Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Regulatory Mechanisms of Bone Development and Function

Monir Ali El-Ganzuri[1], Rasha Rashad Ahmed[2], Eslam Muhammad Bastawy[1]

1. Department of Zoology, faculty of Science, Ain Shams University,
2. Department of Zoology, faculty of Science, Beni-seuif University, Egypt

Manuscript Info

Manuscript History:

Received: 22 August 2015
Final Accepted: 25 September 2015
Published Online: October 2015

Key words:

Bone, Osteoblasts, Osteoclasts, Bone remodeling.

***Corresponding Author**

Monir Ali El-Ganzuri

Abstract

Bone is metabolically active organ where 10% of it is normally and constantly replaced comprising two types of cells, osteoblasts (the bone forming cells) and osteoclasts (the bone-resorbing cells). These two cells are involved in a vital process called, bone remodeling. Bone remodeling is a dynamic, lifelong process in which old bone is removed from the skeleton and new bone is added. A variety of biochemical assays that reflect the activity of osteoblasts and osteoclasts have been developed for clinical use. They have helped increase our understanding of the bone remodeling cycle the pathogenesis of skeletal disorders, and the response of these disorders to therapy. However some regulatory mechanisms are working in order to maintain healthy bone on different levels. Summarizing that bone holds an outstanding and striking intrinsic capacity to regenerate.

Copy Right, IJAR, 2015,. All rights reserved

INTRODUCTION

1- The structural Organization of Bone:

Bone is metabolically active organ where 10% of it is normally and constantly replaced. Bone constitutes the trabecular or spongy bone (25%) and cortical or compact bone (75%). Trabecular bone is metabolically active and forms the internal supportive elements of the bone. The outer capsule is made up of cortical bone and is particularly present in the shafts of long bones. At the structural level, two different forms of bone can be distinguished, i.e. cortical or compact bone– which forms the diaphysis of long bones and provides protection for the medullary cavity– and trabecular, cancellous, or spongy bone, which is found at the ends of the long bones (epiphyses). The total skeleton comprises about 20% of trabecular bone. Trabecular bone has a porosity of 50%–90%, while cortical bone of 10% (1).

The high surface-to-volume ratio of the trabecular bone involves its metabolic function, whereas the cortical bone has mainly a structural and protective role. The outer bone surface is in contact with the surrounding soft tissue *via* the periosteum. The inner bone surface faces the medullary cavity and is covered with the endosteum. Both the

periosteum and endosteum are connective tissues organized in layers. The cambium layer is in intimate contact with the mineralized structure and provides a pool of mesenchymal cells with the potential to differentiate into the chondrogenic and the osteogenic lineage. Trabecular bone and cortical bone are composed of the same microstructural elements: **cells**, **organic matrix**, **crystalline inorganic matrix** and **soluble factors** (2).

The calcified matrix of adult bone is built up in multiple layers of oriented collagen fibres, giving rise to the typical structure of lamellar bone. The lamellae are arranged parallel to each other, as on the surface of flat bones and trabecular structures, or in concentric layers around blood vessels and nerves, forming the Haversian system. The Haversian canals are connected to the periosteum and the endosteum by Volkmann's canals. Blood flow in bone totals 200–400 ml/min in adult humans, indicating that bone is a highly vascularized tissue. Under conditions in which rapid formation of new bone is required, e.g. during skeletal growth in early childhood and in periods of bone regeneration, or in particular metabolic bone disorders, instead of lamellar bone, immature "woven" bone is formed, in which the collagen fibres are randomly oriented and the degree of mineralization is relatively low. Woven bone is later remodelled into lamellar bone, which has better mechanical properties. Formation, remodelling, and repair of the structural elements require the coordinated action of the bone cells: osteoblasts, osteocytes, lining cells, and osteoclasts (3, 4, 5).

Bone strength is defined by the parameters mass, geometry, material properties, and microstructure. The bone mass accounts for about half of the decrease in bone strength in the elderly. The diameter of the corticalis is a parameter of bone geometry. Material properties are modulated by the mineral crystals and the correct synthesis of the single components of the bone matrix. The diameter of the trabeculae as well their horizontal and vertical interconnectivity are defined as bone strength (6, 7).

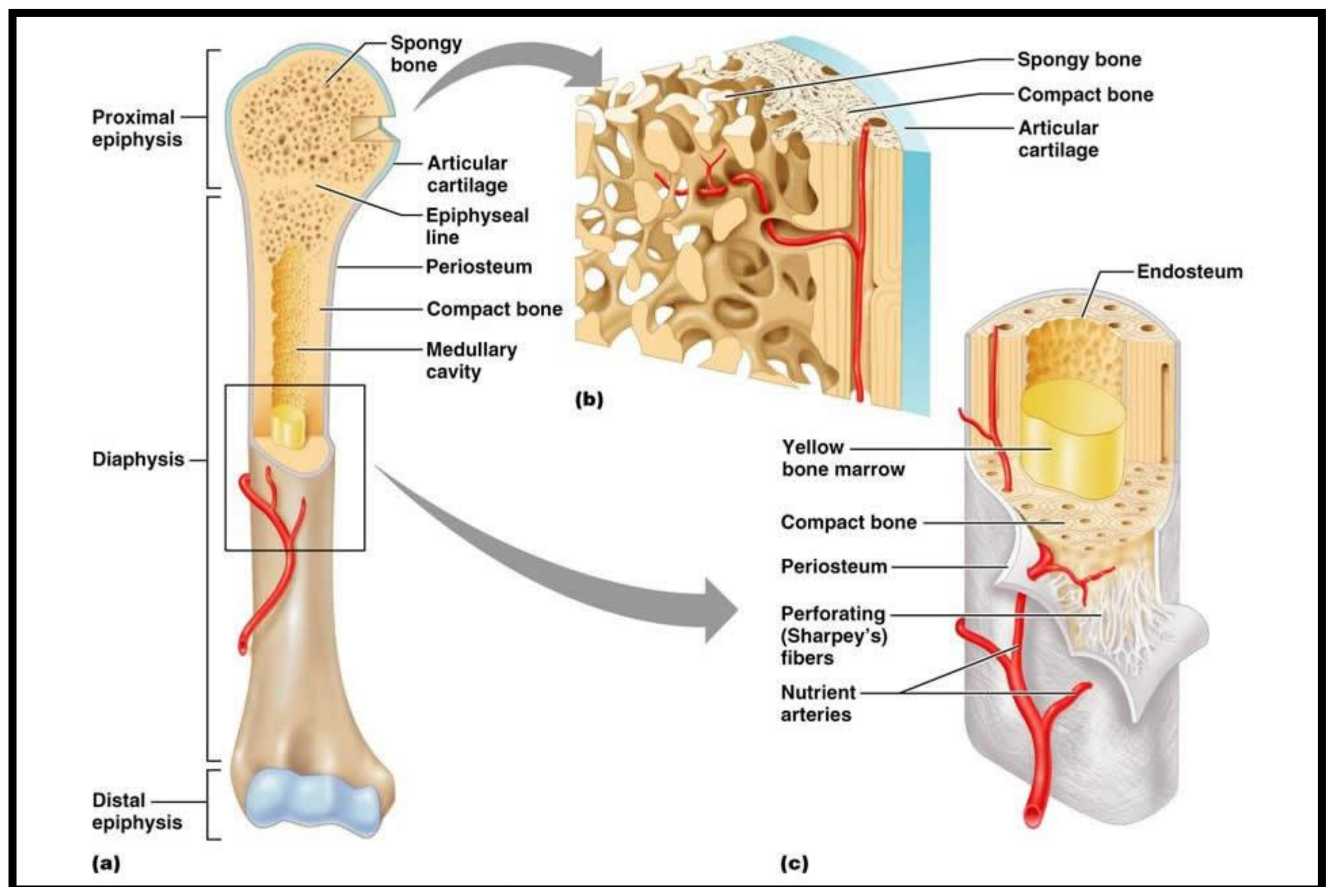


Fig. 1: Structure of bone: Cortical bone together with its vascular system surrounds the trabecular network (Bilezikian, 2008).

1.1**-** The Bone Cells**:**

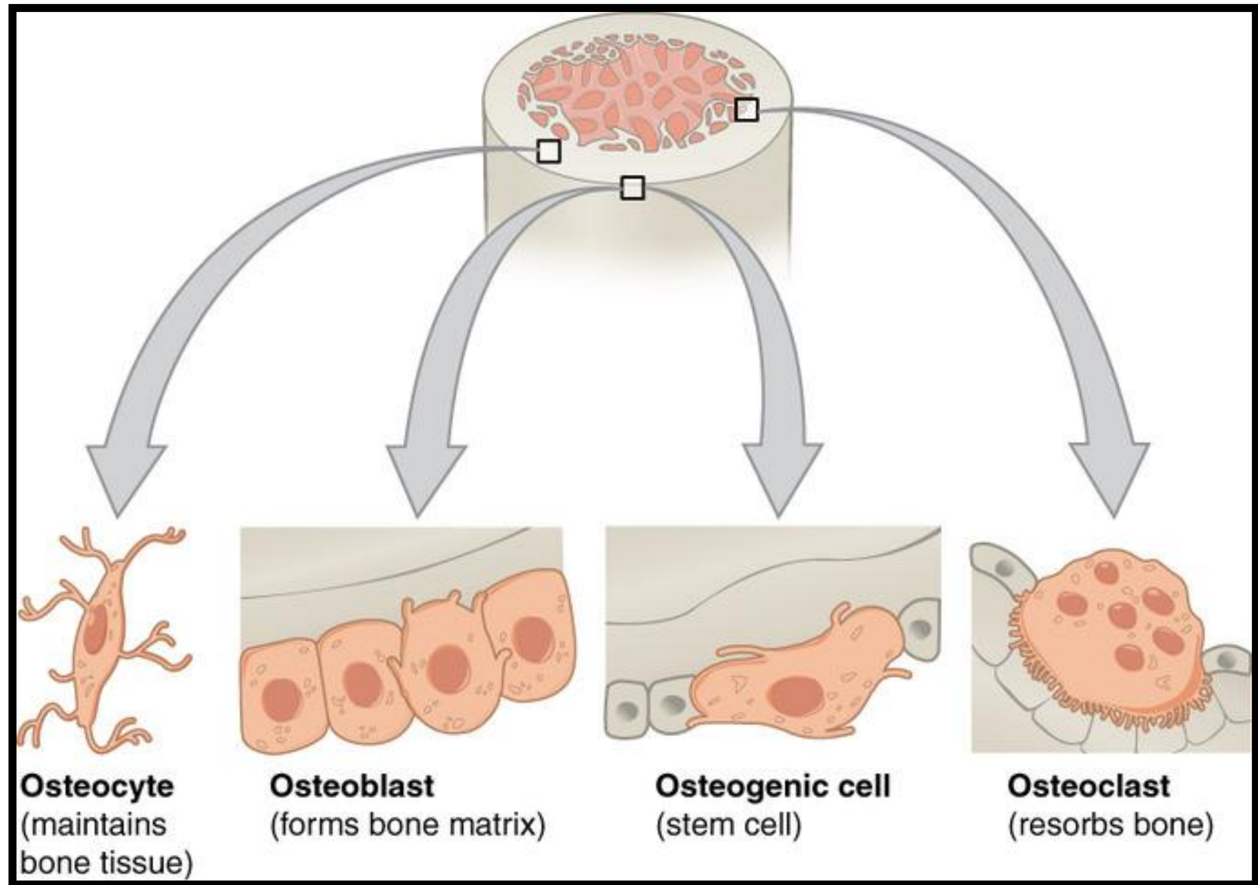
The osteocytes are cells that are embedded in the mineralized matrix of both woven and lamellar bone. They emerge at the end stage of osteoblast differentiation and become trapped within the mineralized matrix. The osteocytes reside singly in small lacunae but are connected to neighboring cells through cytoplasmic protrusions, which form a dense network of canaliculi. This syncytium permits direct communication between neighbouring osteocytes, as well as with lining cells and osteoblasts on the bone surface. Nutrition and oxygen transport within this network is limited to the diffusion distance, which is approximately 100 μm which is equivalent to the wall thickness of trabecular structures and the outer diameter of an osteon (8).

