



# Cellular and Molecular Biology in Bone Remodeling

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## Keywords

Osteoblast · Osteoclast · Osteocytes · Cancellous · Cortical · Haversian · Basic multicellular unit · Perilacunar · Bone remodeling

## 1 Clinical Rationale

### 1.1 Introduction

Bone is a dynamic mixture of cells, collagen and hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ). In order to maintain its structural integrity and provide mineral homeostasis bone is constantly remodeled throughout postnatal life [1]. Normal bone remodeling occurs in either bone type (cortical or cancellous) by the coordinated action of bone formation by osteoblasts and bone resorption by osteoclasts. In normal physiological conditions, bone formation and resorption are nearly equal and there is no net change in bone mass. However, under different levels of exercise or pathological conditions bone mass can change due to an imbalance in overall remodeling. When bone mass decreases, skeletal fragility can result, which subsequently increases a patient's risk of fracture. The increased risk of fracture is only partially explained by a reduction in bone mass and is dictated by other parameters such as bone structure and material

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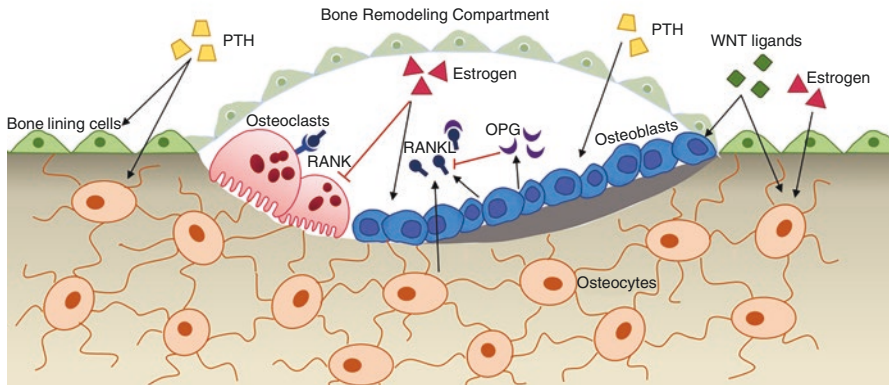
quality [2]. Nonetheless, increased skeletal fragility is found in patients with bone remodeling disorders such as osteoporosis and other pathologies [3, 4].

## 1.2 Bone Remodeling Disorders

*Osteoporosis* is a disease characterized by low bone mass, compromised bone strength, deterioration of bone tissue, and disrupted bone architecture. It has a widespread incidence affecting 1 in 2 women and 1 in 5 men over the age of 50 [5]. The clinical definition of osteoporosis, defined by the World Health Organization (WHO), is bone mineral density (BMD) at the hip or lumbar spine of a patient being less than or equal to 2.5 standard deviations below the young healthy reference population (30 years old) mean BMD (called a T-score) [6]. Osteopenia is a disease in which bone density is below average (T-score between  $-1$  and  $-2.5$ ), though not as severe as osteoporosis [7]. Some causes of low bone mass are estrogen deficiency, as seen in postmenopausal women, glucocorticoids, disuse, and diabetes/obesity [6]. Treatments to alleviate symptoms of osteoporosis include lifestyle changes, such as cessation of smoking, reduction of alcohol use, and increased physical activity. Vitamin D and calcium supplements may also improve bone health but can have unintended drawbacks seen in other organ systems, such as calcium buildup in the cardiovascular system [8]. The most effective pharmaceutical therapies for osteoporosis treatment are anti-resorption drugs, such as bisphosphonates (Chapter “Effects of Osteoporosis Drugs—Morphological Assessment and Adverse Events”) and Denosumab (Chapter “Denosumab in the Treatment of Postmenopausal Women with Osteoporosis: Fracture Outcomes, BMD, and Morphological Assessment”), that minimize bone resorption [8–10].

