1 Bone Health: Basic and Applied Bone Biology

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Introduction

Recently, bone biology and its role in maintaining the bone health integrity has got in focus and has become a vastly growing area of research. Given its intricate systemic and local connections, bone biology merges the traditional fields of anatomy, physiology, and biomechanics together with the increasingly complex fields of developmental biology and molecular genetics. Therefore, it is essential for clinicians who treat bone disorders such as osteoporosis, as well as other metabolic bone disorders to keep themselves updated and develop a working knowledge of this topic. Such studies of the bone biology revealed how the bone structure can be optimized so that it gets strong but, in the meantime, remains relatively light weight. In depth analysis of the bone biology and its fundamental role in preserving bone health revealed how the integrity of the skeleton is maintained through the balanced activities of its constituent cell types. Furthermore, molecular dissection of genetic disorders of highly increased or reduced bone mass has identified many of the crucial proteins controlling the activity of these bone cell types [1]. This information has resulted in both novel ways to treat or diagnose more common bone disorders and a better understanding of the common genetic variants that lead to differences in bone density in the general population.

The skeletal architecture is remarkably adapted to provide adequate strength and mobility without negative impact on the bones themselves; meaning that bones do not break when subjected to substantial impact, or heavy loads are placed on them during vigorous physical activity. Therefore, the bone shape and structure are considered, at least, as important as its mass in providing this strength. In addition, the skeleton act also as a storehouse for two important minerals, namely, calcium and phosphorus. These are essential for the functioning of other body systems, and this storehouse is called upon in times of need. To be able to carry out its dual roles of support and mineral homeostasis, as well as to repair any damage to the skeleton, bones are constantly changing. Old bone breaks down and new bone is formed on a regular basis, subsequently, the skeletal tissue is replaced several times during life. This requires a perfectly controlled regulatory system that involves specialized cells able to communicate with each other. These cells are expected also to respond to several different signals, both internal and external, mechanical, hormonal, systemic (affecting the whole skeleton) as well as local (affecting only a small region of the skeleton) [2]. It is not surprising that with so many different tasks to perform and so many different factors regulating how the

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skeleton grows, adapts, and responds to changing demands; there are many ways that these processes can go astray.

This chapter discusses bone biology, providing the reader with the background required to understand the basis of bone biology including bone structure, cells, and extracellular matrix, the mechanical and chemical stimulants versus inhibitors of bone activity, as well as the interaction among these components both in physiologic situations and in response to injury. It also expands to discuss applied bone biology and its implementation in the prevention, diagnosis, and principles of treatment approaches related to bone disease that are discussed in detail later in this book.

Basic Bone Biology

Bone is a specialized form of connective tissue that serves as both a tissue and an organ system within higher vertebrates. As such, its basic functions include locomotion, protection, and mineral homeostasis.

Cellular Composition

The cellular makeup of bones includes osteoblasts, osteocytes, bone lining cells, and osteoclasts, as well as its matrix which contains an organic and an inorganic component [3, 4]. Another cellular classification has also been developed stratifying the cells into bone forming and bone resorbing cells [5]. Further differentiation of bone cells is based on their origin. Osteoblasts, osteocytes, and bone lining cells originate from mesenchymal stem cells known as osteoprogenitor cells, whereas osteoclasts originate from hemopoietic stem cells. The location of these cells also varies. Bone cells found along the surface of bone include osteoblasts, osteoclasts, and bone lining cells, whereas osteocytes are located in the interior of bone [6, 7]. Downey and Siegel (2006) [6] as well as Rachner and colleagues (2011) [7] provided detailed reports on bone biology.

Osteoblasts

Osteoblasts are cuboidal cells that are located along the bone surface comprising 4–6% of the total resident bone cells and are largely known for their bone forming function. Osteoblasts are derived from undifferentiated mesenchymal cells that are located in the marrow, endosteum, periosteum, and bone canals. These cells, also referred to as "preosteoblasts," can migrate from surrounding tissue or through the vascular system. Mesenchymal cells are stellate in shape, contain relatively small amounts of cytoplasm and organelles, and possess a single nucleus. Differentiation and proliferation of mesenchymal cells into osteoblasts occurs during both intramembranous and endochondral bone formation (Fig. 1.1) [3, 4].

The commitment of mesenchymal cells towards the osteoprogenitor lineage requires the expression of specific genes, following timely programmed steps, including the synthesis of bone morphogenetic proteins (BMPs) and members of the Wingless (Wnt) pathways [8]. The expressions of Runt-related transcription factors 2, Distal-less homeobox 5 (Dlx5), and osterix (Osx) are crucial for osteoblast differentiation [9]. Additionally, Runx2 is a master gene of osteoblast differentiation, as demonstrated by the fact that Runx2-null mice are devoid of osteoblasts [9, 10]. Runx2 has demonstrated to upregulate osteoblast-related genes such as ColIA1, ALP, BSP, BGLAP, and OCN [11]. Once a pool of osteoblast progenitors expressing Runx2 and ColIA1 has been established during osteoblast differentiation, there is a proliferation phase. In this phase, osteoblast progenitors show alkaline phosphatase (ALP) activity, and are considered preosteoblasts [12]. The transition of preosteoblasts to mature osteoblasts is characterized by an increase in the expression of Osx and in the secretion of bone matrix proteins such as osteocalcin (OCN), bone sialoprotein (BSP) I/II, and collagen type I. Moreover, the osteoblasts undergo morphological changes, becoming large and cuboidal cells [13–17].

With the advent of electron microscopy, the structure of the osteoblast has become more

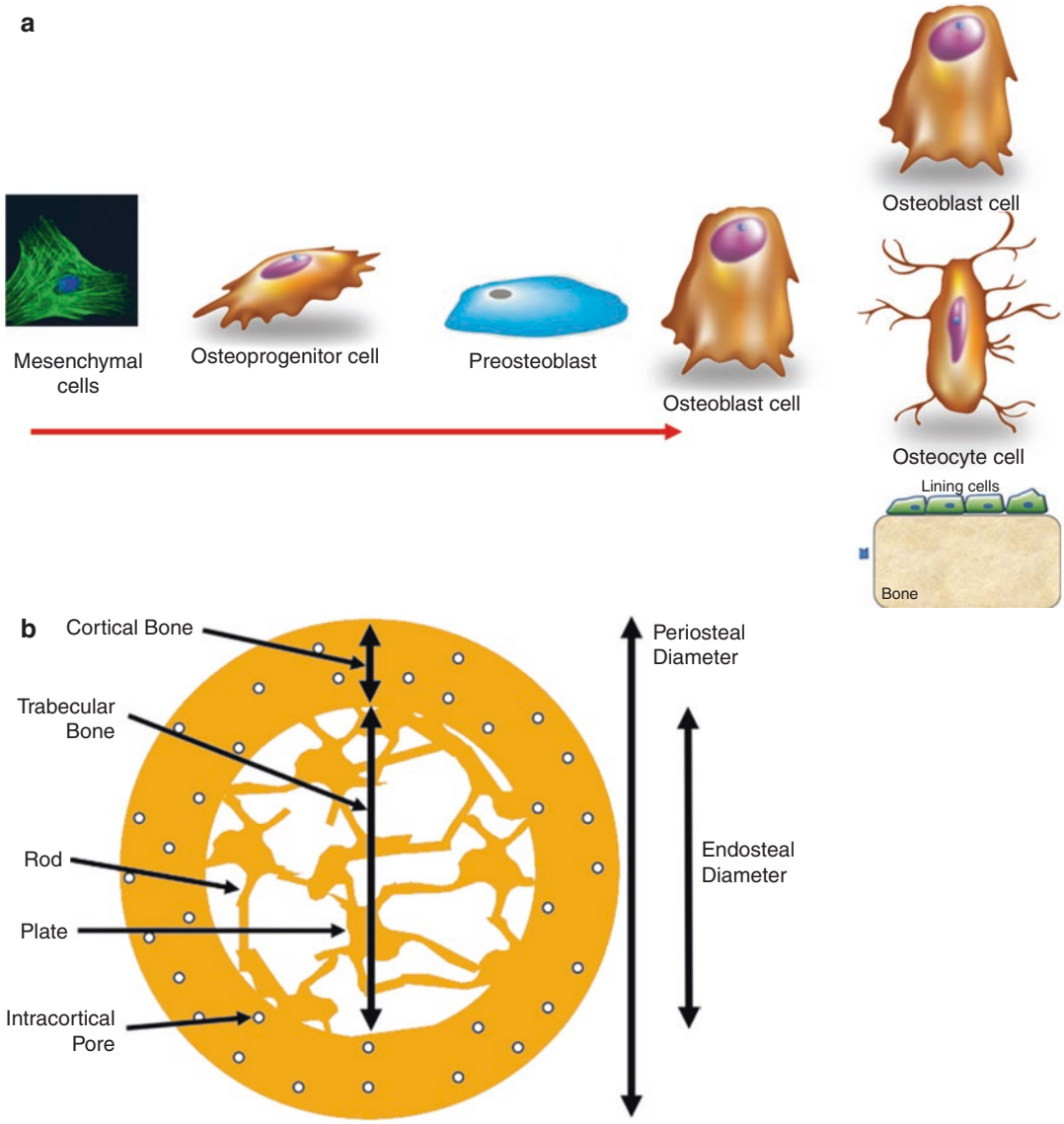


Fig. 1.1 (**a**) Development schema of mesenchymal cell differentiation into mature osteoblasts and its fate. Mesenchymal refers to cells which were deep within the embryo during early development; some of them remain in the bone marrow but do not form blood cells. (**b**) Structural characteristics of bone. Bone is comprised of a dense cortical shell that surrounds a spongy trabecular bone network. The periosteal diameter combined with the endosteal diameter determines cortical thickness. The size of bone along with cortical thickness and porosity significantly contribute to bone strength. The inner trabecular compartment contains a network of plates and rods that also contribute to bone strength. (Quoted under open access scheme from: Choksi et al. [286])

defined. These robust cells are tightly packed along the surface linings of bone. When active, osteoblasts are oval and contain large quantities of rough endoplasmic reticula (RER), mitochondria, and Golgi apparatus. Their single nucleus is found within the center of the cell. Other microscopic components found within these cells include mitochondria, microtubules, microfilaments, lysosomes, glycogen, and lipids. Functionally, the osteoblast is responsible for production of the organic matrix, which is composed of proteins and polysaccharides. Evidence

exists that osteoblasts, under the influence of parathyroid hormone and local cytokines, release mediators that activate osteoclasts [3].

Bone Lining Cells

Eventually, osteoblasts follow 1 of 3 pathways. These cells may (1) remain active osteoblasts, (2) become surrounded by matrix and become osteocytes, or (3) become relatively inactive and form bone lining cells. Bone lining cells are thin, elongated cells that cover most bone surfaces in the mature skeleton. Cytoplasmic extensions or gap junctions often link them to each other or to osteocytes. Because they are metabolically inactive, bone lining cells contain fewer organelles and less cytoplasm than osteoblasts. At times, they are referred to as "resting osteoblasts" or "surface osteocytes." [3–6].

Bone lining cells cover the bone surfaces, where neither bone resorption nor bone formation occurs [18]. The secretory activity of bone lining cells depends on the bone physiological status, whereby these cells can reacquire their secretory activity, enhancing their size and adopting a cuboidal appearance [19]. Several suggestions have been raised regarding the function of these cells. It has been shown that these cells prevent the direct interaction between osteoclasts and bone matrix, when bone resorption should not occur. They also participate in osteoclast differentiation, producing osteoprotegerin (OPG) and the receptor activator of nuclear factor kappa-B ligand (RANKL) [20]. Moreover, the bone lining cells, together with other bone cells, are an important component of the Bone Modeling Unit (BMU), an anatomical structure that is present during the bone remodeling cycle [21]. Buckwalter et al. [3] indicated that, in the presence of parathyroid hormone, these cells secrete enzymes that remove the osteoid covering of the bone matrix in preparation for osteoclastic removal of bone. Other authors [4, 6] reported that bone lining cells may be precursors for osteoblasts, regulate the crystal growth in bone, or function as a barrier between extracellular fluid and bone.

Osteocytes

It is estimated that osteocytes make up more than 90% of the bone cells in an adult skeleton. Osteocytes are derived from mesenchymal stem cells lineage through osteoblast differentiation. In this process, four recognizable stages have been proposed: osteoid-osteocyte, pre-osteocyte, young osteocyte, and mature osteocyte [22]. As immature osteocytes, recently surrounded in bone matrix, they closely resemble osteoblasts. Thus, the cytoplasm contains large amounts of rough endoplasmic reticula (RER) and large Golgi apparatus and mitochondria, with lesser amounts of microtubules, microfilaments, and lysosomes. As these cells mature and more matrix is laid down, osteocytes become located deeper within the bone tissue and eventually become smaller as they lose cytoplasm and get incorporated into the bone matrix. This process is accompanied by conspicuous morphological and ultrastructural changes, including the reduction of the round osteoblast size and the nucleus-to-cytoplasm ratio increases, which correspond to a decrease in the protein synthesis and secretion [23]. This accounts for the enlarged appearance of their nucleus. Furthermore, they are located within a space or lacuna and have long cytoplasmic processes that project through canaliculi within the matrix and facilitate the contact process among the adjacent cells. These connecting processes are thought to be extremely important in cellular communication and nutrition within a mineralized matrix [4–7]. Moreover, this important cellular network is thought to allow cell-mediated exchanges of minerals between the fluids in the bone and the vascular supply. It also is believed that the cellular network senses the mechanical deformation within bone that leads to the coordinated formation and resorption of bone [3].

Once the stage of mature osteocyte totally entrapped within mineralized bone matrix is accomplished, several of the previously expressed osteoblast markers such as OCN, BSPII, collagen type I, and ALP are downregulated. On the other hand, osteocyte markers including dentine matrix protein 1 (DMP1) and sclerostin are highly expressed [24–26]. While the osteocyte cell body

is located inside the lacuna, its cytoplasmic processes (up to 50 per each cell) cross tiny tunnels that originate from the lacuna space called canaliculi, forming the osteocyte lacuna-canalicular system [27] (Figs. 1.2). These cytoplasmic processes are connected, through gap junctions, to other neighboring osteocytes processes, as well as to cytoplasmic processes of osteoblasts and bone lining cells on the bone surface, facilitating the intercellular transport of small signaling molecules such as prostaglandins and nitric oxide among these cells [28]. In addition, the osteocyte lacuna-canalicular system is in close proximity to the vascular supply, whereby osteocytes have access to oxygen and nutrients [17].

It has been estimated that osteocyte surface is 400-fold larger than that of the all Haversian and Volkmann systems and more than 100-fold larger than the trabecular bone surface [29, 30]. The cell–cell communication is also achieved by interstitial fluid that flows between the osteocytes processes and canaliculi [30]. By the lacuna-canalicular system (Fig. 1.6), the osteocytes act as mechanosensors as their interconnected network has the capacity to detect mechanical pressures and loads, thereby helping the adaptation of bone to daily mechanical forces [31]. By this way, the osteocytes seem to act as orchestrators of bone remodeling, through regulation of osteoblast and osteoclast activities [32]. Moreover, osteocyte apoptosis has been recognized as a chemotactic signal to osteoclastic bone resorption [33, 35]. In agreement, it has been shown that during bone resorption, apoptotic osteocytes are engulfed by osteoclasts [36–38].

The mechanosensitive function of osteocytes (Fig. 1.3) is accomplished due to the strategic location of these cells within bone matrix. Thus, the shape and spatial arrangement of the osteocytes are in agreement with their sensing and signal transport functions, promoting the translation of mechanical stimuli into biochemical signals, a phenomenon that is called piezoelectric effect [39] (Fig. 1.7). The mechanisms and components by which osteocytes convert mechanical stimuli to biochemical signals are not well known. However, two mechanisms have been proposed. One of them is through a protein complex formed by a cilium, and its associated proteins PolyCystins 1 and 2, which has been suggested to be crucial for osteocyte mechanosensing and for osteoblast/ osteocyte-mediated bone formation [40]. The second mechanism involves osteocyte cytoskeleton components, including focal adhesion protein complex and its multiple actin-associated proteins such as paxillin, vinculin, talin, and zyxin [41]. Upon mechanical stimulation, osteocytes produce several secondary messengers, for example, ATP, nitric oxide (NO), Ca2+, and prostaglandins (PGE2 and PGI2,) which influence bone physiology [42]. Independently of the mechanism involved, it is important to mention that the mechanosensitive function of osteocytes is possible due to the intricate canalicular network, which allows the communication among bone cells.

