Commun 364:214–219
303. Malone AMD, Anderson CT, Tummala P et al (2007) Primary cilia mediate mechanosensing in bone cells by a calcium-independent mechanism. Proc Natl Acad Sci USA. 104:13325–13330
304. Xiao Z, Zhang S, Mahlios J et al (2006) Cilia-like structures and polycystin-1 in osteoblasts/osteocytes and associated abnormalities in skeletogenesis and Runx2 expression. J Biol Chem 281:30884–30895
305. Leucht P, Monica SD, Temiyasathit S et al (2013) Primary cilia act as mechanosensors during bone healing around an implant. Med Eng Phys 35:392–402
306. Chen JC, Hoey DA, Chua M et al (2016) Mechanical signals promote osteogenic fate through a primary cilia-mediated mechanism. FASEB J 30:1504–1511
307. Coughlin TR, Voisin M, Schaffler MB et al (2015) Primary cilia exist in a small fraction of cells in trabecular bone and marrow. Calcif Tissue Int 96:65–72
308. Smith MA, Hoffman LM, Beckerle MC (2014) LIM proteins in actin cytoskeleton mechanoresponse. Trends Cell Biol 24:575–583
309. Wang N, Ingber DE (1994) Control of cytoskeletal mechanics by extracellular matrix, cell shape, and mechanical tension. Biophys J 66:2181–2189
310. Wang N, Butler JP, Ingber DE (1993) Mechanotransduction across the cell surface and through the cytoskeleton. Science 260:1124–1127
311. del Álamo JC, Norwich GN, Yshuan JL et al (2008) Anisotropic rheology and directional mechanotransduction in vascular endothelial cells. Proc Natl Acad Sci USA 105:15411–15416
312. Hu S, Chen J, Fabry B et al (2003) Intracellular stress tomography reveals stress focusing and structural anisotropy in cytoskeleton of living cells. Am J Physiol 285:1082–1090
313. Silberberg YR, Pelling AE, Yakubov GE et al (2008) Mitochondrial displacements in response to nanomechanical forces. J Mol Recognit 21:30–36
314. Koike M, Nojiri H, Ozawa Y et al (2015) Mechanical overloading causes mitochondrial superoxide and SOD2 imbalance in chondrocytes resulting in cartilage degeneration. Sci Rep 5:11722
315. Khatiwala CB, Peyton SR, Putnam AJ (2006) Intrinsic mechanical properties of the extracellular matrix affect the behavior of pre-osteoblastic MC3T3-E1 cells. Am J Physiol Cell Physiol 290:1640–1650
316. Wen JH, Vincent LG, Fuhrmann A et al (2014) Interplay of matrix stiffness and protein tethering in stem cell differentiation. Nat Mater 13:979–987
317. Ritz U, Götz H, Baranowski A et al (2016) Influence of different calcium phosphate ceramics on growth and differentiation of cells in osteoblast–endothelial co-cultures. J Biomed Mater Res B Appl Biomater 105:1950–1962
318. Olivares-Navarrete R, Rodil SE, Hyzy SL et al (2015) Role of integrin subunits in mesenchymal stem cell differentiation and osteoblast maturation on graphitic carbon-coated microstructured surfaces. Biomaterials 51:69–79
319. Fraioli R, Rechenmacher F, Neubauer S et al (2015) Mimicking bone extracellular matrix: Integrin-binding peptidomimetics enhance osteoblast-like cells adhesion, proliferation, and differentiation on titanium. Colloids Surf B 128:191–200
320. Vatsa A, Breuls RG, Semeins CM et al (2008) Osteocyte morphology in fibula and calvaria—is there a role for mechanosensing? Bone 43:452–458
321. Van Hove RP, Nolte PA, Vatsa A et al (2009) Osteocyte morphology in human tibiae of different bone pathologies with different bone mineral density—is there a role for mechanosensing? Bone 45:321–329
322. Murshid SA, Kamioka H, Ishihara Y et al (2007) Actin and microtubule cytoskeletons of the processes of 3D-cultured MC3T3-E1 cells and osteocytes. J Bone Miner Metab 25:259

323. Adachi T, Aonuma Y, Tanaka M et al (2009) Calcium response in single osteocytes to locally applied mechanical stimulus: Differences in cell process and cell body. J Biomech 42:1989–1995
324. Sugawara Y, Ando R, Kamioka H et al (2008) The alteration of a mechanical property of bone cells during the process of changing from osteoblasts to osteocytes. Bone 43:19–24
325. Prendergast PJ, Huiskes R (1995) The biomechanics of Wolff's law: recent advances. Ir J Med Sci 164:152–154
326. Turner CH (1998) Three rules for bone adaptation to mechanical stimuli. Bone 23:399–407
327. Hsieh Y-F, Turner CH (2001) Effects of loading frequency on mechanically induced bone formation. J Bone Miner Res 16:918–924
328. Zhu J, Zhang X, Wang C et al (2008) Different magnitudes of tensile strain induce human osteoblasts differentiation associated with the activation of ERK1/2 phosphorylation. Int J Mol Sci 9:2322–2332
329. Mosley JR, March BM, Lynch J, Lanyon LE (1997) Strain magnitude related changes in whole bone architecture in growing rats. Bone 20:191–198
330. Cullen DM, Smith RT, Akhter MP (2001) Bone-loading response varies with strain magnitude and cycle number. J Appl Physiol 91:1971–1976
331. Rubin CT, Lanyon LE (1984) Regulation of bone formation by applied dynamic loads. J Bone Joint Surg 66:397–402
