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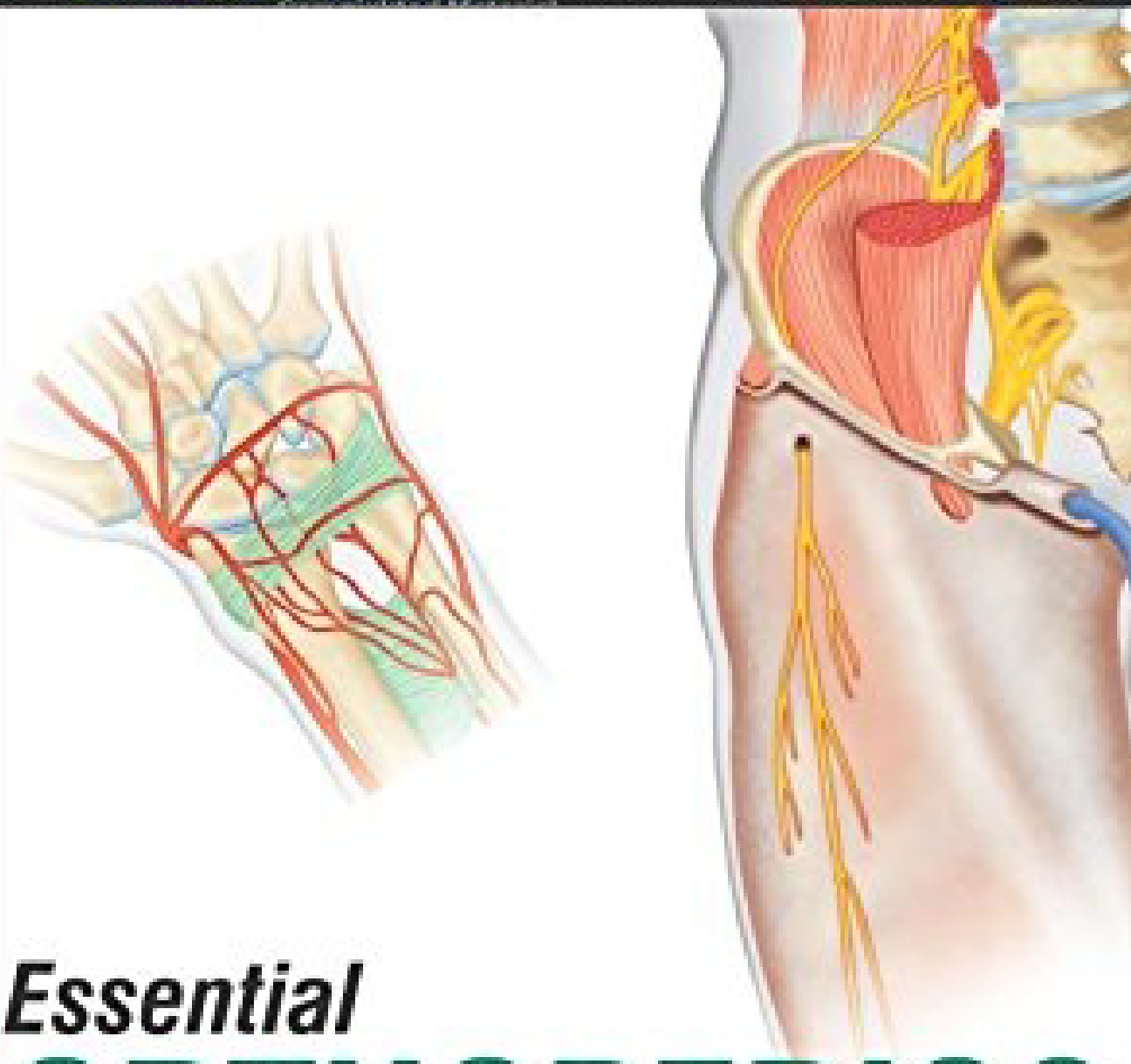

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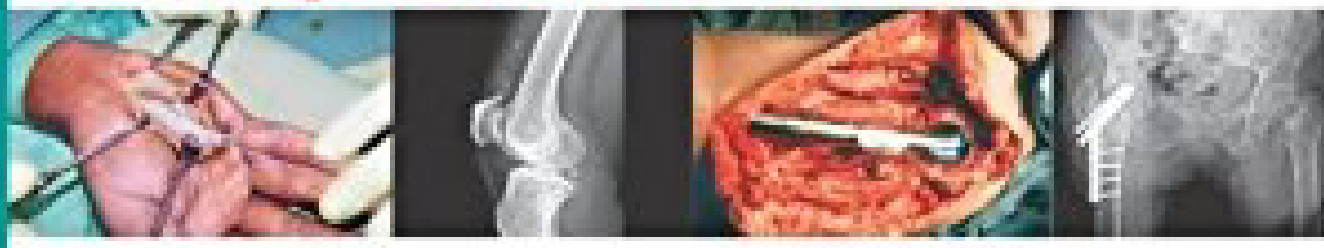

Manish Kumar Varshney

Essential

ORTHOPEDICS

(Principles and Practice)

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ORTHOPEDICS
(Principles and Practice)

2 Volumes

Editor

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The Health Sciences Publisher

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Essential Orthopedics (Principles and Practice) (2 Volumes)

First Edition: **2016**

ISBN: 978-93-5250-175-5

Printed at

Dedicated to

My loving children Siddhant and Mrigank
and my life partner giving strength
to my devotion, Neeta Verma.
The blessings of my parents and my sister have always helped
in maintaining the spark of learning and dissemination.

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PREFACE

Orthopedics like other medical faculties is a growing branch. The growth is, however, much faster than other fields. Till the beginning of last century, the orthopedics as a discrete was nonexistent to this world but the efforts of pioneers in the field gave birth to the baby that boomed possibly the fastest to become one of the most favored surgical branches to new entrants in the postgraduation. Orthopedics is the most glamorous and preferred surgical faculty for all youngsters aspiring to quickly gain sort of "superspecialization" as not much is available or required after one does his postgraduation in orthopedics, the region-wise faculty development into knee, shoulder, hip, pediatric orthopedics, orthopedic oncology and spine surgery are mostly fellowship dependent.

The scenario in orthopedics was not similar when the faculty started and in the beginning most of the evidences were mere "possibilities" as propounded in the replete literature of the older times written by the eminent persons in the field. Soon it came to be realized that opinions do not count and are often deleterious to general practice that may be true only for certain circumstances, so the era of "evidence-based practice" gradually evolved. With a few hitches then dawned the time where evidence was sought for most of the prevailing practices that were repeatedly questioned by surgeons around the world. This was pioneered by efforts of Association for Osteosynthesis (AO) foundation and others and did help to some extent. In the beginning, however, the evidence was more of confusion rather than leading and the laid down facts had to be constantly changed and adjusted more due to poverty in exactness of the previously laid principles and less also to the emerging evidence as is evident from changing AO philosophy and the glaring failure of metal-on-metal hips. As a postgraduate, it became difficult for me to comprehend the historical errors and practice, changing philosophy and emerging new evidence and importantly remember them all. It was even more difficult to be accurate in orthopedic examinations to rationalize my understanding and teachings to that of the senior examiners who had started the practice even in a more bleak and confused environment prevailing at their time. However, I did realize during the course that some fundamentals were constantly getting lost in these times of evolution that could not be passed on to the youngsters and clinical examination has by now become a mere formality due to higher dependence/pervasiveness on advanced imaging and some illicit activities. The beginning of this century was marked by two major developments I feel—the development of improved implants and instrumentation (particularly the locked plate screw constructs) and higher importance laid down for minimally invasive surgery. It became quickly evident to me that we need a text that encompasses all these facts so that the student gets a "One Stop" text where he can refer to most of his queries. This encouraged me to write down the current text and incorporate the most significant of previous practices and the current developments, some attempts have been made to incorporate the futuristic techniques but it is limited by unavailability of "evidence-based practice", so I have personally restricted that.

I feel that the future of orthopedics lies in correct and prudent diagnosis (aided solidly by sound knowledge and clinical examination) and ethical practice (not just money making as is considered an important practice nowadays); the basis of which should be correct and true literature and not polluted one. I found some youngsters in the race of just publishing even resort to unethical practice and publish skewed and incorrect findings that pollute the sacred literature which has access to all and even amateur people who may not be able to rationalize and fall prey to wrong practice. This, I feel, also is the reason that newer meta-analysis and even systematic reviews are unable to yield evidence in favor of one or the other method rather often end in saying "no statistically significant different in practice".

The current text will help a practicing surgeon to acquaint himself with the alternatives available for a particular condition. I have tried to integrate most of the methods that are in practice or known currently for different orthopedic conditions and also organize the text in the form of answers to common theory questions for use to a postgraduate student. I tried to make a sincere effort in producing a text that can give an orthopedic surgeon insight into the orthopedic practice importantly the basic sciences and how diseases are based on faults in them. The textbook has been organized in the form of regional disposition to capture the region-specific conditions. There is vast emerging evidence and will keep emerging even at the time of this writing in the form of modern and new molecular markers that aim to diagnose

disorders correctly and fast. The future for treatment of some of these disorders might also lie in the molecular level by using microinstruments like nanotechnology and gene therapy, so I tried to cover that topic also comprehensively.

I would like to thank from my heart to all those who have contributed to the text. Particularly Prof Alok Sud from Lady Hardinge Medical College and Associated Hospitals, New Delhi, India, who stood by the commitment once made and put constant efforts in providing me the text in pediatric orthopedics and helped till end in organizing some photographs despite ourselves being only formally introduced and worked for a short duration together. The other person who has helped in preparing the text is Dr S Pavan, who stood always by my side whenever required and also relieved me of my duties often at even hectic times to prepare the text. My juniors Dr Jaydip Patel, Dr Amit Singh and Dr Swapnil Sharma have provided help at difficult times when I needed them for text correction and improvement despite some of them being engaged in their own academic work. Especially, I would thank Dr Jaydip, who even woke-up untiringly at nights despite his duties to correct the text and add to the base work done by others and Dr Swapnil, who provided support regardless of his examinations going on. The contribution of others is not nevertheless small and Dr Ashok Jadon, Dr Sanjay L Srivastav, Dr Ankit Data and Dr Sachin Bharti have contributed thoroughly to the nonorthopedic topics and Dr Aditya Soral, Dr Ram Kinkar Jha and Dr S Dutta, without their help the text could never be completed. My family had been very supportive in displaying immense courage to face social restrictions and my untimely absence from home due to writing work, sometimes when my kids needed me most to play, read, learn and enjoy and my wife missing me sometimes altogether in important festivals and ceremonies. There are, however, some sour memories also and it is also important to mention that the writing had not been easy at all, with minimal help coming from persons I thought would contribute the most. The most respected and dear ones from my earlier relations in the field got too busy or otherwise to provide any help but this was to my best use and possibly advantage that it made me write all the topics by myself, which gave me further knowledge and confidence in areas where I was lacking in my training.

Lastly, I am highly thankful to Shri Jitendar P Vij (Group Chairman), Mr Ankit Vij (Group President), Mr Tarun Duneja (Director–Publishing), Ms Samina Khan (Executive Assistant to Director–Publishing), the artists and all the team members who untiringly worked to bring out this wonderful text despite various delays and hitches from my end. It is only with the help of the publishing team at M/s Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, India, that I could produce the text with confidence. Especially Shri Jitendar P Vij has grown in respect and stature in my thoughts who gave me the opportunity and initiated me for writing giving timely broad outlines, similarly Mr Tarun Duneja has constantly assisted me in sorting out nitty-gritty problems that an author would usually encounter.

Providing an updated and correct text is always a challenge and some concepts would keep changing over time—is a fact. To this end, I reiterate all the readers of this text to kindly keep posting me in case they find fundamental errors in the text or some critical updates that should be incorporated so that we can help all others in their future reading. I am open to all the criticism as they are bound to arise by difference of opinion that should be freely communicated to me at my e-mail—drmkvarshney@gmail.com. One may also reach me at the so-called in fashion social sites like Facebook® or WhatsApp. In the name of Almighty I present the text as an endeavor to promote the proficiency of both the neophyte and experienced orthopedic surgeon, bearing in mind to the truest of an attempt to present contradictory accumulation of discernment into logical concepts and the fact that condensing the mountain of knowledge may not be possible in the lifetime with ever-evolving concepts.

Manish Kumar Varshney

ACKNOWLEDGMENTS

I heartily acknowledge the contribution of my teachers Dr PP Kotwal and Dr S Rastogi (Professors, Department of Orthopedics, All India Institute of Medical Sciences, New Delhi, India) for providing clinical photographs and radiographs representative of typical disorders as a learning resource used at various places in the chapters on pediatric upper and lower limb disorders, rheumatoid arthritis, etc. and various clinical and operative learning representative photographs and radiographs for chapter on bone neoplasia respectively.

I also acknowledge the contribution from Dr Neelam Jain (Radiologist, Discovery Imaging Center, Jamshedpur, Jharkhand, India) for providing representative MRI pictures for chapter on magnetic resonance imaging (MRI).

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SECTION 1

Bone Anatomy, Physiology, Pathology and Diseases

Chapter 1

Structure and Function of Bones and Joints

Manish Kumar Varshney

BONE STRUCTURE

Bone is a composite tissue (in engineering sense discussed below) consisting of organic matrix, inorganic minerals, cells and water. Biologically, it is a dynamic mesenchymal (specialized connective) hard tissue that undergoes continuous formation and remodeling throughout life. The size of bone increases by growth (*skeletal modeling*) during initial life till physeal closure and the shape of bone changes through *remodeling*. The remodeling process occurs in adulthood and is essentially a mechanism that differentiates living tissue from non-living tissue. Remodeling gives capacity to bone to repair itself and renew the lost internal structures from wear and tear process. It also enables bone to adapt itself to changing environment resulting from altered activity levels and aging. Both modeling and remodeling occur via "coupling" of bone resorption and bone formation that occurs simultaneously. The bone per se consists of (Fig. 1) predominant inorganic component (60%) and organic component (40%).

- The inorganic portion comprises of crystalline calcium phosphate salts, present in the form of hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ with minor contribution from carbonates, fluorides and other magnesium salts.
- The organic component is dominated by type I collagen that forms the basic architecture of bone on which inorganic portion is deposited. Support to collagen is provided by derived protein components like proteoglycans, glycoproteins, phospholipids and phosphoproteins that serve specific functions (discussed below).