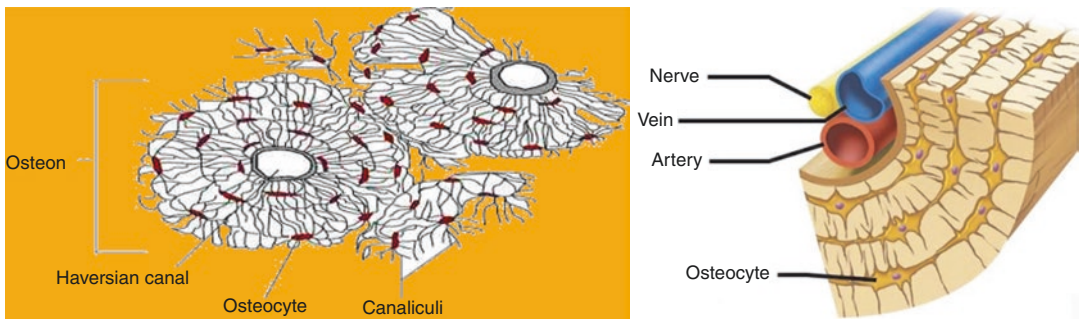


Fig. 1.2 The Haversian system. Bone can be thought of as a skyscraper with an elevator: The entire skyscraper is the osteon. The elevator of the building is like the Haversian Canal of the bone. Each floor of a building is like the Volkmann's Canal. Each office of the building represents an osteocyte

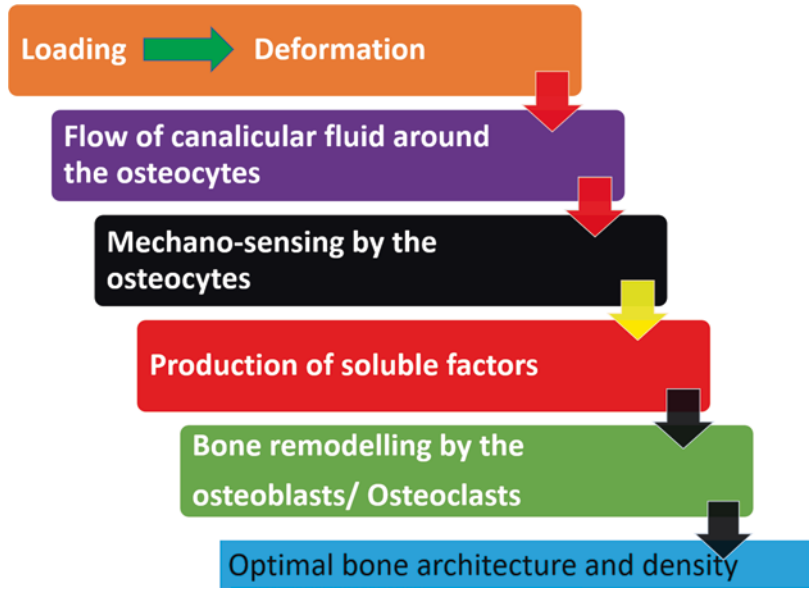


Fig. 1.3 The mechanosensitive function of osteocytes promoting the translation of mechanical stimuli into biochemical signals

Osteoclasts

Osteoclasts are terminally differentiated, multinucleated, giant cells that are responsible for bone resorption under both normal and pathological conditions, such as osteoporosis. Morphologically, osteoclasts tend to be much larger than other bone cells and are generally located on the surface of bones. They are known to be very mobile, moving from various sites and along the bone surface, and this motility is thought to account for the varied appearance of these cells [43]. In bone, osteoclasts are found in pits in the bone surface which are called resorption bays, or Howship's lacunae (Fig. 1.4).

Osteoclasts originate from mononuclear cells of the hematopoietic stem cell lineage, under the influence of several factors. Among these factors are the macrophage-colony stimulating factor (M-CSF), secreted by osteoprogenitor mesenchymal cells and osteoblasts [44]; and RANK ligand, secreted by osteoblasts, osteocytes, and stromal cells (Fig. 1.5) [45]. Together, these factors promote the activation of transcription factors [44, 46] and gene expression in osteoclasts [47, 48].

Macrophage-colony stimulating factor (M-CSF) binds to its receptor (cFMS) present in osteoclast precursors, which stimulates their proliferation and inhibits their apoptosis [46, 49]. RANKL is a crucial factor for osteoclastogenesis and is expressed by osteoblasts, osteocytes, and stromal cells. When it binds to its receptor RANK in osteoclast precursors, osteoclast formation is induced [50]. On the other hand, another factor called osteoprotegerin (OPG), which is produced by a wide range of cells including osteoblasts, stromal cells, and gingival and periodontal fibroblasts [51–53], binds to RANKL, preventing the RANK/RANKL interaction and, consequently, inhibiting the osteoclastogenesis [51] (Fig. 1.8). Thus, the RANKL/RANK/OPG system is a key mediator of osteoclastogenesis [50, 53].

Despite these osteoclastogenic factors having been well defined, it has recently been demonstrated that the osteoclastogenic potential may differ depending on the bone site considered. It has been reported that osteoclasts from long bone marrow are formed faster than in the jaw. This different dynamic of osteoclastogenesis possibly could be due to the cellular composition of the bone-site specific marrow [54].

Osteoclasts are characterized by having multiple nuclei, which average between 3 and 20, tend to be oval and concentrated mid-cell. There is less RER present than in osteoblasts, which is consistent with decreased production and secretion of proteins. Mitochondria are more numerous within osteoclasts than any other cell type within

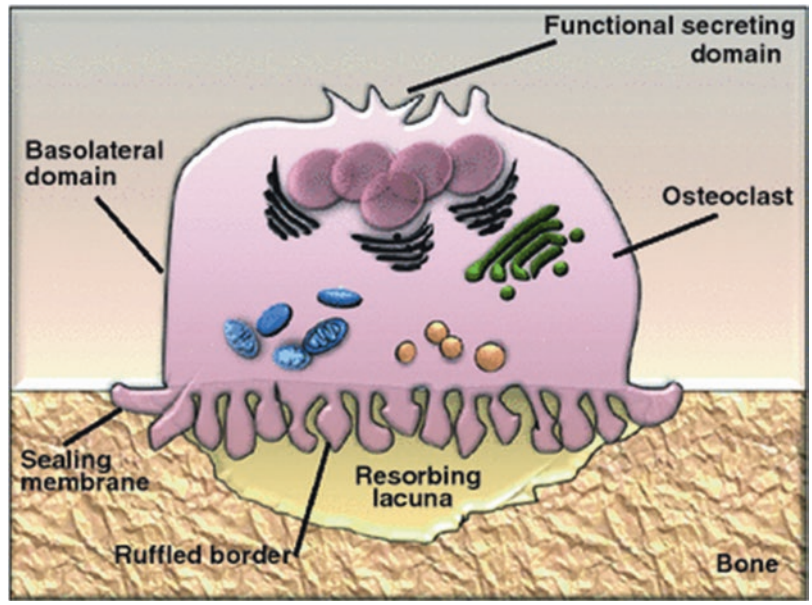


Fig. 1.4 Schema showing the resorption lacuna (Howship's lacuna): osteoclasts are found in pits in the bone surface which are called resorption bays, or Howship's lacunae

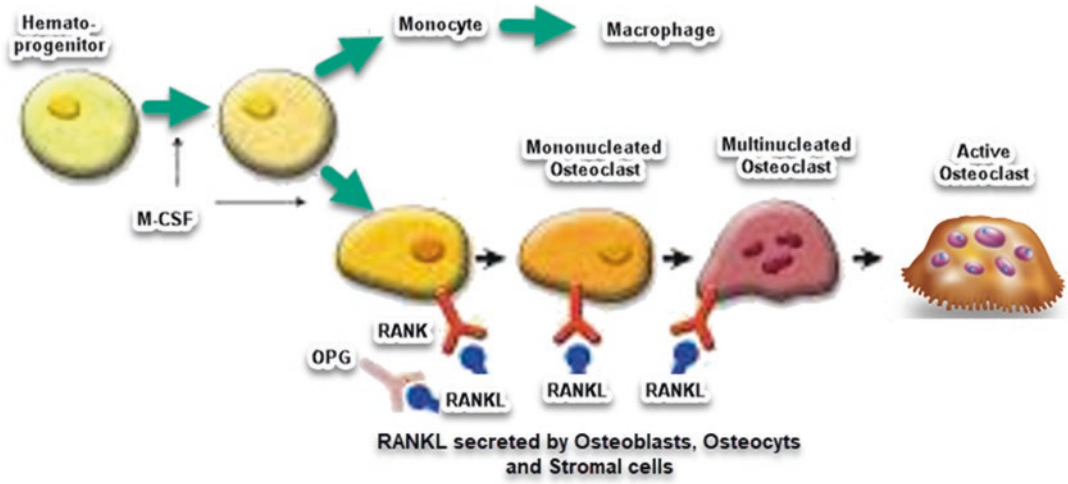


Fig. 1.5 Osteoclastogenesis: Development schema of hematopoietic precursor cell differentiation into mature osteoclasts. The hematopoietic cells form the liquid part of the bone marrow, and some of them circulate with the blood

the body. Between the nuclei are vesicles of Golgi material, which are relatively small in number. Many lysosomal types of vacuoles are present, leading to the common description of the cytoplasm as being "foamy." [55, 56]. The plasma membrane of the active osteoclast has an infolded appearance known as a ruffled border. The deep infolds of this border result in appendage-like projections of the cell that can wrap around bony prominences or lie along the surface. The large membrane surface area potentially permits extensive exchange between the intracellular and extracellular environments [3, 55].

During bone remodeling osteoclasts polarize; then, four types of osteoclast membrane domains can be observed: the sealing zone and ruffled border that are in contact with the bone matrix as well as the basolateral and functional secretory domains, which are not in contact with the bone matrix [57, 58]. These domains are only formed

when osteoclasts are in contact with extracellular mineralized matrix, in a process which $\alpha v\beta 3$-integrin, as well as the CD44, mediates the attachment of the osteoclast podosomes to the bone surface [59–62]. Ultrastructurally, the ruffled border is a membrane domain formed by microvilli, which is isolated from the surrounded tissue by the sealing zone, also known as clear zone. The sealing zone is an area devoid of organelles located in the periphery of the osteoclast adjacent to the bone matrix [61]. This sealing zone is formed by an actin ring as well as several other proteins [58]. The $\alpha v\beta 3$-integrin binds to noncollagenous bone matrix containing-RGD sequence such as bone sialoprotein, osteopontin, and vitronectin, establishing a peripheric sealing that delimits the central region, where the ruffled border is located [61].

The maintenance of the ruffled border is also essential for osteoclast activity; this structure is formed due to intense trafficking of lysosomal and endosomal components. In the ruffled border, there is a vacuolar-type H^+-ATPase (V-ATPase), which helps to acidify the resorption lacuna and hence to enable dissolution of hydroxyapatite crystals (Fig. 1.6) [45, 63, 64]. In this region, protons and enzymes, such as tartrate-resistant acid phosphatase (TRAP), cathepsin K, and matrix metalloproteinase-9 (MMP-9), are transported into a compartment called Howship lacuna leading to bone degradation [57, 64–67] (Fig. 1.3). The products of this degradation are then endocytosed across the ruffled border and transcytosed to the functional secretory domain at the plasma membrane [68].

Abnormal increase in osteoclast formation and activity leads to some bone diseases such as osteoporosis, where resorption exceeds formation causing decreased bone density and increased bone fractures [68]. In some pathologic conditions including bone metastases and inflammatory arthritis, abnormal osteoclast activation results in periarticular erosions and painful osteolytic lesions, respectively [47, 68, 69]. On the other hand, in osteopetrosis, which is a rare bone disease, genetic mutations that affect formation and resorption functions in osteoclasts lead to decreased bone resorption, resulting in a disproportionate accumulation of bone mass [70]. These diseases demonstrate the importance of the normal bone remodeling process for the maintenance of bone homeostasis.

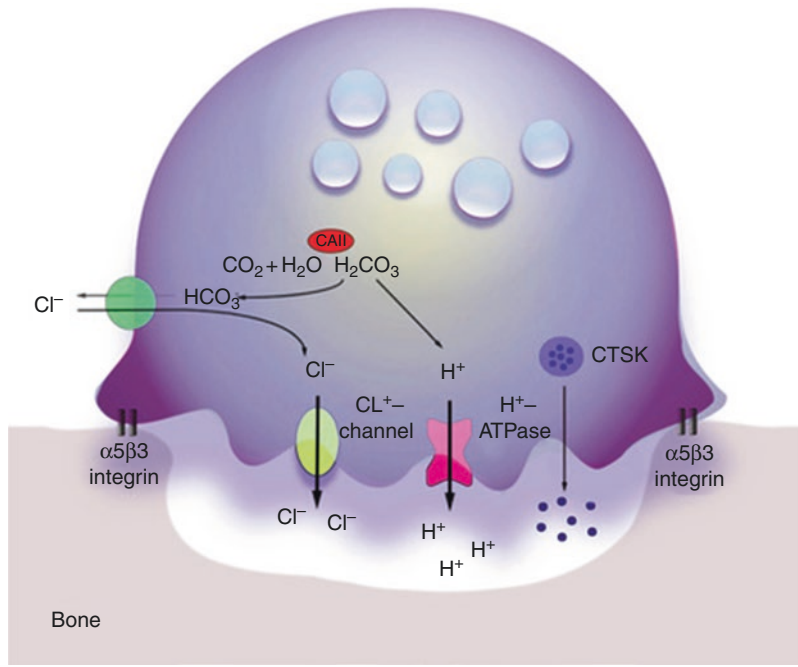


Fig. 1.6 Osteoclast bone resorption site: In the ruffled border, there is a vacuolar-type H + -ATPase (V-ATPase), which helps to acidify the resorption lacuna and hence to enable dissolution of hydroxyapatite crystals

Furthermore, there is evidence that osteoclasts display several other functions. For example, it has been shown that osteoclasts produce factors called clastokines that control osteoblast during the bone remodeling cycle. Furthermore, earlier studies revealed that osteoclasts may also directly regulate the hematopoietic stem cell niche [71]. These findings indicate that osteoclasts are not only bone resorbing cells but also a source of cytokines that influence the activity of other cells.

Bone Structure

Bone is a combination of osteoid matrix and hydroxyapatite [$Ca^{10}(PO4)^6(OH)^2$] crystal but bone also contains water, noncollagenous proteins, lipids, and specialized bone cells [72].

The type 1 collagen bone matrix gives bone elasticity, flexibility, and tensile strength. The collagen fibers are made up of three helical chains and combine together to form fibrils. Fibrils are then interwoven and bound by crosslinks [73]. Noncollagenous proteins, adsorbed from the serum, also make up the matrix. The role of such proteins is becoming increasingly clear and their major functions include strengthening the collagen structure and regulating its mineralization. Bone mineral, in the form of hydroxyapatite crystals, is an essential store of calcium and phosphate required for mineral homeostasis and provides the skeleton with mechanical rigidity and compressive strength. Recently, Nuclear Magnetic Resonance (NMR) spectroscopy has given new insights into the detailed composition of bone matrix and mineral [74].

Bones fulfill a protective and supportive role, but are also essential for locomotion; they are therefore required to be strong yet light. Consequently, bones are made up of two, structurally distinct, types– cortical and trabecular (cancellous) (Fig. 1.7). Cortical bone is solid with penetrating vascular canals and makes up the outer dense shell. It has an outer periosteal surface containing blood vessels, nerve endings, osteoblasts and osteoclasts and an inner, endosteal surface adjacent to the marrow [75]. On the endosteal surface of cortical bone is the honeycomb-like trabecular bone, which is made up of a fine network of connecting plates and rods [76].