The osteocytes probably act as mechanosensors that signal the need for bone remodelling for adapting the bone to functional load according to Wolff's law (8) and remodelling for repairing microstructural changes within the bone matrix. The osteocytes can detect changes in the levels of hormones, such as oestrogen and glucocorticoids that influence their survival rate. Since the osteocytes form a network spanning the skeletal system, they may play─ through their residual metabolic activity─ a role in bone turnover (9, 5, 10, 11).

The osteoblasts are mesenchymal cells located on the surface of the mineralized matrix and are responsible for formation of new bone; i.e., they synthesize and regulate the deposition and mineralization of the extracellular matrix. The osteoblasts form a dense monolayer clustering at each bone-forming site. They exhibit a prominent Golgi apparatus and a well-developed rough endoplasmic reticulum. The osteoblasts secrete mainly type I collagen as well as a large number of non-collagenous proteins. The layer of unmineralized bone matrix, termed osteoid, serves as a template for the initiation and propagation of mineralization. During development and the early stages of bone regeneration, woven bone is produced by pinching of matrix vesicles from osteoblasts similar to mineralized hypertrophic cartilage (12).

The osteoblasts lay down lamellar bone onto the previously- formed woven bone (13). After ceasing matrix-forming activity, the osteoblasts can undergo apoptosis, or remain in inactive form as so called lining cells on the bone surface (5). In addition, endosteal osteoblasts provide the microenvironment, or niches, for haematopoietic stem cells, which give rise to cells of the myeloid lineage, including osteoclasts (14).

The osteoclasts are large polykaryon cells containing between 3 and 30 nuclei, and are considered to be the exclusive bone-resorbing cells. This cell type contains large numbers of lysosomes, mitochondria, and an extensive Golgi complex. The osteoclasts are located at bone surfaces within Howship's lacunae, also called resorption lacunae. Under normal conditions, the osteoclasts are rarely found in bone, but they appear in increased numbers at sites of high bone turnover, such as in the metaphysis of growing bone or in trabecular bone in postmenopausal osteoporosis. A characteristic feature of active osteoclasts, which distinguishes them from polynuclear macrophages, is the development of a ruffled border surrounded by an organelle-free area, termed the "sealing zone". Through the finger-like structures of the ruffled border, the osteoclasts secrete proteolytic enzymes, such as cathepsin K and matrix metalloproteinase nine, as well as hydrogen ion. The acidic pH of approximately 4.5 provides the environment for the mobilization of hydroxyapatite and optimal conditions for the proteolytic enzymes to degrade the bone matrix. The degraded matrix components are released in the periosteoclastic environment by transcytosis (15, 16, 17, 5)**.**



(Fig. 2): Schematic diagram of different types of bone cells (Bilezikian, 2008).

1.2- The Organic Matrix:

The organic matrix contains approximately 90% type I collagen; the remaining 10% consist of non-collagenous components. Type I collagen serves as the main structural element which has the ability to bind a large number of non-collagenous proteins. Type I collagen is composed of two α_1 chains and one α_2 chain, which share a common amino acid sequence consisting of Glycine (Gly-X-Y repeats). X represents a proline residue, Y is in most cases modified by a post-translational reaction into hydroxyproline; the latter is essential for stabilization of the triple-helical structure. Before the procollagen molecules assemble to form a triple-helical structure, the N- and C-terminal propeptides are removed by proteolytic cleavage and serve as metabolic markers of bone formation. The single triple-helical structures are interconnected by the formation of covalent cross-links. The corresponding cleavage products, pyridinoline and deoxypyridinoline, as well as the not completely digested N- and C-terminal telopeptides, are metabolic markers of bone resorption (18, 19).

Proteoglycans and glycoproteins are the non-collagenous components of the organic bone matrix. Proteoglycans are large macromolecules composed of glycosaminoglycans (GAGs) covalently linked to a core protein from which the molecule derives its name, e.g. decorin, biglycan, versican and fibromodulin. Each GAG, such as chondroitin sulphate or heparan sulphate, is made up of repeating disaccharide units that contain a sulphated amino sugar. Proteoglycans not only serve as anchoring sites for collagen fibres, but also contain binding sites for growth factors (20). Gene deletion of biglycan in mice causes a phenotype characterized by a reduced growth rate and decreased bone mass (21).

Glycoproteins, which are produced at different stages of osteoblast maturation, are post-translationally modified proteins with N- or O-linked oligosaccharides. Thrombospondin, fibronectin, osteopontin, and bone sialoprotein are glycoproteins which constitute binding domains for cell membrane receptors of the integrin class. Osteocalcin and

matrix gamma-carboxyglutamic acid (Gla) protein are characterized by their content of multiple γ-carboxy glutamic acid residues which exhibit high affinity for mineral ions such as Ca^{2+}. Mice deficient in osteocalcin show a higher bone mass when is compared to wild-type animals (22). Mice deficient in matrix (Gla) protein are normal at birth, but develop severe calcification of their arteries (23). It seems likely that both glycoproteins are involved in the regulation of matrix mineralization. The expression of the genes coding for matrix molecules is linked to different stages of osteogenic differentiation (24).

The extracellular matrix is also an important storage pool of growth factors that are believed to play a role during bone remodelling (20).

1.3- Crystalline inorganic matrix:
The inorganic part of bone is made of calcium hydroxyapatite (HA) which has a chemical formula of $Ca_{10}(PO_4)_6(OH)_2$; it is mostly responsible for the stiffness of bone material. The plate-shaped apatite crystals include some impurities, mostly of carbonate, which increase the solubility of bone. The solubility is important for mineral homeostasis, and thus for bone adaptation to exercise loading among other things (25, 26).

1.4- Soluble factors:
Polypeptide growth factors appear to play an important role in the development and growth of osseous tissue. Polypeptide growth factor effects on bone are necessarily divided into two areas of study: exogenously- produced endocrine factors, which act on specific bone target cells, and endogenously- produced “local” factors with possible autocrine or paracrine action. Known growth factors of extraosseous origin have been investigated for their effects on bone in organ culture (27). Examples of exogenous factors, epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and insulin-like growth factor (IGF-1) generally stimulate cellular proliferation. EGF and FGF depress formation of the collagenous extracellular matrix, whereas PDGF and IGF-1 (somatomedin-C) stimulate collagen synthesis (27). EGF, PDGF, and transforming growth factors (TGF-a, TGF-8) may also play a role in bone resorption, as they stimulate calcium release by a prostaglandin-mediated mechanism (28).

Examples of Endogenous or local growth factors, bone-derived growth factor, transforming growth factor beta (TGF-β), cationic cartilage-derived growth factor (CDGF), skeletal growth factor (hSGF), bone morphogenetic protein. Other local factors affecting bone cells include interleukin-1 (29), macrophage-derived factors (30, 31), and an endothelial cell-derived growth factor (32).

2- Bone Remodelling:
Bone remodelling is a dynamic, lifelong process in which old bone is removed from the skeleton and new bone is added. It consists of two distinct stages – resorption and formation – that involve the activity of special cells called osteoclasts and osteoblasts (33).

Usually, the removal and formation of bone are in balance and maintain skeletal strength and integrity. The bone turnover is a cyclic process of destruction and reconditioning of bone as a result of the concerted effects of osteoclasts (bone-resorbing cells) and osteoblasts (bone-forming cells) (33).

The objective is to replace microdamage and to adapt bone shape and density to patterns of usage and the attendant forces exerted. These cell types occur in areas referred to as bone remodelling units (BMUs) (33).

Bone remodelling is the result of the coordinated action of bone-resorbing osteoclasts and bone-forming osteoblasts. The osteoblasts and osteoclasts interact within a spatial structure known as the basic multicellular unit (BMU) in cortical bone and in analogy, bone structural units in trabecular bone. BMUs are functional in the developing and growing skeleton during the process of modelling and during remodelling of mature bone. Histological examinations of BMUs demonstrate that osteoclasts excavate a resorption canal in the corticalis and a cavity in the trabecular structures (Fig. 3a). It is hypothesized that growth factors released from the bone matrix and the osteoclasts can attract mesenchymal progenitor cells provided by the blood capillaries and stimulate their differentiation into functional osteoblasts (34).

The osteoblasts deposit osteoid onto the previously-resorbed channel or cavity that is later mineralized (Fig. 3b, 3c, 3d). In cortical bone, the resorption canal is continuously filled with layers of lamellar bone with a blood vessel

remaining in the middle. The end-stage product is the secondary osteon. An estimated one million BMUs exist at any time in the adult skeleton. The life span of a BMU is much longer than the life span of osteoblasts and osteoclasts. Hence, to replace old cells, their respective progenitors have to be recruited and caused to differentiate into their mature phenotypes. Potential sources of progenitor cells are the blood capillaries, centrally located within each BMU. The osteoblast progenitors can originate from pericytes, covering the outer basal membrane of the capillaries, and from a subpopulation of circulating mononucleated cells (35, 36).

The osteoclasts arise from circulating cells of the monocytic lineage, which possibly receive a specific "area code" of adhesion molecules expressed by the endothelial cells (37, 38, 39, 40).

The birth, life, and death of osteoblasts and osteoclasts within the BMU is fundamental to understand the pathophysiology of skeletal diseases such as osteoporosis (40). Osteoblasts, osteoclasts, and their respective progenitors are exposed to a variety of systemic hormones and local factors that regulate the tight balance of bone remodelling. Systemic hormones are brought into the BMU by means of blood capillaries and cells release local factors in an autocrine/paracrine mode of action. Local and systemic factors can influence the activation frequency, which defines the number of BMUs at a given time point, in addition to the remodelling balance of the cells within each BMU (33).

The primary cells partaking in the formation of bone include the osteoclasts, derived from blood (haematopoietic) stem-cell precursors and osteoblasts which differentiate from stromal-cell precursors. The role of osteoblasts is to manufacture a complex extra-cellular matrix, which is capable of undergoing mineralization. Bone strength is a combination of the amount of bone, bone structure, and other aspects of bone quality, which include localized material properties, non-mineralized matrix proteins, and bone turnover (41).

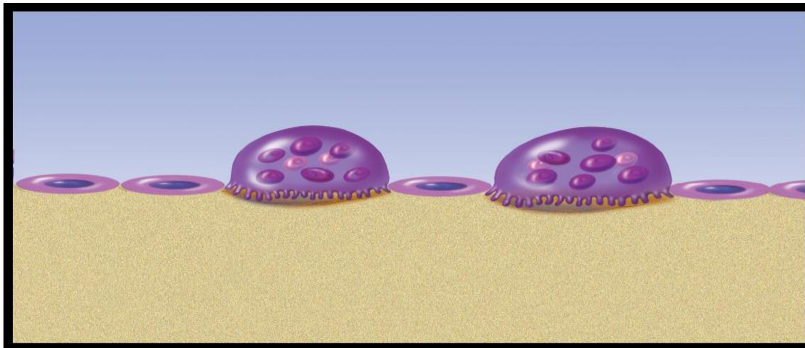


Fig. 3.a: Cells that break down bone – called osteoclasts – act on the trabecular bone surface to erode the minerals and matrix (*Swaminathan*, 2001).

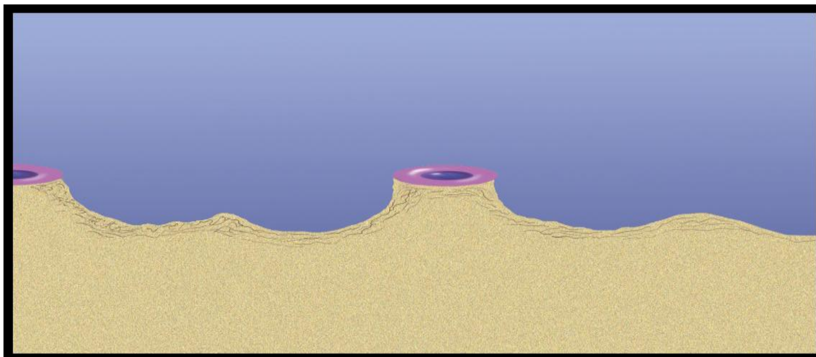


Fig. 3.b: This phase is complete when small cavities are created on the surface of the trabecular bone (bone has been removed) (*Swaminathan*, 2001).