Both the components give bone its unique mechanical, biological and electrical properties. Loss or inadequacy of mineral component (osteomalacia or rickets) or organic component (like osteogenesis imperfecta) produces structurally weak bones that fail easily. The bones comprise (Figs 2A to E) of typical distribution of hard ("compact") bone outside, supported internally by biologically more active ("cancellous") bone that has nine times the metabolic activity (Table 1). This distribution

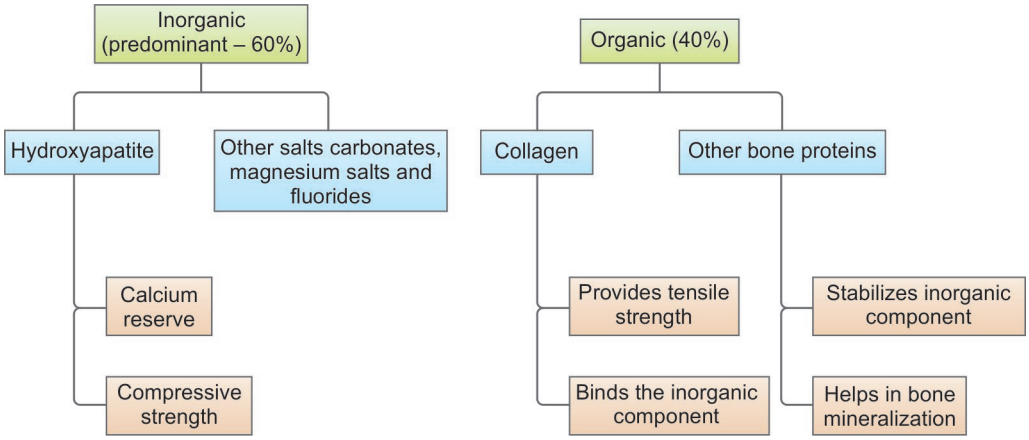
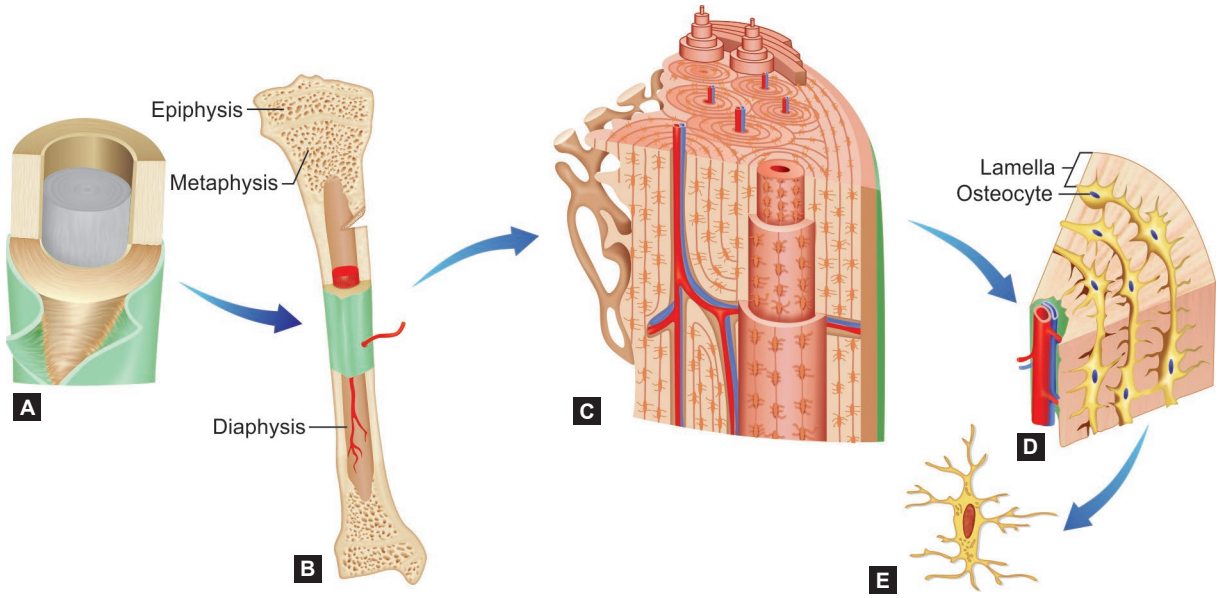


Fig. 1: Broad constituents of bone



Figs 2A to E: The gross appeareance (A to C) to microstructure (D and E) of bone. The cortex forms a cylinder encircling the medullary cavity (A) and is in turn covered by the periosteum (green envelop here). Illustrated are the dispositions of cortical and cancellous components (B) of human bone in a typical long bone (tibia here), the cancellous bone typically occupies the epiphysis and metaphysis of a long bone while cortical bone predominates in the diaphysis. The microstructure of mature cortical (compact) bone comprises of concentric rings around Haversian (longitudinal) and Volkmann (transverse) canal system within the osteoid (C), also shown are the osteocytes arranged in the lamellations (D) that form the largest network of cells connecting skeletal system to the outside environment through their processes (E) and also maintains homeostasis within bone

TABLE 1: Difference between cortical and cancellous bone

Cortical bone	*Cancellous bone*
Forms the outer part or "shell" of bone	Contained within shell
Predominantly found in diaphyseal region	Predominantly seen in metaphyseal and epiphyseal regions
Concentric lamellar structure around Haversian system—osteonal formation	Contains lamellae, but osteons are missing
Provides compressive strength to bone	Provides tensile strength to bone and resilience
Provides attachment to tendons, ligaments and periosteum	Provides scaffolding to marrow cavity and space for osteoprogenitor cells
Metabolically less active	Nine times more metabolically active than cortical bone
Slow remodeling. Thickening occurs on the concave side (compression), while convex side (tensile side) undergoes thinning and resorption	Complete trabecular structure changes with continuous remodeling. Trabecular hypertrophy under compressive forces and even with tensile forces. They atrophy and disappear with reduction of these forces
Usually minimal change in osteoporosis	Undergoes great amount of resorption in osteoporosis
When used as a graft mainly provides strut support and compressive strength—less osteogenic potential	Preferred in bone grafting for higher osteogenic potential and remodels by creeping substitution

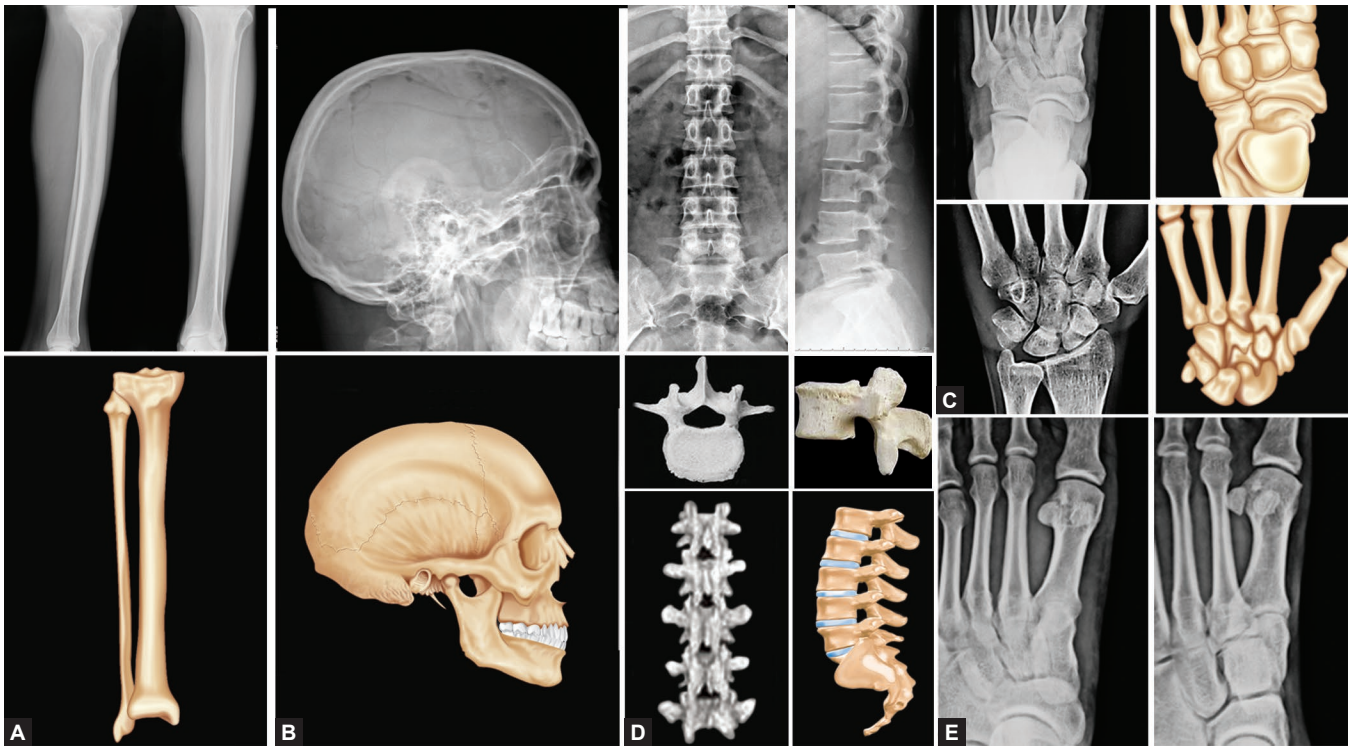
of hard and weak components gives bone mechanical advantage of discrete rigidity and flexibility. Such a structure is called a "composite" mentioned initially.

CLASSIFICATION OF BONE BY APPEARANCE

The human body has five different types of bones (Figs 3A to E):

Long Bones

The long bones are formed by endochondral ossification. The bones of arm, forearm, thigh and legs, *viz.* femur, ulna, tibia, radius, humerus and fibula, are typical examples. These have two ends (*epiphysis*), a cylindrical tube in the middle (*diaphysis*) and a transitional zone between them (*metaphysis*). The long bones develop from cartilage enlage through a process of endochondral ossification. The ossification centers for epiphysis and the diaphysis



Figs 3A to E: The five types of bones in human body. Long bones here exemplified by tibia and fibula (A) have expanded metaphysis at both the ends capped by epiphysis that is separated by physis from metaphysis in an immature bone, and a cylindrical shaft in middle. Flat bones are typified by skull (B) bones that have an outer and an inner table. The carpal and metatarsal bones (C) represent the small irregular bones like pebbles of different shapes. Vertebrae (D) are best examples of irregular bones with multiple irregular processes and parts. Sesamoid bones develop later in life commonly at the wear and tear sites of tendons or where the tendons need some mechanical advantage for their action, here are shown the sesamoid bones in the tendon of flexor hallucis brevis beneath the first metatarsal of foot (E)

are different and are separated by *growth plate also known as physis* which is basically a layer of hyaline cartilage organized into different layers.

Flat Bones

The flat bones as against long bones develop from a discrete process called intramembranous ossification. Scapula and sternum are the representative examples. The flat bones have an inner and an outer table of cortical bone intervened by trabecular bone as is exemplified by skull bones.

Short Bones

Carpal and tarsal bones are representative examples. They are predominantly composed of trabecular bone that is shelled by a thin layer of cortical bone.

Irregular Bones

They are like short bones in cut sections, but unlike them they have no smooth structure resembling any geometrical shape (hence irregular). Vertebrae are classic examples.

Sesamoid Bones

These bones also resemble short bones, but form without ossification center (except patella) due to undue stress in the region. They are found embedded in tendons or ligaments and serve specific functions.

The bone functions to:

- Provide a rigid framework of all vertebrates to support the body
- Act as levers for muscles
- Give shape to soft tissues and protect vital organs of body by forming rigid or flexible cavities
- Provide minerals in time of need as it has mineral reserve for calcium and phosphate.

GROSS FEATURES OF BONE

As evident from the above classification the bones are either shaped as a hollow tube (long bones) or bilaminar plate of bone (flat bones) containing variable cortical or trabecular structures. *Cortical (compact) bone* is dense and calcified bone forming hard outer structure of bone providing most of the mechanical strength. It is also

referred to as "cortex" commonly by surgeons and consists of aggregations of concentric lamellar bone in the form of osteons. Osteons at their center contain Haversian and Volkmann vascular canal systems, individual nerves and one or two lymphatic channels (discussed below). One can say that well developed osteons define cortical or compact bone and its presence is the hallmark. The marrow cavity is the space inside the cortical walls that contains hematopoietic marrow tissue, fat and bony spicules. *Trabecular bone* consists of these slender spicules and trabeculae (not more than 0.2–0.4 mm) that are separated by marrow spaces. Trabeculae of bone support the marrow elements by increasing surface area and providing scaffold, also they lighten the bone. It fills the metaphyseal and epiphyseal region of bones. They are composed of lamellar bone with longitudinal arrangement of lamellae, but the osteons are not formed (Table 1). These spongier regions develop according to the lines of stress giving bone ability to deform (elastic nature) before failing (tensile strength). By virtue of outer cortex bone resists compression also avidly (composite design).

The bone can be divided into following parts (anatomically and functional distinct units, Fig. 2):

- Epiphysis—Part of bone that lies between physis or physeal scar and articular cartilage. The part is usually intra-articular and takes part in joint formation and function. The periosteum in intra-articular region lacks the cambium layer that has totipotential cell rests.
- Physis—The growing structure consisting of flat portion adding length to bone and circumferential portion adding to width of bone and physis itself as it grows (discussed below for detailed structure).
- Metaphysis is the funnel-like part at ends of diaphysis that predominantly comprises of trabecular bone. The metaphysis lies between physis and diaphysis and is quite susceptible to osteoporotic fractures being deficient in cortical bone that undergoes less resorption. Being metabolically active it is also susceptible to remodeling defects like multiple osteochondromatosis. Radiologically, the extent of metaphysis is defined by a square drawn from epiphysis from a line having greatest horizontal dimension at metaphysis.
- Diaphysis—This is the predominant central tubular portion of long bones giving them the characteristic form. Most cortical bone is found on this region giving tensile and compressive strength to the region. It is the strongest part of bone and susceptible to fractures by virtue of extreme levered forces being transmitted through it. Also, it is subject to direct trauma in the center of limb.
- Bone marrow—It fills the medullary cavity and is responsible for most of the hematopoietic activity from the contained progenitors. The marrow gradually changes from red (hematopoietic) in adolescents to yellow or white (fatty) in adults. The red marrow persists in the vertebrae, some metaphyseal regions and flat bones in adults.
- Periosteum—It is a thick fibrous membrane that covers the bone like a laminating membrane (except articular cartilage and dense tendon attachments). The membrane is divided histologically into outer fibrous (collagen) and inner cellular layer. The latter is important structure responsible for bone repair and is referred to as "cambium" layer. It contains totipotent (young children) or multipotent (adolescents and adults) cells that serve as osteoprogenitor cells capable of forming new bone and callus with traumatic disruption. Periosteum also serves to add thickness to bone by appositional bone deposition; this is especially true at the sites of tendon attachment through sharpey's fibers that give a traction force on bone. Sharpey's fibers are thick collagen bundles that anchor the periosteum to circumferential lamellae and dominate in the regions of tendon attachment.
- Endosteum—There is no microscopic or macroscopic structure distinctly seen inside the bone that can be referred to as endosteum. The outer resting layer of marrow and its interface with bone is what is referred to as endosteum. Electron microscopically there is a thin arrangement of highly cellular osteoblastic and osteoclastic elements devoid of characteristically distinguishable membrane.

MICROSCOPIC ANATOMY

Based on collagen fiber arrangements, bones have two distinct histological appearances—woven bone and the lamellar bone.