The structural differences between cortical and trabecular bone underlie their diverse functions. The majority of the mature skeleton (80%)

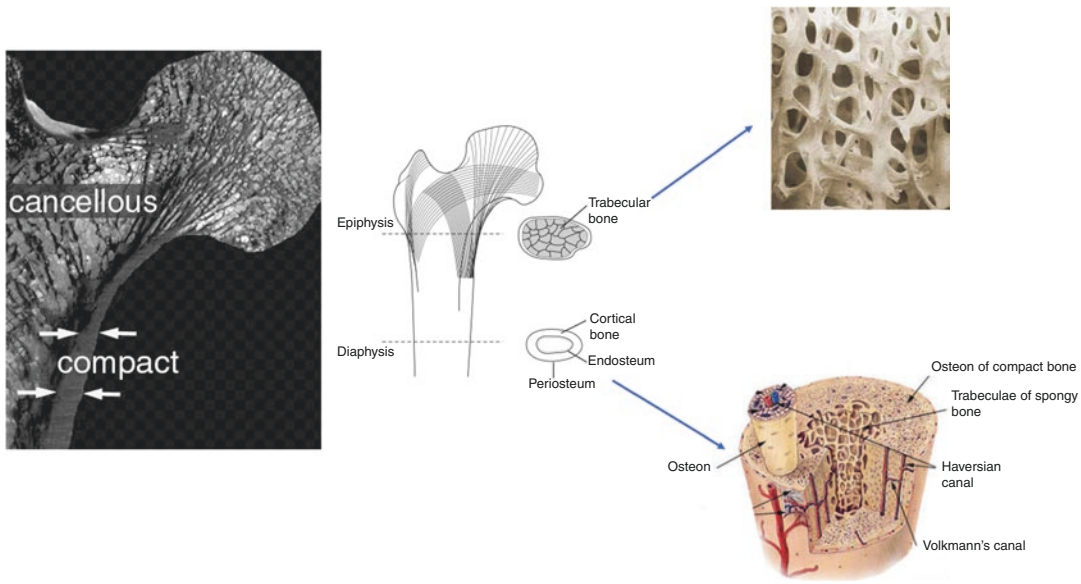


Fig. 1.7 Structural arrangement of cortical Bone and cancellous bone

is dense cortical bone that has a high torsional resistance and a lower rate of turnover. Nevertheless, it can release mineral in response to a significant or long-lasting deficiency. By contrast, trabecular bone, which is less dense, more elastic, has a higher turnover rate, and high resistance to compression makes up the rest of the skeleton. It serves to provide mechanical support, helping to maintain skeletal strength and integrity with its rods and plates aligned in a pattern that provides maximal strength. Trabecular bone has a large surface area for mineral exchange and is more metabolically active than cortical bone, rapidly liberating minerals in acute insufficiency [77]. Consequently, trabecular bone is also preferentially affected by osteoporosis [78].

The proportions of cortical and trabecular bone present are dependent on the individual bone's function. In vertebrae, trabecular bone predominates to resist compressive forces. By contrast, long bones, which principally act as levers, are mostly composed of cortical bone to allow them to resist both compressive and torsional forces [78, 79].

Although bone exhibits significant mechanical strength at a minimum weight, its biomechanical properties allow for significant flexibility without compromising this mechanical strength. Within these classifications, cortical and cancellous bone can consist of either woven (primary) or lamellar (secondary) bone. Comparison of cortical and cancellous bone demonstrates a similar matrix structure and composition, but vastly different masses, with cortical bone having a greater mass-to-volume ratio [3].

Cortical bone surrounds the marrow cavity and the trabecular plates of the cancellous bone. It accounts for 80% of the mature skeleton and forms the diaphysis, or shaft, of long bones. The metaphysis and epiphysis of long bones have thinner cortical walls, with the epiphysis forming a bulbous end surrounding the inner cancellous bone. Short bones (e.g. the tarsals and carpals), the vertebrae, skull, and pelvic bones also tend to have thinner cortical walls but contain a greater percentage of cancellous bone compared with long bones [17].

The differences in mechanical properties between cortical and cancellous bone are due to the differences in architecture, even though the composition and materials are the same. The thick, dense arrangement of the diaphysis of long bones allows cortical bone to have a much higher resistance to torsional and bending forces, whereas cancellous bone provides greater resilience and shock absorption, such as in the epiphyseal region of long bones. Cancellous bone generally has a higher metabolic rate and appears to respond quicker to changes in mechanical loading and unloading, such as seen with prolonged immobilization. This may be due, in part, to the greater exposure of bone cells within cancellous bone to the adjacent bone marrow cells and vascular supply, whereas cells within cortical bone tend to be embedded deeper within the bone matrix [3].

Woven and lamellar bone are the terms based on the microscopic differentiation of the bone. Lamellar bone represents the main type of bone in a mature skeleton. Woven bone is composed of loosely and randomly arranged collagen bundles containing numerous osteocytes which lie in lacunar that vary in size and shape, whereas lamellar bone is characterized by an orderly arrangement of collagen bundles and their cells. Lamellar bone is secondary bone created by remodeling of woven bone. Cortical and cancellous bone can be made up of either woven or lamellar bone. Woven bone, sometimes referred to as primary bone, is seen in embryonic bone that is later resorbed and replaced by lamellar, or secondary, bone by 4 to 5 years of age. Woven bone, however, also is seen during the initial stages of fracture healing, within cranial sutures, ear ossicles, and epiphyseal plates. Exemplified by the relatively quick turnover rate during deposition and resorption, woven bone has a greater rate of metabolic activity compared with lamellar bone. Due to its composition, woven bone has a scattered, irregular appearance, whereas lamellar bone has a very orderly arrangement [17].

Histologically, the osteocytes seen in woven bone also are more randomly scattered than those in lamellar bone, where the osteocytes are uniform in size and shape and are oriented in line

with the other cells and structures within the bone [80]. When lamellar bone is viewed microscopically in cross-section, the organization of the layers appears in parallel units or sheets with densely packed collagen fibrils. Concentric rings of lamellae form osteons, which are also known as haversian systems. Osteons surround central canals (haversian canals), which contain blood, lymph vessels, and, occasionally, nerves. Between the central canals and the surrounding cells are the cell processes of osteocytes, which travel within tunnel-like structures known as canaliculi. They extend out in a radial manner between the central canals and surrounding osteocytes (Fig. 1.4). This allows for diffusion of nutrients in a system that is surrounded by a hard, mineralized matrix. The central canals also branch and anastomose with obliquely oriented vascular branches known as Volkmann canals. These structures allow for extended communication from the periosteum to the endosteum [81].

Primary osteons undergo resorption and new osteons form, leaving behind boundaries known as cement lines. The constant resorption and deposition of new bone is the basis for the dynamic process of bone turnover. Histologically, it is possible to see areas within a cross-section of bone where remnants of primary osteons exist along with secondary osteons [81, 82].

The complex and dynamic network of lacunae and canals within bony tissue form an extravascular space where, adjacent to a mineralized matrix, fluids and ions can flow relatively unrestricted, and mechanical bone deformations can be converted to electrical signals and transmitted to other areas of the tissue. Some authors [83, 84] have hypothesized the role of electrical signals in the regulation of bone function based on this interdependent network.

Cells Gaps

The normal development and maintenance of skeletal tissue is dependent on the tightly coordinated activity of osteoblasts, osteoclasts, and osteocytes. This coordination balances the bone forming function of the osteoblasts, the bone resorption led by the osteoclasts, and the osteocytes which seem to coordinate the activation of these two cell types. In order for the bone embedded osteocytes (Fig. 1.8) to control and facilitate the bone formation and resorption on the bone surfaces, there is an obvious need for these cells

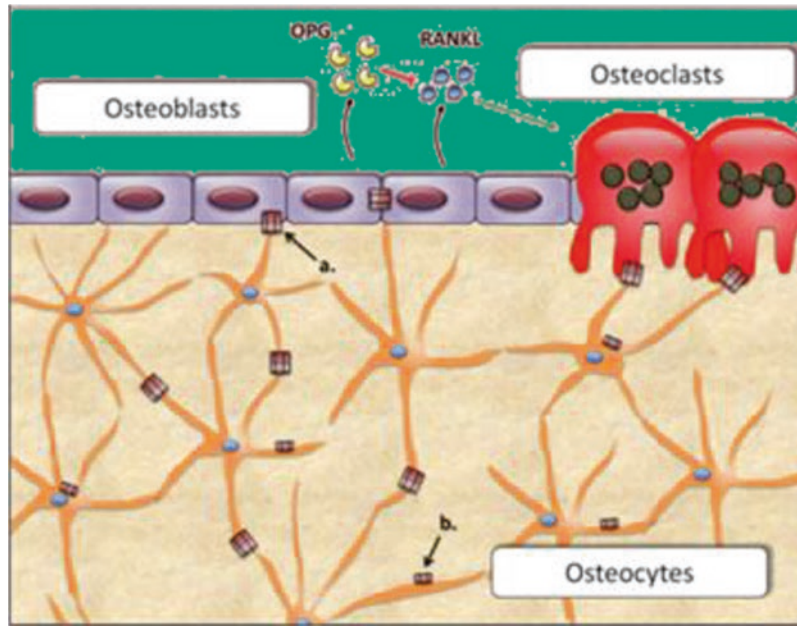
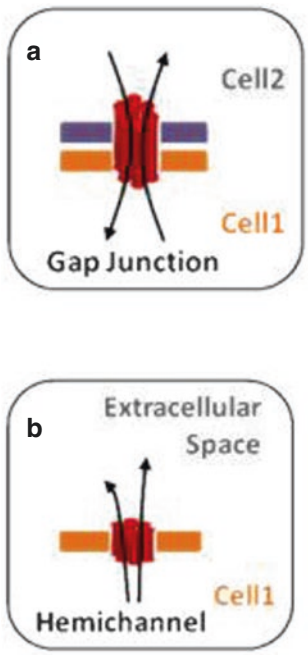


Fig. 1.8 Illustration of osteocytes embedded in bone. Long dendritic-like processes, enable contact between osteocytes and surface osteoblasts

to signal over a substantial distance, impeded by the presence of a mineralized matrix. This is accomplished both by the release of soluble signals (e.g., RANKL, osteoprotegerin and sclerostin) and by direct cell-to-cell communication through gap junctions. Osteocytes have an extensive network of long, dendritic-like cell processes that extend through the bone canaliculi, where they physically interconnect with adjacent osteocytes and with osteogenic cells on the bone surface via connexin-containing gap junctions [85] .

Gap junctional communication has been hypothesized to play a critical role in the coordination of bone remodeling. Osteoblasts and osteocytes have been shown to express three major gap junction proteins, connexin43 (Cx43), connexin45 (Cx45), and connexin46 (Cx46). Likewise, surface osteoblasts, osteoprogenitors, and bone lining cells express Cx43 and form functional gap junctions among each other as with osteocytes. Chondrocytes, the cells that form cartilage, have also been shown to express Cx43; as do the bone resorbing osteoclasts. Gap junctions are aqueous conduits that are formed by the docking of two hemichannels on juxtaposed cells (Fig. 1.9). They permit diffusion of ions, metabolites, and small signaling molecules (e.g., cyclic nucleotides and inositol derivatives). The result is a functional syncytium of interconnected cells throughout bone that acts in concert to orchestrate the formation and turnover of bone [86]. In addition to classic gap junctional intercellular communication, unopposed gap junction hemichannels exist at the membrane, where they function as direct conduits between the cytosol and extracellular milieu [87].

Depending upon the expressed connexin genes, the resultant gap junction channels will exhibit specific charge and size permeability. For example, Cx43 permits the diffusion of relatively large signal molecules <1.2 kDa molecular mass, with a preference for negatively charged molecules.



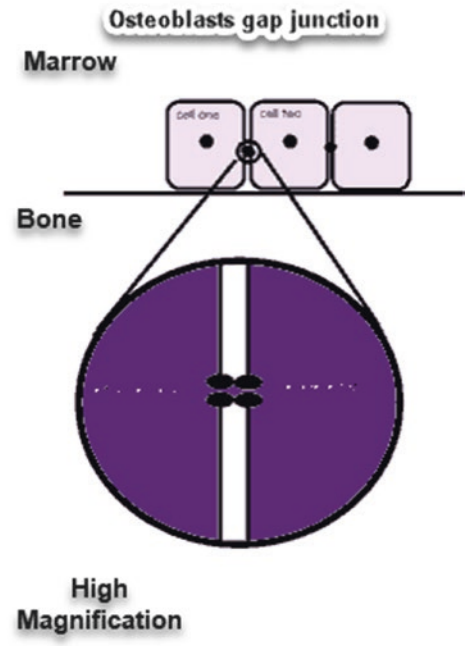


Fig. 1.9 Gap junctions with neighboring osteoblasts allow cells to communicate with each other or to extracellular space. Cx43 containing gap junctions form between the osteocytes and osteoblasts, (**a**) which allows the exchange of molecules between the cells. Osteocytes are also known to express gap junction hemichannels (**b**), that allow for the release of factors into the extracellular space. The regulation of bone resorption by osteoclasts is mediated by osteoblast/osteocyte produced RankL and OPG. The balance of these factors in the control of osteoclast formation is a target of Cx43

Inositol derivatives [88–93] and cADP-ribose [89, 94] are capable of diffusion through gap junctions and can elicit a $Ca2^{+}$ response in coupled cells. In contrast, Cx45 forms a smaller pore, permitting diffusion of molecules <0.3 kDa, with a preference for positively charged molecules. Interestingly, connexins can be present as a homomeric or heteromeric hemichannel, and the connexin isotypes that forms the gap junction hemichannels dictate the molecular size and permeability of the resulting gap junction channel [95–99]. For example, Cx43 and Cx45 are two such connexins that can assemble into a single hemichannel composed of both monomeric units. In the resultant Cx43/Cx45 heteromeric channel, the biochemical properties of Cx45 dominate and chemical and electrical coupling among cells is markedly reduced [95, 100, 101]. In addition, some connexin (hemichannel) pairs can form heterotypic interactions dependent upon the compatibility of the extracellular loops of the opposing hemichannels (e.g., one cell expressing monomeric Cx43 hemichannels may dock with an adjacent cell expressing monomeric Cx45 hemichannels).

These properties provide the gap junction great plasticity in dictating the size permeability and selectivity of the resultant communicative channel, restricting or allowing signaling only to coupled cells. Further, gap junction channels are regulated in a similar fashion as other membrane channels, with open/closed states sensitive to transmembrane voltage and posttranslational modification of the connexin subunits. Activation of extracellular signal regulated kinase (ERK) and protein kinase C has been shown to dynamically regulate Cx43 channel open/ closed state by phosphorylation of the C-terminal tail of the connexin monomers [102–104].

Accumulating evidence from many model systems consistently suggests that the unique profile of connexins expressed by a particular cell type can dictate the types of signals, second messengers, and metabolites that are propagated among cells. In this way, the cells can form a "functional syncytium" within which the cells communicate, with the advantage that the type of signals that can be diffused can be regulated. Thus, not all cells in the network share every signal; while some signals that diffuse through the gap junctions are rapidly distributed, propagation of others may be limited to serve specific functions [86].

Gap Junctions and Skeletal Development

The involvement of Cx43 in the processes that control bone cell function and ultimately bone quality is conspicuously complex, with differential responses based on the context of the effect. For example, loss of Cx43 differentially modulates the response of bone cells on the periosteal and endosteal surface of bone in response to mechanical loading [105]. Somewhat paradoxically, loss of Cx43 reduces the anabolic effect of mechanical load and yet also blunts the effects of mechanical unloading or perhaps even aging induced bone loss [106, 107]. This implies that Cx43 transmits signals that can be either osteoanabolic or osteo-catabolic, depending on the context such as aging, mechanical loading or unloading, or even location (i.e., differential effects on the periosteal and endosteal surfaces of bone) [108]. This complexity underscores the need to understand the specific details of how Cx43 affects bone cells and bone remodeling and raises several important questions. What are the second messengers and effectors of the osteoanabolic effects of Cx43 on bone? How do these differ from the effectors of the osteo-catabolic actions? Can we selectively regulate the ability to communicate and/or respond to some signals passed through gap junctions but not others? Understanding the molecular mechanisms by which Cx43 can modulate bone cell function in a context-dependent manner is critical to the development of treatments that modulate these connexin-regulated pathways to enhance or maintain bone quality.

Bone Remodeling

While the skeleton may seem an inert structure, in fact, it is a dynamic organ, comprised of tissue and cells in a continual state of activity through-

out a lifetime. The skeleton regulates its own maintenance and repair by remodeling. This process also provides a mechanism for rapid access to calcium and phosphate to maintain mineral homeostasis [109, 110]. Bone remodeling was recently reviewed by Kendre and Basset (2018) [110].