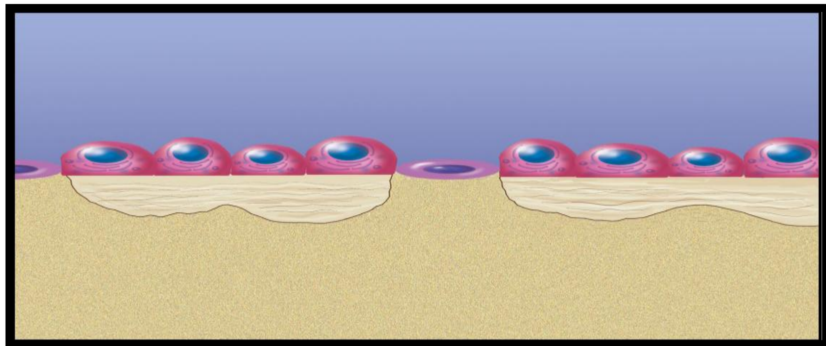
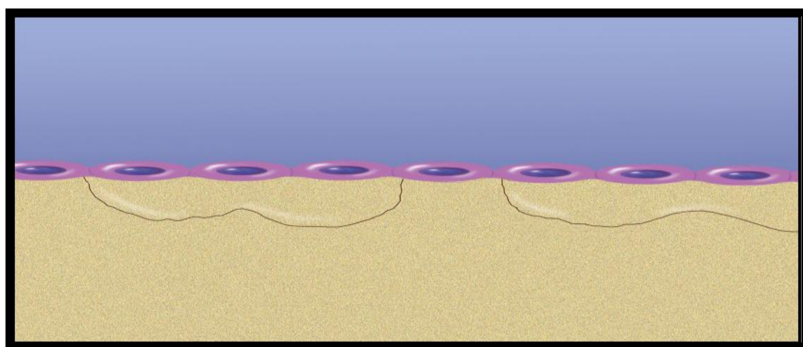


Fig. 3.c: Cells that form new bone – called osteoblasts – work to repair the surface and fill the eroded cavities with new bone that then has to be mineralized (calcified) (*Swaminathan*, 2001).



(Fig. 3.d): The bone surface is restored and covered with a layer of protective bone cells called lining cells. The new bone is calcified and the remodelling process is completed (*Swaminathan*, 2001).

Changes in the bone remodelling rate can be monitored by biochemical parameters. Bone mineral density imaging with Dual X-ray absorptiometry (DXA) provides the current definition of osteoporosis by the world health organization WHO. However, biochemical parameters and DXA's usefulness for making predictions about the fracture risk is limited (42).

High-resolution imaging techniques such as computed tomography (CT) and magnet resonance imaging (MRI) that take the three-dimensional bone architecture into account are evaluated to assess the fracture risk. Moreover, finite element analysis comprises mathematical models where the 3D structural information provides the basis for calculating how much load bone can bear (43).

2.1- Biochemical markers of bone remodelling**:**

A variety of biochemical assays that reflect the activity of osteoblasts (the bone forming cells) and osteoclasts (the bone-resorbing cells) have been developed for clinical use. They have helped increase our understanding of the bone remodelling cycle, the pathogenesis of skeletal disorders, and the response of these disorders to therapy (44, 45, 46)**.**

Biomarkers of bone formation and resorption reflect the overall osteoblastic and osteoclastic activity in the skeleton and in some situations may serve as surrogates for histologic examination of bone (47).

2.1.1- Biochemical markers of bone formation:

Markers of bone formation are measured in serum. Some are enzymes or other proteins secreted by osteoblasts; others are byproducts of type I collagen deposition (48).

The osteoblasts are mononuclear cells that attach to bone surfaces and form new bone, most commonly at sites that recently underwent resorption. They produce type I collagen and other matrix components of osteoid, and they also mineralize the osteoid with hydroxyapatite (49).

Table "1": Showing the biochemical markers of **bone formation**:

S. NO	MARKER	COMMENTS
1	**Alkaline Phosphatase**	Bone-specific alkaline phosphatase (bAP) is a constituent of osteoblast membrane. Its principal role is phosphate hydrolysis, which permits growth of hydroxyapatite crystals (49).
2	**Osteoclacin (OC)**	Sensitive and specific marker of bone formation. OC is synthesized by osteoblasts and odontoblasts and is incorporated directly into bone matrix, but some circulates in blood (49).
3	**Procollagen I extension peptides**	N-terminal (called PINPs) and C-terminal (called PICPs) extension peptides are cleaved during the extracellular processing of type I collagen, prior to fibril formation. Procollagen I carboxy-terminal peptide can be measured in plasma and correlates with growth velocity and with bone mineral acquisition. The level of each of the propeptides in blood is thought to reflect the amount of newly-synthesized collagen (50).

2.1.2- Biochemical markers of bone resorption

Markers of bone resorption are measured in serum or urine. The most direct indicators are fragments of bone collagen produced by osteoclast activity (48).

The osteoclasts are multinucleated cells that resorb bone. They initiate bone remodelling and help shape the growing bone and so are more numerous in children.

Table "2" showing the biochemical markers of **bone resorption:**

S. NO	MARKER	COMMENT
1	**Tartrate-resistant acid phosphatase**	Enzyme present in the osteoclast and released during osteoclastic activity; however serum TRAP is not bone-specific (51).
2	**Hydroxyproline (HP)**	HP is a product of post-translational hydroxylation of proline in the procollagen chain and reflects bone resorption. HP is an amino acid common to– and characteristic of– all forms of collagen, and urinary hydroxyproline excretion is the oldest test of bone resorption. However, this test lacks specificity for bone resorption because excreted hydroxyproline also comes from other tissues, particularly from skin collagen (which can turn over rapidly in certain disorders), from newly synthesized collagen that is not incorporated into tissue, and from dietary collagen and gelatin. Because it is less specific than newer tests, it is no longer widely used (52).
3	**Collagen pyridinium cross-links (more specific markers)**	Pyridinoline (Pyr) and deoxypyridinoline (Depyr) are generated from hydroxylysine during post-translational modification of collagen. (Pyr) and (Dpyr) are released during matrix resorption and are excreted in urine. (Dpyr) is more specific for bone (44) .Pyridinolines are cross-linking amino acids that strengthen collagen fibrils in the extracellular matrix. They are found in the main fibril-forming collagens (types I, II, and III) of many tissues. Pyridinoline is the major chemical form, but deoxypyridinoline is also unusually abundant in bone collagen and hence is a relatively selective bone marker. Since pyridinolines are not metabolized and are largely excreted as small peptides when produced by osteoclastic bone resorption. Immunoassays have been developed that selectively measure cross-link-containing peptide fragments in urine and serum. The first was an assay that recognizes N-telopeptide of collagen type I (NTx) in urine (53) and serum (50). The recognized feature in this

		sequence is fully generated during the process of osteoclastic proteolysis and so requires no further metabolism by the liver or kidney for its production. Results from second-morning urine collections correlate well with those from 24-hour collections, which simplifies patient evaluation. Several other assays target structural variants of a peptide sequence that originates from the carboxy-terminal cross-linking region of collagen type I (CTx) (54, 55)
4	**Cathepsin-K enzyme**	This is of interest because it is the primary proteolytic enzyme used by osteoclasts to degrade bone type I collagen during resorption. Several studies suggest it may be valuable as a marker of bone resorption (56), but more studies are required to evaluate its performance relative to established bone resorption markers.
5	**Receptor activator of nuclear factor kappa *(RANK)*, *RANK* ligand, and its decoy receptor osteoprotegerin**	These are the pivotal regulators of osteoclast recruitment and activity (57). They may eventually be used as markers of bone metabolism, though the broad role of *RANK* ligand signalling in the immune system may limit its specificity.

3- Bone Regeneration:

Bone holds the intrinsic capacity to regenerate without the formation of a scar. The basic mechanisms of bone regeneration are very similar to those during bone development, except that early stages are linked with an inflammatory response. Bone injury− e.g. fractures, reconstruction surgery, placement of dental or orthopedic implants and tooth extraction, blood vessel disruption − immediately leads to the development of a blood clot that fills the defect site with a provisional fibrin-rich extracellular matrix. Accumulating platelets and the immigrating neutrophils and macrophages provide a source of growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and a broad spectrum of other bioactive molecules that target mesenchymal progenitor cells with their osteochondrogenic potential. Blood capillaries sprout into the blood clot by a process termed angiogenesis (58, 59). In parallel, endothelial progenitor cells that originate from the bone marrow are transported *via* the blood stream to the site of blood vessel formation where they contribute to capillary sprouting. This process is termed vasculogenesis (60).

Once the blood clot is replaced by the blood vessel-rich granulation tissue, differentiation of mesenchymal progenitor cells into functional osteoblasts is initiated, Low oxygen tension and the instability of a fracture, however, favour the differentiation of mesenchymal progenitor cells into the chondrogenic lineage. Cartilaginous tissue, which stabilizes the defect site, is then replaced by bone *via* endochondral ossification. Lamellar bone is laid appositionally onto immature woven- and pre-existing bone, as well as onto osteoconductive surfaces of implants and grafting materials (4; 61, 62).

Bone regeneration also involves the process of adaptive modelling and remodelling. This mechanism is based on the sensing of mechanical extension and mechano-transduction. Conversion of mechanical forces into cellular response involves activation of intercellular signals, which are induced by stretched cytoskeletal molecules and piezoelectric potentials. (8, 63).

4- Regulatory Mechanisms of Development and Function of bone (Fig. 4):

Bone development, remodelling and regeneration basically depend on the same elements: mesenchymal progenitor cells that differentiate into osteoblasts or chondrocytes, haematopoietic progenitors that give rise to osteoclasts, local clues that attract the cells and control their proliferation and differentiation into the mature phenotypes, and a provisional matrix, which defines the area for these dynamic processes.

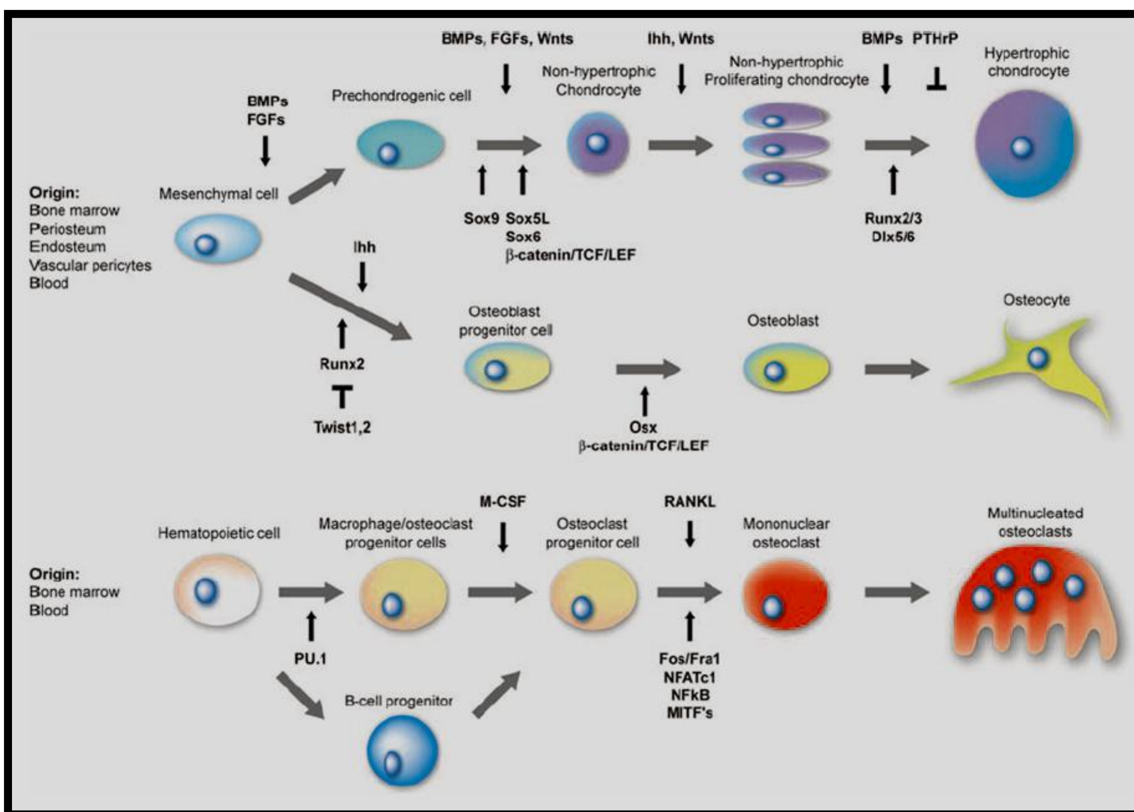


Fig. 4: Bone formation during development, remodelling and repair (Karsenty and Wagner, 2002).