## 2 Bone Cells and Associated Functions (Fig. 1)

*Osteoclasts* are multinucleated cells, derived from monocytes of the hematopoietic lineage that function to resorb bone. The differentiation of monocyte lineage cells into mature osteoclasts is dependent on receptor activator of NF- $\kappa$ B ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) [11]. M-CSF binds to its receptor (c-Fms) on osteoclast precursors and mature cells to stimulate their proliferation and survival [11]. However, RANKL, which is expressed by both osteoblasts and osteocytes, is the primary osteoclast differentiation factor [11]. RANKL binds to its receptor, RANK, present on osteoclasts, to induce transcription factors and enzymes that promote bone resorption. Osteoprotegerin (OPG), an endogenous antagonist to RANKL, inhibits RANKL binding to RANK by acting as a decoy receptor [8, 12]. OPG is secreted by osteoblast lineage cells to aid in the tight regulation of osteoclastogenesis [12, 13]. Osteoclasts are able to resorb bone due to their motile cytoskeleton, adhesion molecules, and a ruffled border at the bone surface [8, 14]. These qualities of osteoclasts allow for their attachment to bone, largely via  $\alpha$ V $\beta$ 3 integrins, and the creation of a sealed-off microenvironment between the osteoclast ruffled border and the bone surface. At the ruffled border active carbonic anhydrase



**Fig. 1** Overview of cellular and molecular mediators of bone remodeling. The Bone Remodeling Compartment (BRC) is a specialized environment where bone remodeling occurs through osteoclasts resorbing old or damaged bone tissue, and osteoblasts forming new bone matrix. Bone remodeling is tightly regulated and is driven by local osteocytic factors such as RANKL, OPG, and WNT ligands or via endocrine signaling by estrogen and PTH. Specifically, estrogen acts on osteoblasts and osteocytes to promote bone formation, while inhibiting osteoclastic bone resorption. PTH has pleiotropic effects on bone. It acts on the osteoblasts, osteocytes, and bone lining cells to directly promote bone formation or to cause osteoblasts and osteocytes to release RANKL. RANKL then binds to RANK receptors on osteoclasts, to promote osteoclastogenesis. However, osteoblasts and osteocytes release a decoy receptor, OPG, that tightly regulates osteoclastogenesis by inhibiting RANKL-RANK binding. WNT ligands, part of the  $\beta$ -catenin canonical signaling pathway, play a major role in bone remodeling by acting on osteoblasts and osteocytes to promote osteoblastogenesis and indirectly control osteoclastogenesis. Abbreviations: receptor activator of NF- $\kappa$ B ligand, OPG, osteoprotegerin, PTH, parathyroid hormone

converts  $\text{CO}_2$  and  $\text{H}_2\text{O}$  to bicarbonate ( $\text{HCO}_3^-$ ) and protons ( $\text{H}^+$ ) that are secreted into the resorption compartment creating an acidic environment. This acidic environment allows for activation of osteoclast specific matrix metalloproteinases, including tartrate resistant acid phosphatase (TRAP), and the collagenase cathepsin K, which facilitates breakdown of the extracellular matrix and resorption of bone matrix [8, 15, 16]. Cathepsin K aids in removing poor quality bone where micro-cracks have accumulated and hole-like lacunae have formed [8]. TRAP is highly expressed by mature osteoclasts during bone resorption and is critical for skeletal development [14, 17]. Another highly expressed enzyme produced by mature osteoclasts is Src kinase [8]. Src kinase's main role is to mediate multiple pathways that regulate osteoclast activity, but not number, such as formation of a ruffled border critical for bone resorption. Without the presence of Src kinase there will be an increase in osteoblastic bone formation, but no effect on overall osteoclast number.