First defined by Frost, the bone remodeling cycle is a tightly regulated process that replaces old and damaged bone with new [111]. Anatomically, the cycle takes place within a Basic Multicellular Unit (BMU), which is composed of osteoclasts, osteoblasts, and a capillary blood supply [112]. The BMU lasts longer than the lifespan of the osteoblasts and osteoclasts within it and so requires constant replenishment of these cells, and is critically controlled by the osteocyte. The structure and composition of the BMU vary depending on whether it is located within trabecular or cortical bone. In trabecular bone, the BMU is located on the surface such that a "trench" of bone, called Howship's lacunae, is resorbed and then refilled. By contrast, in cortical bone, the osteoclasts within the BMU form a cutting cone that "tunnels" into the cortex (osteoclastic tunneling), removing damaged bone. Behind the cutting cone, new bone is then laid down concentrically on the tunnel walls by differentiated osteoblasts to leave a vascular supply within the Haversian canal of the new osteon [113]. In both instances, the BMU is covered by a canopy of cells which delineate the bone remodeling compartment (BRC).

The Bone Remodeling Compartment

Although macroscopically the skeleton seems to be a static organ, it is an extremely dynamic tissue at the microscopic level. Its ability to sustain the tremendous loads placed on it in everyday life depend on, among other factors, being able to remodel and repair the constant microcracks that develop both in cancellous bone — the "spongy" bone present in the vertebrae, pelvis, and ends (metaphyses) of long bones — and in cortical bone — the compact bone present in the shafts (diaphyses) of the long bones and surrounding cancellous bone in the vertebrae and pelvis. Since remodeling sites in cancellous bone in the vertebrae and pelvis are close to red marrow, which is known to contain osteoprogenitor cells (4), whereas remodeling sites in cortical bone are distant from red marrow, it had been assumed that the mechanisms of bone remodeling were likely to be different in cancellous versus cortical bone. Specifically, the assumption was that the cells needed for bone remodeling traveled directly from the red marrow to bone surfaces in cancellous bone, whereas they accessed cortical bone via the vasculature. However, it now seems that the fundamental mechanisms of bone remodeling might be very similar in both bone compartments, occurring in what has been termed the basic multicellular unit (BMU), which comprises the osteoclasts, osteoblasts, and osteocytes within the bone-remodeling cavity. Although the existence of the BMU has been established for a long time, the intimate relationship between the BMU and the vasculature, particularly in cancellous bone, was less well appreciated. This intimate relationship was initially described by Burkhardt et al. [114] more than 20 years ago and analyzed in detail in subsequent studies by Hauge and colleagues [115]. These investigators demonstrated that the cells in the BMU, even in cancellous bone, were not directly contiguous to the bone marrow, but rather they were covered by a "canopy" of cells (most probably bone-lining cells) that seem to be connected to bone-lining cells on the quiescent bone surface. In turn, these bone-lining cells on the quiescent bone surface are in communication with osteocytes embedded within the bone matrix. Penetrating the canopy of bone-lining cells, and presumably serving as a conduit for the cells needed in the BMU, are capillaries. Hauge et al. [115] introduced a new concept where he placed the BMU (consisting of osteoclasts, osteoblasts, and osteocytes), both in cancellous and in cortical bone, within the bone remodeling compartment (BRC), which comprises the BMU, the canopy of bone-lining cells, and the associated capillaries.

Therefore, the bone remodeling compartment (BRC) provides a defined area of remodeling

with close anatomical coupling of osteoclasts and osteoblasts [116, 117]. Hauge et al. [115] demonstrated that the cells in the BRC, are covered by a "canopy" of cells forming the outer lining of a specialized vascular structure with the denuded bone surface as the other delineation (Fig. 1.10). The cells of this canopy display all classical markers of the osteoblastic phenotype, and are therefore most probably bone-lining cells, which seem to be connected to bone-lining cells on the quiescent bone surface. The structure has been demonstrated in cortical as well as trabecular bones. In turn, these bone-lining cells on the quiescent bone surface are in communication with osteocytes embedded within the bone matrix. Penetrating the canopy of bone-lining cells, and presumably serving as a conduit for the cells needed in the BRC, are capillaries.

Cells may enter the remodeling space either via diapedesis through the lining cell dome covering the BRC or via the circulation. It is still debatable whether all cells involved in remodeling arrive via the circulation. Circulating osteoclast precursors have been demonstrated several years ago, there is a growing evidence that osteoblast lineage cells are also present in the circulation strengthening the involvement of circulating precursor cells in the process [118, 119].

The BRC is the most probable structure at which coupling between osteoclasts and osteo-

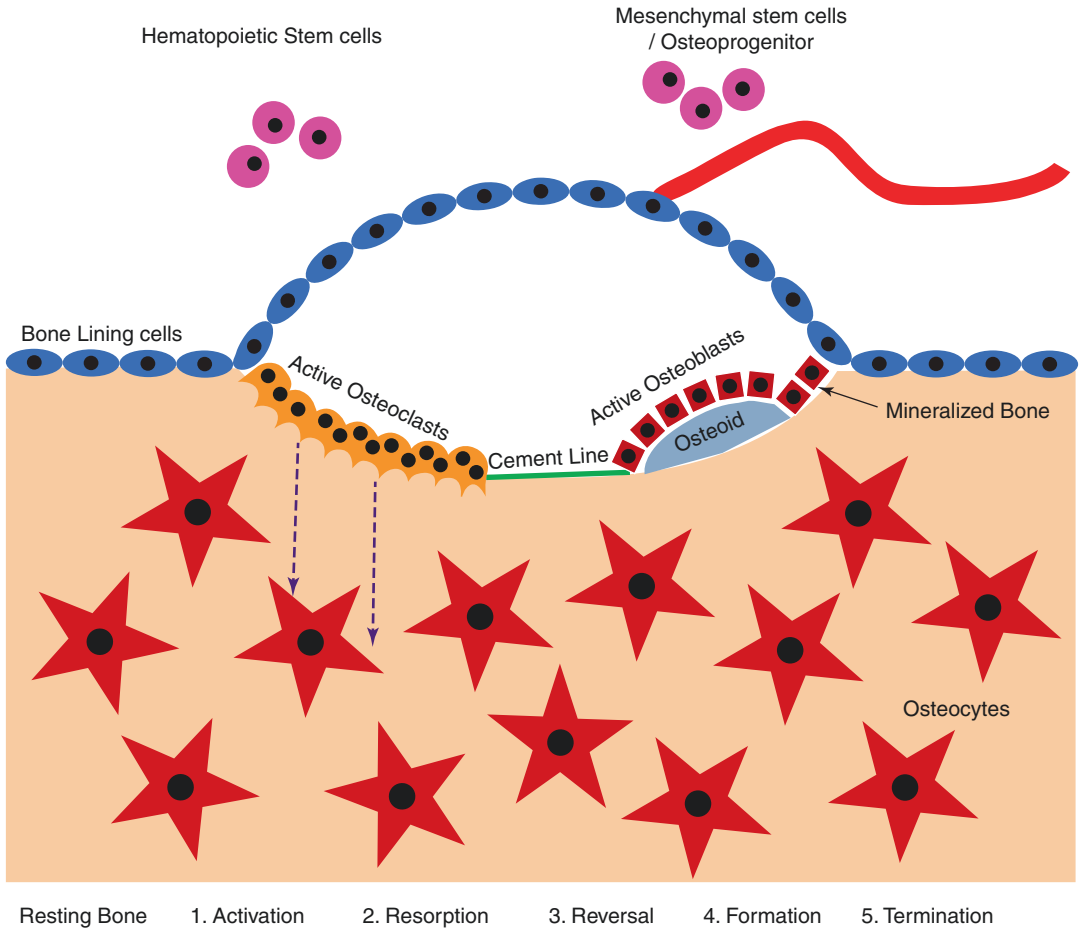


Fig. 1.10 The bone remodeling compartment (BRC) at different phases of the bone remodeling cycle. Schematic diagram of the bone remodeling cycle illustrating the phases of: activation, resorption, reversal, formation and termination. Hemopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). (Quoted with permission from Kendre and Bassett [110])

blasts occurs. It also obviates the need for a "postal code" system ensuring that resorptive and formative cells adhere to areas on the bone surface, where they are needed. Bone surfaces are generally covered by lining cells, which would prevent direct contact between bone cells and integrins or other adhesion molecules known to modulate cell activity. The BRC would be the only place where circulating osteoclasts as well as circulating osteoblast precursors would be in contact with these matrix constituents, because the formation of the BRC involves detachment of lining cells from the bone surface [117].

The Remodeling Cycle – Cellular and Molecular Mechanisms

The remodeling cycle occurs in a highly regulated and stereotyped fashion with five overlapping steps of activation, resorption, reversal, formation, and termination occurring over the course of 120–200 days in cortical and trabecular bone, respectively [120]. The remodeling cycle can be as short as 100 days in thyrotoxicosis and primary hyperparathyroidism and exceed 1000 days in low turnover states like myxedema and after bisphosphonate treatment [121]. Osteocytes orchestrate the bone remodeling by regulating osteoclast and osteoblast differentiation and consequently bone resorption and formation.

Activation

The first stage of bone remodeling involves detection of an initiating remodeling signal. This signal can take several forms, e.g. direct mechanical strain on the bone that results in structural damage or hormone (e.g. estrogen or parathyroid hormone [PTH]) action on bone cells in response to more systemic changes in homeostasis.

Daily activity places ongoing mechanical strain on the skeleton, and it is thought that osteocytes sense changes in these physical forces and translate them into biological signals that initiate bone remodeling (Fig. 1.11) [122]. Damage to the bone matrix [123] or limb immobilization [72] results in osteocyte apoptosis and increased osteoclastogenesis. Under basal conditions, osteocytes secrete transforming growth factor β (TGF-β), which inhibits osteoclastogenesis. Focal osteocyte apoptosis lowers local TGF-β levels, removing the inhibitory osteoclastogenesis signals and allowing osteoclast formation to proceed [73].

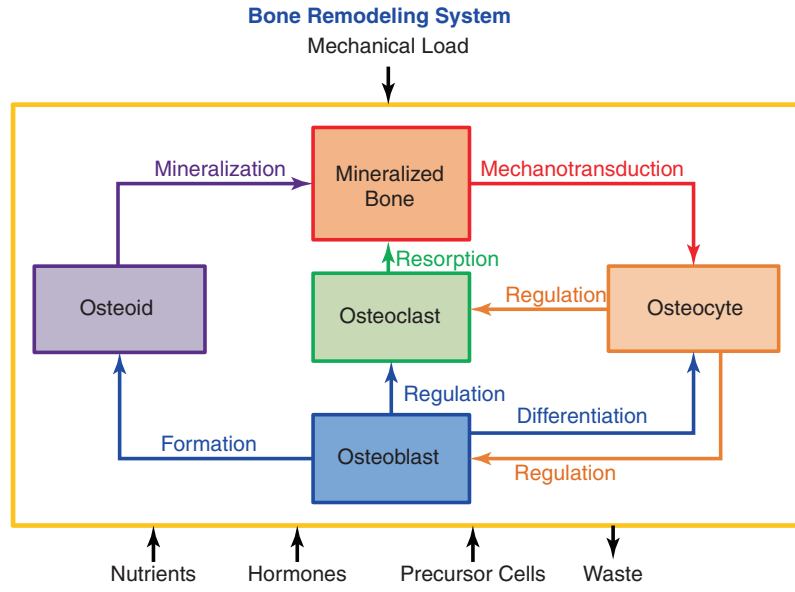


Fig. 1.11 Bone remodeling system in response to mechanical stimuli. In addition to the local factors, other systemic factors play a role in the remodeling process

Osteoclast precursor cells are recruited from the circulation and activated; the bone surface is exposed as the lining cells separate from underlying bone and form a raised canopy over the site to be resorbed [116]. Multiple mononuclear cells fuse to form multinucleated preosteoclasts which bind to the bone matrix to form sealing zones around bone-resorbing compartments, thus isolating the resorption pit from surrounding bone. Initiation of bone remodeling is the first important step ensuring that, in health, remodeling only takes place when it is required. In "*targeted remodeling,*" which refers to removal of a specific area of damaged or old bone, the initiating signal originates from the osteocytes that use their extensive network of dendritic processes to signal to other cells [109, 124–127]. Osteocyte apoptosis, induced for example by the disruption of osteocyte canaliculi caused by bone matrix microdamage, leads to release of paracrine factors that increase local angiogenesis and recruitment of osteoclast and osteoblast precursors [128–130]. In contrast, "*nontargeted remodeling*" refers to remodeling in response to systemic changes in hormones such as parathyroid hormone (PTH), thus allowing access to bone calcium stores and is not directed towards a specific site.

Resorption (Approximately Two Weeks in Duration)

Differentiation and activation of osteoclasts are also regulated by osteocytes. Rearrangement of the osteoclast cytoskeleton results in adherence to the bone surface, formation of a sealing zone and generation of a ruffled border that provides a greatly enhanced secretory surface area. Initially, osteoclasts pump protons, generated by Carbonic Anhydrase II, into the resorbing compartment to dissolve the bone mineral. Specifically, the H^+-ATPase pumps H^+ into resorption lacunae; this is coupled to Cl^- transported via a chloride channel thus maintaining electroneutrality [131]. Subsequently, the collagen-rich bone matrix is degraded by proteases such as cathepsin K and matrix metalloproteinases [132, 133]. The resorption phase is terminated by osteoclasts programmed cell death, ensuring that excess resorption does not occur (Fig. 1.12) [134].

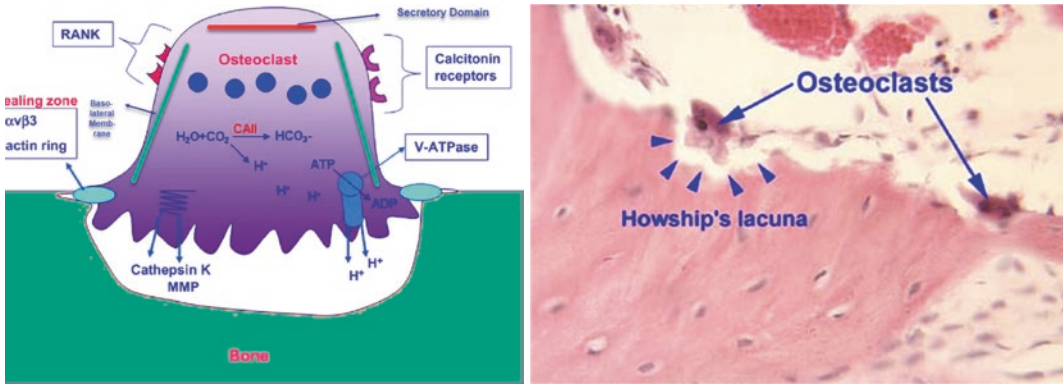


Fig. 1.12 Rearrangement of the osteoclast cytoskeleton results in adherence to the bone surface, formation of a sealing zone and generation of a ruffled border that provides a greatly enhanced secretory surface area. Consequently, four types of osteoclast membrane domains are observed: the sealing zone and ruffled border that are in contact with the bone matrix as well as the basolateral and functional secretory domains, which are not in contact with the bone matrix. In the ruffled border, there is a vacuolar-type H + -ATPase (V-ATPase), which helps to acidify the resorption lacuna and hence to enable dissolution of hydroxyapatite crystals. In this region, protons and enzymes, such as tartrate-resistant acid phosphatase (TRAP), cathepsin K, and matrix metalloproteinase-9 (MMP-9) are transported into a compartment called Howship lacuna leading to bone degradation. The products of this degradation are then endocytosed across the ruffled border and transcytosed to the functional secretory domain at the plasma membrane

Reversal (Approximately Four to Five Weeks in Duration)

The reversal phase, where bone resorption switches to formation. There are two key events occurring. Firstly, the freshly resorbed bone surface is prepared for deposition of new bone matrix and further signaling occurs that couples resorption to formation, ensuring that there is no net bone loss [135, 136]. Preparation of the bone surface is carried out by cells of an osteoblastic lineage which remove unmineralized collagen matrix, and a noncollagenous mineralized matrix "cement-line" is then deposited to enhance osteoblastic adherence [137].

The exact signal that couples bone resorption to subsequent formation is not yet fully understood. However, it is likely that the cells of the reversal phase are involved in sending or receiving these signals [138–140]. It has been postulated that osteoclasts may be the source of the coupling factor, either secreting cytokines such as interleukin 6 (IL-6), or via a regulatory receptor on their surface such as the Ephrin receptor family and their membrane bound ligand, Ephrins, present on osteoblasts [141]. Other signaling pathways may include matrix-derived factors such as BMP-2, transforming growth factor ßb and insulin-like growth factor [142, 143].

Formation (Approximately Four Months in Duration)

New bone formation can be divided into two parts. Firstly, osteoblasts synthesize and secrete a type-1 collagen-rich osteoid matrix. Secondly, osteoblasts play a part in regulating osteoid mineralization [125, 144].