3.4.1- Regulatory Mechanisms of Chondrocytes:

Chondrocyte proliferation is positively regulated by the parathyroid hormone-related protein (PTHrP)/Indian Hedgehog (*Ihh*) feedbackloop. PTHrP is produced by cells of the periarticular perichondrium and by early stages of proliferating chondrocytes and acts as a mitogen for late proliferating and prehypertrophic chondrocytes. With increasing distance from the PTHrP source, the mitogenic activity fades and prehypertrophic chondrocytes can develop. Prehypertrophic chondrocytes are the main source of the soluble factor Ihh, which stimulates the production of PTHrP by an unknown mechanism. Ihh is also a mitogen for proliferating chondrocytes directly and through BMP signalling. Thus, PTHrP expression regulated by Ihh can control the length of the zone of proliferating chondrocytes and their transition into the prehypertrophic phenotype. Moreover, mice without a functional *Ihh* gene lack the bony collar because *Ihh* regulates the master gene of osteogenic differentiation, the transcription factors Runx-2 (64). Ihh is not required for osteoblastogenesis in bones formed through intramembranous ossification. Runx-2 is further expressed by chondrocyte condensations, prehypertrophic and hypertrophic chondrocytes. The transcription factor drives differentiation into hypertrophic chondrocytes. Even though in some growth plates hypertrophic chondrocytes can be detected in the absence of functional Runx-2; they fail to be mineralized (65, 66, 67).

Bone morphogenetic proteins (BMPs) belong to the transforming growth factor (TGF) superfamily. At least 15 different BMPs have been characterized at the molecular level. During development, BMPs are expressed within mesenchymal condensations (BMP-5), in the perichondrium (BMP-2, BMP-4, BMP-5, BMP-7), and in the future zones of joint formation growth and differentiation factor-5 (GDF-5). BMP-7 is expressed in proliferating chondrocytes and BMP-2 and BMP-6 are found in hypertrophic chondrocytes. Overall, BMPs are mitogenic for chondrocytes and negatively regulate their terminal differentiation (68,69, 70).

BMPs bind to serine-threonine receptors termed BMP-receptor type IA or IB, each of which forms a heterodimer with a type II receptor. BMPRIA controls the pace of chondrocyte proliferation, whereas BMPRIB is involved in

cartilage formation and apoptosis (71). At the extracellular level, the biologic activity of BMPs is modulated by a number of antagonists known as noggin, chordin, gremlin, and follistatin. Of these, noggin and chordin have been shown to bind to BMP-2 and BMP-4 with high affinity, and noggin-null mutations indicate the involvement of this molecule in patterning the neural tube, somites, and cartilage. Overexpression of noggin in the osteoblastic lineage causes a decreased trabecular bone volume and impaired osteoblastic function (72, 73). During the postnatal phase, BMPs and their corresponding receptors are expressed during fracture repair (74, 75, 65, 76, 66, 67).

Once the chondrocytes have undergone hypertrophy, they express the key angiogenic molecule vascular endothelial growth factor (VEGF) in response to transcriptional activation by hypoxia-inducible factor-1. Conditional knock out of VEGF in chondrocytes results in delayed blood vessel invasion. Matrix metalloproteinase-9 (MMP-9)− highly expressed in chondroclasts and osteoclasts− is involved in the release of VEGF from the mineralized extracellular matrix. Interestingly, *Runx-2* is required for vascular invasion, *via* regulation of VEGF expression. VEGF also plays a key role in fracture healing (65, 77, 66, 67).

The transcription factor *Sox-9*, which has a high mobility-group (HMG)-box DNA-binding domain, is required for the transition of mesenchymal progenitors into chondrocytes and their condensation within the skeletal anlagen, as concluded from mouse models and genetic studies of humans. In addition, chondrocytes in *Sox-5* and *Sox-6* double knock-out mice failed to undergo hypertrophy, and consequently columnar organization of the growth plate is affected (65, 66, 67).

Genetic studies further suggest the involvement of the functional axis fibroblast growth factor (FGF) - 18/FGF receptor 3 (FGFR-3) in chondrocyte proliferation. Constitutive activation of the FGFR-3 tyrosine kinase receptor causes the virtual absence of prehypertrophic chondrocytes, whereas gene deletion models show larger zones of proliferating chondrocytes. *FGF-18* produced by the perichondrium binds to FGFR-3 on proliferating chondrocytes. *FGF-18* gene ablation similarly results in larger zones of proliferating chondrocytes, suggesting that *FGF-18* is the predicted ligand for FGFR-3, which inhibits chondrocyte proliferation (65, 66, 67).

4.2- Regulatory Mechanisms of Osteoblasts:

Runx-2, also termed CBFA1/PEBP2αA/AML2, encodes a member of the (runt) family of transcription factors whose expression precedes the appearance of osteoblasts. Null mutation causes the formation of a cartilaginous skeleton without any evidence of bone (78, 79). Heterozygote mutations of *Runx-2* have been detected in mice and humans, correlating with the phenotype of cleidocranial dysplasia, hypoplastic clavicles and nasal bones and retarded ossification of parietal, interparietal, and supraoccipital bones (80, 81, 79).

Runx-2 is also important for the continuing activity of osteoblasts in adults. In addition, the transcription factor plays a role in the transition of proliferating chondrocytes into pre- and hypertrophic chondrocytes (66, 82). *Runx-2* activity is transiently inhibited at the posttranscriptional levels by Twist proteins, basic helix-loop-helix transcription factors, which are expressed in *Runx2*-positive cells throughout the skeleton early in development. The interaction between a C-terminal region of Twist and the Runt domain of *Runx-2* prevents DNA binding and gene activation (83).

Runx-2 activity is further modulated by co-activator core binding factor β (CBFβ), and other transcription factors such as the Smads, Rb, and MAP kinases and co-repressors (84, 85, 86). Among the factors that control *Runx-2* transcription are BMPs and Ihh (87, 64). Moreover, BMPs and *Runx-2* are also involved in the transition of mesenchymal cells into a phenotype with the potential to support osteoclastogenesis (88, 89, 90).

Another transcription factor that controls osteoblast fate is called osterix (91). Osterix knockout animals express *Runx-2* but not vice-versa, suggesting that osterix acts downstream in the signalling cascade. Osterix-deficient mice show all stages of cartilage development up to mineralized hypertrophic chondrocytes. The catabolic effects of immunosuppression on bone metabolism may involve the decreased nuclear factor of activated T (NFAT) that is required to form a complex with osterix to activate target genes (92).

Distal-less homeobox 5 *(Dlx5)*, msh homeobox homologue 2 *(Msx2)* and members of the Fos family are additional transcription factors that regulate bone development (65, 93). Signalling *via* the Wnt pathway is also involved in the regulation of bone remodelling. Wnt ligands bind to a receptor complex composed of low-density lipoprotein receptor protein (LRP) 5 and LRP6, and a member of the frizzled family of seven transmembrane-spanning proteins.

Activation of the frizzled receptor complex causes stabilization of intracellular β-catenin by inhibition of its phosphorylation. Unphosphorylated β-catenin accumulates in the cytoplasm and translocates to the nucleus where it associates with LEF/TCF transcription factors and controls gene transcription. Phosphorylated β-catenin is rapidly degraded. Heterozygous gain-of function mutations in the gene coding for LRP5 are associated with a high bone mass (94, 95).

The mutation reduced the affinity of LRB5 for the inhibitor Dickkopf-1 (96). Loss of function mutation causes the osteoporosis pseudoglioma syndrome, which is characterized by low bone density (97). Both phenotypes could be confirmed in mouse models (97). Genetic mouse models further indicate that β-catenin is essential in determining that mesenchymal progenitors become osteoblasts and not chondrocytes, regardless of regional locations or ossification mechanisms (98, 99).

Animal models based on stabilization and destabilization of β-catenin in osteoblasts showed that these mutations primarily affect bone resorption rather than bone formation (100). Whether modulation of Wnt signalling, for example by blocking binding of Dkk-1 to LRP-5, will be a therapeutic opportunity in osteoporosis remains to be determined (93, 101, 102).

Sclerostin is an inhibitor of osteoblast function, causes generalized progressive bone overgrowth termed osteosclerosis. Sclerostin binds LRP5 and 6 and inhibits canonical Wnt signalling, suggesting that increased Wnt signalling is linked with osteosclerosis (103, 104). PTH can inhibit sclerostin transcription (105, 106). Its unique expression by osteocytes and action on osteoblasts suggest that sclerostin may be an osteocyte-derived factor that is transported to osteoblasts at the bone surface and modulates bone formation (107).

4.3- Regulatory Mechanisms of Osteoclast:

Osteotropic hormones such as 1, 25-dihydroxyvitamin D3 [1, 25(OH)2D3] and PTH, and local factors such as prostaglandins or cytokines [interleukin (IL)-1, IL-6, IL-11, tumour necrosis factor (TNF)] can induce the expression of a membrane-associated osteoclast differentiation factor in osteoblasts/stromal cells. Osteoclast precursors recognize this factor through cell/cell interaction, thereby receive the signal necessary for differentiation into mature osteoclasts (108).

Factors that are involved in osteoclast differentiation are members of the TNF/TNF receptor superfamily; the receptor activator of nuclear factor-kB *(RANK)*, the RANK ligand *(RANKL)*, and osteoprotegerin (OPG) (109, 110). *RANK* is expressed on mononuclear osteoclast precursors of the monocyte/macrophage lineage, while *RANKL*, which is identical with the fore-mentioned osteoclast differentiation factor, is expressed on stromal/osteoblast cells as well as on activated T lymphocytes.

RANK/RANKL interaction and the presence of macrophage colony-stimulating factor (M-CSF) are sufficient to induce the formation of osteoclasts, and are also important for their activation and survival. *OPG*− a soluble member of the TNF-receptor superfamily− acts as a decay receptor that competes with *RANK* for *RANKL* (Fig. 4). This explains why overexpression of *OPG* in mice leads to osteopetrosis, whereas *OPG* knockout mice develop severe osteoporosis. This illustrates the importance of the balance between *RANKL* and *OPG* in the control of bone remodelling.

Other synonyms used in the literature for *RANKL* are osteoclast differentiation factor (ODF), osteoprotegerin ligand (OPG-L) and TNF related activation-induced cytokine (TRANCE). The decoy receptor *OPG* is also known as osteoclastogenesis inhibitory factor (OCIF) and TNF receptor like molecule 1 (TR1) (109, 17, 110). *RANKL*, *RANK* and *OPG*, although considered the bottleneck of osteoclastogenesis, represent approximately one-tenth of genes and loci that can control osteoclastogenesis and activation (110).

On the basis of knockout models, it is possible to delineate a genetic cascade that controls osteoclast differentiation. The *PU.1* gene apparently acts at the earliest stages in this cascade, since mice with a loss of function mutation lack both osteoclasts and macrophages. *PU.1* is suggested to regulate the expression of the M-CSF receptor on cells of the monocyte/macrophage lineage (111). Mice with a severe defect in their *M-CSF* gene (*op/op* mice) generate only immature macrophages and are deficient in osteoclasts (112).

