CARTILAGE AND BONE

BIOCHEMISTRY AND PHYSIOLOGY

P. J. Roughley

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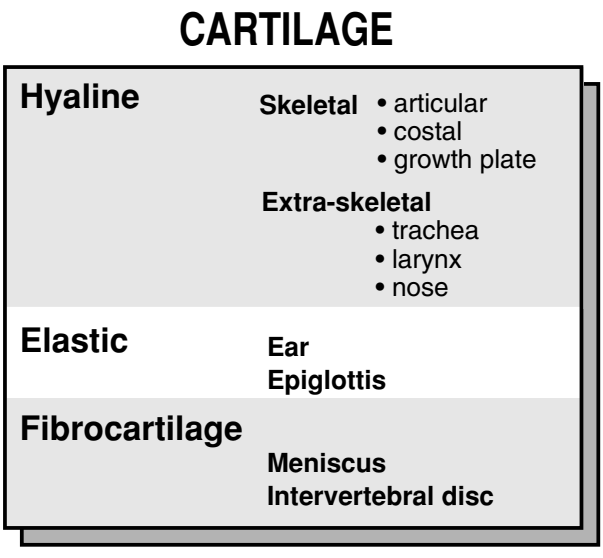
CARTILAGE

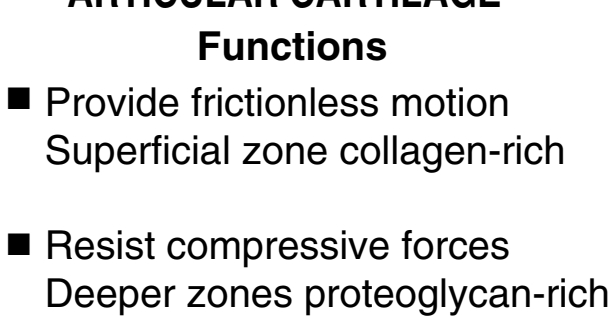
Types

Cartilage exists in the body as three types: hyaline, elastic and fibrocartilage. **Hyaline cartilage** is found mainly in association with the skeletal system, as articular cartilage, costal cartilage or growth plate, though it does also occur in extra-skeletal sites, such as the larynx, trachea, bronchi and nose. It is characterized by its type II collagen and high concentration of aggregating proteoglycan. **Elastic cartilage** is found mainly in the ear and epiglottis, and differs from hyaline cartilage by the presence of substantial amounts of elastic fibers. **Fibrocartilage** is also associated with the skeletal system, with the best examples being the meniscus of the knee and the anulus fibrosus of the intervertebral discs. Unlike the other cartilage types, it is composed principally of type I collagen and has a lower proteoglycan content.

Articular cartilage

Articular cartilage covers the surface of bones where they meet in movable joints. In the healthy young individual, it has a white, lustrous and smooth appearance. It is composed of only one cell type - the chondrocyte. Histologically, it may be divided into **two regions**: a superficial collagen-rich layer, and a deeper proteoglycan-rich layer which stains with cationic dyes such as Safranin O. The tissue is designed to fulfill **two major functions**: to provide a smooth surface compatible with frictionless motion, and to resist the compressive forces encountered across the joint under loading. The proteoglycan content of the tissue is an essential contributor to this latter function.



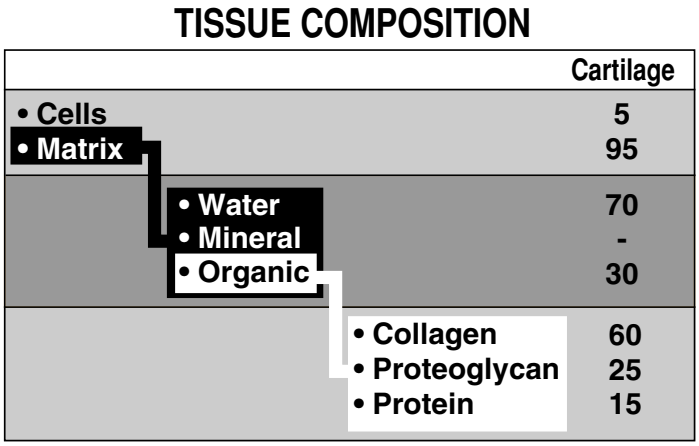


Composition

Mature articular cartilage contains about 5% of its volume as **cells**, the remainder being **extracellular matrix**. There is normally no mineral, and organic material accounts for about 30% of the matrix, with the remainder being water. About 60% of the organic material is collagen, 25% is proteoglycan and the remainder is a variety of matrix proteins.