- *Woven Bone* is also called immature bone, coarse bundled bone or sometimes fiber bone. It is made from randomly oriented collagen fibers in interlacing or "burlap" fashion, with numerous osteoblasts and osteoprogenitor cells (so-called immature). When viewed under polarized light, it shows haphazard structural organization. Woven bone is much more cellular than the organized lamellar bone and has higher number of cells per unit area. Woven bone is the major bone type in the developing fetus that matures to lamellar bone in adult. In adults, immature bone is still found at remodeling sites, in the alveolar socket (mouth), fracture repair (callus) and at tendinous intersection. It occurs pathologically in osteosarcoma, fibrous dysplasia and several other tumors. The synthesis of woven bone is triggered by platelet-derived growth factor (PDGF A

and PDGF B) and insulin-like growth factor (IGF I and IGF II) and is seen in areas of fast bone growth.

- *Lamellar (thin plate) bone (mature bone)* on polarized light microscopy has characteristic well-organized arrangement of collagen fibers seen as parallel bundles (2–4 μm) of deposited bone. Lamellar bone develops during remodeling of immature bone by replacement of the latter. Continuous secondary organization (remodeling) is pathognomonic of mature lamellar bone. Lamellar bone is deposited in slow growing regions, but the control mechanisms have not been fully understood. In the cortex, the lamellae have concentric tubular arrangement containing 5–15 concentric lamellae. *Outer circumferential lamellae* (Fig. 2) lie next to the periosteum, while *inner circumferential lamellae* lie near endosteum. The *interstitial lamellae* (Fig. 2) represent archaic remnants of old concentric lamellae. These variable size (thick or thin) tubes of concentric lamellar arrangement are called *osteon* and a number of them are closely packed with few gaps, if any to form compact bone. The fibers of each lamella run in a spiral fashion rather than concentric cylinders around the canal. The osteons (*Haversian systems*—after Clopton Havers who defined it in 1691) are cylindrical units that surround a central *Haversian canal* (Fig. 2) that contains vascular bundle of capillaries and venules and also nerves, lymphatic canals and a loose connective tissue encompassing osteoprogenitor cells. It is a *branching system* of cylinders arranged longitudinally in the bone. *Volkmann's canals (transverse perforating canal system)* are vascular channels that interconnect Haversian canals and also the Haversian system to periosteal blood vessels and intramedullary vascular supply (Fig. 2). Osteocytes are located in the interlaminar regions with their processes arranged in a radial pattern into the canaliculi. The osteons act like fibers of bamboo that resist deformation.

THE CELLULAR ELEMENTS OF BONE

Osteoblasts, osteocytes and osteoclasts are predominant cells in bone. Osteoblasts serve the purpose of bone formation (osteogenesis), while osteoclasts are mainly accountable for bone resorption; their combined action contributes to progressive mineralization and remodeling. The osteocytes maintain the milieu of bone and its homeostasis through vast network of canalicular system that communicates with external environment through Haversian system. The three types of cells have different origins; while osteoblasts and osteocytes originate from mesenchymal stem cells, the osteoclasts are related to monocyte or macrophages, and hence derived from hematopoietic stem cells. Bone mineralization is a chemical process that is facilitated by the cellular elements, so we can call it biochemical process. The bone resorption and bone formation are to be tightly coupled with favorable chemical milieu (controlled by various extraneous and intrinsic processes) to result in proper physiological mineralization.

Osteoblasts

Osteoblasts are derived from pluripotent mesenchymal stem lineage. These mesenchymal progenitors can differentiate into various cell types including fibroblast, chondrocytes, adipocyte (PPARγ2 stimulant), myoblasts (MyoD stimulant) and bone marrow stromal cells. Under appropriate stimulation [by Cbfa1 (core-binding factor α1) and/or runt-related transcription factor 2 (Runx2)], the stem cells first differentiate into osteoprogenitor cells (pluripotent cells) and then into osteoblasts (Fig. 4). The osteoblast pathway can be induced by bone morphogenetic protein (BMP) 2, 4 and 7 that upregulate the Cbfa1 mRNA.

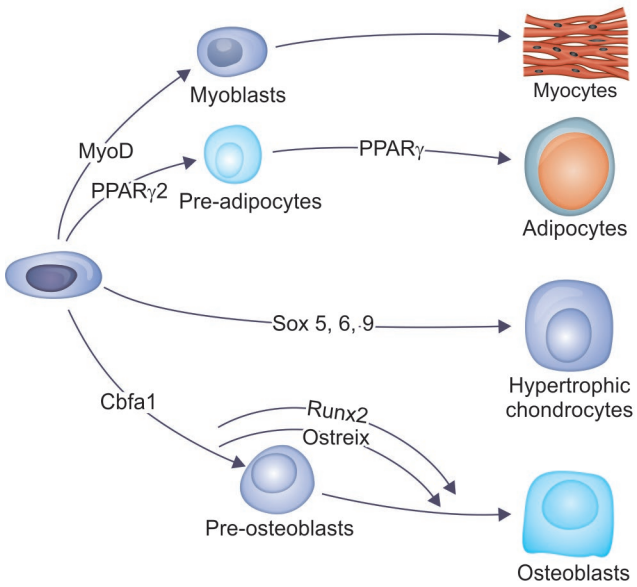


Fig. 4: The differentiation pathway for mesenchymal stem cell and generation of osteoblasts. Note the stem cell can transform into different cells under appropriate influences. For generation of osteoblasts the stem cells first differentiate into osteoprogenitor cells (pluripotent cells) induced by BMP 2,4,7 that upregulate the Cbfa1 mRNA. There is also a possible flux mechanism that operates simultaneously and it has been found that inhibitor of PPARγ that reduces adipocytogenesis increases osteoblastogenesis, and hence bone formation. This may find therapeutic utility in future management of osteoporosis.

The osteoprogenitor cells are disperced into various bone elements and this has a purpose. They are found in:

- The inner layer of the periosteum (predominant source)—responsible for callus formation
- Bone marrow (regenerative and intramedullary callus formation)
- The endosteum—endosteal callus formation
- Haversian and Volkmann's canals—direct or primary healing of bone and remodeling
- Perivascular tissue adjacent to bone—ill-defined function, but may form ectopic bone as after surgery or pathologic bone formation in myositis ossificans.

Osteoblasts measure 15–20 μm in diameter and contain copious cytoplasm. They are cuboidal to columnar, and form a single layer of cell over bone surface where new matrix is being laid down. The cells deposit new bone or new osteoid (*the osteoid seam*) along the surface adjacent to bone only—a property called polarization, bone is not deposited at the free or superficial surface. The *mineralization front* lies deeper to the osteoid seam, where organized mineralization of the newly formed osteoid is being carried out. Osteoblasts are connected to each other by adherens type tight junctions (established by major transmembrane protein cadherins) that also help in communication between cells (communicating junctions) other such junctions include the desmosomes and tight junctions. The high metabolic activity of osteoblasts is suggested by presence of abundant rough endoplasmic reticulum and bulky Golgi apparatus (involved in protein synthesis) and abundant mitochondria required for fulfilling energy requirements and staining basophilic with hematoxylin and eosin stain.

Osteoblasts serve two *main functions:*

1. They produce the organic component of bone matrix—the osteoid by synthesizing and secreting type I collagen along with proteoglycans or glycosaminoglycans. Each new layer is laid down upon existing layer of osteoid (appositional growth) separated by a distinct cementing or watermark line.
2. Osteoblasts facilitate subsequent mineralization of osteoid by secreting matrix vesicles. They create a conducive milieu for deposition of calcium and phosphate in the organic matrix. Osteocalcin secretion is at its peak during mineralization.

The *accessory functions* (also important) of osteoblasts include:

- Production of *non-collagenous proteins* including the osteocalcin, osteopontin, bone sialoprotein and osteonectin that takes part in bone mineralization and maintenance
- Regulating bone metabolism—this is made possible by responding to alteration in levels of hormones involved in calcium metabolism through receptors for parathyroid hormone (PTH) and 1, 25-dihydroxyvitamin D3 present on mature cells
- Paracrine activity by secretion of various cytokines like IL-6 and IL-11, and granulocyte colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) (thus play a role in myelopoiesis also). The osteoblasts also secrete a number of growth factors like transforming growth factor-β (TGF-β), IGF, BMP and PDGF
- Differentiation of osteoclasts—this is now considered a primary function with increasing understanding of osteoporosis. The osteoblasts secrete receptor activator of nuclear factor kappa B (RANK) ligand to regulate the activation and differentiation of osteoclasts that then effect remodeling. The pathway is also the culprit for increased bone resorption in osteoporosis and drugs targeting the same are increasingly becoming popular.

The Process of Mineralization as a Function of Osteoblasts (Fig. 5)

Alkaline phosphatase (ALP) produced by the osteoblast acts as a pyrophosphatase and is the primer for initiation of the mineralization process. The matrix vesicles secreted actively by osteoblasts (discussed above) are the centers for synthesis of crystalline hydroxyapatite from amorphous calcium phosphate though this also takes place outside of vesicles after mineralization has been initiated. The crystals within vesicles act as needle to rupture the membrane of vesicles when they come in contact. These free crystals induce further the precipitation of crystalline hydroxyapatite over the entire organic matrix surface which is lying in a supersaturated solution of calcium and phosphate.

Markers of Osteoblastic Activity

- Alkaline phosphatase enzyme levels and activity is increased with osteoblast activity
- The non-collagenous proteins (discussed above) also mark osteoblast phenotype and are expressed uniquely during osteoblast differentiation.

Growth and evolution of osteoblast activity: With growth and proliferation the molecular activity and development pathways keep changing as the cell is destined to achieve a unique functionality. The progressive changes can be described in a flow diagram as follows:

Expression of cell cycle and histone genes (initial proliferative phase) → upregulation of genes linked to formation of bone matrix (viz. for type I collagen and ALP) → expression of genes for osteocalcin and bone sialoprotein that are associated with mineralization (final stage of osteoblast maturation).

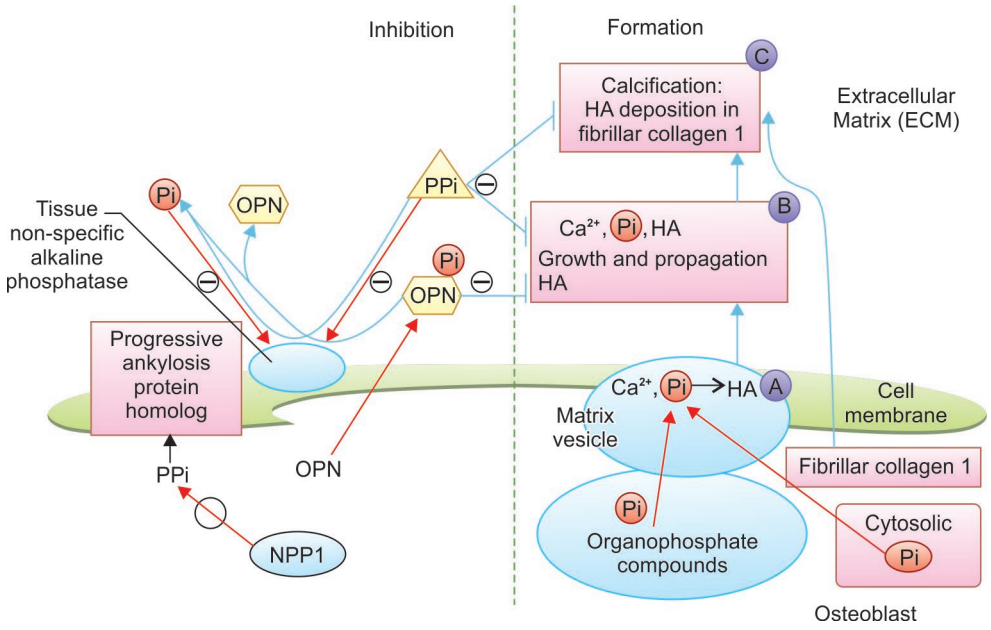


Fig. 5: Role of osteoblasts in bone mineralization. Also depicted is the complex interaction of the alkaline phosphatase and pyrophosphate along with the role of vesicles

It is observed that with osteoblast maturation the proliferative capacity keeps constantly decreasing.

Osteoblast Differentiation and Regulation

Osteoblast proliferation or differentiation is under paracrine and endocrine control, with the predominant control of the former. There is a fine control of relative strengths of opposing signaling pathways within a complex system. Osteoblasts respond to chemical and mechanical stimuli (Wolff's law). The chemical regulators include growth factors (usually proliferation and maturation factors) like TGF-β, BMPs, fibroblast growth factors (FGFs) and transcription factors (differentiation factors) like Runx2/cbfa-1 [core-binding factor alpha (1)] and osterix predominantly.

- Runx2 transcription pathway target genes include osteocalcin, bone sialoprotein, osteopontin and collagen a1 that are responsible for mineralization front mainly and also somewhat for production of cartilage anlage. Mutations causing dysfunction or loss of function of the Runx2 gene causes cleidocranial dysplasia. The disease is characterized by absent or hypoplastic clavicles and prolonged opening of cranial sutures (delayed ossification). Osteoblasts fail to differentiate in mice with targeted deletion of the Runx2 gene and the skeleton comprises exclusively of unossified cartilage. Due to lack of any stimulation from absent osteoblasts these mice also lack osteoclasts.
- Osterix basically acts further downstream of Runx2 pathway and is also responsible for osteoblast differentiation affecting mineralization.

Other regulators of osteoblast differentiation and function include:

- Dickkopf (Dkk1)—This is a negative regulator of bone formation. Reduced Dkk1 (gene deletion) increases trabecular and cortical bone thickness and volume.
- Osteocalcin (gamma-carboxyglutamic acid protein), osteopontin (secreted phosphoprotein 1) and osteonectin (secreted acidic cysteine rich protein)—osteopontin and osteocalcin are negative regulators of bone formation deficiency of former leads to ectopic calcification of medial layer of arteries and resistance to estrogen deficient bone resorption, while deficiency of latter produces higher bone mass of improved functional quality without impairing bone resorption. Osteonectin is a positive regulator and its deficiency produces severe osteopenia, cataracts and weak lens capsule.
- Wnt/β-catenin pathway: Signaling by the Wnt family of secreted glycolipoproteins via the transcription coactivator β-catenin controls embryonic development and adult homeostasis (canonical pathway). This pathway promotes osteoblast commitment and proliferation, finally culminating into its differentiation. The survival of osteoblast and osteocyte is also improved by Wnt/β-catenin pathway even in adverse conditions. Wnt binds to a coreceptor low-density lipoprotein

receptor-related protein (LRP5 or LRP6) activating the pathway which is comediated by one of the frizzled family member (Fz). The activity and binding of LRP5/6 is antagonized by sclerostin (product of osteocytes) and the Dickkoppf (Dkk) family, thus they are now being targeted for osteoporosis therapy and prevention by inhibiting their action.