The next steps of osteoclast differentiation are impaired by the absence of cytoplasmic tumour necrosis factor receptor-associated factor (TRAF) - 2, -5, and -6, which interact with the intercellular domain of RANK. Among the six major downstream signalling pathways are NFATc1, NF-kB, Akt/PKB, and the three mitogen-activated protein kinases (MAPK): extracellular signal-regulated kinase (ERK), p38, and c-Jun NH (2)-terminal kinase (JNK), which plays distinct roles in osteoclast differentiation, function and survival.

Intracellular signalling pathways can be modulated by interferon (IFN)-γ, which promotes degradation of TRAF-6, and IFN-β which down-regulates one of the master genes of osteoclastogenesis, *c-fos*. Activation of the co-stimulatory receptors Fc receptor common gamma chain (FcRγ) and DNAX-activating protein 12 (DAP12), both of which harbor the immune-receptor tyrosine-based activation motif (ITAM), can activate calcium signalling, thereby mediating activation of NFATc1 (113).

Mice in which the genes for either $\alpha_V\beta_3$ integrin or the tyrosine kinase c-Src were knocked out generate differentiated but functionally-impaired osteoclasts. Finally, failure to produce functional proteins such as H^+-ATPase, carboanhydrase II, or cathepsin K renders osteoclasts incapable of bone resorption.

Human phenotypes caused by mutation show the same characteristics; for example juvenile Paget's disease is associated with a mutation in the gene coding for OPG (114), and mutation of carboanhydrase II produces osteopetrosis (115). Inflammatory cytokines provide co-stimulatory signals that directly modulate osteoclastogenesis, besides their indirect activity *via* increasing the expression of *RANKL* by stromal cells/osteoblasts. Hence, IL-1 and TNF-α, which are highly abundant at sites of chronic inflammation, can amplify the process of osteoclastogenesis under permissive levels of *RANKL* (116, 117). Both cytokines are pathologic factors in the progression of bone resorption in rheumatoid arthritis, and neutralization of their bioactivity by intravenous infusion of antagonists or antibodies (118, 119).

However, neither cytokine appears to be required for bone development, since disruption of either of the cytokine receptors in mice results in a minimal bone phenotype (120, 121). Bisphosphonates, which are considered first-line therapies for osteoporosis, also target osteoclasts.

4.4- Regulation of remodelling:

The control of bone remodelling is complex and orchestrated by numerous local and systemic factors, and their expression and release is controlled by regulatory loops. Furthermore, the number and the responsiveness of the bone cells have an impact on bone remodelling. Local and systemic factors can affect bone remodelling by directly or indirectly targeting mature cells and their respective progenitor cells. The following schematics are a brief report on some of the main actions of local factors and a summary of the physiologic function of systemic factors that regulate bone mass (Figs. 5 and 6).

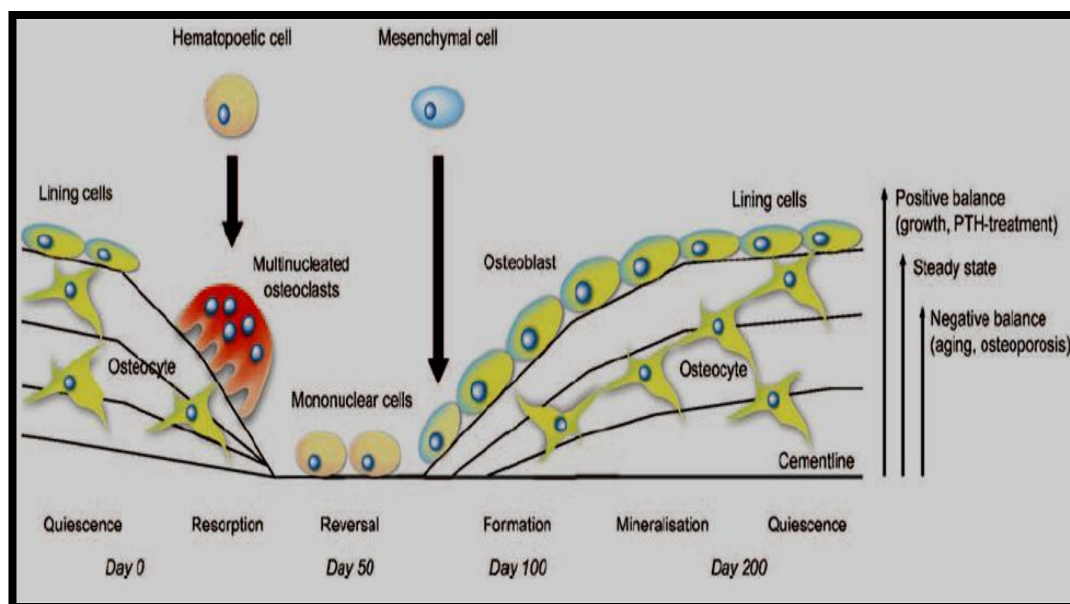


Fig. 5: Local factors that regulates bone remodelling (Liedert *et al*., 2005).

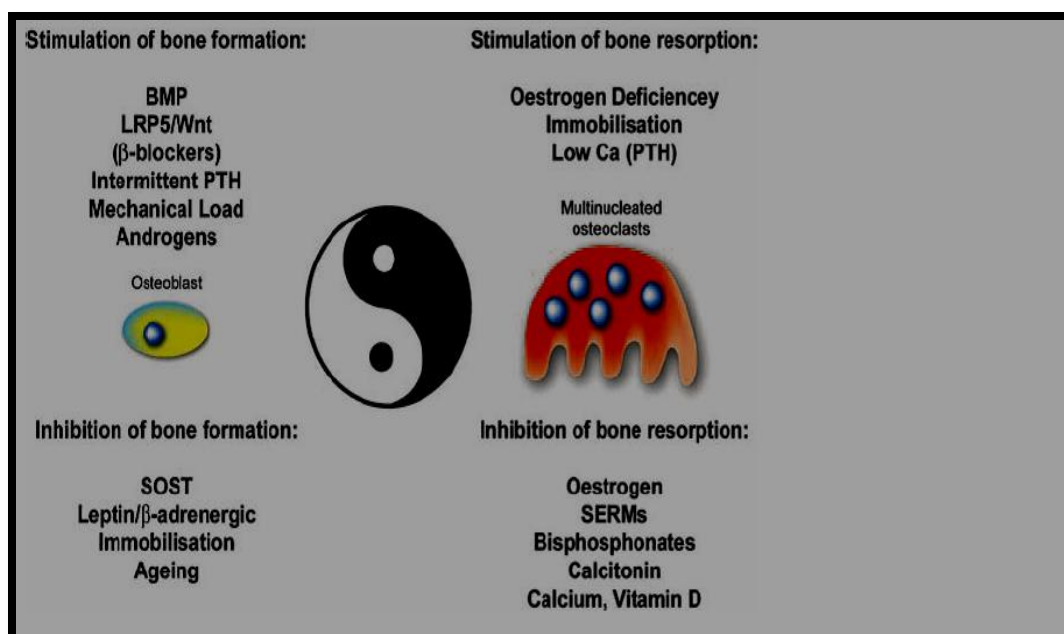


Fig. 6: Systemic factors that regulate bone mass (Jakob *et al*., 2008).

Members of the BMP family, Wnt family, hedgehog family and growth factors such as transforming growth factor β, platelet-derived growth factor, fibroblast growth factor, and insulin-like growth factor can modulate the various steps of osteoblastogenesis, e.g. commit pluripotent mesenchymal cells to the osteoblastic lineage, stimulate migration and proliferation and promote their further differentiation into mature cells (122, 123, 124).

Moreover, local growth factors can also enhance or inhibit osteoclast formation and function. Interleukin-1 (IL-1), IL-3, IL-6, IL-11, leukaemia inhibitory factor (LIF), ciliary neutrophilic factor (CNTF), tumour necrosis factor (TNF), granulocyte macrophage-colony stimulating factor (GM-CSF), M-CSF, and c-kit ligand are among those

growth factors that enhance osteoclast activity, whereas cytokines such as IL-4, IL-10, IL-18, and interferon γ inhibit the development of osteoclasts.

Prostaglandins are also centrally involved in bone remodelling and repair and target osteogenic cells as well as osteoclasts (125, 126). Deviations from the coordinated expression of these cytokines and growth factors in various cell types such as osteoblasts/stromal cells, monocytes/macrophages, lymphocytes, and malignant cells have been implicated in several pathologic conditions associated with increased bone loss such as osteoporosis, Paget's disease, and tumour hypercalcaemia. Moreover, it should be clearly stated that most of the local factors influence both the osteoblastic and the osteoclastic lineage (15, 9, 127).

4.4.1- Factors Regulating Bone remodelling:

- Parathyroid Hormone:

Parathyroid hormone (PTH) is an 84-amino acid peptide and the main factor responsible for the short-term regulation of calcium homeostasis. Extracellular calcium levels provide the signals for PTH secretion, a process controlled by the calcium-sensing receptor on the parathyroid cell. PTH increases renal tubular absorption of calcium, whereas it inhibits the tubular reabsorption of phosphate. PTH also stimulates the renal synthesis of 1, $25(OH)_2D_3$, thereby increasing calcium and phosphate absorption through the intestinal tract. Moreover, PTH enhances bone resorption (128).

- 1, 25-Dihydroxyvitamin D_3:

Sources of vitamin D are either dietary or endogenously produced in the skin from 7-dehydrocholesterol under the influence of ultraviolet light in the epidermis. Bound to a specific vitamin D binding protein in the blood, it is transported to the liver where it is hydroxylated at the C-25 position, thus being converted to $25(OH)_2D_3$. A second hydroxylation at the C-1 position takes place in the kidney, resulting in the formation of the most potent metabolite, 1, $25(OH)_2D_3$. In addition, the local production of 1, $25(OH)_2D_3$ by the extrarenal 1-hydroxylase has been described. When serum calcium levels are high, the kidney increases the 24-hydroxylase activity, which ensures the synthesis of the relatively inactive metabolites 24, $25(OH)_2D_3$ and 1, 24, $25(OH)_2D_3$ (129).

A complex of 1, $25(OH)_2D_3$ together with the vitamin D receptor (VDR) binds to a vitamin D-responsive element on the DNA, which has been identified in− for example− the promoter region of osteocalcin, 24-hydroxylase and calbindin D28k. Vitamin D metabolites are also considered to have rapid actions mediated *via* membrane receptors (130). Since 1, $25(OH)_2D_3$ has potent effects on calcium homeostasis, its production has to be strictly regulated. High levels of PTH as a result of hypocalcaemia stimulate 1-hydroxylase activities in the kidney, whereas high levels of calcium and 1, $25(OH)_2D_3$ have inhibitory effects. The enzymatic activity of the 24-hydroxylase is reciprocally regulated. The classical target tissues of 1, $25(OH)_2D_3$ are bone, intestine, and kidney (131).

- Calcitonin:

Calcitonin is a 32-amino-acid peptide hormone produced by the C cells of the thyroid gland. In contrast to PTH and 1, $25(OH)_2D_3$− both of which increase calcium release from the mineralized matrix− calcitonin is an inhibitor of osteoclast activity. Calcitonin is not secreted until plasma calcium levels reached approximately 9.5 mg/dl in humans. In addition, dopamine, and oestradiol stimulate calcitonin secretion (132).

- Oestradiol:

Oestrogens can regulate calcium homeostasis, which is an essential requirement during fetal development and postnatal lactation. Oestrogen deficiency, after menopause or following ovariectomy is associated with a progressive loss of trabecular bone structure, also increasing the perforation of cortical bone. Oestrogen loss causes high bone remodelling rates, where bone formation cannot compensate for the increased resorption. Also, nongenotropic effects of sex steroids by synthetic ligand can prevent bone loss induced in ovariectomized and orchidectomized mice, without affecting the reproductive organs (133).