Nerve and vascular supply

Articular cartilage is characterized by having **no nerves** and **no vascular system**. The former property allows pain-free motion of the joints during normal use, though it can also be viewed as detrimental as it results in injury to the tissue not being perceived by the individual. The absence of a vascular supply means that nutrition and hydration for the tissue must arise from the synovial fluid by diffusion. Tissue exposed during surgery will rapidly dry with resulting cell death unless bathed in fluid. Also, the usual source of connective tissue repair arising via the microvasculature is absent from this tissue, and incisions in the cartilage do not repair, unlike those in a vascular connective tissue like skin. Because of their non-reliance on vascular oxygen, chondrocytes undergo anaerobic metabolism and survive for several days following death.



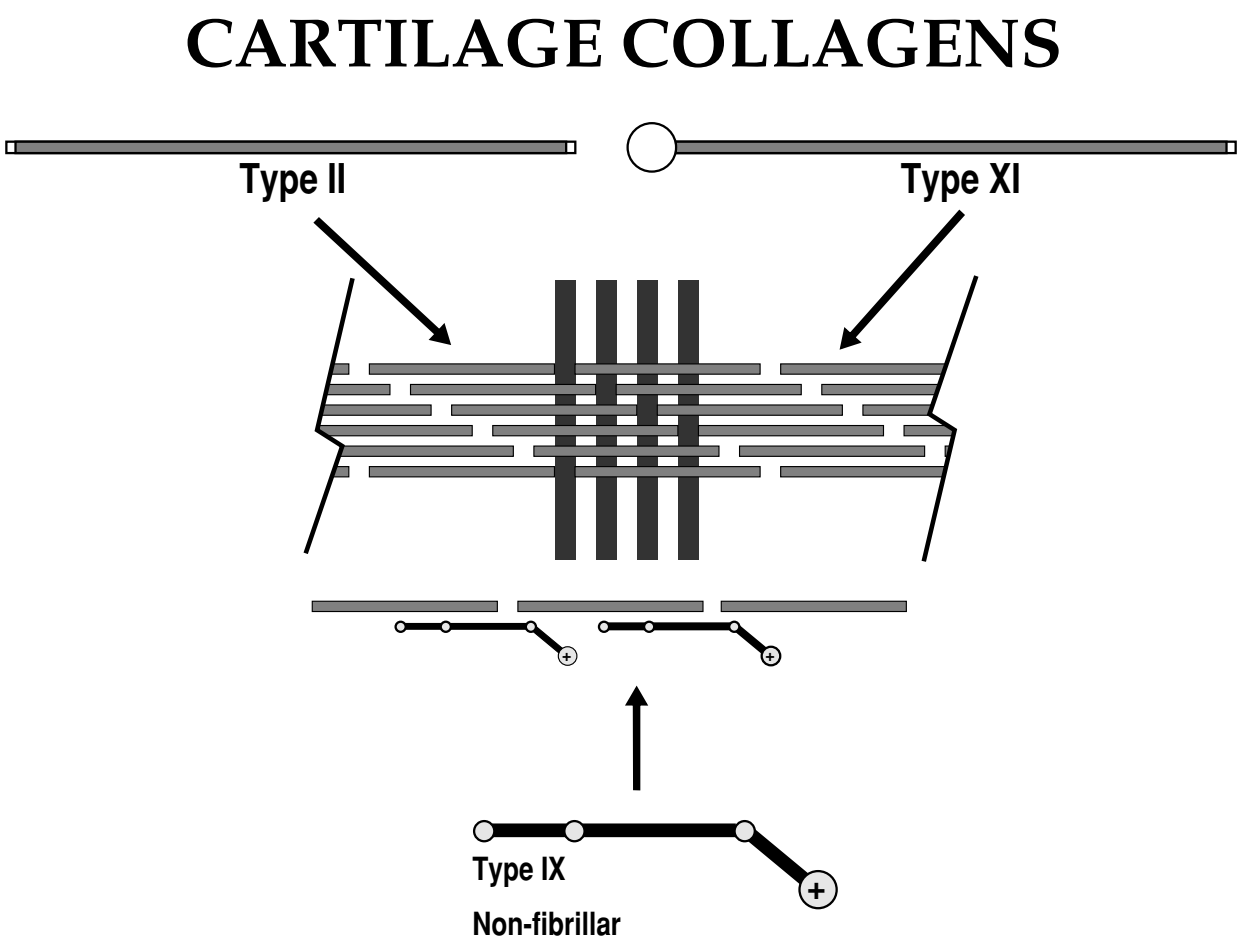
ARTICULAR CARTILAGE

Structure

- No nerves
  - Pain-free use
  - Non-perception of injury
- No blood vessels
  - No bruising
  - Nutrition from synovial fluid
  - Hydration from synovial fluid
  - Source of repair absent
  - Viable after death