- Leptin has long known to be synthesized by adipocytes. It is a peptide having its receptor in the hypothalamus. Leptin mediates its effects of osteoblast differentiation and mineralization by inhibiting glycogen synthase kinase-3β (GSK-3β).This mechanism seems to be centrally regulated, but overall effect is negative regulation on bone formation. The leptin-hypothalamic axis control pathway is not fully elucidated as to how it controls bone deposition or bone mass. It is a common finding that patients with generalized lipodystrophy (absence of adipocytes and white fat) develop osteosclerosis and accelerated bone growth, this has also been reproduced in laboratory by producing leptin or leptin receptor deficient mice that develop higher bone mass.

Mechanical Regulation of Osteoblast Function

Osteoblasts also respond to mechanical stress to mediate changes in bone size and shape, a property that has been exploited in some treatment forms like Ilizarov osteosynthesis and possibly also in electrical or sonological stimulation of bone formation. Calcium hydroxyapatite crystals have a piezoelectric effect possibly modulating the osteoblast activity, but complete understanding and science behind this effect is lacking. This process is essential component of bone remodeling.

In adult and aging bone where many of the bone surfaces are inactive, osteoblasts become flattened resembling squamous cells lining bone surfaces (quiescent osteoblasts). This quiescent reservoir gets reactivated into functional forms during remodeling, fracture repair and neoplasia when active bone formation occurs.

Role of Osteoblasts in Various Disorders

Osteoarthritis (OA)—subchondral bone metabolic changes have been suggested as a major pathogenic factor for development of osteoarthritis. There is five-fold increase in leptin expression in osteoarthritis that modulates the osteoblasts to actively produce reactive bone at the degenerated ends. This effect is observed in the form of elevated levels of bone formation markers (osteocalcin and ALP) seen in osteoblasts of osteoarthritic bone. Type I collagen levels synthesis is also increased in osteoarthritis producing subchondral sclerosis that is a radiological hallmark of osteoarthritis.

Rheumatoid arthritis (RA)—Osteoclasts are the major culprit cells for rheumatoid arthritis and cause three types of bone changes:

1. Focal bone loss—seen at the joint margins producing the characteristic erosive changes and cysts.
2. Periarticular osteopenia—seen more prominently around inflamed small joints of hands and feet.
3. Generalized bone loss—this involves the axial and appendicular skeleton and is cytokine mediated—property of systemic involvement. Tumor necrosis factor-α (TNF-α) is the predominant inflammatory cytokine in rheumatoid arthritis. This is responsible for reduction in ALP activity, decreased osteocalcin expression and perturbed collagen type I synthesis that prohibits mineralization of tissue, while bone resorption is continued. It also directly inhibits the osteoblast function in RA.

Osteoporosis and glucocorticoid related to osteoblast function: osteoprotegerin (OPG)/RANK/Receptor activator of nuclear factor-kappa B ligand (RANKL) system represents the main regulatory factors of bone remodeling and are involved in the pathogenesis of osteoporosis though all the mechanism are not fully clear. DKK-1 mRNA is overexpressed in osteoblasts treated with glucocorticoid suppressing the mineralization function.

Smoking and alcohol effect on osteoblast—Moderate amount of nicotine and alcohol both seem to stimulate osteoblast to produce bone. Moderate alcohol consumers have low levels of osteoporotic fractures. Excess of everything though is bad and leads to increased bone resorption.

Osteocytes

Around 10% of the embryonic osteoblastic population is lost by getting trapped and enclosed in their own synthesized matrix. These then become osteocytes (Fig. 6). Their cytoplasm also contains spherical granules stainable with periodate-leukofuchsin like osteoblasts suggesting common origin. The spaces which they occupy are known as lacunae. The lacunae *(L. "pit or depression"*) are flat to oval cavities containing fine apertures called canaliculi *(L. "tiny dust")* through which cytoplasmic processes of osteocytes pass (Fig. 2). Baud and Auil classified the lacunae into four types as follows:

1. Inactive: Small lacunae with smooth borders largely seen in cortical bone.
2. Osteolytic: Large lacunae with irregular borders present in cancellous bone.
3. Osteoplastic: Large lacunae with recently formed matrix present at sites of remodeling and fracture repair.
4. Empty: Lacunae only containing cellular debris following death of osteocytes.

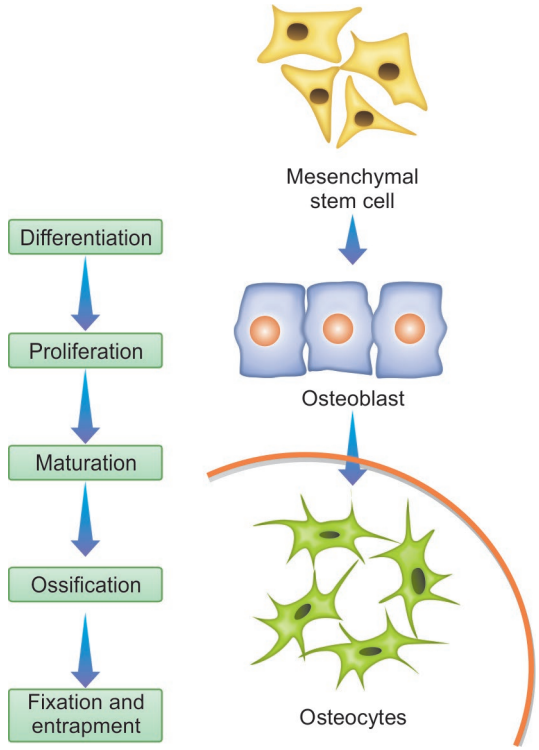


Fig. 6: Formation of osteocytes: These cells are none other than osteoblasts that get entrapped in the bone matrix with deposition of it around the cells. The cells then develop and communicate with other cells through processes

Osteocytes are not dead or nonfunctional cells and their vitality is essential to the maintenance of bone. When the osteocyte dies, the bone around it also becomes nonfunctional and is eventually removed. Osteocytes live within the substance of bone unlike surface cells such as osteoblasts and comprise 90–95% of all bone cells. The processes of osteocytes intercommunicate and these cells are also connected to surface osteoblasts through the network of canaliculi, hence in all they form a large network inside the bone. Unlike osteoblasts the processes of osteocytes are joined by gap junctions which help them "talk" to each other and with cells far off and outside of the bone matrix. They serve following functions:

- Cell signaling (because of vast network) and maintaining the viability of bone matrix. Osteocytes also have N-methyl-D-aspartate (NMDA) neural receptors and may be involved in central mechanisms of bone mineral metabolism like bone resorption in reflex sympathetic dystrophy (RSD)
- Regulates the mineral exchange between the extracellular fluid (ECF) and bone by means of the widespread canalicular system
- Osteocytes express osteoblast-specific factor-1 (OSF-1) that serves to stimulate osteoblasts. The secreted OSF-1 accumulates on bone surface and binds to N-syndecan (receptor for OSF-1) located on osteoblast progenitor cells
- Osteocytes similar to osteoblasts are also known as *mechanosensory cells*. The thin layer of unmineralized matrix around osteocyte cell body and processes mediate the mechanical influence by loading derived flow of interstitial fluid across the osteocyte membrane. This affect translation of mechanical stress to cellular events culminating in bone formation and remodeling.

Regulation of Bone Metabolism and Mineralization as a Function of Osteocytes

- The bone-renal axis for bone mineral metabolism: Patients with autosomal recessive hypophosphatemic rickets display a hypomineralized bone phenotype manifesting as rickets or osteomalacia. There is isolated renal phosphate wasting associated with elevated fibroblast growth factor 23 (FGF23, a phosphatonin) levels and normocalciuria. Similar, phenotype is displayed in animal models having deficient dentin matrix protein 1 (DMP1) which is otherwise highly expressed in osteocytes. In patients with hypophosphatemic rickets there is a mutation affecting the DMP1 start codon, while some patients display a seven base pair deletion damaging the functional C terminus of DMP1. These findings suggest close relation of renal function and osteocyte in bone mineral metabolism.
- Osteocytes possess receptors for PTH, which regulates mineral ion homeostasis
- Human osteocytes secrete sclerostin that inhibits bone formation. In the absence of sclerostin a disorder called sclerosteosis develops in which the skeleton develops high bone mass characterized by increased osteoblast activity. Uninhibited osteoblastic activity results from loss of the SOST gene product, sclerostin.

Osteoclasts

Osteoclasts are multinucleated cells related to the monocyte/macrophage lineage found at bone remodeling site. Their cytoplasm is acidophilic and contains β-glucuronidase. They are derived from *hematopoietic progenitor* cells unlike the osteocytes or osteoblasts. Despite having discrete origin for osteoclasts their differentiation requires the presence of osteoblasts at various steps. Differentiation and maturation of osteoclasts also need a variety of hematopoietic cytokines, such as TNF, interleukins 1, 3, 6 and 11, stem cell factor and colony stimulating factors (CSF). Development of osteoclasts and their maturation (the osteoclastogenesis) needs hormonal support from PTH

and 1,25-dihydroxyvitamin D3 and cytokines like TGF-α, and epidermal growth factor (EGF). Osteoclastogenesis is inhibited by calcitonin, estrogen and TGF-β (Fig. 7). The main function of osteoclasts is bone resorption that is in contrast to osteoblasts at first glance, but in fact both processes are complementary in normal physiology of bone. The characteristic features of osteoclasts are as follows:

- They are found within pits called *Howship's lacunae*—These are the sites of active bone resorption or may represent quiescent cavities where bone resorption has already occurred.
- Osteoclasts like osteoblasts are highly polarized cells with only one site of activity where bone resorption is occurring. The nuclei gather away from the resorbing bone surface (Fig. 8) as the space near to resorptive site will be occupied by vesicles and organelles involved in active resorption.
- "Ruffled border"—It is the cell surface in direct apposition to the bone with numerous infoldings of the plasma membrane (Fig. 8). Ruffled border disappears when the cell is in the resting state. The cytoplasmic region between the conglomerated nuclei and the ruffled border (site of bone resorption) is rich in carbonic anhydrase and in tartrate resistant acid phosphatase (TRAP), lysosomes, mitochondria, vesicles and free ribosomes.
- Clear zone—At the site of active bone resorption osteoclast attach to bone matrix in a ring-like fashion sealing the area. This ring-like area of the cell membrane that forms the perimeter of the ruffled border is called "clear zone" or "sealing zone". This attachment to the bone matrix involves participation of actin filaments and the alpha-v beta-3 (αVβ3) integrin.

The Osteoclast-mediated Bone Resorption

Bone resorption is a systematic process that involves sequential steps as follows:

Mineral resorption: Bone resorption requires the creation of acidic media via secretion of hydrogen ions (aided by ATP driven proton pump) around the ruffled border of osteoclasts in the sealed off clear zone. The process requires the enzyme carbonic anhydrase II to generate hydrogen ions. Acid phosphatase is produced by osteoclasts. These dissolve the alkaline mineral phase of bone.

Removal of organic matrix: Lysosomes release acid hydrolases into the acidified extracellular space and collagenase. Degradation of collagen may also be helped by oxygen-derived free radicals. There is disruption of mineralized matrix to a depth of 1–2 μm. Osteoclasts migrate over the bone surface (migration front lead by osteoclasts followed by osteoblasts), creating many resorption pits in their path which is followed by mineralization front lead by osteoblasts recreating bone (*coupled bone resorption and formation*).

Regulation of osteoclastic bone resorption: The process is highly regulated otherwise all the bone of body will dissolve away in an uncontrolled manner. Osteoclasts are stimulated primarily by IL-6 and RANKL (and are the targets for antiresorptive therapy). These cytokines are

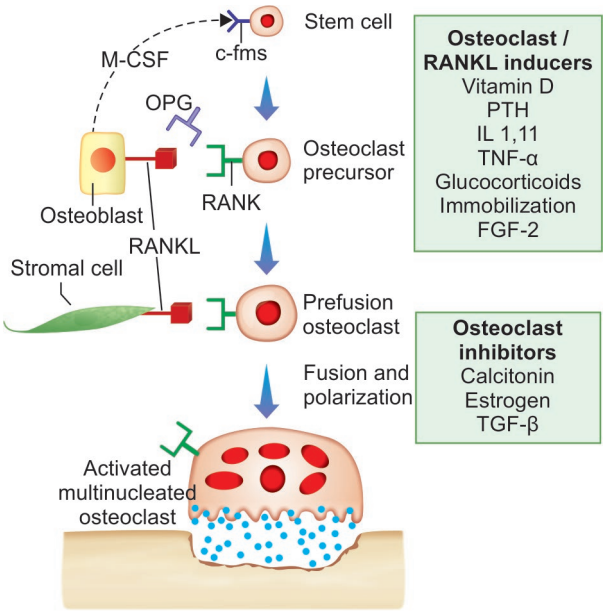


Fig. 7: Role of various cytokines and factors in production of osteoclasts (osteoclastogenesis). Osteoclastogenesis is inhibited by calcitonin, estrogen and TGF-β

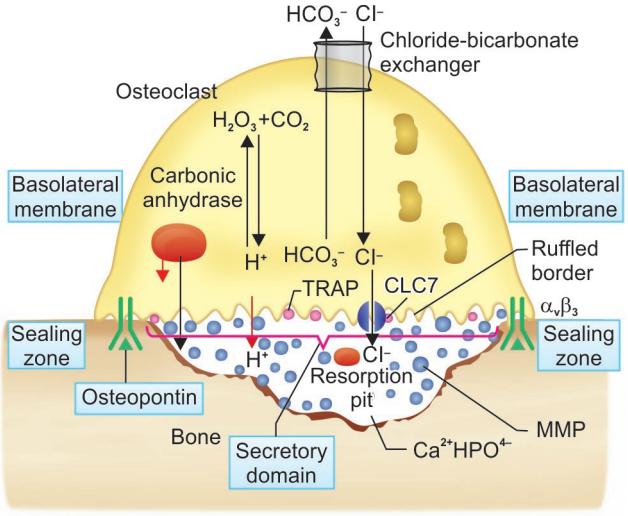


Fig. 8: Illustration depicting the structure and function of osteoclasts

produced locally by the osteoblast under the influence of PTH, Vitamin D3, TGF-β, IL-1 and TNF-α. Osteoclasts have calcitonin receptors that can directly influence the cells, but PTH or vitamin D receptors are missing from osteoclasts so their influence is indirect. Osteoclastic stimulation resulting in bone resorption is also influenced by interactions of the cell membrane integrins and bone matrix proteins that contain amino acids RGD (arginine, glycine, asparagine) like the type I collagen, fibronectin, bone sialoprotein II and osteopontin. These proteins bind to cell membrane integrins and initiating outside-in signaling pathways that finally culminate in bone resorption. In pathological state like in giant cell tumor (osteoclastoma), the osteoclasts are stimulated for bone resorption by IL-6, this has been documented by perturbation of osteoclastic activity in osteoclastoma by anti-IL-6 antibodies; however, physiological role of IL-6 antibodies has not been documented for osteoclastogensis.