Androgen receptor (AR)-deficient mouse models have also increased bone turnover, which is associated with low testosterone levels. Interestingly, substitution of testosterone− but not dihydrotestosterone which cannot be converted to oestradiol by the aromatase− can compensate for orchiedectomy-induced bone loss. These findings further suggest that the ERs are of critical importance for bone preservation in male mice (134). In humans,

testosterone is more potently converted into oestradiol by the aromatase expressed in bone and adipose tissue, leading to oestradiol levels of about 30 pg/ml in men (135).

The effects of oestrogen on bone cells is complex and occurs at multiple sites: Oestrogen deficiency is associated with increasing medullary numbers of mesenchymal progenitor cells and progenitors of osteoclasts, in mice (136,137). Monocytes, T cells and B cells from individuals with postmenopausal osteoporosis have a higher density of RANKL on their cell membrane (138), and oestrogen can positively regulate OPG expression (139). Oestrogen levels can also regulate the expression of cytokines of blood and marrow-derived cells, e.g. IL-1 and TNF- α, which in turn can influence the formation, activation and survival of osteoclasts (140, 141, 116, 117). Cytokines and other bioactive molecules, which are regulated by oestradiol presumably, act in cooperation; they control each other's expression and may have synergistic effects on their target cells (137). Blocking of each single cytokine, IL-1, IL-6 and TNF-α, can counteract bone loss in the ovariectomy mouse model (142, 142). T-cell-derived TNF-α is suggested to be a key factor of bone loss in this model, involving the TGF-β-mediated mechanism (144, 145).

Also B cells, which provide a subset of osteoclast progenitors and the cytokine IL-7, are considered to be players in the process of ovariectomy-induced bone loss (146, 147). Oestrogen and androgen are also a proapoptotic factor for osteoclasts, implying that steroid deficiency extends the lifetime of the bone-resorbing cell (148, 149). Oestradiol deficiency can lower the lifespan of osteoblasts and osteocytes, thereby signalling the need for increased bone remodelling. Oestradiol can trigger the differentiation of progenitor cells into the osteogenic *vs* the adipogenic lineage (150) and can stimulate the expression of *RUNX-2*, which parallels the increased osteogenic differentiation of *in vitro* cell cultures.

References

1. Sikavitsas V I, Temenoff J S and Mikos A G. (2001). Biomaterials and bone mechanotransduction. Biomaterials.22(19): 2581-2593

2. Henneicke H, Gasparini SJ, Brennan-Speranza TC, Zhou H and Seibel MJ. (2014). Glucocorticoids and bone: local effects and systemic implications. Trends Endocrinol Metab. 25(4):197-211.

3. Baron R, Weir EC, Horowitz MC, Centrella M, Kacinski BM and Insogna KL.(1993). Macrophage colony-stimulating factor release and receptor expression in bone cells. J Bone Miner Res. 8(12):1507-18.

4. Gruber, R., P. Pietschmann, and M. Peterlik. (2008). Introduction to bone development, remodelling and repair in radiology of osteoporosis. Springer. 1-23.

5. Currey JD. (2012).The structure and mechanics of bone. Journal of Materials Science. 47(1):41-54.

6. Paris O, Zizak I, Lichtenegger H, Roschger P, Klaushofer K and Fratzl P. (2000). Analysis of the hierarchical structure of biological tissues by scanning X-ray scattering using a micro-beam. Cell Mol Biol. 46:993– 1004.

7. Riggs BL and Parfitt AM. (2005). Drugs Used to Treat Osteoporosis: The Critical Need for a Uniform Nomenclature Based on Their Action on Bone Remodeling. Journal of Bone and Mineral Research. 20(2):177-184.

8. Frost HM. (2004). A 2003 update of bone physiology and Wolff's Law for clinicians. The Angle Orthodontist. 74(1):3-15.

9. Manolagas SC. (2000). Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocrine reviews. 21(2):115-37.

10. Nijweide PJ., De Rooij KE and Westbroek I. (2002). Osteocyte-specific monoclonal antibody MAb OB7.3 is directed against Phex protein. Journal of bone and mineral research. 17(5):845-53.

11. Knothe Tate ML, Adamson JR, Tami AE, and Bauer TW. (2004). Int J Biochem Cell Biol. 36(1):1-8.

12. Anderson HC, Garimella R and Tague SE. (2005).The role of matrix vesicles in growth plate development and bio-mineralization. Front Biosci. 10:822-837.

13. Rakhmatia YD, Ayukawa Y, Furuhashi A and Koyano K. (2013). Current barrier membranes: titanium mesh and other membranes for guided bone regeneration in dental applications. Journal of prosthodontic research. 57(1):3-14.

14. Taichman RS. (2005). Blood and bone: two tissues whose fates are intertwined to create the hematopoietic stem-cell niche. Blood. 105(7):2631-9.

15. Roodman GD. (1996). Advances in bone biology: the osteoclast. Endocr Rev. 17(4):308-32.

16. Salo J, Lehenkari P, Mulari M, Metsikkö K and Väänänen HK. (1997). Removal of osteoclast bone resorption products by transcytosis. Science. 276(5310):270-3.

17. Teitelbaum SL. (2000). Osteoclasts, integrins, and osteoporosis. J Bone Miner Metab.18(6):344-9.

18. Swaminathan R. (2001). Biochemical markers of bone turnover. Clin Chim Acta. 313(1-2):95-105.

19. Bou-Gharios G and De Crombrugghe B. (2008). Type I collagen structure, synthesis and regulation. Principles of bone biology JP Bilezikian, LG Raisz and TJ Martin (Eds) Elsevier, Amsterdam, The Netherlands. 285-318.

20. Antonarakis GS, Moseley R and Waddington RJ. (2014). Differential influence of fluoride concentration on the synthesis of bone matrix glycoproteins within mineralizing bone cells in vitro. Acta Odontologica Scandinavica. 72(8):1066-9.

21. Xu T1, Bianco P, Fisher LW, Longenecker G, Smith E, Goldstein S, Bonadio J, Boskey A, Heegaard AM, Sommer B, Satomura K, Dominguez P, Zhao C, Kulkarni AB, Robey PG and Young MF.(1998). Targeted disruption of the biglycan gene leads to an osteoporosis-like phenotype in mice. Nat Genet. 20(1):78-82.

22. Ducy P, Desbois C, Boyce B, Pinero G, Story B, Dunstan C, Smith E, Bonadio J, Goldstein S, Gundberg C, Bradley A and Karsenty G.(1996). Increased bone formation in osteocalcin-deficient mice. Nature.382(6590):448-52.

23. Luo G, Ducy P, McKee MD, Pinero GJ, Loyer E, Behringer RR and Karsenty G.(1997). Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. Nature. 386(6620):78-81.

24. Stein GS and Lian JB. (1993). Molecular mechanisms mediating proliferation/differentiation interrelationships during progressive development of the osteoblast phenotype. Endocrinol Rev. 14:424–442.

25. Gkiatas I, Lykissas M, Kostas-Agnantis I, Korompilias A, Batistatou A and Beris A. (2015). Factors affecting bone growth. American journal of orthopedics (Belle Mead, NJ). 44(2):61-7.

26. Morgan B, Dickerman RD, Reynolds A, Tackett J and Bennett M. (2008). Bone morphogenic protein. J Neurosurg Spine. 9(4):401.

27. Centrella M, McCarthy TL and Canalis E. (1987). Transforming growth factor beta is a bifunctional regulator of replication and collagen synthesis in osteoblast-enriched cell cultures from fetal rat bone. Journal of Biological Chemistry. 262(6):2869-74.

28. Hurley MM, Fall P, Harrison JR, Petersen DN, Kream BE and Raisz LG. (1989). Effects of transforming growth factor α and interleukin-1 on DNA synthesis, collagen synthesis, procollagen mRNA levels, and prostaglandin E2 production in cultured fetal rat calvaria. Journal of Bone and Mineral Research. 4(5):731-6.

29. Yokota K, Sato K, Miyazaki T, Kitaura H, Kayama H and Miyoshi F. (2014). Combination of Tumor Necrosis Factor α and Interleukin-6 Induces Mouse Osteoclast-like Cells With Bone Resorption Activity Both In Vitro and In Vivo. Arthritis & Rheumatology. 66(1):121-9.
30. Živković JM, Najman SJ, Vukelić MĐ, Stojanović S, Aleksić MV and Stanisavljević MN. (2014). Osteogenic effect of inflammatory macrophages loaded onto mineral bone substitute in subcutaneous implants. Archives of Biological Sciences. (00):20.

31. Wynn TA, Chawla A and Pollard JW. (2013). Macrophage biology in development, homeostasis and disease. Nature. 496(7446):445-55.

32. Gehrke I, Gandhirajan RK, Poll-Wolbeck SJ, Hallek M and Kreuzer K. (2011). Bone Marrow Stromal Cell–Derived Vascular Endothelial Growth Factor (VEGF) Rather Than Chronic Lymphocytic Leukemia (CLL) Cell–Derived VEGF Is Essential for the Apoptotic Resistance of Cultured CLL Cells. Molecular Medicine. 17 (7-8):619.

33. Bellido T. (2014). Osteocyte-driven bone remodeling. Calcif Tissue Int. 94(1):25-34.
34. Martin TJ and Sims NA. (2005). Osteoclast-derived activity in the coupling of bone formation to resorption. Trends Mol Med. 11:76–81.

35. Doherty MJ, Ashton BA, Walsh S, Beresford JN, Grant ME and Canfield AE. (1998). Vascular pericytes express osteogenic potential in vitro and in vivo. J Bone Miner Res. 13:828– 838.

36. Eghbali-Fatourechi GZ, Lamsam J, Fraser D, Nagel D, Riggs BL and Khosla S. (2005). Circulating osteoblast-lineage cells in humans. N Engl J Med 352:1959–1966.

37. Parfitt AM. (1998). Osteoclast precursors as leukocytes: importance of the area code. Bone 23:491–494.

38. Parfitt AM. (2000). the mechanism of coupling: a role for the vasculature. Bone 26:319–323.

39. Parfitt AM. (2002).Targeted and non-targeted bone remodeling: relationship to basic multicellular units origination and progression. Bone 30:5-7.

40. Manolagas SC. (2000). Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocrine reviews. 21(2):115-37.

41. Bilezikian J, Raisz L and Martin J. (2008). Principles of bone biology, 3rd edn. Academic Press, SanDiego

42. Stewart A and Reid DM. (2002). Quantitative ultrasound in osteoporosis.Semin Musculoskelet Radiol. 6(3):229-32.

43. Srinivasan S and Peh WC. (2013). Radiography in Osteoporosis. Osteoporosis and Bone Densitometry Measurements. Springer. p. 15-30.

44. Calvo MS, Eyre DR and Gundberg CM. (1996). Molecular basis and clinical application of biological markers of bone turnover. Endocr Rev. 17(4):333-68.

45. Seibel MJ. (2005). Biochemical markers of bone turnover: part I: biochemistry and variability. Clin Biochem Rev. 26(4):97-122.

46. Seibel MJ. (2006). Biochemical markers of bone turnover part II: clinical applications in the management of osteoporosis. Clin Biochem Rev. 27(3):123-38.

47. Szulc P and Delmas PD. (2008). Biochemical markers of bone turnover: potential use in the investigation and management of postmenopausal osteoporosis. Osteoporos Int. 19(12):1683-704.

48. Srivastava AK, Vliet EL, Lewiecki EM, Maricic M, Abdelmalek A, Gluck O and Baylink DJ. (2005). Clinical use of serum and urine bone markers in the management of osteoporosis. Curr Med Res Opin. 21:1015–1026.

49. Cremers S and Garnero P. (2006). Biochemical markers of bone turnover in the clinical development of drugs for osteoporosis and metastatic bone disease: potential uses and pitfalls. Drugs. 66:2031–2058.

50. Clemens JD, Herrick MV, Singer FR and Eyre DR. (1997). Evidence that serum NTx (collagen-type I N-telopeptides) can act as an immunochemical marker of bone resorption. Clin Chem 43(11):2058–2063.