**Osteoblasts**, derived from mesenchymal stem cells, are primarily responsible for the synthesis of bone extracellular matrix. This process, often referred to as osteogenesis, is crucial for the proper development and maintenance of bone. In adult skeletons, osteoblasts are recruited to regenerate, or remodel, areas of bone with depleted matrix or defects [8, 18]. To synthesize bone at sites of resorption, osteoblasts secrete bone matrix proteins, such as collagen type 1 (Col1) and

noncollagenous proteins such as osteocalcin (OCN) and osteopontin (OPN). Through osteoblast derived alkaline phosphatase (ALP) [8, 19] the synthesized bone extracellular matrix (called osteoid) undergoes mineralization to form hydroxyapatite [18–20]. Osteoblast differentiation is controlled by two well-studied transcription factors: Runt-related transcription factor 2 (RUNX2) and Osterix (Osx). RUNX2 is an essential bone-specific transcription factor that causes the upregulation of osteoblast differentiation marker genes and induces osteogenesis [8, 21]. Osx is located downstream of RUNX2 and is required for osteoblast maturation and bone mineralization [18, 21, 22]. Osteoblast growth and differentiation are also mediated by different classical signaling pathways such as transforming growth factor-beta (TGF- $\beta$ ), bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), and the WNT/  $\beta$ -catenin pathway [23]. Furthermore, osteoblasts are sensitive to mechanical stimuli resulting from mechanical loading [8, 18, 20].

**Osteocytes** are the most abundant cell type in bone and comprise about 90–95% of all the cells in bone [24]. They are terminally differentiated, post-mitotic osteoblasts that have become embedded in mineralized bone matrix. The osteocyte has two major components: (1) a central cell body in a bone cavity called a lacuna (15–20 microns in humans) and (2) small dendritic projections (50–100 per cell) [25] coming off the cell body that protrude through microscopic (50–300 nm) [26] channels called canaliculi. This vast network of lacunae and canaliculi make up the lacunar-canalicular system of bone, which is thought to serve as a biochemical transport system between osteocytes, vascular channels, and bone surfaces as well as a mechanical amplification system. Through these expansive networks, osteocytes can connect and transport small molecules to neighboring bone cells via gap junctions (see section “Important Molecules in Bone Remodeling”). Due to these features, osteocytes have been implicated as the key mechanosensitive and endocrine regulated cell in bone that regulates bone mass. Osteocytes express higher levels of key bone matrix and phosphate regulatory molecules, including osteocalcin (OCN, dentin-matrix protein 1 (DMP1), phosphate-regulating neutral endopeptidase (PHEX), matrix extracellular, phosphoglycoprotein (MEPE) and fibroblast growth factor 23 (FGF23), than osteoblasts [27]. They also express molecules that directly inhibit osteoblast formation and activity, such as dickkopf-related protein 1 (Dkk1) and sclerostin, and regulate osteoclast formation via RANKL and its decoy receptor OPG. Osteocytes can live the entire lifetime of the host (mean half-life 25 years) [28] or undergo apoptosis (i.e., programmed cell death) due to various environmental stimuli such as bone microdamage (linear microcracks), estrogen deficiency, unloading, or glucocorticoid use [29]. It was first shown by Cardoso et al. that osteocyte apoptosis, which occurs in microdamaged regions of the bone following fatigue loading (which results in microcracks), is a key event necessary for targeted intracortical remodeling [30]. Subsequent *in vitro* and *in vivo* work has suggested that apoptotic osteocytes near fatigue damage microcracks in bone ( $\leq 300$  microns) do not secrete any signals and therefore this response is

fairly targeted to the damaged bone region. This same effect is apparent in estrogen withdrawal after ovariectomy, where osteocyte apoptosis is upregulated 4–7 times in the posterior diaphyseal cortex, resulting in activation of endosteal resorption [33]. Estrogen signaling is necessary for continued mitigation of mitogen-activated protein kinases (MAPKs) activation, which neutralizes reactive oxygen species (ROS) in osteocytes thereby preventing apoptosis [29].