Pathological bone resorption: TNF-α and IL-1 secreted by T-cells and macrophages in rheumatoid arthritis and other pathological conditions also stimulate bone resorption. These cytokines bypass the normal cell-to-cell contact required for osteoclast formation (in physiological state) instead they directly stimulate osteoclast progenitors to differentiate and mature into osteoclasts. This type of bone resorption leads to loss of bone mass and produces uncoupling of the process of bone resorption and formation.

Regulation of Osteoclastogenesis

- Interferon-gamma (IFN-γ) suppresses osteoclastogenesis. The T-cell mediated osteoclastogenesis is supported by suppressor of cytokine signaling 1 (SOCS1) which inhibits cytokine signaling. SOCS1 counteracts inhibitory cytokines such as IFN-γ so it is a positive regulator for osteoclastogenesis, but not always. The osteoclast precursor cells that lack SOCS1 are more susceptible to the inhibitory effects of IFN-γ. SOCS1 is induced by RANKL stimulation during osteoclastogenesis. It is interesting to note that the osteoclast precursor cells develop tolerance or resistance to IFN-γ mediated inhibition only if they are first stimulated by RANKL that induces SOCS1. This order of stimulation suggests that the ultimate fate of osteoclast precursor cells is determined not only by the balance of cytokines, but also by the cytokine first encountered.
- Osteoclastogenesis is negatively regulated by interferon-β (IFN-β). RANKL induces IFN-β in osteoclast precursors. IFN-β inhibits the expression of c-Fos which is an essential transcription factor for osteoclastogenesis. It is interesting to note that the influence of IFN-β on cells and RANKL mediated induction of IFN-β is further negatively modulated by the c-Fos that sets up a negative feedback loop, thus RANKL induced IFN-β induces its own inhibitor.

Regulation of Osteoclast Differentiation

The RANK/RANKL/OPG axis: The proliferation and survival of osteoclast precursors, cells of the monocyte-macrophage lineage is dependent on M-CSF secreted by osteoblasts (Figs 7 and 9) and marrow stromal cells. Osteoblasts under the influence of PTH, vitamin D, PGE2 or IL-11 express RANKL mRNA. Both osteoblasts and stromal cells produce RANKL that binds to the RANK receptor on osteoclast precursors. M-CSF primes hematopoietic progenitor cells to become osteoclasts that are activated by RANKL to differentiate into mature or functional osteoclasts. The RANK and RANKL interaction on osteoclast precursors and on osteoblasts or stromal cells, respectively, requires cell-to-cell contact for further development of osteoclast precursors and maturation (Fig. 9). In the cytoplasm, RANK undergoes complex interaction with TNF receptor associated factor (TRAF). TRAF have different effects, while TRAF2 induces osteoclast differentiation TRAF6 is involved in osteoclast activation. Osteoblasts also secrete soluble protein OPG that prevents osteoclast activation by interfering with above RANK/RANKL interaction as it falsely attaches to RANKL (hence it acts as a decoy receptor). OPG, thus, modulates the process of osteoclastogenesis and it strongly blocks osteoclastic bone resorption.

Clinical Implication

As the RANKL/RANK interaction is so important to osteoclast activation, differentiation and maturation, it is a hot target to prevent increased bone resorption in metabolic bone diseases such as rheumatoid arthritis and osteoporosis

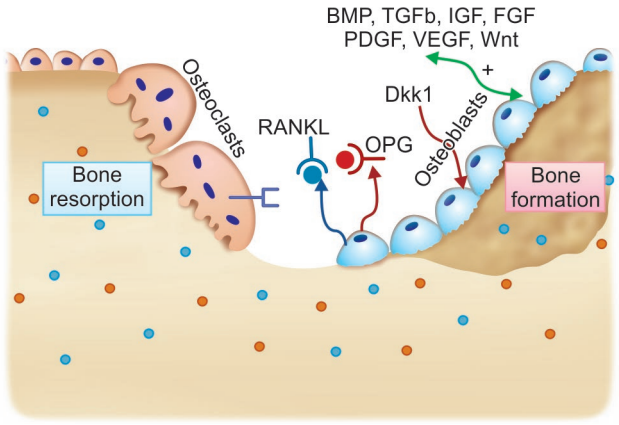


Fig. 9: The bone resorption-synthesis coupling due to interaction between osteoclasts and osteoblasts mediated by RANK-RANKL interaction and OPG protein that is essential for remodeling

and even neoplasia (like GCT). In recent studies, it has been also found to be involved in bone resorption in osteoclastoma. It has been conjured that inhibiting the RANKL/RANK interaction or RANK mediated signals would prevent pathological bone loss by tipping the balance to mineralization. In laboratory mouse knockouts for RANK and RANKL have been found to develop osteopetrosis. These mice have absence of osteoclasts and there is complete failure of tooth eruption. The other models are transgenic mice that overexpress OPG (discussed above) in the liver. These mice also develop severe osteopetrosis due to prevention of RANK/RANKL interaction. Patients from familial Paget's disease of bone have been found to have altered first exon of RANK. Denosumab, a novel drug is found to be effective in preventing attachment of RANKL to RANK receptor, hence opening the possible role in above mentioned diseases.

THE COMPOSITION AND STRUCTURE OF BONE MATRIX

Collagen

Bone is a connective tissue. Like all other connective tissues it also contains varying amounts and combination of collagen, elastin (a related fibrous protein), glycosaminoglycans and proteoglycans. Collagen is the most abundant protein component in all these. Collagens have a unique triple helix composed of three component polypeptide alpha chains. There are several subtypes each produced by a different gene and differ in their biochemical structure. Some 28 different types of collagen have been identified, important ones are listed in detail in Table 2. Type I collagen is the most abundant type of collagen in bone (easy to remember; b-one). Type I collagen, also known as alpha-1 type I collagen, is a protein that in humans is encoded by the *COL1A1* gene. It is a *fibrillar type collagen* that is also present in other tissues like skin, menisci, tendon and ligaments, intervertebral disk annulus fibrosus and synovial joint capsules. Most (≈90%) of bone organic matrix is made up of type I collagen. Type I collagens have several subtypes. The type I collagen specific to bone has predominantly galactosyl-hydroxylysine amino acid configuration in contrast to glucosyl-galactosyl-hydroxylysine predominant conjugate found in dermal collagen. Also hydroxylation and glycosylation as post-translational modifications of collagen are found only in bone specific collagen that partly explains mineralization property of this tissue and not at other places where type 1 collagen is found. The type I collagen is composed of basic structure comprising of repeating tripeptide sequence (Gly-X-Y). The X and Y are commonly proline and hydroxyproline and only to a lesser extent lysine/hydroxylysine. The Gly-X-Y is organized in *a left handed supertwisted helix* (the "α" chain) contributing majorly to the strength of collagen. As a comparison of strength collagen fibers are said to have tensile strength greater than steel wire of equivalent cross-section. Collagen synthesis is controlled by over 20 genes. The single collagen fibril is made of three polypeptide chains arranged in a helical fashion making up the fundamental units. Aggregate of three units of these fundamental collagen fibril forms *tropocollagen.* This tropocollagen then aggregates in a staggered fashion to form a collagen microfibril. A collagen fibril is formed by removal of N- and C-propeptides from collagen microfibrils causing their rearrangement. Between two tropocollagen molecules there is electron microscopically identified "dark area" which is termed a

TABLE 2: Different types of collagen and characteristics

Type of collagen	*Remarks*	*Disorder*
I	Most abundant, bone, teeth, tendon, skin, vessels, cornea and fibrocartilage	Osteogenesis imperfecta, Ehlers-Danlos syndrome, Caffey's disease
II (fibrillar)	Hyaline cartilage, vitreous humor of eye and nucleus pulposus of intervertebral disk disorder (IVD)	Chondrodysplasias and collagenopathy type II and XI
III (fibrillar)	Granulation tissue, skin, intestines and large vessels (30%)	Ehlers-Danlos syndrome, Dupuytren's contracture
IV	Lens of eye, renal glomeruli and basement membranes	Alport syndrome and Goodpasture disease
V (fibrillar)	Interstitial tissue associated with collagen I and large vessels (5%)	Ehlers-Danlos syndrome
VI	Short chain collagen of interstitial tissue	Atopic dermatitis and ulrich myopathy
VII	Anchoring fibrils of dermoepidermal junctions	Epidermolysis bullosa dystrophica
VIII	Endothelial cells	Corneal dystrophy type II
IX	Fibril associated collagens with interrupted triple helix (FACIT) collagen, cartilage (10–20%)	Multiple epiphyseal dysplasia type 2 and 3
X	Mineralizing cartilage	Schmid metaphyseal dysplasia
XI (fibrillar)	Cartilage	Collagenopathy type II and XI
XII	FACIT collagen	
XIII	Transmembrane collagen	
XIV	Undulin	
XVII	Transmembrane collagen	Bullous pemphigoid and junctional epidermolysis bullosa

"hole". It measures about 41 nm and is considered to be the site where mineralization is initiated (Fig. 10). The cross-linking of collagen fibrils imparts stability and improves structural integrity. Cross-linking in collagen is a chemical process involving aldol reaction. Initially, aldehyde is generated by an amino oxidase enzyme which condenses with a lysyl or hydroxyl group to produce a Schiff base forming a cross-link. The aldehyde may also condense with a similar aldehyde in an aldol reaction to generate a stronger bond. The amino oxidase enzyme can be blocked by nitriles. These nitriles are alkyl cyanide substances responsible for producing the disorder lathyrism. Poor collagen quality of collagen in lathyrism leads to development of various spinal coronal and sagittal plane deformities, demineralized bone, recurrent joint dislocations, aortic aneurysm and various nervous system manifestations. On the other hand, extensive "cross-linking" between α-chains as is found in aging individuals gives a rigid and brittle character to the connective tissue. Penicillamine prevents collagen cross-linking and is administered to patients with scleroderma. Defective cross-linking renders collagen susceptible to collagenases. The genetic collagen defects produce various disorders like:

- Osteogenesis imperfecta [clinically presenting with brittle bones that fracture easily, characterized by a glycine to cystine change though various other varieties are found (discussed later)]
- Ehler-Danlos syndrome (clinically present with loose joints that frequently dislocate and also relocate, characterized by a glycine to serine substitution).

Clinical marker—For estimating bone turnover, urinary excretion of hydroxyproline (found exclusively in collagen) and other products of collagen degradation (such as pyridinoline and deoxypyridinoline) are assessed in osteoporosis. These are markers of collagen breakdown and the level of collagen degradation by these degradation products in urine or serum indirectly reflect amount of bone turnover.

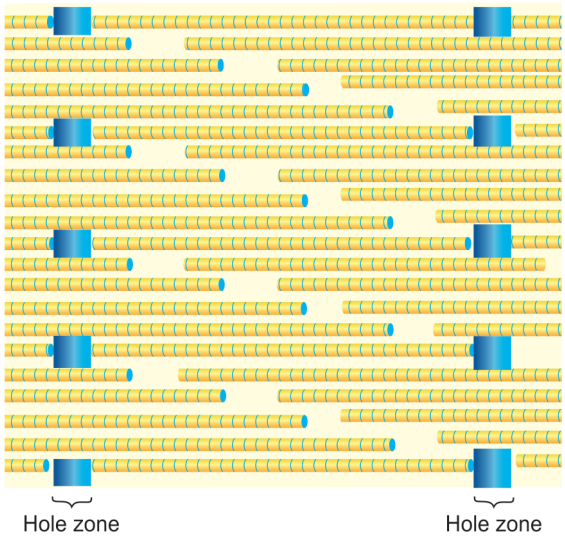


Fig. 10: Illustration of the structure of collagen fibril distribution (organic phase of bone) and concept of hole zone that is the space available for mineralization (inorganic phase)

Other Non-collagenous Matrix Proteins

Calcium Binding Proteins

Osteopontin: Osteopontin is a sialylated and highly phosphorylated phosphoprotein, which exists in multiple forms and is important in cell attachment. This protein is regulated by substances such as 1, 25-dihydroxyvitamin D, TGF-β, PTH, etc. Osteopontin binds to the integrin receptor on osteoclasts and activates the phospholipase C pathway resulting in increased intracellular calcium through Src tyrosine kinase. Osteopontin is found in high amounts in the extracellular matrix of developing intramembranous and endochondral bones, and is present in good amount in osteoblasts, osteocytes, osteoclasts and chondrocytes.

Bone sialoprotein II (BSP II): BSP II is a bone specific protein having cell attachment properties due to its RGD sequence (but less than osteopontin).

Osteonectin: This is a 32 kilodalton (kD) phosphorylated glycoprotein that regulates the extracellular calcium hydroxyapatite formation and mineralization. *Osteonectin* has various other names like SPARC that stands for its description—"secreted protein acidic rich in cysteine", culture shock protein or basement membrane-40 (BM-40), and is encoded by SPARC gene. Osteonectin links mineral to collagen (by binding to both to Ca^{2+}, collagen type I and hydroxyapatite) and thrombospondin. It also promotes mineralization by initiating hydroxyapatite crystal growth. Recently, osteonectin has been found to have involvement in pleotropic functions like morphogenesis, tissue remodeling, angiogenesis and cell migration. The last function may be explained by its function as an anti-adhesive protein by virtue of involvement in cell matrix interactions that is possibly linked to prostate carcinoma metastasis. Osteonectin is expressed in various tissues, but its concentration is particularly high in osseous tissue (up to 10,000 times compared to other tissues). The second peculiarity is that it is the most abundant non-collagenous bone protein and quantitatively it increases with bone maturity. The functions of this protein for bone tissue are:

- Mineralization of nascent bone
- Support to osteoblasts in development, maturation and survival
- Crystallization of inorganic solutes and binding to collagen matrix

- Cell migration, proliferation and possibly differentiation.

Gamma-carboxyglutamic acid proteins ("Gla" proteins): Osteocalcin (bone Gla protein) comprises significant portion of about 20% of the total non-collagenous proteins in bone. It is the second most predominant non-collagenous protein of bone. Osteocalcin is an osteoblast-specific protein with characteristic 3-gamma carboxyglutamic acid residues (Gla) that serves to negatively regulate osteoblasts itself (self-check mechanism). Its synthesis is vitamin K-dependent and is enhanced by 1, 25-hydroxyvitamin D3. The circulation and excreted protein concentration indirectly reflects metabolic cellular activity. Osteocalcin serves following functions:

- Regulates crystal growth and osteoclast recruitment
- Inhibitor of osteoblast function
- May attract osteoclast progenitors in the area for maturation into osteoclasts, thus acting as chemoattractant.

Osteocalcin is encoded by BGLAP gene. It is synthesized by osteoblasts following stimulation from 1, 25-hydroxyvitamin D3 regulated by TGF-β and secreted into the osteoid during mineralization. Osteocalcin is required to stimulate bone mineral maturation. Hence, it serves as a marker for mineralized tissue (like ALP) and increased bone turnover. Apart from ALP it is a good and specific marker for increased osteoblastic activity. It should, however, be clearly understood that both these markers do not correlate with each other as they are synthesized by osteoblasts during different stages of development. The early differentiation marker is ALP, while osteopontin and osteocalcin are late differentiation markers. Clinical utility lies in following the progress of patients with osteosarcoma, and as a marker for its recurrences or metastases in patients with anabolic therapy for osteoporosis the serum levels correlate well with bone turnover and increases in bone marrow density (BMD). Other than bone osteocalcin acts as a hormone that stimulates pancreatic β cells to secrete insulin and increases synthesis of testosterone that may have a role in male fertility.