51. Halleen JM, Alatalo SL, Janckila AJ, Woitge HW, Seibel MJ, Väänänen HK. (2001). Serum tartrate-resistant acid phosphatase 5b is a specific and sensitive marker of bone resorption. Clin Chem 47:597–600

52. Eyre D. (1992). New biomarkers of bone resorption. J Clin Endocrinol Metab 74(3):470A–470C.

53. Hanson DA, Weis MA, Bollen AM, Maslan SL, Singer FR and Eyre DR. (1992). A specific immunoassay for monitoring human bone resorption: quantitation of type I collagen cross-linked N-telopeptides in urine. J Bone Miner Res. 7(11):1251-8.

54. Garnero P, Shih WJ, Gineyts E, Karpf DB and Delmas PD.(1994). Comparison of new biochemical markers of bone turnover in late postmenopausal osteoporotic women in response to alendronate treatment. J Clin Endocrinol Metab.79(6):1693-700.

55. Christgau S, Rosenquist C, Alexandersen P, Bjarnason NH, Ravn P, Fledelius C, Herling C, Qvist P and Christiansen C. (1998). Clinical evaluation of the Serum CrossLaps One Step ELISA, a new assay measuring the serum concentration of bone-derived degradation products of type I collagen C-telopeptides. Clin Chem. 44(11):2290-300.

56. Meier C1, Meinhardt U, Greenfield JR, De Winter J, Nguyen TV, Dunstan CR and Seibel MJ. (2006). Serum cathepsin K concentrations reflect osteoclastic activity in women with postmenopausal osteoporosis and patients with Paget's disease. Clin Lab. 52(1-2):1-10.

57. Boyce BF and Xing L. (2007). The RANKL/RANK/OPG pathway. Curr Osteoporos Rep. 5(3):98-104.

58. Risau W. (1997). Mechanisms of angiogenesis. Nature. 386(6626):671-4.

59. Carmeliet P. (2000). Mechanisms of angiogenesis and arteriogenesis. Nat Med.6(4):389-95.

60. Asahara T and Kawamoto A. (2004). Endothelial progenitor cells for postnatal vasculogenesis. Am J Physiol Cell Physiol. 287(3):C572-9.

61. Barnes GL, Kostenuik PJ, Gerstenfeld LC, and Einhorn TA.(1999). Growth factor regulation of fracture repair. J Bone Miner Res. 14(11):1805-15.

62. Gerstenfeld LC, Barnes GL, Shea CM and Einhorn TA. (2003). Osteogenic differentiation is selectively promoted by morphogenetic signals from chondrocytes and synergized by a nutrient rich growth environment. Connect Tissue Res. 44(1):85-91.

63. Frost HM. (2004). A 2003 update of bone physiology and Wolff's Law for clinicians. The Angle Orthodontist. 74(1):3-15.

64. St-Jacques B, Hammerschmidt M and McMahon AP. (1999). Indian hedgehog signaling regulates proliferation and differentiation of chondrocytes and is essential for bone formation. Genes Dev. 13:2072–2086.

65. Karsenty G and Wagner EF. (2002). Reaching a genetic and molecular understanding of skeletal development. Dev Cell. 2:389–406.

66. Kronenberg HM. (2003). Developmental regulation of the growth plate. Nature 423:332–336.

67. Provot S and Schipani E. (2005). Molecular mechanisms of endochondral bone development. Biochem Biophys Res Commun. 328:658–665.

68. Thomas JT, Lin K, Nandedkar M, Camargo M, Cervenka J and Luyten FP.(1996). A human chondrodysplasia due to a mutation in a TGF-beta superfamily member. Nat Genet 12:315–317.

69. Thomas JT, Kilpatrick MW, Lin K, Erlacher L, Lembessis P, Costa T, Tsipouras P and Luyten FP. (1997). Disruption of human limb morphogenesis by a dominant negative mutation in CDMP1. Nat Genet 17:58–64.

70. Francis-West PH, Parish J, Lee K and Archer CW. (1999). BMP/ GDF-signalling interactions during synovial joint development. Cell Tissue Res 296:111–119.

71. Zou H, Wieser R, Massague J and Niswander L. (1997). Distinct roles of type I bone morphogenetic protein receptors in the formation and differentiation of cartilage. Genes Dev 11:2191–2203.

72. Devlin RD, Du Z, Pereira RC, Kimble RB, Economides AN, Jorgetti V and Canalis E. (2003). Skeletal overexpression of noggin results in osteopenia and reduced bone formation. Endocrinology 144:1972–1978.

73. Wu XB, Li Y, Schneider A, Yu W, Rajendren G, Iqbal J, Yama- moto M, Alam M, Brunet LJ, Blair HC, Zaidi M and Abe E.(2003). Impaired osteoblastic differentiation, reduced bone formation, and severe osteoporosis in noggin-over- expressing mice. J Clin Invest 112:924–934.

74. Onishi T, Ishidou Y, Nagamine T, Yone K, Imamura T, Kato M, Sampath TK, ten Dijke P and Sakou T. (1998). Distinct and overlapping patterns of localization of bone morphogenetic protein (BMP) family members and a BMP type II receptor during fracture healing in rats. Bone 22:605– 612.

75. Reddi AH. (1998). Role of morphogenetic proteins in skeletal tissue engineering and regeneration. Nat Biotechnol 16:247–252.

76. Canalis E, Economides A and Gazzerro E. (2003). Bone morphogenetic proteins, their antagonists, and the skeleton. Endocr Rev 24:218–235.

77. Carano RA and Filvaroff EH. (2003). Angiogenesis and bone repair. Drug Discov Today 8:980–989.

78. Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Degu- chi K, Shimizu Y, Bronson RT, Gao YH, Inada M, Sato M, Okamoto R, Kitamura Y, Yoshiki S and Kishimoto T. (1997). Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. Cell 89:755–764.

79. Otto F, Thornell AP, Crompton T, Denzel A, Gilmour KC, Rosewell IR, Stamp GW, Beddington RS, Mundlos S, Olsen BR, Selby PB and Owen MJ. (1997) .Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. Cell 89:765–771.

80. Lee B, Thirunavukkarasu K, Zhou L, Pastore L, Baldini A, Hecht J, Geoffroy V, Ducy P and Karsenty G. (1997). Missense mutations abolishing DNA binding of the osteoblast-specific transcription factor OSF2/CBFA1 in cleidocranial dysplasia. Nat Genet 16:307–310.

81. Mundlos S, Otto F, Mundlos C, Mulliken JB, Aylsworth AS, Albright S, Lindhout D, Cole WG, Henn W, Knoll JH, Owen MJ, Mertelsmann R, Zabel BU and Olsen BR. (1997). Mutations involving the transcription factor CBFA1 cause cleidocranial dysplasia. Cell 89:773–779.

82. Zelzer E and Olsen BR. (2003). The genetic basis for skeletal dis- eases. Nature. 423:343–348.

83. Bialek P, Kern B, Yang X, Schrock M, Sosic D, Hong N, Wu H, Yu K, Ornitz DM, Olson EN, Justice MJ and Karsenty G. (2004). A twist code determines the onset of osteoblast differentiation. Dev Cell. 6:423–35.

84. Kronenberg HM. (2004).Twist genes regulate Runx2 and bone formation. Dev Cell. 6:317–318.

85. Kundu M, Javed A, Jeon JP, Horner A, Shum L, Eckhaus M, Muenke M, Lian JB, Yang Y, Nuckolls GH, Stein GS and Liu PP. (2002). Cbfbeta interacts with Runx2 and has a critical role in bone development. Nat Genet. 32:639–644.

86. Yoshida CA, Furuichi T, Fujita T, Fukuyama R, Kanatani N, Kobayashi S, Satake M, Takada K and Komori T. (2002). Core-binding factor beta interacts with Runx2 and is required for skeletal development. Nat Genet. 32:633– 638.

87. Ducy P, Zhang R, Geoffroy V, Ridall AL and Karsenty G. (1997). Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. Cell 89:747–754.

88. Hoshi K, Komori T and Ozawa H. (1999). Morphological characterization of skeletal cells in Cbfa1-deficient mice. Bone 25:639–651.

89. Abe E, Yamamoto M, Taguchi Y, Lecka-Czernik B, O'Brien CA, Economides AN, Stahl N, Jilka RL and Manolagas SC. (2000). Essential requirement of BMPs-2/4 for both osteoblast and osteoclast formation in murine bone marrow cultures from adult mice: antagonism by noggin. J Bone Miner Res. 15:663–673.

90. Geoffroy V, Kneissel M, Fournier B, Boyde A and Matthias P. (2002). High bone resorption in adult aging transgenic mice overexpressing cbfa1/runx2 in cells of the osteoblastic lineage. Mol Cell Biol 22:6222–6233.

91. Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Beh- ringer RR and de Crombrugghe B. (2002). The novel zinc finger containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell 108:17–29.

92. Koga T, Matsui Y, Asagiri M, Kodama T, de Crombrugghe B, Nakashima K and Takayanagi H. (2005). NFAT and Osterix cooperatively regulate bone formation. Nat Med. 11:880– 885.

93. Harada S and Rodan GA. (2003). Control of osteoblast function and regulation of bone mass. Nature. 423:349–355.

94. Boyden LM, Mao J, Belsky J, Mitzner L, Farhi A, Mitnick MA, Wu D, Insogna K and Lifton RP. (2002). High bone density due to a mutation in LDL-receptor-related protein 5. N Engl J Med. 346:1513–1521.

95. Little RD, Carulli JP, Del Mastro RG, Dupuis J, Osborne M, Folz C, Manning SP, Swain PM, Zhao SC, Eustace B, Lappe MM, Spitzer L, Zweier S, Braunschweiger K, Benchekroun Y, Hu X, Adair R, Chee L, FitzGerald MG, Tulig C, Caruso A, Tzellas N, Bawa A, Franklin B, McGuire S, Nogues X, Gong G, Allen KM, Anisowicz A, Morales AJ, Lomedico PT, Recker SM, Van Eerdewegh P, Recker RR and Johnson ML. (2002). A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant high-bone-mass trait. Am J Hum Genet. 70:11–19.

96. Ai M, Holmen SL, Van Hul W, Williams BO and Warman ML. (2005). Reduced affinity to and inhibition by DKK1 form a common mechanism by which high bone mass-associated missense mutations in LRP5 affect canonical Wnt signaling. Mol Cell Biol 25:4946–4955.

97. Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Regi- nato AM, Wang H, Cundy T, Glorieux FH, Lev D, Zacharin M, Oexle K, Marcelino J, Suwairi W, Heeger S, Sabatakos G, Apte S, Adkins WN, Allgrove J, Arslan-Kirchner M, Batch JA, Beighton P, Black GC, Boles RG, Boon LM, Borrone C, Brunner HG, Carle GF, Dallapiccola B, De Paepe A, Floege B, Halfhide ML, Hall B, Hennekam RC, Hirose T, Jans A, Juppner H, Kim CA, Keppler-Noreuil K, Kohlschuetter A, LaCombe D, Lambert M, Lemyre E, Letteboer T, Peltonen L, Ramesar RS, Romanengo M, Somer H, Steichen-Gers- dorf E, Steinmann B, Sullivan B, Superti-Furga A, Swo-boda W, van den Boogaard MJ, Van Hul W, Vikkula M, Votruba M, Zabel B, Garcia T, Baron R, Olsen BR and Warman ML. (2001). LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell. 107:513–523.

98. Day TF, Guo X, Garrett-Beal L and Yang Y. (2005). Wnt/beta- catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. Dev Cell. 8:739–750.

99. Hill TP, Spater D, Taketo MM, Birchmeier W and Hartmann C. (2005). Canonical Wnt/beta-catenin signaling prevents osteoblasts from differentiating into chondrocytes. Dev Cell. 8:727–738.

100. Glass DA 2nd, Bialek P, Ahn JD, Starbuck M, Patel MS, Cle- vers H, Taketo MM, Long F, McMahon AP, Lang RA and Karsenty G .(2005). Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. Dev Cell 8:751–764.