The process of bone mineralization, whereby hydroxyapatite crystals are deposited among collagen fibrils, is complex and its regulation is incompletely understood. Control is exerted by systemic regulation of calcium and phosphate concentrations, local concentration of calcium and phosphate within extracellular matrix vesicles and by local inhibitors of mineralization, including pyrophosphate and noncollagenous proteins such as osteopontin. The ratio of inorganic pyrophosphate to phosphate is a critical regulator of mineralization, and the relative activities of tissue nonspecific alkaline phosphatase and ectonucleotide pyrophosphatase are the key determinants of this ratio [145–147].

Termination

Once mineralization is complete, osteoblasts undergo apoptosis, change into bone-lining cells or become entombed within the bone matrix and terminally differentiate into osteocytes. Osteocytes play a key role in signaling the end of remodeling via secretion of antagonists to osteogenesis, specifically antagonists of the Wnt signaling pathway such as SOST [76].

The Remodeling Cycle – Major Signaling Pathways

The remodeling cycle is tightly regulated to achieve balanced resorption and formation. While systemically released factors play a regulatory role, the fact that remodeling occurs at multiple, anatomically distinct sites at the same time indicates that local regulation is critical to achieving this fine balance. Accordingly, two key pathways, RANKL/RANK/OPG and Wnt, transduce systemically and locally produced signals. Their regulatory role in determining the balance and timing of bone resorption and formation within the remodeling cycle makes them potentially important targets for pharmacological interventions in disease states such as osteoporosis.

Receptor Activator of Nuclear Factor Kappa-B Ligand Signaling Pathway (RANKL/RANK/OPG Signaling)

Identification of the receptor activator of Nf-κb ligand (RANKL/RANK/OPG) Signaling Pathway in the 1990s was a crucial breakthrough in understanding the regulation of osteoclasto-

genesis in the remodeling cycle and provided the pharmacological target for the novel anti-resorptive denosumab [148].

A permissive concentration of macrophage-colony stimulating factor (M-CSF), which is expressed by osteocytes and osteoblasts and stimulates RANK expression, is required prior to the action of RANKL [149, 150].

RANKL binding to its receptor, RANK, on osteoclastic precursor cells, drives further osteoclast differentiation and facilitates fusion, activation, and survival. RANKL/RANK binding induces downstream signaling molecules including mitogen-activated protein kinase, tumor necrosis factor (TNF)-receptor-associated factor 6, NF-κB, and c-fos and ultimately activation of key transcription factors, including nuclear factor-activated T cell cytoplasmic 1 (NFATc1), a master transcription factor of osteoclast differentiation as it regulates the expression of osteoclast genes [151–154].

While RANKL can be produced by osteoblasts, osteocytes, and chondrocytes, it is the osteocytes, within the bone matrix, able to sense changes in load and microdamage that are thought to stimulate osteoclastogenesis via production of RANKL at the initiation of the bone remodeling cycle [155, 156].

Osteoprotegerin (OPG), a decoy receptor for RANKL, was identified prior to the discovery of RANK/RANKL. It is secreted by osteoblasts and osteocytes and is able to inhibit osteoclastic bone resorption by binding to RANKL and preventing its binding to RANK [156, 157]. Thus, the RANKL:OPG ratio is key in the regulation of bone resorption, bone mass, and skeletal integrity and is modulated by a number of systemic factors; RANKL expression is induced by bone-resorbing factors such as 1α,25-dihydroxy vitamin D3, interleukin 6, and parathyroid hormone (Fig. 1.13).

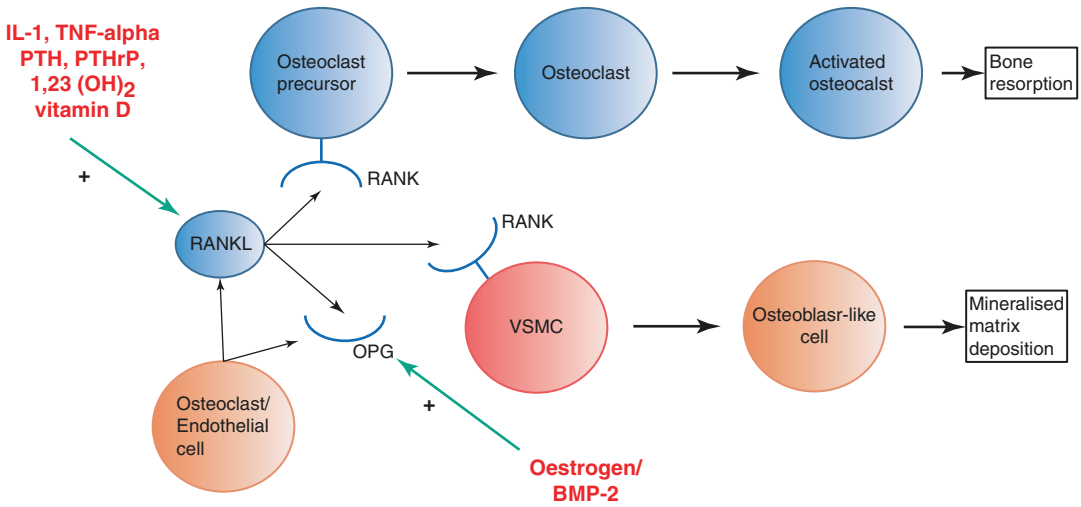


Fig. 1.13 Simplified diagram showing potential RANK/RANKL/OPG involvement in bone remodeling and in vascular calcification. Receptor activator of nuclear factor kappa-B ligand (RANKL) from osteoblasts or endothelial cells binds to the Receptor Activator of Nuclear Factor kappa-B (RANK) of osteoclast precursors, or vascular smooth muscle cells (VSMCs). This leads to differentiation into mature osteoclasts in the bone, which are involved in bone resorption, whereas in vascular calcification, VSMCs undergo a phenotypic transition into osteochondrogenic cells that can deposit mineralized matrix. Osteoprotegerin (OPG) is the decoy receptor for RANKL, and a potential inhibitor for mineralization. Factors affecting the RANK/RANKL/OPG signalling pathway. Oestrogen and Bone morphogenic Protein-2 (BMP-2) induce osteoprotegerin (OPG) expression whereas 1,25(OH)2 Vitamin D3, PTH, PTHrP, IL-1 and tumor necrosis factor a (TNFa) induce RANKL. OPG is a decoy receptor for RANKL blocking its binding to RANK. Thus, it is the RANKL: OPG ratio that determines the rate of osteoclastogenesis. (Quoted with amendment under open access scheme from Tsang [287])

Wnt Signaling

Wnt is a cytokine involved in the development and homeostasis of various organs. In 2001, low-density lipoprotein receptor-related protein 5 (LRP5) was identified as the gene responsible for osteoporosis pseudoglioma syndrome and regulation of bone mass. Since LRP5 belongs to the low-density lipoprotein receptor family, this finding garnered the attention of researchers in the bone, mineral, and Wnt research fields. In bone, Wnt signaling dominate osteoblast differentiation pathways and act via binding to a receptor complex consisting of LDL receptor-related protein 5 (LRP5) orLRP6 and one of ten Frizzled molecules (The Frizzled family is composed of seven-transmembrane-spanning receptors) [158, 159]. The so-called canonical Wnt signaling pathway is active in all cells of the osteoblastic lineage and involves the stabilization of β-catenin and regulation of multiple transcription factors [160, 161]. Wnt/β-catenin signaling is also important for mechanotransduction, fracture healing, and osteoclast maturation [162–164]. The terminology of canonical vs. noncanonical is historic (Canonical means the overarching and most significant, it refers to specific pathways" as those specific of tissues, cell lines, etc. Noncanonical pathways are those that deviate from the canonical paradigm. The noncanonical pathway refers to the β-catenin-independent pathway). In the classical example of the Wnt pathway, canonical refers to the pathway components that lead to stabilization of beta-catenin in response to certain Wnt ligands. Any other biological outcomes of Wnt signaling are termed noncanonical.

The activation of canonical Wnt-signaling promotes osteoblast differentiation from mesenchymal progenitors at the expense of adipogenesis, which leads to improved bone strength, while suppression causes bone loss [165] (Fig. 1.14). Canonical Wnt signaling in osteoblast differentiation is modulated by Runx2 and osterix [166].

Different Wnt ligands and Frizzled receptors can engage various signaling responses. Wnt5a binds to Ror2 receptors and activates noncanonical signaling pathways, thereby promoting osteoclast differentiation and bone-resorbing activity. In contrast, Wnt16 activates non-canonical Wnt signaling in osteoclast precursor cells and suppresses the Rankl-induced activation of Nf-κb and Nfatc1, thereby inhibiting osteoclast differentiation [158].

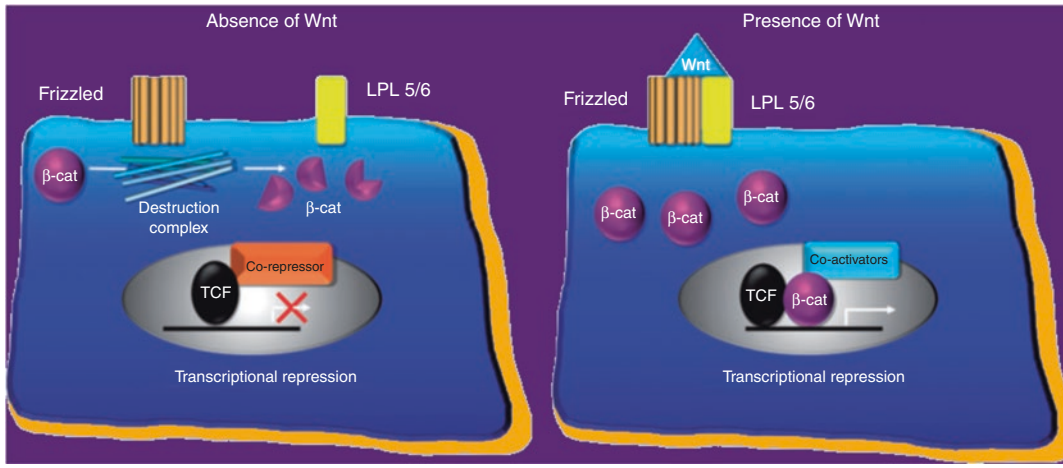


Fig. 1.14 Schematic illustration of canonical Wnt signaling. In the absence of Wnt, Frizzled and its coreceptors LPL5/6 do not interact. The destruction complex, present in the cytoplasm, degrades b-catenin and target gene expression is repressed. In the presence of Wnt, Frizzled binds to its coreceptors and blocks the action of the destruction complex. b-catenin accumulates in the cytoplasm, translocates to the nucleus displacing transcriptional corepressors and recruiting coactivators leading to an increased expression of key target genes involved in osteoblast differentiation. (Quoted with permission from Kendre and Bassett [110]

Wnt signaling is a prime target for bone active drugs and the approach include inhibition of Wnt antagonist like Dkk1, sclerostin, and Sfrp1 with neutralizing antibodies and inhibition of glycogen synthase kinase 3 β (GSK3 β), which promotes phosphorylation and degradation of β-catenin. One of the most promising approaches, which will be discussed later in this book, is the inhibition of the osteocyte protein sclerostin, which exerts tonic inhibition of osteoblast activity [167]. Sclerostin is the product of the *SOST* gene, which is mutated and downregulated in patients with sclerosteosis and van Buchem disease [168], which are diseases characterized by high bone density. Expression levels of sclerostin are repressed in response to mechanical loading and intermittent PTH treatment [169]. Preliminary studies with a humanized monoclonal antibody against sclerostin have shown bone anabolism in both animals as well as humans [117, 170].

Hormonal Impact on Bone Remodeling

Parathyroid Hormone (PTH)

PTH is a polypeptide hormone secreted by the chief cells of the parathyroid glands. It acts to raise the level of calcium in the bloodstream with direct actions on bone and the kidneys, and indirectly on the intestine via the influence on vitamin D. The hormone has a physiological, negative feedback loop that is influenced by the amount of calcium present in the blood. When there is a decreased concentration of plasma calcium, there is less binding to calcium-sensing receptors (CaSR) on the parathyroid gland. This will lead to an increased release of PTH to raise the levels of calcium. PTH has an indirect action on the osteoclasts by increasing the activity of receptor activator of nuclear factor kappa ligand (RANKL), which regulates the osteoclastic activity of bone resorption and leads to more calcium released into the plasma. In contrast, high levels of plasma calcium bind to the CaSR on the parathyroid gland and inhibit the release of PTH. Stimulating the CaSRs causes a conformational change of the receptor and stimulates the phospholipase C pathway. This ultimately leads to higher intracellular calcium, thereby inhibiting exocytosis of PTH from the chief cells of the parathyroid gland. This is only one piece to the calcium homeostasis as PTH has actions at the kidneys and intestines to regulate the levels of calcium and phosphate [171, 172].

Estrogen

A deficiency of estrogen leads to increased bone remodeling, where bone resorption outpaces bone formation and leads to a decrease in bone mass. It is believed, based on animal studies, that estrogen may influence local factors that regulate the precursors of osteoblasts and osteoclasts. Estrogen may block the production and action of interleukin-6 (IL-6), which would hinder bone resorption. Also, it is believed that the survival of osteoclasts thrives in the deficiency of estrogen, where the degree of bone turnover would be greater [173].

Calcitonin

Calcitonin, a polypeptide hormone, is released from thyroid C cells in response to elevated calcium levels. Regarding bones, calcitonin binds to calcitonin receptors on osteoclasts to inhibit bone resorption. It is believed that calcitonin does not play a prominent role in calcium homeostasis in adults, but it may be more important in skeletal development. However, calcitonin is clinically used as a treatment option to treat osteoporosis [174].

Growth Hormone

Growth hormone (GH), a peptide hormone secreted by the pituitary gland, acts through insulin-like growth factors to stimulate bone formation and resorption. Growth hormone acts directly and indirectly via insulin-like growth factor (IGF) to stimulate osteoblast proliferation

and activity, but it also stimulates the bone resorption activity of osteoclasts; however, the cumulative net effect of this dual activity favors bone formation [175].

Glucocorticoids

Glucocorticoids decrease bone formation by favoring the survival of osteoclasts and causing the cell death of osteoblasts. There is an increase in RANKL action and a decrease in osteoprotegerin (OPG). OPG is a cytokine receptor and member of the tissue necrosis factor superfamily that acts as a decoy receptor for RANKL, so it would normally hinder RANKL–RANK interaction and activity.

Thyroid Hormone

Thyroid-stimulating hormone (TSH), thyroxine (T4), and triiodothyronine (T3) cause bone elongation at the epiphyseal plate of long bones through chondrocyte proliferation and also stimulate osteoblast activity. In states of hypothyroidism or hyperthyroidism, the degree of bone turnover is low and high respectively. The rate of bone turnover is due to the effect of T3/T4 on the number and activity level of osteoblasts as well as osteoclasts. For example, the high metabolic state of thyrotoxicosis causes increased osteoblast function and increased osteoclastic number and activity and leads to a higher bone turnover [176]. Fig. 1.15 shows the major endocrine influences on bone remodeling.

Bone Modelling Vs. Remodeling

Bone Modeling

Bone modeling describes the process whereby bones are shaped or reshaped by the independent action of osteoblasts and osteoclasts. The activities of osteoblasts and osteoclasts are not necessarily coupled anatomically or temporally as is the case in bone remodeling. Bone modeling defines skeletal development and growth and is responsible for the shaping of bones and their movement through space. Even in adults, adaptation to permanently changed strain leads to modeling of bone, an example of which is tibial modeling after harvesting fibula for reconstructive surgery [177]. Abnormalities in bone modeling cause skeletal dysplasias or dysmorphias.

One important example of modeling is to preserve skeletal shape during linear growth. In the metaphysis, below the growth plate, there is osteoclastic resorption on the periosteal surface, while there is new bone formation on the inner endosteal surface thus converting the shape of the epiphysis into the diaphysis [178, 179]. When these processes are disrupted, for example, following antiresorptive (bisphosphonate) treatment of childhood osteogenesis imperfecta, a dramatic inhibition of normal metaphyseal modeling "Metaphyseal inwaisting" is seen [180]. Modeling is also responsible for radial growth of the diaphysis of long bones. Here, osteoclastic resorption occurs on the endosteal surface, while osteoblastic bone formation occurs at the periosteal surface thus increasing the overall diameter with age.