332. Srinivasan S, Ausk BJ, Poliachik SL et al (2007) Rest-inserted loading rapidly amplifies the response of bone to small increases in strain and load cycles. J Appl Physiol 102:1945–1952
333. Pereira AF, Shefelbine SJ (2014) The influence of load repetition in bone mechanotransduction using poroelastic finite-element models: the impact of permeability. Biomechan Model Mechanobiol 13:215–225
334. Stavenschi E, Corrigan MA, Johnson GP et al (2018) Physiological cyclic hydrostatic pressure induces osteogenic lineage commitment of human bone marrow stem cells: a systematic study. Stem Cell Res Ther 9:276
335. Warden SJ, Turner CH (2004) Mechanotransduction in the cortical bone is most efficient at loading frequencies of 5–10 Hz. Bone 34:261–270
336. Verbruggen SW, Vaughan TJ, McNamara LM (2014) Fluid flow in the osteocyte mechanical environment: a fluid–structure interaction approach. Biomechan model mechanobiol 13:85–97
337. Wittig NK, Laugesen M, Birkbak ME et al (2019) Canalicular junctions in the osteocyte lacuno-canalicular network of cortical bone. ACS Nano 13:6421–6430
338. Gatti V, Azoulay EM, Fritton SP (2018) Microstructural changes associated with osteoporosis negatively affect loading-induced fluid flow around osteocytes in cortical bone. J Biomech 66:127–136
339. Nauli SM, Alenghat FJ, Luo Y et al (2003) Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. Nature Genet 33:129–137
340. Praetorius HA, Frokiaer J, Nielsen S, Spring KR (2003) Bending the primary cilium opens Ca^{2+}-sensitive intermediate-conductance K^+ channels in MDCK Cells. J Membr Biol 191:193–200
341. Waychunas GA (2014) Disrupting dissolving ions at surfaces with fluid flow. Science 344:1094–1095
342. Gross D, Williams WS (1982) Streaming potential and the electromechanical response of physiologically-moist bone. J Biomech 15:277–295
343. Frijns A, Huyghe J, Wijlaars M (2005) Measurements of deformations and electrical potentials in a charged porous medium. In: Gladwell GML, Huyghe J, Raats PA, Cowin SC (eds) IUTAM symposium on physicochemical and electromechanical interactions in porous media, vol 125, pp 133–139. Springer, Dordrecht
344. Hong J, Ko S, Khang G, Mun M (2008) Intraosseous pressure and strain generated potential of cylindrical bone samples in the drained uniaxial condition for various loading rates. J Mater Sci Mater Med 19:2589–2594

345. Pienkowski D, Pollack SR (1983) The origin of stress-generated potentials in fluid-saturated bone. J Orthop Res 1:30–41
346. Iatridis J, Laible J, Krag M (2003) Influence of fixed charge density magnitude and distribution on the intervertebral disc: applications of a poroelastic and chemical electric (PEACE) model. J Biomech Eng 125:12–24
347. Fukada E, Yasuda I (1957) On the piezoelectric effect of bone. J Phys Soc Jpn 12:1158–1162
348. Elmessiery MA (1981) Physical basis for piezoelectricity of bone matrix. IEE Proc A 128:336–346
349. Halperin C, Mutchnik S, Agronin A et al (2004) Piezoelectric effect in human bones studied in nanometer scale. Nano Lett 4:1253–1256
350. Marino AA, Becker RO (1974) Piezoelectricity in bone as a function of age. Calcif Tissue Int 14:327–331
351. Wang T, Feng Z, Song Y, Chen X (2007) Piezoelectric properties of human dentin and some influencing factors. Dent Mater 23:450–453
352. Reinish GB, Nowick AS (1975) Piezoelectric properties of bone as functions of moisture content. Nature 253:626–627
353. Ahn AC, Grodzinsky AJ (2009) Relevance of collagen piezoelectricity to "Wolff's Law": a critical review. Med Eng Phys 31:733–741
354. Ramtani S (2008) Electro-mechanics of bone remodelling. Int J Eng Sci 46:1173–1182
355. Frias C, Reis J, e Silva FC et al (2010) Polymeric piezoelectric actuator substrate for osteoblast mechanical stimulation. J Biomech 43:1061–1066
356. Reis J, Frias C, Canto e Castro C, Botelho ML, Marques AT, Simões JA, Capela e Silva F, Potes J (2012) A new piezoelectric actuator induces bone formation in vivo: a preliminary study. BioMed Res Int 613403
357. Zhang Y, Chen L, Zeng J et al (2014) Aligned porous barium titanate/hydroxyapatite composites with high piezoelectric coefficients for bone tissue engineering. Mater Sci Eng C 39:143–149
358. Liu J, Gu H, Liu Q, Ren L, Li G (2019) An intelligent material for tissue reconstruction: The piezoelectric property of polycaprolactone/barium titanate composites. Mater Lett 236:686–689
359. Jacob J, More N, Kalia K, Kapusetti G (2018) Piezoelectric smart biomaterials for bone and cartilage tissue engineering. Inflamm Regen 38:2
360. Damaraju SM, Shen Y, Elele E, Khusid B, Eshghinejad A, Li J, Jaffe M, Arinzeh TL (2017) Three-dimensional piezoelectric fibrous scaffolds selectively promote mesenchymal stem cell differentiation. Biomaterials 149:51–62