## 2.1 Bone Types and Haversian System

Structurally, bone is composed of two distinct types, cancellous and cortical. **Cancellous bone** is found at the end of long bones (epiphysis/metaphysis) or in flat bones, such as the pelvis, clavicle and cranium, and is made up of highly arranged trabecular struts. These trabecular struts, in humans, can be dominated by a rod- or plate-like architecture, are 150–300 microns thick and interspaced every 0.5 to 1.5 mm [34, 35]. Therefore, cancellous bone has a large surface area to volume ratio (porosity of 0.5–0.95) and its remodeling serves a primary metabolic function in the body. Cancellous bone remodeling starts at the surface of the trabeculae and usually takes about 200 days to complete [36]. In contrast, **Cortical bone**, which is denser and more compact, makes up the outer covering of all bone and is sometimes referred to as compact bone. Cortical bone's structure and a usually lower rate of turnover enable it to serve a primarily load-bearing function. Human cortical bone is initially formed and made up of discrete units called osteons that make up the Haversian system of bone. These osteons are cylindrical in nature and consist of blood vessels and nerves at their center (Haversian canal) surrounded by rings of concentric layers of compact bone called lamellae. Interspersed between these layers of lamellar bone are osteocytes and their lacunar-canalicular system. Osteons are typically 200  $\mu\text{m}$  in diameter and 3 mm in length [37]. Both cancellous and osteonal remodeling occurs by the stochastic and coordinated action of multiple cell types, termed basic multicellular units (BMU's), that remodels bone [38, 39].

## 2.2 “Basic Multicellular Unit” BMU-Based Remodeling

The replacement of old osteons in cortical bone or trabecular struts to form secondary (i.e. new) osteons or new trabeculae, respectively, occurs by the coordinated action of a group of bone cells termed the “Basic Multicellular Unit” (BMU), which creates a structural feature of bone tissue called the “Bone Remodeling Unit” (BRU) [40]. These processes occur in a specialized environment, that is enclosed by canopy cells and is vascularized and innervated, called the “Bone Remodeling Compartment” (BRC) [41] (see Chapter “Cellular and Molecular Biology in Bone Remodeling”; Fig. 1 and Chapter “Mechanism Reversing Bone Resorption to Formation During Bone Remodeling”). Bone remodeling in total occurs across

several discrete phases named in order of their occurrence; Activation-Resorption/Reversal-Formation (A-R-F) (see Chapter “Bone Remodeling and Modeling: Therapeutic Targets for the Treatment of Osteoporosis”). In the **activation phase**, hormonal (PTH) or mechanical remodeling signals (RANKL, microdamage) sensed by the osteocyte and osteoblasts, signal to pre-osteoclasts to undergo osteoclast formation and resorptive activity [42–44]. This activation phase is quick and lasts 1–3 days. Once osteoclasts have formed, the resorption phase begins with the osteoclasts forming an ellipsoidal “cutting cone” in cortical bone or Howship’s lacunae in cancellous bone [45]. The osteoclasts begin to resorb bone along the anatomical axis of the osteon or trabeculae at a rate of 20–40  $\mu\text{m}$  per day for about 3 weeks [46], leaving behind demineralized and partially digested bone matrix. Following osteoclast-mediated resorption, a **reversal stage** begins that initiates bone formation (see Chapter “Significance of Reversal Resorption Phase in Bone Loss”). Although the cell type responsible for initiating bone formation is contested [1, 47–49], it is generally accepted that this specialized cell prepares the bone surface and potentially secretes coupling factors to promote osteoblast formation and activation. In the last phase of BMU based bone remodeling, the **formation phase**, osteoblasts deposit a new unmineralized bone matrix, called osteoid, into the prepared resorption spaces. This osteoid is later mineralized with the help of specialized metalloenzymes, such as alkaline phosphatase, from the osteoblast [50, 51]. These osteoblasts can then either become entrapped in minerals to form osteocytes, undergo apoptosis, or become quiescent to form lining cells that cover the new bone surface to aid in the next remodeling cascade [52, 53].