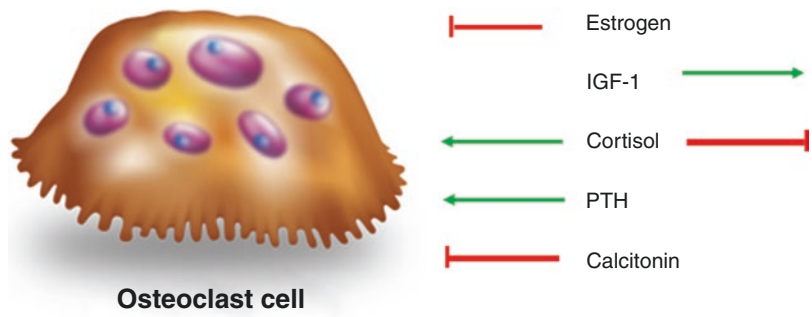


Fig. 1.15 Schema showing the major endocrine influences on bone remodeling. IGF-1, insulin-like growth factor-1; PTH, parathyroid hormone

The majority of bone modeling is completed by skeletal maturity but modeling can still occur even in adulthood such as in an adaptive response to mechanical loading and exercise and in renal bone disease [181–184]. Bone modeling has been demonstrated in aging humans. Modeling-based bone formation contributes to the periosteal expansion, just as remodeling-based resorption is responsible for the medullary expansion seen at long bones and ribs with aging [185].

How is bone modeling controlled? Physical activity can stimulate bone modeling. This is seen for example in tennis players where the arm used for tennis has a higher bone mass than the other arm [186]. Bone modeling is also controlled by other factors as modeling-based bone formation was also seen at the ribs, which are not axially loaded, in the denosumab nonhuman primate study [187]. It is therefore likely that bone modeling is controlled by genetic factors in combination with environmental factors such as physical strain and probably hormonal factors, as it has been demonstrated that the parathyroid hormone (PTH) and inhibition of sclerostin can stimulate modeling-based bone formation [188, 189].

Bone Remodeling

The purposes of remodeling are many including the replacement of old and damaged bone with new bone and calcium homeostasis (long-term homeostasis). Bone remodeling is most prominent on cancellous bone surfaces and it is estimated that 80% of bone remodeling activity takes place in cancellous bone, although cancellous bone only comprises 20% of bone. The relative importance of cortical remodeling increases with age as cancellous bone is lost and the remodeling activity in both compartments increases [190]. Disturbance of bone remodeling, such as in osteoporosis, with a net bone loss passes in three phases: (1) A reversible bone loss because of increase in the remodeling space, i.e., the amount of bone resorped but not yet reformed during the remodeling cycle. This mechanism leads to decrease in average trabecular thickness and cortical width, and to increase in cortical porosity. (2) An irreversible bone loss caused by negative bone balance, where the amount of bone formed by the osteoblasts is exceeded by the amount of bone resorbed by the osteoclasts at the same remodeling site. Consequently, progressive thinning of trabecular elements, reduced cortical width and increased cortical porosity is seen. (3) Finally, perforation of trabecular plates by deep resorption lacunae leads to complete irreversible removal of structural bone components [191]. In the cortical bone, remodeling takes place at both the periosteal and endocortical surfaces, but it also occurs inside the compact cortical bone [192, 193]. At the cortical surfaces remodeling is a surface-based process similar to the process in cancellous bone (Fig. 1.16), whereas intracortical remodeling is characterized by osteoclasts drilling through the compact bone in the cutting cone followed by osteoblasts filling the cylindrical void in the closing cone (Fig. 1.17) [194, 195]. This is called a Haversian remodeling system.

By removing old and damaged bone targeted remodeling plays a key role in maintaining the mechanical strength of bone. However, excessive remodeling and repair poses a risk to bone strength as it destabilizes bone and introduces stress concentrators [195]. Even targeted remodeling may be harmful. For example, excessive strain causes regional microdamage, which leads to targeted remodeling removing the damaged bone and a larger volume of the surrounding undamaged bone, this temporary volume deficit increases the strain in neighboring bone and the potential establishment of a vicious cycle between damage and repair [196]. Furthermore, bone is an important player in calcium homeostasis. There are several examples of bone being a dynamic part of calcium homeostasis, for example, during pregnancy and lactation or when male deer grow antlers, the latter being an extreme example in which sufficient calcium can only be attained by temporarily removing it from the skeleton [197]. The potential conflict between preserving bone strength and providing calcium to the rest of the body becomes more obvious with aging when vitamin D production and, thereby calcium absorption, decreases and sec-

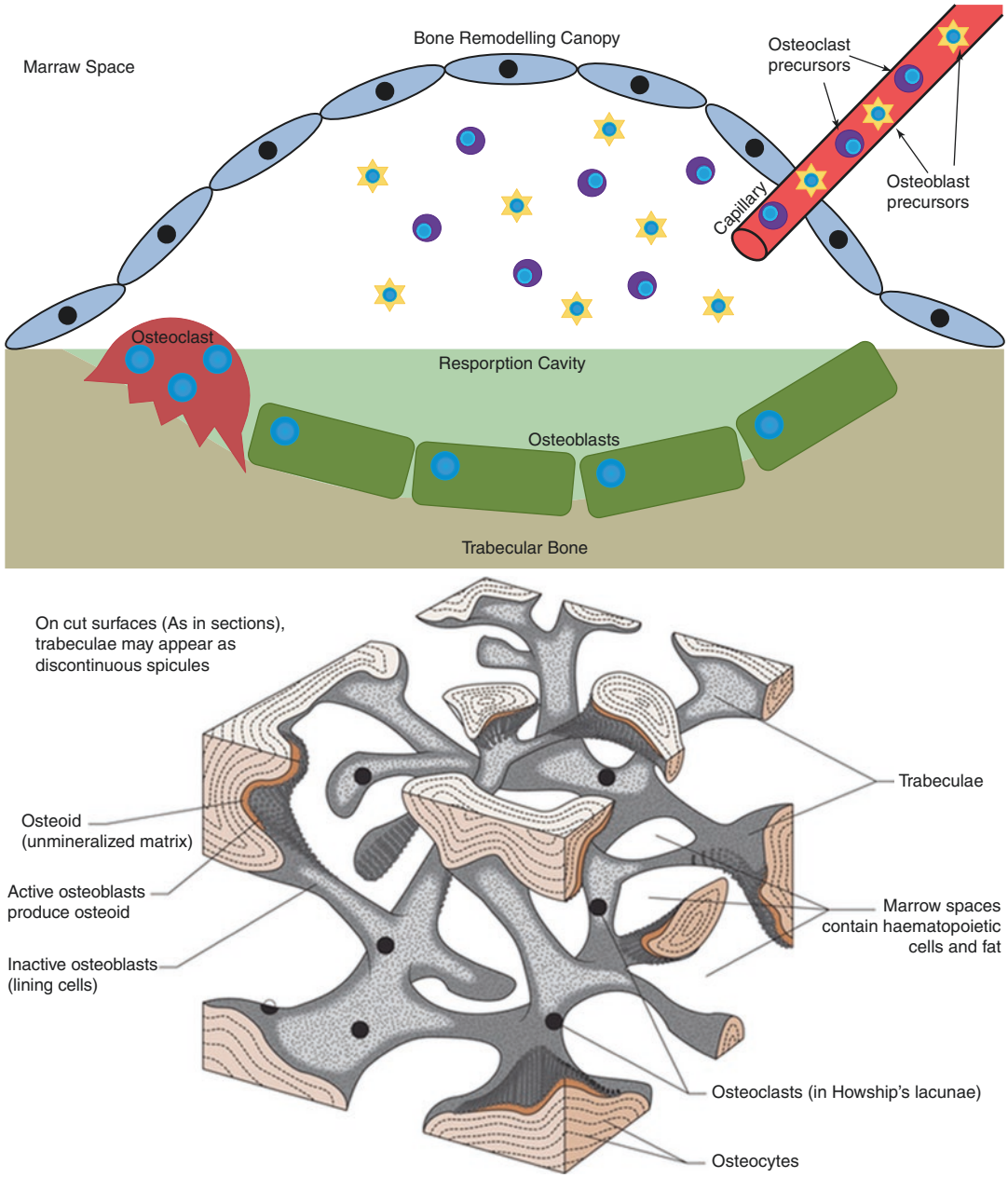


Fig. 1.16 Trabecular remodeling is a surface-based process. Osteocyte apoptosis, induced for example by the disruption of osteocyte canaliculi caused by bone matrix microdamage, leads to release of paracrine factors that increase local angiogenesis and recruitment of osteoclast and osteoblast precursors. (Quoted under open access scheme Creative Commons Attribution License (CC BY) from: Owen and Reilly [288])

ondary hyperparathyroidism develops in order to maintain adequate serum calcium levels by increasing bone resorption. Furthermore, the estrogen insufficiency in postmenopausal women also leads to increased remodeling activity. Increased resorptive activity in a young individual is accompanied by complementary increased formation and the balance at each bone resorption unit is neutral, therefore the bone loss is merely reflecting an opening of the remodeling

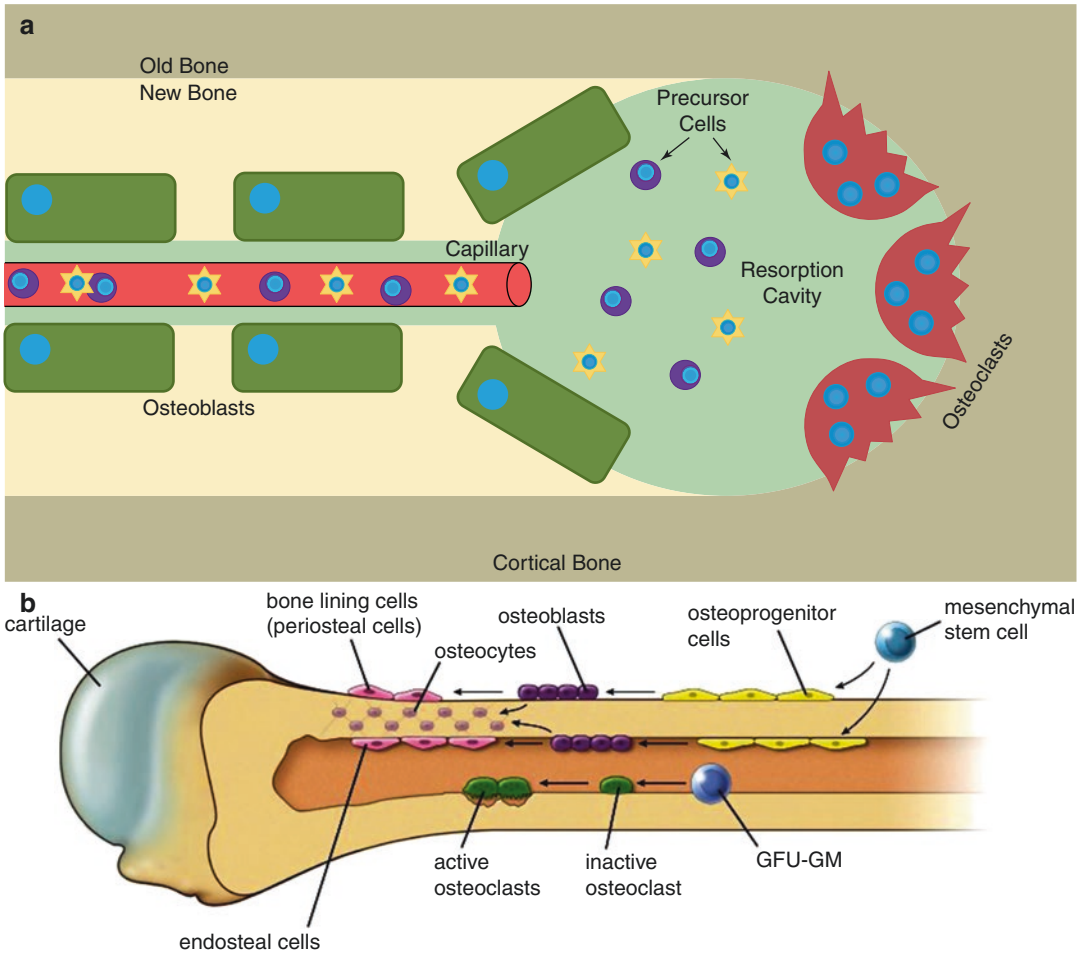


Fig. 1.17 A and B: Cortical bone remodeling: In the cortical bone, remodeling takes place at both the periosteal and endocortical surfaces, as well as inside the compact cortical bone. (Quoted under open access scheme Creative Commons Attribution License (CC BY) from: Owen and Reilly [288])

space and is therefore reversible. The situation in postmenopausal women and elderly men is very different. The balance between resorption and subsequent formation at each bone resorption unit is negative with increased resorptive activity, leading, therefore, to bone loss that is irreversible due to thinning of the trabeculae, loss of trabeculae, and thinning of the cortex (Fig. 1.18).

Bone remodeling also plays a role in the maintenance of acid/base balance, and the release of growth factors embedded in bone. Moreover, it provides a reservoir of labile mineral (short-term homeostasis) and it is the only mechanism by which old, dying, or dead osteocytes can be replaced [198].

Applied Bone Biology

Abnormalities of the Bone Remodeling Cycle

In the bones of healthy adults, the remodeling cycle displays tight coupling between bone resorption and bone formation. Accordingly, several metabolic bone diseases including osteoporosis, hyperparathyroidism, Paget's disease, and osteopetrosis are characterized by loss of such coupling.

The cellular pathophysiology of osteoporosis is heterogeneous and differs according to the underlying pathogenesis. In postmenopausal

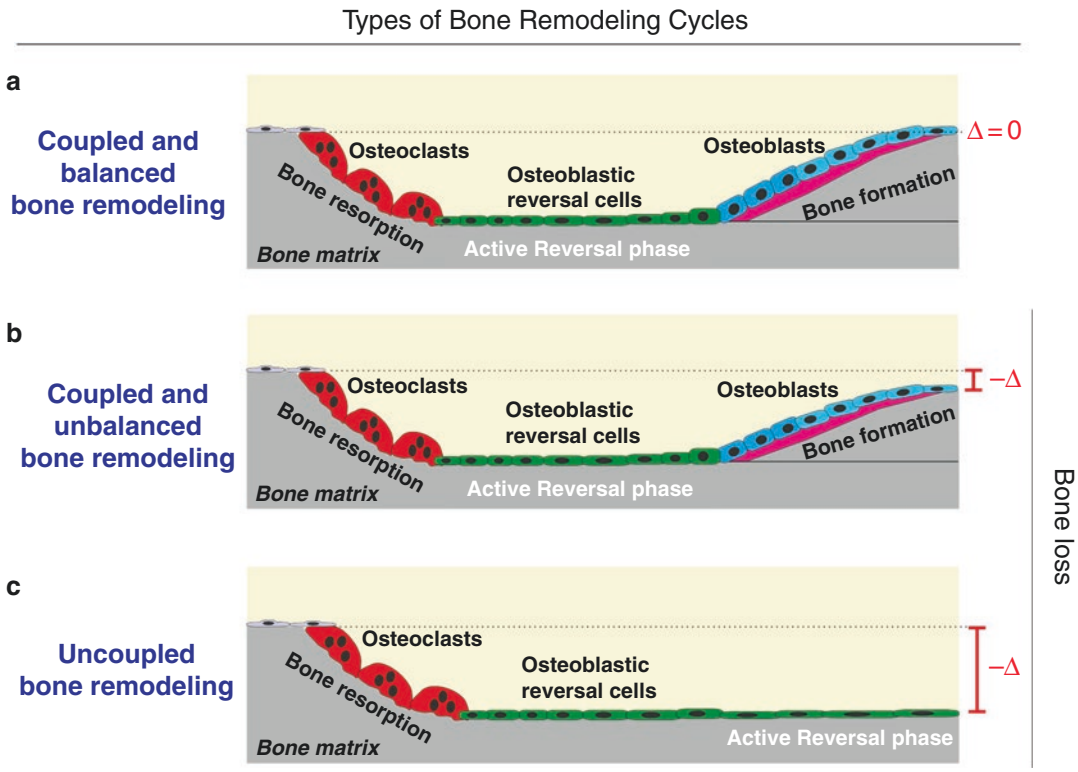


Fig. 1.18 Types of remodeling cycles: Three types of bone remodeling cycles. According to the present hypothetical model, bone loss in PMO depends on the relative abundance of three concurrent types of bone remodeling cycles. They all start with bone resorption, but differ greatly by the degree of restitution of bone matrix. A: the cavity is completely refilled. It is the prevailing type of bone remodeling cycle occurring in physiological conditions and in PHPT. B: the cavity is only partially refilled, as a result of a failure of the bone formation process. It is the type commonly considered responsible for bone loss in PMO. C: the cavity remains completely unfilled, as a result of an arrest of the reversal phase, so that bone formation is not even initiated. Its contribution to bone loss in PMO is most often overlooked but is highlighted in the present study. (Quoted with permission from Andersen et al. [289] (license number: 4879510361059))

osteoporosis, the most common abnormality is an increase in remodeling rate accompanied by reduced bone formation at the level of the individual bone remodeling unit, resulting in increased bone turnover and a negative remodeling balance. However, in some postmenopausal women with osteoporosis, bone turnover appears to be reduced, even when no secondary cause is apparent [199]. Where osteoporosis is due to underlying disease, changes in bone remodeling vary according to the underlying etiology but many forms of secondary osteoporosis are characterized by low bone turnover and negative remodeling balance, with episodes of increased bone turnover during periods of disease activity [200]. In glucocorticoid-induced osteoporosis, the most common cause of secondary osteoporosis, there is an initial transient phase of increased bone turnover superimposed on reduced bone formation at the tissue and cellular level that persists throughout the duration of glucocorticoid use [201]. The changes in bone remodeling determine the associated structural changes. In contrast to increased bone turnover with a net result of bone microarchitecture disruption; bone structure is relatively well preserved in low turnover states [202]. In addition, changes in other determinants of bone strength, such as the degree and heterogeneity of mineralization, matrix and mineral structure, and microdamage repair, are largely dependent on the underlying alterations in bone remodeling.