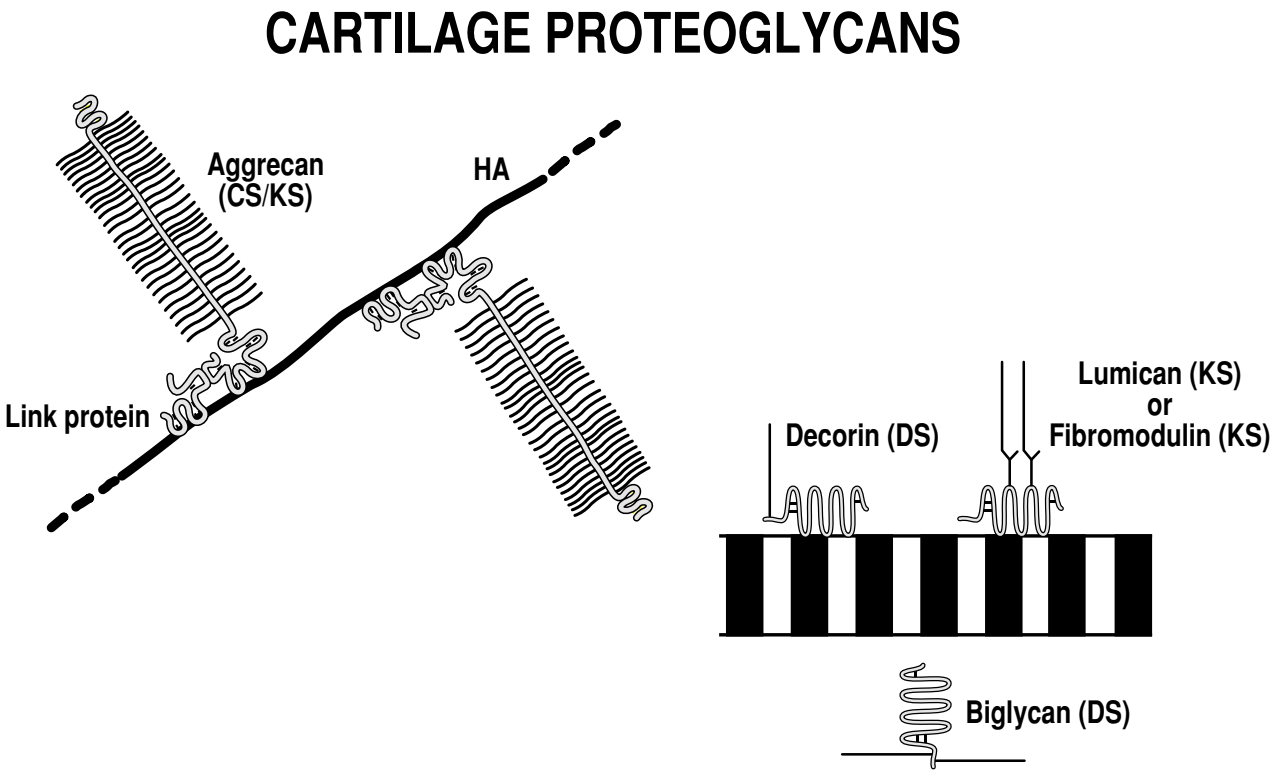
Collagens

Collagens consist of 3 polypeptide chains that form a **triple helix** along at least part of their length. They can be divided into **fibrillar collagens** (types I, II, III, V and XI), which form the framework of the tissue, and **non-fibrillar collagens**. The framework of connective tissues is composed of **collagen fibrils**, which consist of triple helical collagen molecules arranged head to tail in linear arrays and side by side in a staggered manner. This staggered lateral arrangement gives the collagen fibril its characteristic cross-striated appearance when viewed in the electron microscope. Different connective tissues contain different collagen types, reflecting their varied functions. The collagens of hyaline and elastic cartilage are distinct from other connective tissues. They are the fibrillar **types II and XI collagen**, with the former being most abundant, and the non-fibrillar **type IX collagen**. Types II and XI collagen occur in the same fibrils, and the presence of type XI collagen limits fibril diameter. Type IX collagen resides on the surface of the fibrils, and is thought to facilitate interaction between the collagenous framework of the tissue and the interspersed proteoglycan. Growth plate cartilage is unique in also containing the non-fibrillar **type X collagen**, which is thought to play an integral role in the mineralization process. Fibrocartilages contain **type I collagen** as their predominant fibrillar collagen, in common with most other connective tissues.



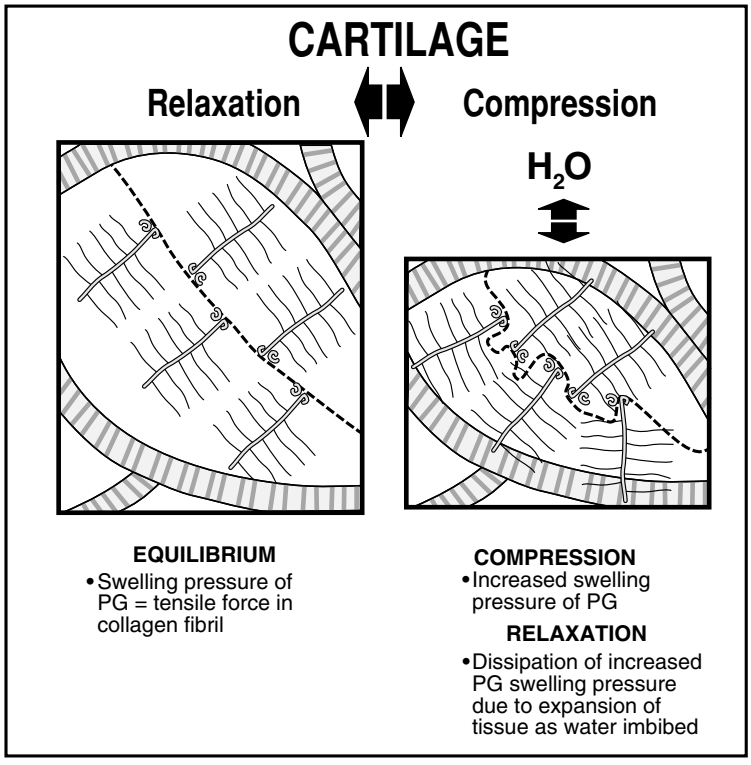
Proteoglycans