Fibronectin and thrombospondin: These proteins contain an arginine-glycine-aspartic acid (RGD) amino acid sequence (like osteopontin) and mediate the attachment to integrins, located on cell surfaces. *Fibronectin (FN)* is prominent and versatile extracellular matrix glycoproteins. FN is involved in cell adhesion, development and growth, proliferation, differentiation and cell migration. It is responsible for wound healing, development of carcinoma lung, embryonic growth and development of various tissues. FN mutation and deficiency are incompatible with life. *Thrombospondin (TSP 1, 2, 3, 4 and 5)* is an antiangiogenic protein first isolated from α-granules of platelets. TSP acts as an autocrine growth factor and takes part in prominent role in organization of the extracellular matrix by binding substrates at various binding sites. It also mediates platelet aggregation. TSP is deposited into the bone matrix, where it regulates the extracellular matrix proteins. It is also expressed by osteoblasts and chondrocytes besides platelets.

Connective tissue growth factor: Connective tissue growth factor (CTGF) also called CCN2 is matricellular protein of the CCN family of extracellular matrix associated with heparin binding proteins that regulates various cellular functions like cell adhesion, migration, proliferation and differentiation. It also regulates matrix production and cell survival. CTGF is involved in bone cell (especially osteoblast) differentiation and maturation. The angiogenic activity (chemotaxis of endothelial cells and vascular smooth muscle cells) of CTGF is responsible for neovascularization of the mineralized cartilage in the process of endochondral ossification. CTGF stimulates the production of extracellular matrix (ECM) proteins in fibroblasts and osteoblasts like type I collagen and fibronectin. It is also mitogenic for fibroblasts and chondrocytes and also promotes their differentiation. CTGF blocks apoptosis where cell adhesion is prevented so supports cell migration and improves cell survival. This feature especially gathers importance in various tumorigenesis (cartilaginous tumors), development of atherosclerosis and other fibrotic diseases.

Osteoactivin: Osteoactivin (OA) expression increases during matrix maturation and mineralization. OA is expressed in various malignant tumors such as in glioma and hepatocellular carcinoma facilitating tumor invasiveness.

Alkaline Phosphatase: This enzyme is a hydrolase that causes dephosphorylation in alkaline medium. It is produced by osteoblasts in bone and has three related isozymes. The isozymes are tissue related and are associated with three separate genes. These are:

1. The placental (regan isozymes)
2. Intestinal form (ALP-3) is seen in a variety of tissues such as bone (ALP-2), liver (ALP-1), kidney (proximal convoluted tubules) and skin.
3. Tissue nonspecific form—Found in bone osteoblasts is associated with a single gene at chromosome 1. ALP is adhered to the cell membrane, via. phosphatidylinositol that can be broken by phospholipase C and the enzyme is released free from cell membrane.

All three isozymes require zinc and magnesium ions for their activity. ALP is a glycoprotein that catalyzes the splitting (by hydrolysis) of phosphates (such as pyridoxal-5'-phosphate) at an alkaline pH between 8 and 10. This makes enzyme inactive in blood. Bone specific ALP reflects the biosynthetic activity of osteoblasts. The synthesis of tissue nonspecific ALP is increased by Vitamin D and thyroxin,

whereas glucocorticoids and PTH inhibit its production. In physiological states, the expression of ALP is cell cycle dependent, where its activity is high in G1 through S phases, and reduces in G2 through M phases. The most interesting fact is that despite such a long period of identification of this enzyme the exact role of ALP in bone mineralization is still not fully understood and most explanations are theories. The enzyme has been demonstrated in matrix vesicles, but its role is elusive and may involve degradation of pyrophosphate that is otherwise an endogenous inhibitor of apatite crystal formation by precipitation. Serum ALP activity is raised in various orthopedic and non-orthopedic conditions as follows:

Orthopedic conditions where serum ALP is raised:

- Growing children (physiological rise)
- Primary and secondary hyperparathyroidism
- Rickets and osteomalacia
- Healing fractures
- Neoplasias like osteosarcoma
- Paget's disease
- Osteoblastic metastasis from prostate
- Treatment of osteoporosis by anabolic agents
- Hyperthyroidism
- Herpes zoster

Non-orthopedic causes of raised ALP:

- Neoplasias—Leukemia and hodgkin's lymphoma
- Pregnancy
- Oral contraceptives
- Hepatitis
- Hepatic malignancies
- Amyloidosis
- Inflammatory bowel disease
- Septicemia
- Sarcoidosis
- Myocardial and pulmonary infarctions (acute injuries)
- Pancreatitis.

The ALP serum levels are reduced in hypophosphatasia.

BLOOD SUPPLY OF BONE

Bone receives around a fifth (10–20%) of the cardiac output. The two predominant vascular systems for blood supply include (Fig. 11):

1. The periosteal system.
2. The endosteal system (misnomer as there is no endosteum, better called *intramedullary system*).

Minor contributions come from:

1. Epi-metaphyseal system.
2. Articular ligaments (like obturator ligament in hip).

The Periosteal System (also called accessory nutrient arterioles) supplies only the outer third of the cortex. Entire long bone except its cartilage ends is covered by periosteum with deficiencies only in the region of capsular attachments and nutrient vessels. The periosteum has an inner cellular osteogenic layer (*cambium*) containing osteoprogenitor cells. This layer serves to increase bone girth by appositional bone deposition before skeletal maturity. The outer layer of periosteum consists of fibrous tissue that is predominantly supportive and imparts stiffness to it. The periosteum is thick in children due to the presence of active cambium (cellular layer) with blood supply in the form of *longitudinal arterioles* incorporated within. However, with aging the cambium gets hypotrophied and becomes thin, also the vascularity reduces with absence of prominent longitudinal arterioles. This has important surgical implications with respect to higher incidence of nonunion and delayed union in fractures fixed after ripping periosteum, especially in adults. Over most of the surface of long bones in an adult the periosteum is loosely attached beneath muscle bellies. The only periosteal blood vessels in those areas are venules and capillaries. Periosteal supply is a low pressure system compared to intramedullary system of blood supply. So, the normal flow in mature bone is *centrifugal* with excess blood exiting from the periosteal venules. In vascular stress situations like acute embolism of the intramedullary system or reaming of intramedullary canal, the blood flow of the periosteum increases many fold compensating for the loss in blood supply. In such situations, the blood flow becomes *centripetal*.

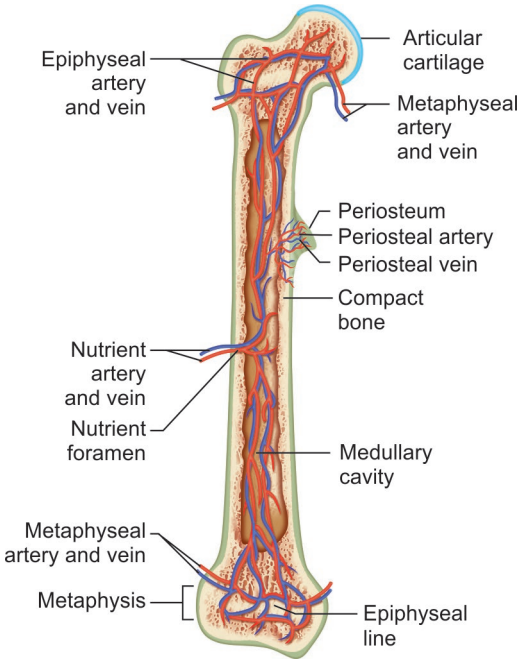


Fig. 11: Illustration depicting the blood supply of bone, nutrient artery—diaphyseal circulation, metaphyseal and epiphyseal vessels supply the respective zones; periosteum supplies the peripheral one-third of the cortex

Intramedullary system: Diaphyseal nutrient arteries (one or two) enter through the cortical bone often in an oblique direction. The common ports of nutrient artery entry are the fascial regions firmly attached to the diaphysis of long bones or along anatomical bony ridges like linea aspera of the femur. Perivascular fat supports and protects the afferent blood vessels in these regions so that the vessels can approach directly the cortical surface and enter. The principal nutrient artery to bone is formed of these afferent vessels. The afferent vessels after entering the intramedullary system divide into ascending and descending branches that supply inner two-thirds of the cortex (outer thirds being supplied by periosteal supply, discussed above) and whole of the medullary cavity. The above system holds true for most long bones, except large irregular bones, flat bones and some short bones that receive a major blood supply from the periosteum superficially or otherwise from large nutrient arteries that directly penetrate into the medullary bone. The intramedullary and periosteal systems of blood supply anastomose freely.

Metaphyseal and epiphyseal arteries: This system supplies blood to the ends of bones, diaphysis being supplied by the above mentioned systems. They arise as principle branches from adjacent articular supply or periarticular plexus. This system also freely anastomoses with the diaphyseal or intramedullary system terminating in bone marrow, trabecular bone, cortical bone and articular cartilage. In immature skeleton, growth plate (end plates of cartilage component of physis) separates these arteries from intramedullary system. Near the growth plate (physis) a few vessels make hairpin bend and retreat back upon themselves, while most enter into an open circulation. In the past, this arrangement of hairpin bends was considered to reduce the rate of blood flow to cause localization of blood-borne bacteria and serve as focus of onset of hematogenous osteomyelitis, especially in children. This concept is challenged now (discussed under bone infection section). After closure of growth plate in adult, the entire expanded end of the long bone becomes the metaphysis. This metaphysis receives superficial blood supply from periosteal arteriolar vessels entering all over, except in regions covered by articular cartilage. The longitudinal afferent arterioles seen in periosteum of immature skeleton in metaphysis and diaphysis disappear with age related atrophy of the cambium layer of the periosteum. Throughout life of individual this layer remains dormant and atrophic until activated by specific stimulus like trauma.

Venous and Lymphatic Drainage

From bone the blood is drained by collateral venous system that accompanies the afferent arteries. These veins leave through foramina near the articular ends of the bones commonly. Lymphatic vessels are also abundant in the periosteum.

Direction of Blood Flow

The intramedullary system of vessels represents a high pressure system within the long bone. This system is derived from the nutrient artery that is a branch of systemic circulation, so the intravascular pressure is higher in the medulla or marrow of bone than in the periosteal system, where vasculature mainly comprises of venules and capillaries. As is commonly known from laws of physics fluid that current flows from high to low pressure region, consequently, the *direction of normal blood flow* in physiological state in bone through the diaphyseal cortex of a long bone is *centrifugal,* i.e. from medulla to periosteum (inside-out). Under some pathologic conditions the intramedullary vascular pressure may get diminished resulting in reversal of blood flow through the vascular channels of the diaphyseal cortex, so that it becomes outside-in or centripetal. This flow reversal can happen with:

- Occlusive vascular disease
- Osteoarthritis
- Displaced fracture
- Reaming of intramedullary canal.

Under above conditions, the blood flow reversal primarily occurs through existing normal vascular channels though studies demonstrate opening up and development of new vascular channels.

NERVE SUPPLY OF BONE

Bones have rich nerve supply, especially at the articular ends of the long bones, the vertebrae and larger flat bones. The nerve fibers supplying bones accompany nutrient blood vessels to reach the interior of bones and Haversian system. Also, accompanying the arteries inside the Haversian system are vasomotor nerves that control blood flow through them as in physiological state in most other body systems by vascular constriction or dilation. The periosteal nerves have nociceptive ends so it is pain sensitive (common experience is needed for anesthetizing periosteum, while placing Steinman pin). Bones are also innervated by sympathetic fibers originating from the sympathetic ganglion that again enters bone along with nutrient vessels. Blood flow in bones reduces by 80% in stressful conditions and shock. Neurotransmitters released by various nerve endings not only regulate the blood flow, but also they have a role in bone development and remodeling. Dopamine transporter gene DAT (-/-) deletion mice demonstrated 30% reduction in bone mass and strength.

The endogenous cannabinoids (anandamide, 2-arachidonoylglycerol) have been found to regulate bone remodeling to some extent. They activate the G protein-coupled; central and peripheral cannabinoid receptor type 1 (CB1) and type 2 (CB2), respectively. CB1 is responsible for the typical cannabinoid associated psychotropic and analgesic effects, but CB2 is of interest and plays role in liver fibrosis and atherosclerosis. Endocannabinoids inhibit lipogenesis (effect opposite that of corticosteroids and alcohol). So they have trophic effect on bone formation and remodeling (Fig. 12). CB2 receptor null mice show accelerated age-related trabecular bone loss with minimal change in cortical thickness (osteoporosis like changes). This is partly explained by increased osteoclast number in the trabecular bone. CB2-specific agonist increases osteoblast number and activity, while simultaneously restraining osteoclastogenesis in trabecular bone. They inhibit proliferation of osteoclast precursors directly and also restrict the differentiation and maturation of osteoclasts by suppressing expression of receptor activator of NF-κβ ligand in bone marrow-derived osteoblasts or stromal cells. Thus, it appears that endocannabinoid system maintains normal bone mass by CB2 signaling. In addition to other modalities being tried, CB2 receptor system may serve a molecular target for the diagnosis and treatment of osteoporosis in future.

BONE DEVELOPMENT

In human embryo bone appears after 7th week. Typically two forms of bone formation are evident in the system. The bones of the entire axial and appendicular skeleton develop through either intramembranous or endochondral bone formation (ossification). The two processes differ in absence or presence of a cartilaginous intermediary. A cartilage model is first formed and which ossifies in latter process (Fig. 13), while it is conspicuously absent in former. A third mode of physiological bone formation during development is appositional ossification.

Non-physiological bone formation: Bone can also form in various other forms and processes, but not involving the skeletal development. Callus formation and regenerate development are physiological forms of bone formation that occur in specific conditions and are not a part of developmental ossification process. Similarly, ectopic ossification and myositis ossificans are pathological forms of bone formation.