### 2.3 Perilacunar Remodeling

The lacunar-canalicular system is vast. In humans, it makes up an area of bone nearly 215  $\text{m}^2$  [2] and therefore represents a large reservoir of available minerals and bioactive molecules. Osteocytic osteolysis, or the newer term, perilacunar remodeling (also called perilacunar turnover to distinguish it from bone remodeling involving the BMU which requires both osteoclasts and osteoblasts), is now recognized as a distinct form of bone turnover that is carried out directly by the osteocyte. Typically, perilacunar remodeling is associated with conditions that place rapid demands on the skeleton, such as lactation, calcium restriction, and space flight. In fact, experiments have shown that at any one time 15–20% of osteocytes are associated with new bone-forming surfaces and can produce the same enzymes used by osteoclasts to resorb bone including matrix metalloproteases (MMPs), proton pumps, carbonic anhydrase, and cathepsin K [24, 54, 55]. More recent evidence has shown that perilacunar remodeling is coordinated via TGF $\beta$ -1 and PTH receptor signaling in osteocytes as well as the downstream transcriptional regulators Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) [54, 56, 57]. More importantly, these same studies show that perilacunar remodeling has a direct effect on the local bone matrix quality and therefore can directly influence fracture resistance.

### 3 Important Molecules in Bone Remodeling

#### 3.1 Local (Paracrine and Autocrine)

**Receptor activator of nuclear factor-kappa- $\beta$  ligand (RANKL), its receptor RANK and Osteoprotegerin (OPG)**—RANKL is expressed in the local bone microenvironment by osteoblast lineage cells (osteoblasts and osteocytes) and signals to its receptor RANK on osteoclastic precursor cells to control their fusion, survival, and differentiation into mature osteoclasts [58]. RANKL is highly upregulated in bone cells, especially osteocytes exposed to PTH, after bone damage, and disuse [59]. People with mutations in the RANKL gene (TNFSF11) demonstrate osteopetrosis or excessively brittle bone due to hypermineralization [60]. On the other hand, OPG is a decoy receptor for RANKL that is expressed by osteoblast lineage cells and blocks osteoclastogenesis through competitive inhibition of RANKL-RANK binding [61]. OPG is upregulated due to bone loading, estrogen, and transforming growth factor  $\beta$  (TGF- $\beta$ ) [62]. Therefore, RANKL/OPG pathways play a key role in coordinating bone resorption by various local and systemic regulators.

**WNT/ $\beta$ -Catenin Signaling and Sclerostin**—The WNT/ $\beta$ -catenin signaling pathway has emerged as a major pathway controlling bone remodeling. In canonical WNT signaling, WNT ligands (19 distinct proteins in vertebrates) bind to the frizzled receptor with co-activators LRP4/5/6. This results in  $\beta$ -catenin stabilization, nuclear translocation and binding to TCF/LEF in the cell nucleus. This stabilization increases transcriptional activation of genes that increase osteocyte/osteoblast proliferation, viability, and bone formation while simultaneously decreasing bone resorption via decreased RANKL/OPG signaling [63, 64]. Therefore, modulating WNT/ $\beta$ -catenin has become a key target to improve bone mass and quality by increasing bone formation while downregulating resorption. One key approach used to modulate WNT/ $\beta$ -Catenin signaling is by inhibiting sclerostin, the product of the SOST gene and a WNT/ $\beta$ -Catenin antagonist that binds LRP5/6. Sclerostin is primarily produced by osteocytes, is regulated by loading, and mutations in the gene lead to high bone mass disorders such as Sclerosteosis or Van Buchem's disease [65–68].

**Gap Junctional Intercellular Communication (GJIC)**—Gap junctions are membrane-spanning protein channels that allow for the passage of small molecules such as ATP, calcium, prostaglandin, and microRNAs between bone cells [69]. The predominate gap junction protein in bone is connexin 43 (Cx43), which is encoded by the GJA1 gene and highly expressed by osteoblast lineage cells, especially osteocytes. It is found at the connections between osteocytes and other bone cells and is necessary for osteocytic regulation of osteoblasts via mechanical stimulation [70]. Mutations in the GJA1 gene in humans cause oculodentodigital dysplasia and are associated with high turnover osteopenia and increased intracortical porosity [71–73]. This is likely because Cx43 is necessary for normal osteoblast lineage cell differentiation, development, and function. Cx43 has also been shown to play a large role in skeletal homeostasis and skeletal adaptation to mechanical loading. For example, loss of Cx43 in osteoblast lineage cells (osteoblasts and osteocytes) attenuates bone loss seen with unloading [74, 75]. Interestingly, the same conditional

deletion of Cx43 leads to greater bone formation with skeletal loading [76, 77]. These studies and recent reviews suggest that these beneficial skeletal results are due to Cx43's regulation of osteocyte apoptosis and specific factors such as PGE2, sclerostin, and RANKL/OPG, respectively, that are critical for normal bone remodeling [74, 78–81].