101. He X, Semenov M, Tamai K and Zeng X. (2004). LDL receptor- related proteins 5 and 6 in Wnt/beta-catenin signaling: arrows point the way. Development 131:1663–1677.

102. Logan CY and Nusse R. (2004). The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol. 20:781– 810.

103. Winkler DG, Sutherland MK, Geoghegan JC, Yu C, Hayes T, Skonier JE, Shpektor D, Jonas M, Kovacevich BR, Staeh- ling-Hampton K, Appleby M, Brunkow ME and Latham JA. (2003). Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. EMBO J 22:6267–6276.

104. Li X, Zhang Y, Kang H, Liu W, Liu P, Zhang J, Harris SE and Wu D .(2005). Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. J Biol Chem. 280:19883–19887.

105. Bellido T, Ali AA, Gubrij I, Plotkin LI, Fu Q, O'Brien C A, and Manolagas SC and Jilka RL. (2005).Chronic elevation in PTH in mice reduces expression of sclerostein by osteocytes: a novel mechanism for hormonal control of osteoblastogenesis. Endocrinology. 146:4577-4583.

106. Keller H and Kneissel M. (2005). SOST is a target gene for PTH in bone. Bone. 37:148–158.

107. van Bezooijen RL, Roelen BA, Visser A, van der Wee-Pals L, de Wilt E, Karperien M, Hamersma H, Papapoulos SE, ten Dijke P and Lowik CW. (2004). Sclerostin is an osteocyte- expressed negative regulator of bone formation, but not a classical BMP antagonist. J Exp Med. 199:805–814.

108. Suda T, Takahashi N and Martin TJ. (1992). Modulation of osteoclast differentiation. Endocr Rev. 13:66–80.

109. Suda T, Takahashi N, Udagawa N, Jimi E, Gillespie MT and Martin TJ. (1999). Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocr Rev. 20:345–357.

110. Boyle WJ, Simonet WS and Lacey DL. (2003). Osteoclast differentiation and activation. Nature. 423:337–342.

111. Tondravi MM, McKercher SR, Anderson K, Erdmann JM, Quiroz M, Maki R and Teitelbaum SL. (1997). Osteopetrosis in mice lacking haematopoietic transcription factor PU.1. Nature. 386:81–84.

112. Cecchini MG, Hofstetter W, Halasy J, Wetterwald A and Felix R. (1997). Role of CSF-1 in bone and bone marrow development. Mol Reprod Dev. 46:75–83.

113. Takayanagi H. (2005). Mechanistic insight into osteoclast differentiation in osteoimmunology. J Mol Med. 83:170–179.

114. Whyte MP, Obrecht SE, Finnegan PM, Jones JL, Podgornik MN, McAlister WH and Mumm S. (2002). Osteoprotegerin deficiency and juvenile Paget’s disease. N Engl J Med. 347:175–184.

115. Sly WS, Hewett-Emmett D, Whyte MP, Yu YS and Tashian RE. (1983). Carbonic anhydrase II deficiency identified as the primary defect in the autosomal recessive syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification. Proc Natl Acad Sci USA. 80:2752–2756.

116. Lam J, Takeshita S, Barker JE, Kanagawa O, Ross FP and Teitelbaum SL. (2000). TNF-alpha induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. J Clin Invest. 106:1481–1488.

117. Wei S, Kitaura H, Zhou P, Ross FP and Teitelbaum SL. (2005). IL-1 mediates TNF-induced osteoclastogenesis. J Clin Invest. 115:282–290.

118. Fleischmann RM, Iqbal I and Stern RL. (2004). Considerations with the use of biological therapy in the treatment of rheumatoid arthritis. Expert Opin Drug Saf. 3:391–403.

119. Mihara M, Nishimoto N and Ohsugi Y. (2005). The therapy of autoimmune diseases by anti-interleukin-6 receptor antibody. Expert Opin Biol Ther. 5:683–690.

120. Lorenzo JA, Naprta A, Rao Y, Alander C, Glaccum M, Widmer M, Gronowicz G, Kalinowski J and Pilbeam CC. (1998). Mice lacking the type I interleukin-1 receptor do not lose bone mass after ovariectomy. Endocrinology 139:3022–3025.

121. Peschon JJ, Torrance DS, Stocking KL, Glaccum MB, Otten C, Willis CR, Charrier K, Morrissey PJ, Ware CB and Mohler KM .(1998). TNF receptor-deficient mice reveal divergent roles for p55 and p75 in several models of inflammation. J Immunol. 160:943–952.

122. Marie PJ. (2003). Fibroblast growth factor signaling controlling osteoblast differentiation. Gene. 316:23–32.

123. Roelen BA and Dijke P. (2003) .Controlling mesenchymal stem cell differentiation by TGFBeta family members. J Orthop Sci. 8:740–748.

124. Govoni KE, Baylink DJ and Mohan S. (2005). The multi-functional role of insulin-like growth factor binding proteins in bone. Pediatr Nephrol. 20:261–268.

125. Okada Y, Lorenzo JA, Freeman AM, Tomita M, Morham SG, Raisz LG and Pilbeam CC. (2000). Prostaglandin G/H syn- thase-2 is required for maximal formation of osteoclast- like cells in culture. J Clin Invest 105:823–832.

126. Zhang X, Schwarz EM, Young DA, Puzas JE, Rosier RN and O'Keefe RJ. (2002). Cyclooxygenase-2 regulates mesenchymal cell differentiation into the osteoblast lineage and is critically involved in bone repair. J Clin Invest. 109:1405–1415.

127. Takayanagi H. (2005). Mechanistic insight into osteoclast dif- ferentiation in osteoimmunology. J Mol Med. 83:170–179.

128. Lombardi G, Di Somma C, Rubino M, Faggiano A, Vuolo L, Guerra E, Contaldi P, Savastano S and Colao A.(2011). J Endocrinol Invest. 34(7):18-22.

129. Trautvetter U, Neef N, Leiterer M, Kiehntopf M, Kratzsch J and JahreisG. (2014) Nutr J. 17:13-19.

130. Norman AW, Mizwicki MT and Norman DP. (2004). Steroid-hormone rapid actions, membrane receptors and a conformational ensemble model. Nat Rev Drug Discov. 3:27–41.

131. Masuyama R. (2014). J Bone Miner Metab. 32(1):1-9.

132. Belova SV. (2011). Biological role and functional characteristics of calcitonin in human and animal organisms. Usp Fiziol Nauk. 42(2):83-9.

133. Kousteni S, Chen JR, Bellido T, Han L, Ali AA, O'Brien CA, Plotkin L, Fu Q, Mancino AT, Wen Y, Vertino AM, Powers CC, Stewart SA, Ebert R, Parfi tt AM, Weinstein RS, Jilka RL and Manolagas SC .(2002). Reversal of bone loss in mice by nongenotropic signaling of sex steroids. Science. 298:843–846.

134. Kawano H, Sato T, Yamada T, Matsumoto T, Sekine K, Watanabe T, Nakamura T, Fukuda T, Yoshimura K, Yoshizawa T, Aihara K, Yamamoto Y, Nakamichi Y, Metzger D, Chambon P, Nakamura K, Kawaguchi H and Kato S. (2003). Suppressive function of androgen receptor in bone resorption. Proc Natl Acad Sci USA. 100:9416–9421.

135. Khosla S, Melton LJ 3rd, Atkinson EJ and O'Fallon WM. (2001). Relationship of serum sex steroid levels to longitudinal changes in bone density in young versus elderly men. J Clin Endocrinol Metab. 86:3555–3561.

136. Jilka RL, Passeri G, Girasole G, Cooper S, Abrams J, Broxmeyer H and Manolagas SC. (1995). Estrogen loss upregulates hematopoiesis in the mouse: a mediating role of IL-6. Exp Hematol. 23:500–506.

137. Jilka RL, Takahashi K, Munshi M, Williams DC, Roberson PK and Manolagas SC. (1998). Loss of estrogen upregulates osteoblastogenesis in the murine bone marrow. Evidence for autonomy from factors released during bone resorption. J Clin Invest. 101:1942–1950.

138. Eghbali-Fatourechi G, Khosla S, Sanyal A, Boyle WJ, Lacey DL and Riggs BL. (2003). Role of RANK ligand in mediating increased bone resorption in early postmenopausal women. J Clin Invest. 111:1221–1230.

139. Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Spelsberg TC and Riggs BL. (1999). Estrogen stimulates gene expression and protein production of osteoprotegerin in human osteoblastic cells. Endocrinology. 140:4367–4370.

140. Bismar H, Diel I, Ziegler R and Pfeilschifter J. (1995). Increased cytokine secretion by human bone marrow cells after menopause or discontinuation of estrogen replacement. J Clin Endocrinol Metab. 80:3351–3355.

141. Zheng SX, Vrindts Y, Lopez M, De Groote D, Zangerle PF, Collette J, Franchimont N, Geenen V, Albert A and Reginster JY. (1997). Increase in cytokine production (IL-1 beta, IL- 6, TNF-alpha but not IFN-gamma, GM-CSF or LIF) by stimulated whole blood cells in postmenopausal osteoporosis. Maturitas. 26:63–71.

142. Jilka RL, Hangoc G, Girasole G, Passeri G, Williams DC, Abrams JS, Boyce B, Broxmeyer H and Manolagas SC. (1992). Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science. 257:88–91.

143. Kitazawa R, Kimble RB, Vannice JL, Kung VT and Pacifici R. (1994). Interleukin-1 receptor antagonist and tumor necrosis factor binding protein decrease osteoclast formation and bone resorption in ovariectomized mice. J Clin Invest. 94:2397–2406.

144. Roggia C, Gao Y, Cenci S, Weitzmann MN, Toraldo G, Isaia G and Pacifici R. (2001). Up-regulation of TNF-producing T cells in the bone marrow: a key mechanism by which estrogen deficiency induces bone loss in vivo. Proc Natl Acad Sci USA. 98:13960–13965.

145. Gao Y, Qian WP, Dark K, Toraldo G, Lin AS, Guldberg RE, Flavell RA, Weitzmann MN and Pacifici R. (2004). Estrogen prevents bone loss through transforming growth factor beta signaling in T cells. Proc Natl Acad Sci USA. 101:16618–16623.

146. Miyaura C, Onoe Y, Inada M, Maki K, Ikuta K, Ito M and Suda T. (1997). Increased B-lymphopoiesis by interleukin 7 induces bone loss in mice with intact ovarian function: similarity to estrogen deficiency. Proc Natl Acad Sci. USA. 94:9360–9365.

147. Toraldo G, Roggia C, Qian WP, Pacifici R and Weitzmann MN. (2003). IL-7 induces bone loss in vivo by induction of receptor activator of nuclear factor kappa B ligand and tumor necrosis factor alpha from T cells. Proc Natl Acad Sci USA. 100:125–130.

148. Hughes DE, Dai A, Tiffee JC, Li HH, Mundy GR and Boyce BF. (1996). Estrogen promotes apoptosis of murine osteoclasts mediated by TGF-beta. Nat Med. 2:1132–1136.

149. Kameda T, Mano H, Yuasa T, Mori Y, Miyazawa K, Shiokawa M, Nakamaru Y, Hiroi E, Hiura K, Kameda A, Yang NN, Hakeda Y and Kumegawa M .(1997). Estrogen inhibits bone resorption by directly inducing apoptosis of the bone- resorbing osteoclasts. J Exp Med. 186:489–495.

150. Verma S, Rajaratnam JH, Denton J, Hoyland JA and Byers RJ. (2002). Adipocytic proportion of bone marrow is inversely related to bone formation in osteoporosis. J Clin Pathol. 55:693–698.

151. Swaminathan R. (2001). Biochemical markers of bone turnover. Clinica Chimica Acta. 313:95-105.

152. Jakob F, Seefried L and Ebert R. (2008). Pathophysiology of bone metabolism. Internist (Berl). 49(10):1159-60

153. Liedert A, Kaspar D, Augat P, Ignatius A and Claes L. (2005). Mechanobiology of Bone Tissue and Bone Cells. Academia.