Intramembranous Bone Formation

In this process like the endochondral ossification a cartilage anlage forms, but it is not ossified and for new bone to be formed the anlage needs to be completely resorbed. The flat bones of the skull and face are typical examples of intramembranous (membranous) ossification. On the preformed scaffold of cartilage the osteoprogenitor cells aggregate at the sites of new bone formation (preosseous condensation) that are usually centrally located and differentiate into osteoblasts that actively synthesize new bone matrix advancing radially peripherally. Osteoblasts then lay the bone successively on this scaffold in layers, a

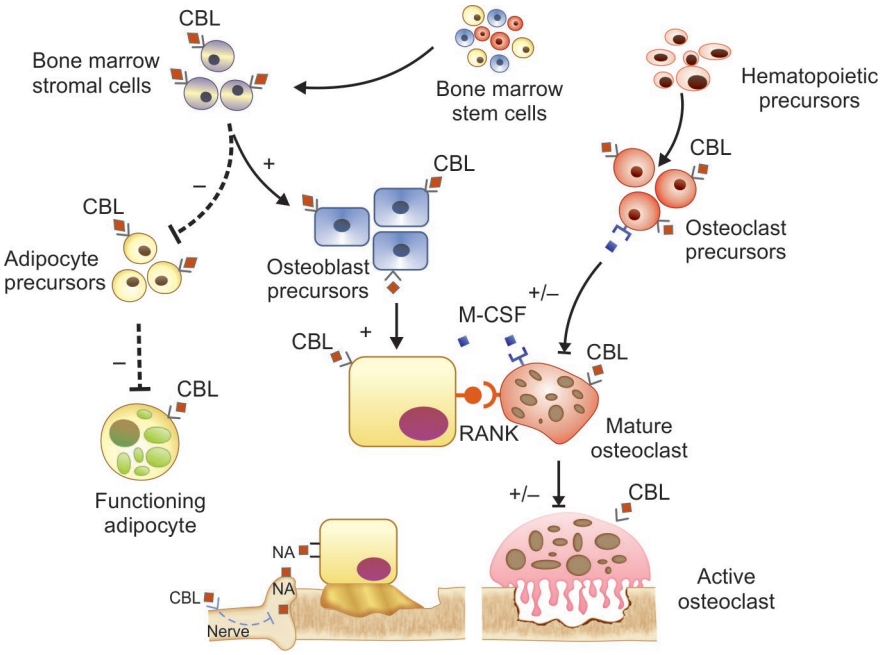


Fig. 12: The endocannabinoid system and its effect on bone formation

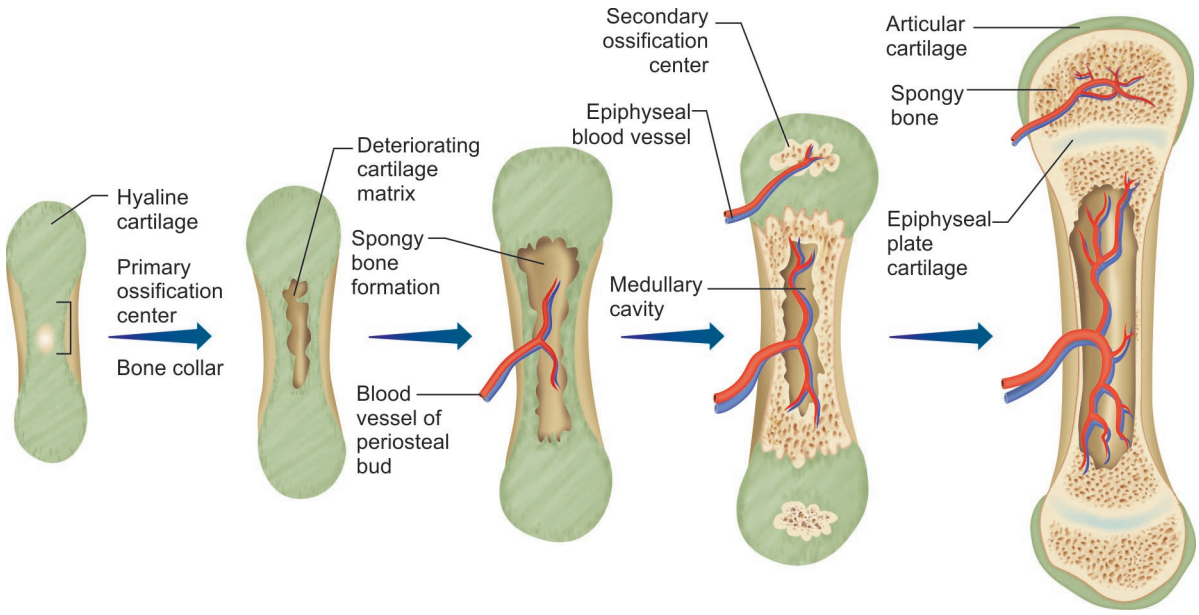


Fig. 13: The illustration depicting process of endochondral bone formation from a cartilage model

process called *apposition* (deposition upon prior bone). Ossification centers develop within the bone and enhance the rates of mineralization. The surrounding mesenchyme condense into periosteum and lays down bone beneath it. The bones take on a *lamellar* character gradually. In the adult, similar process is called Haversian remodeling. Chondroid bones of the skull (wormian bones) that are seen in association with suture closure are developmentally intermediate between cartilage and bone. They contain both types I and type II collagen. Here the scaffold is formed by chondroid bone upon which lamellar bone is deposited. It is not replaced by bone as in the endochondral ossification. Many factors (all not known) play potential roles regulating bone formation. Core-binding factor alpha-1 (cbfa-1) or Runx2 transcription pathway (discussed above) is responsible for osteoblast differentiation and binding to the osteocalcin promoter. This causes osteocalcin expression essential for processing of mineralization front. This pathway is also responsible partly for formation of cartilage anlage. Cbfa-1 mutation causes cleidocranial dysplasia in which there is delayed ossification of cranial sutures and absent or hypoplastic clavicles.

Endochondral Bone Formation

Here a cartilage tissue first forms as a model (cartilage anlage) from aggregated mesenchymal cells and is subsequently ossified. The appendicular skeleton, vertebral column and pelvis develop via endochondoral ossification. This discrete complex process can be divided into five stages.

First stage—this stage begins with differentiation of mesenchymal stem cells to become cartilage progenitors. At molecular level this involves expression of transcription factors, Pax1 and scleraxis by activation of cartilage-specific genes.

Second stage (the precartilaginous state)—this stage involves condensation of the committed mesenchymal stem cells to form compact nodules and these cells differentiate into chondrocytes by the progression of activity of Pax1 and scleraxis in stage one. The condensation of committed cells is affected by N-cadherin. This precartilaginous state also involves expression of SOX9 gene (sex reversal Y-related high-mobility group box protein) that encodes a DNA-binding protein. SOX9 expression is an essential step that is required for proper organization of further complex interactions. Mutations of the SOX9 gene are generally incompatible with life and it has been found that infants with specific mutations of the SOX9 gene die from respiratory failure due to poorly formed tracheal and rib cartilages.

Third stage is marked by chondrocytes proliferation forming the cartilage model (pre-cartilage condensation). Chondrocytes secrete a cartilage-specific extracellular matrix.

In the *fourth stage*, the chondrocytes hypertrophy and produce collagen type X and fibronectin, so that mineralization can proceed by calcium carbonate.

Fifth stage is marked by vascular invasion of the cartilage model and apoptosis of hypertrophic chondrocytes. The

osteoprogenitor cells after proper stimulation differentiate into osteoblasts that begin to lay down osseous matrix on the mineralized cartilage remnants that have been partially degraded. This process occurs in the cartilage model first at the region forming future diaphysis of long bone and is known as the *primary center of ossification*. From the primary ossification center the endochondral ossification spreads vertically along the axis of the developing bone in both directions. *Secondary centers of ossification* form at the ends of each bone (the epiphysis) eventually leaving an area of cartilage between the primary and secondary ossification centers called growth plate or physis. It is here that continued growth in length occurs at both ends of the developing bone.

Structure of Physis (Figs 14 and 15)

The term epiphyseal plate or epiphyseal growth plate so commonly used actually confuses with the term "epiphysis" and should not be used. Rubin introduced the term "physis" or "physeal segment" and is preferable. Two forms of growth plates exist in long bones one horizontal and the other spherical. The horizontal growth plates are responsible for increase in length of bone, while the spherical growth plates take part in growth of epiphysis and the physis itself circumferentially and contributes also to thickness of bone. The fully developed cartilaginous growth plate in the human long bone typically comprises of various anatomically discrete tissues acting together as a composite unit to perform one specialized function, and thus is referred as an organ by many researchers. The physis for physio-anatomical description can be divided into three components (Fig. 14):

1. The *cartilaginous component* of growth plate contains three predominant regions:
   a. Reserve zone—contains spherical, single or paired chondrocytes involved in matrix production. They are full of glycogen and have predominantly anaerobic environment due to low PO_2.
   b. Zone of chondrocyte proliferation—This zone serves three purposes—matrix production, cellular proliferation and longitudinal growth. Latter is equal to the combination of former two. It has flattened chondrocytes arranged in distinct columns. The endoplasmic reticulum occupies progressively increasing percent of the cytoplasmic area that rises from 14.9% at the top of the zone to 40.1% at the bottom of the zone of chondrocyte proliferation. Biochemical analysis reveal that this zone contains the highest content of hexosamine, inorganic pyrophosphate and has highest lysosomal activity. The chondrocytes in this zone are the only cells of growth plate that divide (proliferate). Longitudinal growth in the physis is directly proportional to the

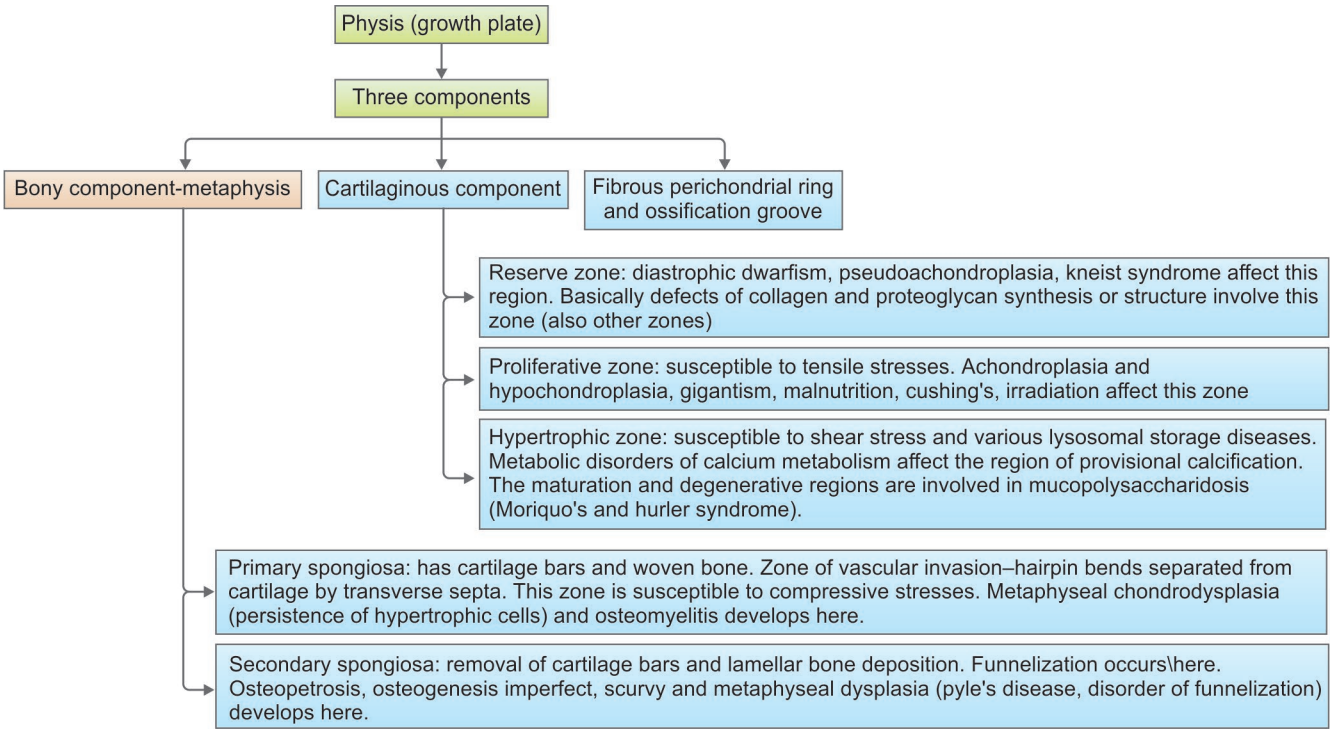


Fig. 14: Structure of physis and involvement in various disorders

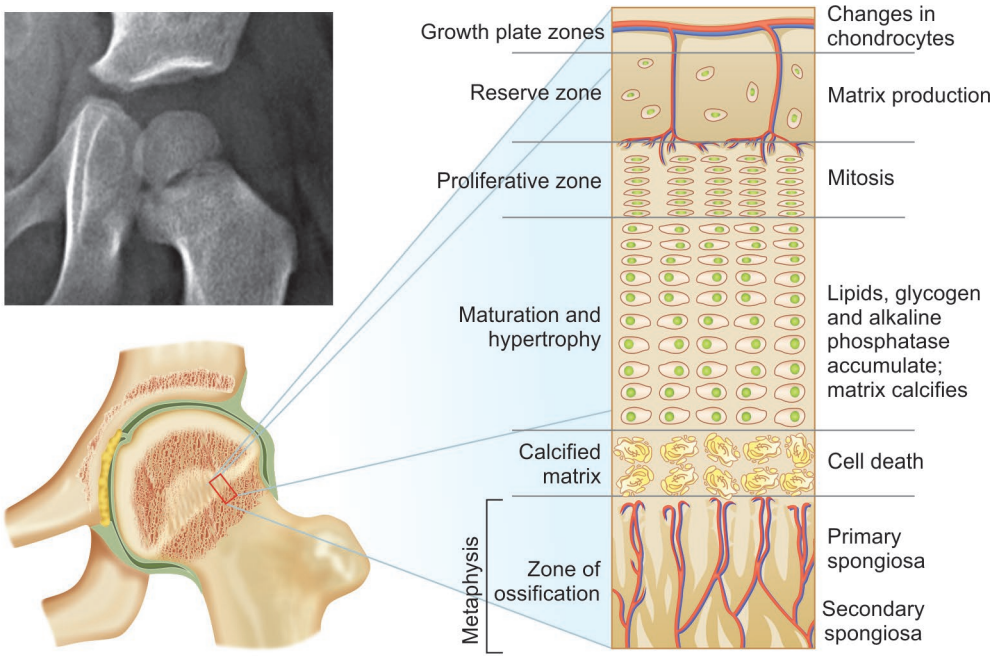


Fig. 15: Structure of growth plate of bone—the physis, the illustration depicts various zones and associated disposition of vasculature

product of rate of production of new chondrocytes at the top of the proliferating zone to maximum size of the chondrocytes in the lowermost layer of the hypertrophic zone. Due to heavy demand and consumption oxygen tension is maintained higher in the proliferating zone of physis at mean of 57 mm Hg (± 5.8 mm Hg) compared to any other zones.

c. Zone of chondrocyte hypertrophy—This zone has three discrete functional and histological regions namely the *maturation zone, degenerative zone* and *zone of provisional calcification*. The function of this zone is to prepare the matrix for calcification and to calcify the matrix. The chondrocytes show progressive vacuolation and increase in size with disintegration. The mitochondria instead of forming adenosine triphosphate (ATP) start to accumulate calcium. The initial calcification ("seeding") occurs at the bottom of the hypertrophic zone (zone of provisional calcification) within physis. This is initiated by matrix vesicles around which then mineralization progresses. As noted above, matrix vesicles are rich in ALP that destroys pyrophosphate which is an inhibitor of calcium phosphate precipitation. Destruction of pyrophosphate tips the balance to precipitation of calcium and phosphate, and hence facilitates mineralization. Matrix vesicles also simultaneously accumulate calcium from the calcium lost through mitochondria at the same level in the middle of the hypertrophic zone. The following sequence of events occurs for final calcification:
   - Mitochondrial calcification
   - Reduction of nutrients and oxygen supply to the hypertrophic chondrocyte with mitochondrial death
   - Anaerobic glycolysis (this occurs due to distance from vascular supply, and hence oxygen tension this hypertrophic zone is very low to a mean of 24.3 ± 2.4 mm Hg). All the stored glycogen is consumed
   - Calcium is released from mitochondria
   - Nucleation of mineralization in matrix vesicles
   - Matrix calcification.