Bone Modeling/Remodeling as Therapeutic Targets

Antiresorptives

Reduction in bone turnover is common to all anti-resorptives regardless of the mechanisms by which they inhibit osteoclast activity. At the cellular level, the predominant effect of antiresorptive drugs is to inhibit the recruitment and activity of osteoclasts, thus decreasing the rate of remodeling and reversing the transient deficit created by resorption cavities in which formation has not yet occurred or been completed, allowing for a modest increase in BMD. The decrease in remodeling rate allows infilling of previously created resorption cavities and stabilises trabecular bone structure. Although the negative remodeling imbalance persists, its impact is limited by the decrease in number of remodeling sites on the bone surface. These drugs probably do not fully correct the negative remodeling balance, but since the number of remodeling units is greatly reduced, the effect of any negative imbalance is decreased. Reduced remodeling is associated with increased secondary mineralization of bone, which further contributes to the increase in BMD [203]. Anti-resorptive agents approved for osteoporosis include the bisphosphonates (alendronate, risedronate, ibandronate and zoledronic acid), denosumab, and raloxifene.

Essentially, antiresorptive therapy preserves existing bone mass and structure and increases the degree and homogeneity of mineralization. In cortical bone, denosumab can improve cortical bone structure at several sites, including the hip, increasing cortical thickness and decreasing porosity [204–207]. A possible explanation for this observation is that denosumab maintains physiological bone modeling [195, 208]. In addition, the accessibility of cortical bone to denosumab might be greater than the accessibility to bisphosphonates, because of differences in pharmacokinetic properties [209].

Suppression of bone remodeling allows a longer time for secondary mineralization to occur, resulting in an increase in both the degree of matrix mineralization and its homogeneity. The differences in mechanisms of action between bisphosphonates and denosumab provide explanations in clinical outcome and opportunities in sequential therapy (Fig. 1.19). Bisphosphonates attach to hydroxyapatite preferably on metabolically active bone surfaces, where they are "ingested" by osteoclasts and promote osteoclast apoptosis. Bisphosphonates can remain in bone tissue for up to 10 years. Denosumab acts by binding to and inhibiting RANKL in circulation, leading to the loss of mature osteoclast formation. Denosumab accesses every bone remodeling unit within circulation, and its distribution does not depend on the activity of bone remodeling [209]. Studies with bisphosphonates have shown that the degree of mineralization increases towards or even above normal, depending on the bisphosphonate administered [210–215]. In postmenopausal women treated for 3 years with annual infusions of zoledronic acid, posttreatment mineralization values were higher than those obtained in a historical reference population [213].

The effects of denosumab on bone matrix mineralization is likely to be similar to bisphosphonates where substantial increases also occur. Changes in other properties of bone matrix and mineral have also been reported in association with bisphosphonate therapy. In women treated with alendronate for 3 years, a higher mineral to matrix ratio in cortical bone was demonstrated compared to untreated controls. Crystallinity, carbonate/protein, and collagen maturity indices were not significantly altered compared to untreated controls [210]. However, higher collagen maturity and crystallinity in iliac crest cortical bone were reported in women who had been treated with alendronate for between 6 and 10 years [211]. In another study in which indices of bone quality were assessed in actively forming trabecular bone surfaces in postmenopausal women treated with alendronate or risedronate, mineral maturity/crystallinity and pyridinoline/divalent collagen cross-link ratio were significantly lower in risedronate-treated women than in those treated with alendronate [215].

The effects of anti-resorptive drugs on cortical bone are of particular interest, given the high

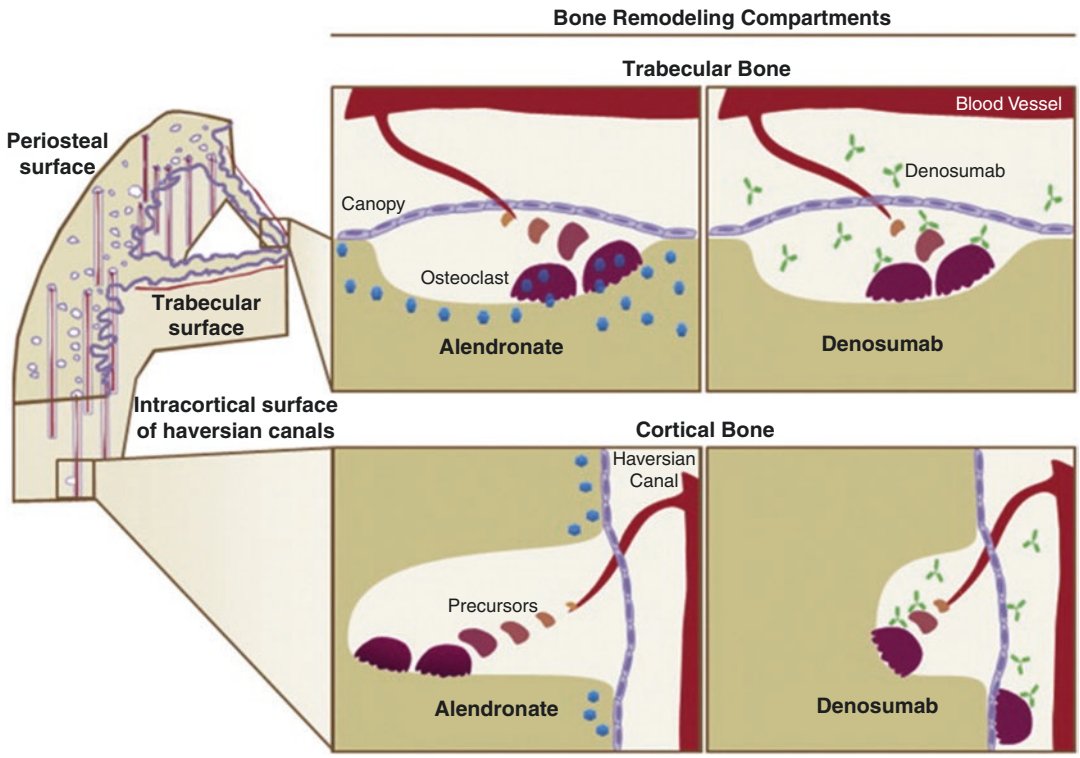


Fig. 1.19 Remodeling is initiated within bone remodeling compartments (BRCs) at points beneath the canopy of cells lining trabecular bone (upper panels) and cortical bone Haversian canals (lower panels). Osteoclast precursors differentiate into bone-resorbing osteoclasts within BRCs. In trabecular bone, alendronate and denosumab inhibit resorption similarly; osteoclasts engulf matrix containing alendronate, and denosumab accesses osteoclasts via the extracellular fluid. In cortical bone, osteoclasts encounter little peri-Haversian canal matrix containing alendronate and so resorb bone but denosumab accesses BRCs as freely as it does in trabecular bone. Available via license: CC BY-NC-ND 3.0

proportion of cortical bone at sites of nonvertebral fractures, the substantial contribution of these fractures to the overall fracture burden and the relatively low anti-fracture efficacy of interventions at these sites. Investigation of these effects is not straightforward, since changes may vary according to skeletal site. Also there have been limitations reported regarding current approaches to the in vivo assessment of cortical bone structure, particularly with respect to measurement of cortical porosity and thickness. Reduced cortical porosity in the distal radius, tibia, and iliac crest has been reported in women treated with bisphosphonates when compared to placebo-treated women [216–219], although this finding has not been universal [220]. Increased tibial cortical thickness was demonstrated after 2 years in a longitudinal study in postmenopausal women randomized to alendronate or placebo, although no significant treatment benefit was seen at the radius.

Earlier studies provided partial insights into the effects of antiresorptive drugs on cortical bone at selected sites, but the available data suggest that the predominant effect of bisphosphonates is to reduce or prevent age-related changes in cortical bone structure, with little evidence for improvement over baseline values. Conversely, there is evidence that denosumab improves cortical bone structure and strength at several sites, including the hip [221–223]. These differences are consistent with the greater increase in hip BMD with denosumab versus alendronate observed in a comparator and the continued increase in spine and hip BMD up to 8 years in denosumab-treated postmenopausal women

[224, 225]. It has been suggested that because of the low surface area/mineralized bone volume in intracortical bone there is less surface to which bisphosphonates can adsorb, whereas circulating denosumab has greater accessibility to intracortical sites [19]. In addition, it is possible that the increase in serum PTH levels that follows profound suppression of bone turnover after injection of denosumab may exert anabolic effects [226]. The recent demonstration in ovariectomized cynomolgus monkeys that modeling-based formation at endocortical and periosteal surfaces in the proximal femur and ninth rib was maintained, despite potent inhibition of remodeling activity, provides another potential mechanism for the effects of denosumab on BMD and bone strength [227]. However, the relevance of these findings to humans is currently unclear, since it is uncertain whether modeling-based bone formation occurs on endosteal surfaces in the normal adult human skeleton. Modeling-based formation was not reported in iliac crest bone from women treated with denosumab [228], although its presence at weight-bearing sites remains a possibility. Finally, whether the differences between denosumab and bisphosphonates in their effects on cortical bone translate into greater antifracture efficacy at nonvertebral sites is unknown, since no head-to-head studies with fracture as the outcome have been conducted.

Anabolic Agents

Anabolic skeletal effects can be achieved through changes in bone remodeling, bone modeling, or a combination of the two. Principally, anabolic agents have been defined by their ability to increase bone formation relative to resorption. This may occur as a result of modeling-based bone formation or when there is a positive remodeling balance due to increased formation at the level of the basic multicellular unit (BMU). In the latter situation, the increase in bone mass depends critically on the remodeling rate; if this is low, changes in remodeling balance will have little impact on bone mass, whereas substantial gains can be achieved when a high remodeling rate is associated with a positive remodeling balance. Anabolic effects on bone may also be achieved if there is uncoupling of bone resorption and formation during bone remodeling. Coupling describes the co-ordination of bone resorption and formation in time and space and refers to tissue-level remodeling (Fig. 1.20) [209].

The available osteoanabolic therapies for osteoporosis are human recombinant PTH peptide [1–34], also known as teriparatide, recombinant human parathyroid hormone (rhPTH 1–84) (which is identical to endogenous parathyroid hormone (PTH) and binds PTH-1 receptors in the bone, kidney, and has an indirect effect on cal-

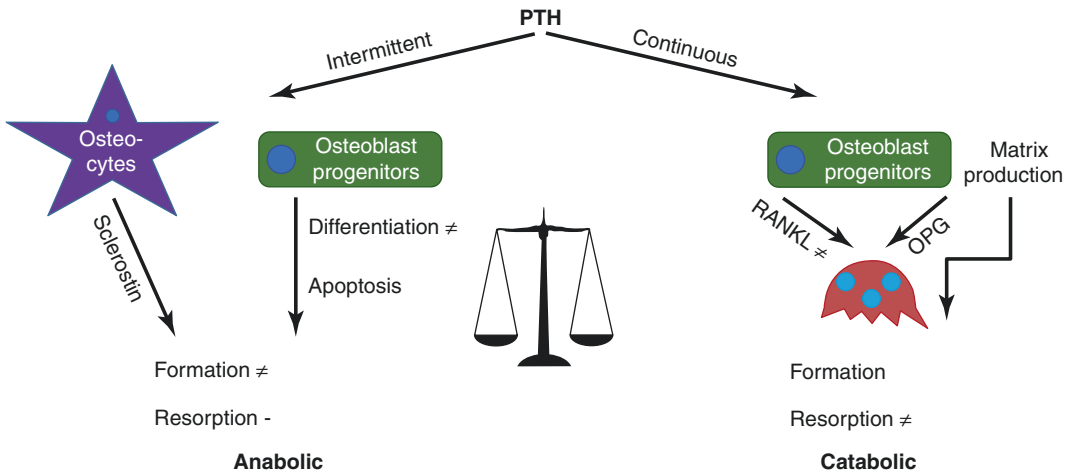


Fig. 1.20 The anabolic or catabolic effects of PTH on bone depends on application modality. (Quoted under open access scheme Creative Commons Attribution License (CC BY) from: Owen and Reilly [288])

cium reabsorption in the intestine), known as Preotact. There is also a highly selective and high affinity parathyroid hormone-related protein (PTHrP) analogue which binds to the PTH1 receptor, known as abaloparatide. Intermittent administration of these PTH peptides is associated with large increases in BMD in the spine and more variable changes in cortical bone depending on the site and the duration of therapy. Bone histomorphometric studies in postmenopausal women treated with teriparatide have demonstrated that increases in bone mass are achieved in trabecular bone by the formation of new bone on quiescent surfaces (modeling), mixed modeling/remodeling in which remodeling units are overfilled and formation extends beyond the limits of the resorption cavity, and increased remodeling rate associated with a positive remodeling balance [229–231]. These changes are associated with increased connectivity of the trabecular bone structure and improvement in the structure model index [232, 233].

Increases in trabecular thickness are small and, in most studies, have failed to achieve statistical significance, possibly as a result of the splitting of thickened trabecular by tunneling osteoclastic resorption [234]. At sites that are rich in trabecular bone, such as the spine, large increases in bone mineral density are seen and in the pivotal clinical trial in postmenopausal women with osteoporosis, a 65–69% reduction in vertebral fractures was demonstrated after a median duration of 21 months [235]. Interestingly, the skeletal response to teriparatide shows evidence of waning after 12–18 months, for reasons that are not currently understood but do not appear to be related to the formation of neutralizing antibodies [236]. Alternative explanations include downregulation of PTH receptors or depletion of bone target cells.

In cortical bone, the changes in BMD with PTH peptide therapy are less consistent and vary according to the skeletal site and the duration of therapy. In the proximal femur, areal BMD shows only small changes over the first 6–12 months of therapy and may even decrease transiently while in the distal radius, significant decreases in areal BMD have consistently been reported [235–238]. Measurement of vBMD in women treated with teriparatide for 12 months demonstrated increases in cancellous bone in the spine and hip, but decreases in cortical bone in the proximal femur, distal radius, and tibia [239]. The likely cause of these latter changes is an increase in cortical porosity and the formation of hypomineralized bone on the endosteum; increased femoral neck bone strength has been reported with longer term treatment (18–24 months) [240, 241]. Histomorphometric analysis of iliac crest bone in teriparatide-treated women indicated that increased intra-cortical porosity is partially or wholly reversed by subsequent bone formation in bone remodeling units [242]. Cortical thickness mapping on CT images of the proximal femur has shown focal increases in cortical thickness following teriparatide therapy in postmenopausal women at sites exposed to normal mechanical loading [243]. Although modeling-based periosteal bone formation in human iliac crest bone has been reported, it has not been demonstrated in cortical bone at other sites as assessed by changes in bone size [240].