### 3.2 Systemic (Endocrine)

**Parathyroid Hormone (PTH)** is an important hormone for maintaining bone homeostasis. It is secreted by the parathyroid glands and plays a key role in coordinating serum calcium and phosphate homeostasis [82]. Specifically, PTH works by binding to its receptor, which is found on all cells of the osteoblast lineage (i.e., bone lining cells, osteoblasts, osteocytes) [83]. PTH receptor activation increases bone formation by converting bone lining cells to active osteoblasts, decreasing osteoblast apoptosis and promoting osteoblastic differentiation by acting as an upstream regulator of RUNX2 [43, 84]. Furthermore, PTH receptor activation of osteocytes increases RANKL mediated osteoclastogenesis and decreases sclerostin expression [85, 86]. However, the effect of PTH on bone is time-dependent. While a constant, elevated level of PTH stimulates bone resorption, an intermittent dose is anabolic and promotes bone formation [18]. Teriparatide, a recombinant form of active PTH, is the oldest and most commonly used bone anabolic drug to treat osteoporosis [87] (see Chapter “Teriparatide”).

**Estrogen (17 $\beta$ -estradiol)** is another important hormone for bone, particularly for women. 17 $\beta$ -estradiol, the most common form of estrogen, has direct effects on bone cells by binding to the estrogen receptors alpha and or beta (ER $\alpha$  and ER $\beta$ ), which are found on osteoblasts, osteoclasts, and osteocytes [88]. ER $\alpha$  and ER $\beta$  activation directly promotes osteocyte and osteoblast function, inhibits their apoptosis, and inhibits osteoclast activation and function [88–90]. All of these estrogen functions work to prevent bone loss and maintain skeletal mineralization. However, postmenopausal women experience a decline in ovarian estradiol levels, which leads to decreased BMD levels [89, 91]. Men also experience a decline of estrogen levels with age, but not to the same extent as women [89]. These decreased serum estradiol levels can be predictive of bone fracture and development of osteoporosis in both men and women [89, 91].

Other systemic factors, including but not limited to **Calcitonin, FGF23, and Insulin** are also important for bone remodeling. Calcitonin is released by thyroid cells and binds to osteoclasts to inhibit bone resorption [92]. FGF23 is produced by mature osteocytes to help the kidneys regulate serum phosphorous levels and maintain mineral homeostasis [93–95]. Insulin is an important hormone in both energy and bone metabolism [96]. Insulin is recruited to bone through the osteoblastic release of osteocalcin, which signals to pancreatic  $\beta$ -cells to release insulin [97]. Insulin acts directly on bone cells by decreasing OPG production from osteoblasts, thereby increasing RANKL concentrations that can activate osteoclasts [97].



**Biomarkers and Assays of Bone Remodeling**—To determine the status of bone turnover, serum biochemical markers, and bone biopsies are used clinically (see Chapter “Metabolic Boneturnover Markers”). The iliac crest is a common biopsy site used to simultaneously observe cortical and trabecular bone mineralization and cellular features histologically [98, 99]. Usually, iliac crest biopsies are taken after systemic administration of a bone chelating agent, such as tetracycline, which can be analyzed histologically to assess rates of bone turnover [98–100] (see Chapter “Histomorphometric Assessment of Remodeling and Modeling-Based Bone Formation”). A noninvasive assay to evaluate bone turnover involves quantifying biochemical markers in blood serum following fasting. Common serum bone formation markers are osteocalcin (OCN) and procollagen type I N-terminal propeptide (P1NP). Common serum bone resorption markers are C-terminal telopeptide (CTX), N-terminal telopeptide (NTX), and TRAP5b [98, 101]. Other serum biochemical markers assessed include PTH, insulin-like growth factor I (IGF-I) (an anabolic hormone for bone), and vitamin D [96, 101].

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