   The cartilaginous matrix gets calcified as the cells hypertrophy. This calcified cartilage then serves as a scaffold for bone matrix deposition by osteoblasts. The lacunae that remain after apoptosis of hypertrophic chondrocytes are utilized by blood vessels. Abnormalities in chondrocyte development or function can disrupt this organized sequence of physeal growth and maturation producing abnormal bones usually stunted in growth and having crooked shape. Achondroplasia is such a condition causing dwarfism and possibly involves a mutation in FGF.
2. The *bony component or metaphysis* serves few important functions—it is involved in vascular invasion of transverse septa at the bottom of cartilaginous

portion of growth plate providing blood supply, the other functions are new bone formation and bone remodeling. It has two predominant components, *the primary spongiosa and the secondary spongiosa*. There is internal (histologic) remodeling with removal of calcified cartilage bars (primary spongiosa) and lamellar bone deposition (secondary spongiosa). The external or anatomic remodeling gives funnel shape to metaphysis (funnelization). Near the transverse septa separating metaphyseal from cartilage component there is low oxygen tension (19.8 ± 3.2 mm Hg) and high degree of rouleaux formation of RBCs due to vascular stasis. High levels of phosphoglucoisomerase (enzyme compatible with anaerobic metabolism) are found in this region. The low oxygen tension inhibits WBC activity which is highly oxygen dependent, while is favorable for pathogens. This may explain the reason for hematogenous osteomyelitis in ends of bone and not vascular stasis per se; the concept is still however challenged).

3. A *fibrous sheath* surrounds the growth plate at periphery that comprises of perichondrial ring of LaCroix and the ossification groove of Ranvier. These two structures are structurally different and serve different functions. It appears that the groove of Ranvier contributes chondrocytes to the physis for the growth in diameter (appositional growth or latitudinal growth) of the plate. There are three distinct cell groups in the Ranvier's ossification groove:
   a. Progenitor cells for osteoblasts—this is a group of densely packed cells that forms the bony band in the perichondrial ring.
   b. Undifferentiated cells and fibroblasts contribute to appositional chondrogenesis and are responsible for diametrical growth of physis.
   c. Fibroblasts cover the groove and serve to firmly anchor the perichondrium of hyaline cartilage to growth plate.

The perichondrial ring provides mechanical support for the otherwise weak bone-cartilage junction of the growth plate. It is a dense fibrous band that encircles the growth plate at the bone-cartilage junction and in which collagen fibers run vertically, obliquely and circumferentially.

Blood Supply of the Physis

The three components of growth plate have distinct blood supply. The proliferative zone receives blood supply from branches of epiphyseal vessels that penetrate the top four to ten columns. These vessels arise perpendicular to the main perichondrial epiphyseal artery and pass through micro spaces in the reserve zone to finally terminate in the proliferative zone at the summit of cell columns. These vessels do not pass across the proliferative zone into hypertrophic zone. The nutrient supply for hypertrophic zone instead comes indirectly from terminal branches of nutrient artery which is also supplied by metaphyseal arteries or plexus of vessels at places. The nutrient artery is the main supply for the central metaphyseal region and as much as four-fifths of the metaphysis receives nutrition and oxygen through it. The metaphyseal blood vessels supply only peripheral portions of the metaphysis, especially through the periosteum. As mentioned earlier also, the nutrient and metaphyseal arteries terminate into vascular loops or capillary tufts. The terminal branches pass vertically toward the bone-cartilage junction of physis and turn back sharply forming hairpin bends just below the last intact transverse septa at the base of the cartilage portion of the plate. The venous branches from hairpin bends descend via several progressively larger veins to finally drain into the large central vein of the diaphysis. Here again we see that no vessels penetrate the bone (metaphysis) cartilage (hypertrophic zone) junction beyond the last intact transverse septa, hence, hypertrophic zone is not directly penetrated by any vessel and most nutrients reach it via diffusion or open circulation. Hence, in a fully developed growth plate, hypertrophic zone is entirely avascular. Compared to above the groove of Ranvier and the fibrous perichondrial ring of LaCroix are richly supplied from perichondrial arteries.

Regulation of Growth Plate (Physis)

The chondrocytes elsewhere in body are not responsible for organ growth as in bone. So the chondrocytes of the growth plate are functionally different from articular cartilage cells. Even the chondrocytes within different parts of the growth plate show different response to similar stimuli. Growth plate is regulated by a host of systemic and local factors.

- Systemic factors regulating the metabolism and development of growth plate include growth hormone, vitamin D and glucocorticoids, IGF I, thyroid hormone and estrogens (in females). These factors affect the linear growth of bone and also maturation of physis (maturation is enhanced by estrogen so females grow longer earlier and physis closes also faster terminating the linear growth earlier)
- PTH, IGF and vitamin C influence the whole cartilage component
- The growth hormone and thyroid hormones are trophic to reserve, proliferative zone and the maturation region of hypertrophic zone
- Gonadal hormones stimulate the hypertrophic and metaphyseal regions

- Local factors that influence the growth and function of physis include TGF-β, PTHrP, Indian hedgehog (IHH) and FGF receptor type 3 (FGFR3) (Fig. 16).
  - TGF-β inhibits chondrocyte proliferation, hypertrophic differentiation and matrix mineralizatio
  - Indian hedgehog (IHH) induces the expression of PTH related protein (PTHrP) in the perichondrium. PTHrP inhibits chondrocyte differentiation, thus acting as a negative feedback loop
  - Proliferative zone is controlled by FGF, PDGF and TGF-β. The maturation of chondrocytes occurs under the influence of prostaglandins and IGF.

Appositional Ossification

The enlargement of bone in diameter occurs by appositional bone growth. The osteoblasts deposit additional bone on the existing bone surface. There is continuous bone resorption from inside until the desired bone thickness is reached.

THE PROCESS OF BONE MINERALIZATION

Calcification of cartilage and osteoid as a physiologic process is called mineralization. The inorganic matrix is laid down in a specific pattern along the organic matrix. The process is complicated and poorly understood. Calcium hydroxyapatite predominates mineral phase in humans (invertebrates contain calcium carbonate, whereas plants have oxalate) that is deposited along the long axis of the collagen fibrils. The inorganic matrix is deposited in the hole zone of collagen matrix created by the space left between staggered arrangement of fibrillar structure. The individual collagen macromolecules are staggered by one-fourth of their length leaving around 400 Å long and 15 Å wide hole zones. The mineral is initially deposited as randomly and poorly oriented amorphous calcium phosphate. The amorphous phase undergoes a series of organized solid phase transformations that ultimately lead to production of crystalline hydroxyapatite which is the stable solid phase. Various factors are important in mineralization (Table 3).

The *initiation of mineralization* is caused by heterogeneous nucleation. There is *active binding* of calcium, phosphate and calcium phosphate complexes at the nucleation site and not just simple precipitation of the mineral. Matrix vesicle provides the requisite environment for this process. The physiologic state of extracellular fluids is supersaturated with respect to octacalcium phosphate. Pyrophosphate and serum proteins act as crystal inhibitors. Phosphatases and proteases are essential to locally remove these inhibitors and facilitate apatite formation. Still crystallization would need local increase in concentration of substrates (calcium and phosphate) far beyond supersaturation levels to overcome the energy of the reaction of crystal formation and real mechanism is elusive. Many theories have been propounded by eminent researchers for explaining mineralization initiation and

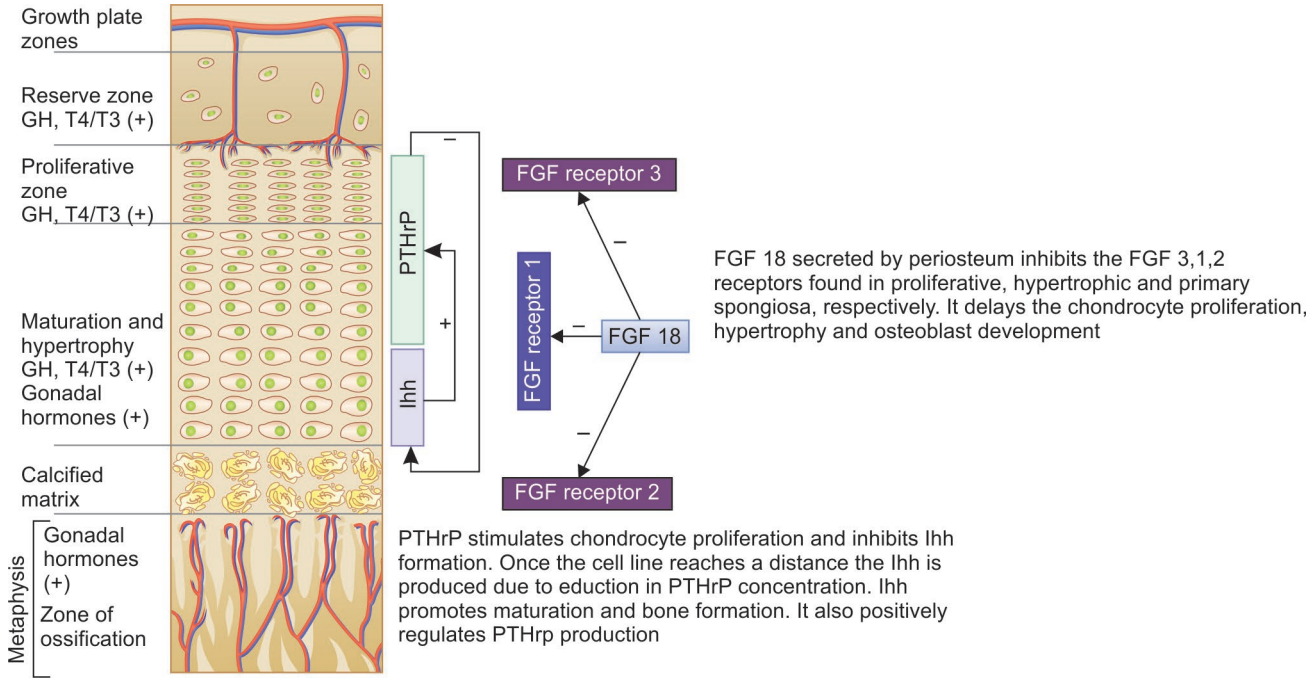


Fig. 16: The effect of various growth factors and cytokines locally on the different zones of physis

TABLE 3: Role of various constituents in bone mineralization

Component	*Remark*
Collagen	Provides support to crystal deposition. Collagen can initiate crystal precipitation. Facilitates formation of solid phase crystals from solution. Does not nucleate crystal deposition
Calcium-binding protein	These phosphoproteins may nucleate crystal deposition and promote polymerization Proteoglycan inhibit calcification by sequestering the calcium ions or shielding collagen
Gla proteins	Osteocalcin and other Gla proteins bind calcium by virtue of Gla residue
Pyrophosphate	Inhibits calcification and increases the solubility of calcium phosphate preventing precipitation
Alkaline phosphatase	Possibly degrades pyrophosphate to aid in crystal precipitation and mineralization

propagation, but for that the role of matrix vesicles should be understood first.

The Role of Matrix Vesicles in Initiation

Matrix vesicles are the membrane bound cell free structures derived from chondrocytes and osteoblasts serve these requisites and initiate mineralization. The trilamellar membrane bound matrix vesicles secreted by chondrocyte seed the calcium phosphate salt in matrix. Probably derived from mitochondria, they can either store calcium or ATP. In the proliferative zone with high oxygen availability, the mitochondria synthesizes ATP for cellular requirement. While progressing down to hypertrophic zone, the oxygen concentration falls and the mitochondria store calcium instead of ATP. These are extruded out at zone of provisional calcification with degeneration of cells and burst releasing microcrystals of calcium phosphate (possibly hydroxyapatite also). Under supersaturated conditions the mineralization is hence initiated.

Mineralization Propagation

After nucleation hydroxyapatite crystal formation undergoes propagation (multiplicative proliferation) leading to progressive ossification of calcification. These are *matrix vesicle mediated* and *collagen mediated* hydroxyapatite precipitation.

For theories regarding bone mineralization one school of thought gives primacy to matrix vesicles. The other school gives bone matrix the primacy for initiating and propagating mineralization. These two theories have tried to explain the intermediate mechanisms between calcium, phosphate and hydroxyl ions in blood stream and eventually formation of hydroxyapatite.

The Urist Triphasic Hypothesis

In first phase, a soluble calcium protein substrate is formed. The calcium disrupts hydrogen bonds in collagen and reacts to form calcium complexes. These anionic complexes in phase two react with phosphate to form soluble protein calcium phosphate complex. In phase three, the neutralized calcium-protein-phosphatase complex reacts with Ca^{2+} and HPO_4^{2-} depending on the solubility product which is kept at metastable state (supersaturated) at the mineralization front.

The Glimcher Hypothesis

Proposes the stereochemical disposition of collagen components to be primarily responsible for nucleation. The nucleation occurs with respect to the physical organization of the collagen physical and chemical properties.

Pathological Calcification

Unlike the physiological calcification of bone described above there are lots of pathological conditions where calcium deposition occurs:

- Damaged tissues have pathologic extracellular or intracellular "dystrophic" calcification. The calcium deposition within the soft tissues (both dystrophic and metastatic) in myeloma, metastases, fat necrosis, trauma, sarcoidosis, scleroderma, hyperparathyroidism, etc. is caused by calcium hydroxyapatite.
- "Metastatic" calcification occurs in association with altered serum levels of calcium and phosphate.
- Crystal deposition in joints—This deposition is rarely massive and simulates tophaceous deposits, hence called tophaceous pseudogout. The linear calcification seen along menisci and articular cartilage or in the intervertebral disk radiographically, is mostly due to calcium pyrophosphate deposition (CPPD disease).

BONE REMODELING

Remodeling is a process that involves tight coupling of bone resorption and formation to adjust to constantly changing requirements with activity and aging (Fig. 17). It is essential for the bone to change its form in response to stress and strain else, it will never leave its infantile form or be able to bear the increasing weight with growth. Persons doing active labor need to have stronger bones supporting muscles so on and so forth. Also, remodeling acts like regular maintenance work healing the microtrauma and fractures that keep occurring in this "hard" tissue. Bone undergoes remodeling throughout life which is primarily a function of trabecular bone arrangement, but is actively also seen in healing