Taken together, the available data indicate that intermittent administration of teriparatide stimulates modeling-based bone formation on cancellous, endosteal, and periosteal surfaces, an effect that is most evident in the early stages of treatment. However, the majority of the anabolic effect in cancellous bone is achieved through remodeling with overfilling of remodeling units. In cortical bone, the effects vary according to site, and may also be modulated by the degree of mechanical loading; increased total bone area, increased cortical porosity, and the formation of hypomineralized new bone can occur in the early stages of treatment, which results in little change, or a decrease in BMD at sites such as the hip and radius. In the Fracture Prevention Trial of teriparatide, the number of nonvertebral fractures was relatively small and although a significant reduction in all fragility nonvertebral fractures was seen, the number of hip fractures was too small (placebo group n = 4, teriparatide 20 μg/day n = 1, teriparatide 40 μg/day n = 3) to enable assessment of efficacy at this site [244, 245]. PTH (1–84) has also been shown to reduce verte-

bral fractures in postmenopausal women but reduction in nonvertebral fractures has not been demonstrated [246]. While preservation of bone strength at the radius and tibia have been reported in women treated with teriparatide, treatment with PTH (1–84) was associated with reduced bone strength at these sites in one small open label nonrandomized study [247]. Further research is required to establish whether there are true differences between these two peptides.

In concordance, in a cohort of 2463 women at high risk of postmenopausal fractures, abaloparatide resulted in an 86% reduction in vertebral and a 43% reduction in nonvertebral fracture. In comparison, daily subcutaneous PTH 1–34 (teriparatide) resulted in an 80% reduction in vertebral and a 30% reduction in nonvertebral fracture. Furthermore, after 18 months of abaloparatide treatment, total hip BMD increased by 3.4% and lumbar spine BMD by 9.2% [248]. Effects of abaloparatide on bone turn over, have not been reported; however, in postmenopausal women treated for 12–18 months with abaloparatide, bone remodeling indices in cancellous iliac crest bone were generally similar to those treated with teriparatide [249].

However, in view of the potential for adverse effects on cortical bone structure at the hip during the early stages of treatment with PTH, these drugs should be used with caution in patients at high risk of hip fracture.

Bone Formation-Sparing Antiresorptive Treatment

Resorbing osteoclasts adhere very tightly to the bone surface, seal off the resorption lacunae, and generate an acidic environment in the resorption lacunae by secreting protons. Bone mineral is dissolved by the acidic environment and the collagen and other noncollagenous proteins are degraded by proteases such as metalloproteinases and cathepsin K [250]. There are no currently available medications fulfilling this role. Odanacatib, an inhibitor of cathepsin K, was once assessed for treatment of osteoporosis and bone metastasis; however, increased stroke risk forced the manufacturing company to scrap the medication. Though there is no current medication available exerting this mechanism of action, we felt it is of value, at least from the research point of view, to share the available data on odanacatib therapy.

Treatment with odanacatib offered a different mechanism of action compared to other biologic anti-resorptive agents such as denosumab; as treatment with odanacatib leaves the osteoclasts alive and unaffected, but inhibits bone resorption by inhibiting cathepsin K activity [250].

The effects of odanacatib on bone was investigated in adult rhesus monkeys. Treatment with odanacatib resulted in increased BMD and bone strength at the lumbar spine and the hip [251, 252]. Histomorphometric analyses of vertebrae, proximal femur, and transiliac bone biopsies demonstrated that odanacatib reduced cancellous bone remodeling in the lumbar vertebrae and hip, and decreased intracortical remodeling at several femoral sites in monkeys. However, treatment with odanacatib preserved or enhanced endocortical bone formation and dose-dependently stimulated modeling-based bone formation at the periosteal surfaces [252]. The effect of odanacatib on cortical bone was also investigated at the central femur. Treatment with odanacatib-stimulated bone formation both at the periosteal surface and at the endocortex. At the endocortex, bone modeling was stimulated whereas bone remodeling was reduced. The intracortical remodeling was also reduced. These changes led to increased cortical thickness and volume [253].

Whether a similar increase of modeling-based bone formation with odanacatib occurs in humans, particularly in estrogen-deprived and older individuals in whom the viability and/or activity of lining cells could be reduced, was subjected to study. An interaction between mechanical loading and cathepsin K inhibition on bone modeling has been postulated, which if true, could explain some differences in bone-mass gain observed with odanacatib at loaded (i.e. hip) compared with less loaded (i.e. radius) sites. The mechanisms by which cathepsin K inhibition, which primarily occurs at remodeling sites, can increase bone modeling, particularly at the peri-

osteal surface, also remains to be elucidated. However, as noted earlier, the phase III trial was stopped by the manufacturing company in 2016, because of increased risk of stroke.

Combined Anabolic and Antiresorptive Treatment

Osteocytes are terminally differentiated osteoblasts which become embedded in newly formed bone matrix and produce sclerostin. As noted earlier in this chapter, sclerostin binds to lipoprotein-related peptide (LRP) 5/6 and thereby inhibits LRP5/6 from binding to the frizzled receptor and activating the Wnt pathway [254, 255]. Activation of the Wnt canonical pathway induces translocation of β-catenin to the nucleus of the osteoblasts and subsequently gene transcription that stimulates bone formation through stimulation of osteoblast differentiation, proliferation, and survival [256]. Osteocytes control bone formation by the release of sclerostin as sclerostin inhibits osteoblastic bone formation. Individuals who produce reduced amounts of sclerostin have a high bone mass and reduced fracture risk [257, 258], and therefore inhibition of sclerostin by antibodies is being investigated as a potential new anabolic treatment of osteoporosis. The anabolic effects of sclerostin inhibition are mediated through an early and transient increase in bone formation combined with a sustained decrease in bone resorption.

Inhibition of sclerostin by romosozumab, a sclerostin antibody, has been investigated in cynomolgus monkeys [259]. BMD and strength increased dose-dependently. Histomorphometric analyses of bone samples revealed increased bone formation on trabecular, periosteal, endocortical, and intracortical surfaces despite decreased resorptive activity. The study also demonstrated that inhibition of sclerostin by romosozumab predominantly stimulates modeling-based bone formation at both cancellous and endocortical surfaces [188].

In iliac crest biopsy samples obtained from postmenopausal women in the fracture study in postmenopausal women with osteoporosis (FRAME) [260], large increases in bone formation were seen in cancellous and endocortical bone after 2 months of treatment with romosozumab although the effect was no longer evident after 12 months of treatment. The eroded surface was significantly reduced at both timepoints, and trabecular bone volume, microarchitecture, and cortical thickness were significantly improved at 12 months. Data from animal studies have shown increased modeling bone formation in response to sclerostin inhibition, but the relative contributions of bone remodeling and modeling to bone formation in humans remain to be established [259].

Drugs that Act on the Bone Mineral/Matrix Composite

Strontium ranelate provides an interesting example of a drug that has little effect on bone remodeling yet increases bone strength and reduces fracture risk [261, 262].

The mechanism by which it exerts these effects has not been clearly established but is likely to be related to the incorporation of strontium into hydroxyapatite crystals in bone mineral [263, 264]. Assessment of bone turnover markers and bone histomorphometry in postmenopausal women demonstrates only a weak anti-resorptive effect and, contrary to earlier expectations based on preclinical studies, no anabolic effect [265, 266]. Although it is now not widely used, strontium ranelate illustrates the potential for targeting treatments directly at the bone mineral/matrix composite rather than at bone remodeling.

Bone Turnover and Fracture Risk

The immediate clinical consequence of osteoporosis is fracture. However, a discrepancy was noted on comparing the occurrence site of osteoporotic fractures. Earlier studies revealed that significant reduction of vertebral fractures occurs early in the course of therapy, typically within 6 months, whereas reduction of nonvertebral fractures and hip fractures specifically has not

been observed before at least 1 year of therapy [267, 268]. This could be explained by the fact that vertebral fragility is primarily determined by focal areas of erosion creating stress risers on trabeculae [269], whereas weakness in the peripheral skeleton results from trabecular and cortical bone loss, particularly cortical porosity, that becomes predominant only in older age [270]. In turn, the elimination of stress risers, which is proportional to the potency of the various antiresorptives, is sufficient to explain the early decrease of vertebral fractures; whereas long-term reversal of the negative bone mineral balance seen in the peripheral skeleton, particularly the progressive restoration of the cortical bone volume, is essential to reduce nonvertebral fractures. As a corollary, spine bone mineral density (BMD) changes have been found to explain less than 50% of vertebral fracture risk reduction [271–275], whereas more recently hip BMD gain with potent parenteral anti-resorptives such as zoledronic acid and denosumab has explained up to 60–90% of nonvertebral fracture risk reduction [276, 277]. Nevertheless, relatively large changes at the hip are needed to significantly influence fracture risk, for example, a 6% BMD gain is equivalent to 1% nonvertebral fracture risk reduction with denosumab [268].

Building Better Bones: Sequential and Combination therapy for Osteoporosis

Unlike most chronic diseases, osteoporosis treatments are generally limited to a single drug at a fixed dose and frequency. Nonetheless, a major challenge in managing patients with established osteoporosis is the increasing reluctance to treat patients with antiresorptive medications for more than 3 to 5 years. This has been attributed to the concern over uncommon but serious side effects such as atypical femur fracture and osteonecrosis of the jaw, as well as the longstanding regulatory 2-year limit on parathyroid-hormone receptor targeted anabolic therapies [278–280]. Furthermore, no approved therapy has been shown to be able to restore skeletal integrity in most osteoporotic patients and the long-term use of osteoporosis drugs is controversial. Thus, it is expected that over a lifetime, the use of more than one medication will be required for many patients with established disease. And consequently, it is imperative that we understand the selective effects of osteoporosis medications when used sequentially or in combination so that we can construct optimal treatment plans in individual patients.

In clinical trials, denosumab given after bisphosphonate continued to increase bone mineral density (BMD) and produced significantly greater gains in BMD at all measured sites when compared to all bisphosphonates. Consequently, denosumab can be given after a bisphosphonate when the treatment goal in BMD gain has not been achieved. However, bisphosphonates also should be given after denosumab discontinuation to prevent BMD loss. Both VERO and ARCH studies proved that anabolic treatment for osteoporosis is more effective than bisphosphonates at preventing vertebral fractures in a high-risk population (with previous vertebral fractures) in both treatment-naïve or bisphosphonate-treated patients [281]. Consequently, anabolic treatment should be considered either as a first-line treatment in patients with previous vertebral fractures or in case a low-traumatic fracture occurs while on bisphosphonate treatment. However, the duration of anabolic treatment is limited and requires antiresorptive medication after discontinuation. The sequential treatment approach in osteoporosis is slightly limited with the result of DATA study, which showed that switching to teriparatide after denosumab led to BMD loss and should be considered with caution. According to the DATA study, teriparatide combined with denosumab gives better BMD gain than both treatments alone [282]. This is the only currently recommended approach using combined treatment in osteoporosis which remains controversial because of the high cost and lack of evidence regarding antifracture benefit. However, in another study, the DATA-Switch study, assessing sequential therapy; results revealed that in postmenopausal osteoporotic women switching from teriparatide to denosumab, bone mineral density

continued to increase, whereas switching from denosumab to teriparatide results in progressive or transient bone loss [283]. These results should be considered when choosing the initial and subsequent management of postmenopausal osteoporotic patients.

Challenges in Developing Treatments for Osteoporosis

In clinical trials conducted in postmenopausal women with osteoporosis, reductions in fracture risk of up to 70% in the spine, 40% in the hip, and 15–20% at nonhip nonvertebral sites have been demonstrated. The limited efficacy at nonvertebral sites is a concern, given the high burden and cost of these fractures [284]. Although poor compliance with osteoporosis management and/or adherence to therapy, as well as continuing falls risk, are likely to contribute to the small effect on nonvertebral fractures provided by currently approved interventions, drug-specific factors may also operate. In particular, failure to improve cortical bone mass and structure adequately, which may be of high relevance. An important challenge, therefore, is to develop drugs that produce greater increases in cortical bone strength throughout the skeleton and provide more effective protection against nonvertebral fractures.

A second challenge is related to the diversity and severity of changes in bone remodeling, mass, microarchitecture and composition in primary and secondary osteoporosis. At present, a "one size fits all" approach is widely used, with anti-resorptive therapy providing the first-line option for the vast majority of patients regardless of the underling pathophysiology and disease severity, but this may be suboptimal in achieving maximum efficacy. As more drugs with differing mechanisms of action are developed, it may become possible to take a more personalized approach to treatment (Fig. 1.21). However, at present the required evidence base to support this approach is lacking.

Finally, increasing concerns about rare but serious skeletal side effects of treatment have emerged, particularly with anti-resorptive drugs. Although suppression of bone turnover is associated with beneficial effects on BMD and fracture risk it has also been implicated on the pathogenesis of atypical fractures and osteonecrosis of the jaw [279, 285]. While the benefit/risk balance for treatment remains positive in patients at high risk of fracture, these adverse effects have been widely publicized and have had a significant impact on prescribing habits and patient uptake. Further studies are required to minimize their occurrence through a better understanding of their pathophysiology and improved identification of risk factors for their development.

In conclusion, to preserve its essential load bearing, protective, and homeostatic functions, the skeleton must undergo continual remodeling and repair. The bone remodeling cycle ensures that old or damaged bone is replaced, and that mineral homeostasis is maintained. Bone remodeling is a highly regulated and stereotyped process characterized by osteoclastic bone resorption followed by osteoblastic bone formation. These two processes are tightly coupled to ensure that bone mass is ultimately preserved.

The osteocyte is the key orchestrator of the bone remodeling cycle. These long-lived, terminally differentiated osteoblasts are entombed within the bone matrix, connected by an extensive dendritic network and act as the skeletal mechanosensor. They respond to microdamage and changes in loading by initiating bone remodeling, and once the repair is complete, they inhibit further bone resorption and formation to maintain bone mass. Furthermore, osteocytes also secrete Fibroblast growth factor-23 (FGF23), respond to hormones such as parathyroid hormone to initiate bone resorption and thus maintain mineral homeostasis.

Recent studies of current and potential therapeutic options for osteoporosis have revealed a range of mechanisms through which bone strength may be improved. Uncoupling of bone remodeling, with suppression of bone resorption and maintenance or stimulation of bone formation provides a new approach that may be more beneficial to cortical bone in particular than currently approved interventions. Whether this translates into greater efficacy in reducing nonvertebral

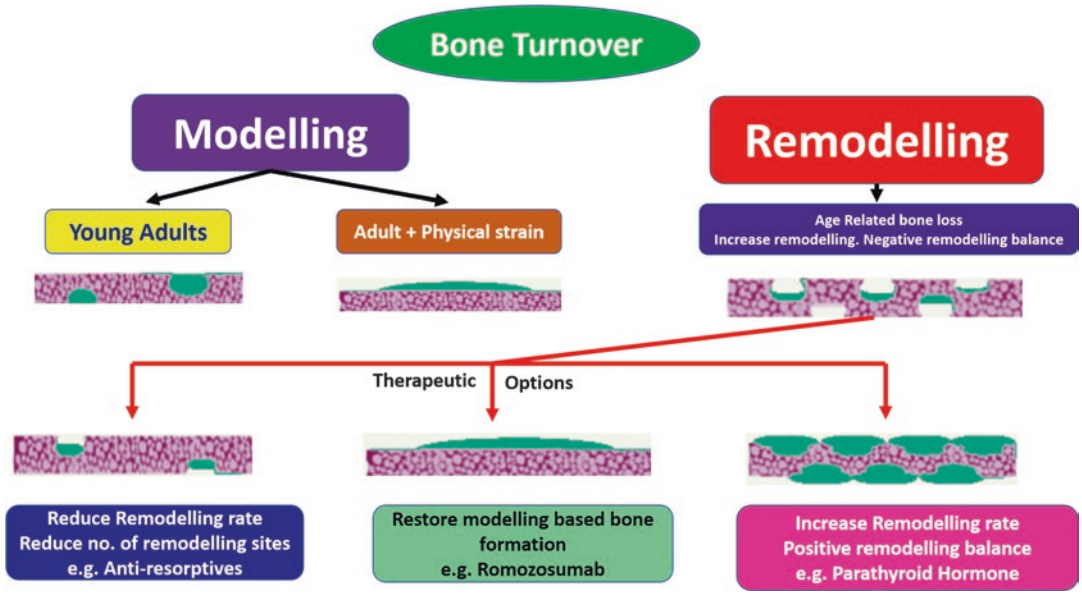


Fig. 1.21 Bone modeling/remodeling as therapeutic targets

fractures and the long-term bone safety of these approaches remains to be established. Nevertheless, the future holds promise for a broad armamentarium of options that should enable a more tailored approach to treatment of the individual patient, based on the underlying changes in bone remodeling, structure, and composition.

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