Proteoglycans are present in the extracellular matrix of all connective tissues. They consist of a central protein core to which sulfated glycosaminoglycans (chondroitin sulfate, dermatan sulfate or keratan sulfate) are covalently attached. The structure of the proteoglycan varies with the different connective tissues. Hyaline and elastic cartilages contain predominantly **aggregating proteoglycans**, which are composed of many **aggrecan** molecules that interact with a central molecule of **hyaluronic acid**. Each interaction is stabilized by the further association of a **link protein**. The aggrecan molecule itself possesses a long core protein with many **chondroitin sulfate** and **keratan sulfate** chains. These sulfated glycosaminoglycans are absent from the terminus of the core protein that interacts with hyaluronic acid. The proteoglycan aggregate provides the tissue with its turgid nature that resists compression. Fibrocartilages do not contain as high an aggrecan content. All cartilages, in common with all soft connective tissues, also contain **non-aggregating proteoglycans**, which interact with collagen fibrils rather than hyaluronic acid. These are **decorin, biglycan, fibromodulin and lumican**. They are much smaller in size than aggrecan and possess only a few **dermatan sulfate** (decorin and biglycan) or **keratan sulfate** (fibromodulin and lumican) chains. They mediate the interactions between adjacent collagen fibrils, or with other matrix components. Cartilage also contains **perlecan**, a **heparan sulfate** proteoglycan normally associated with basement membranes. It's function in cartilage is unknown.



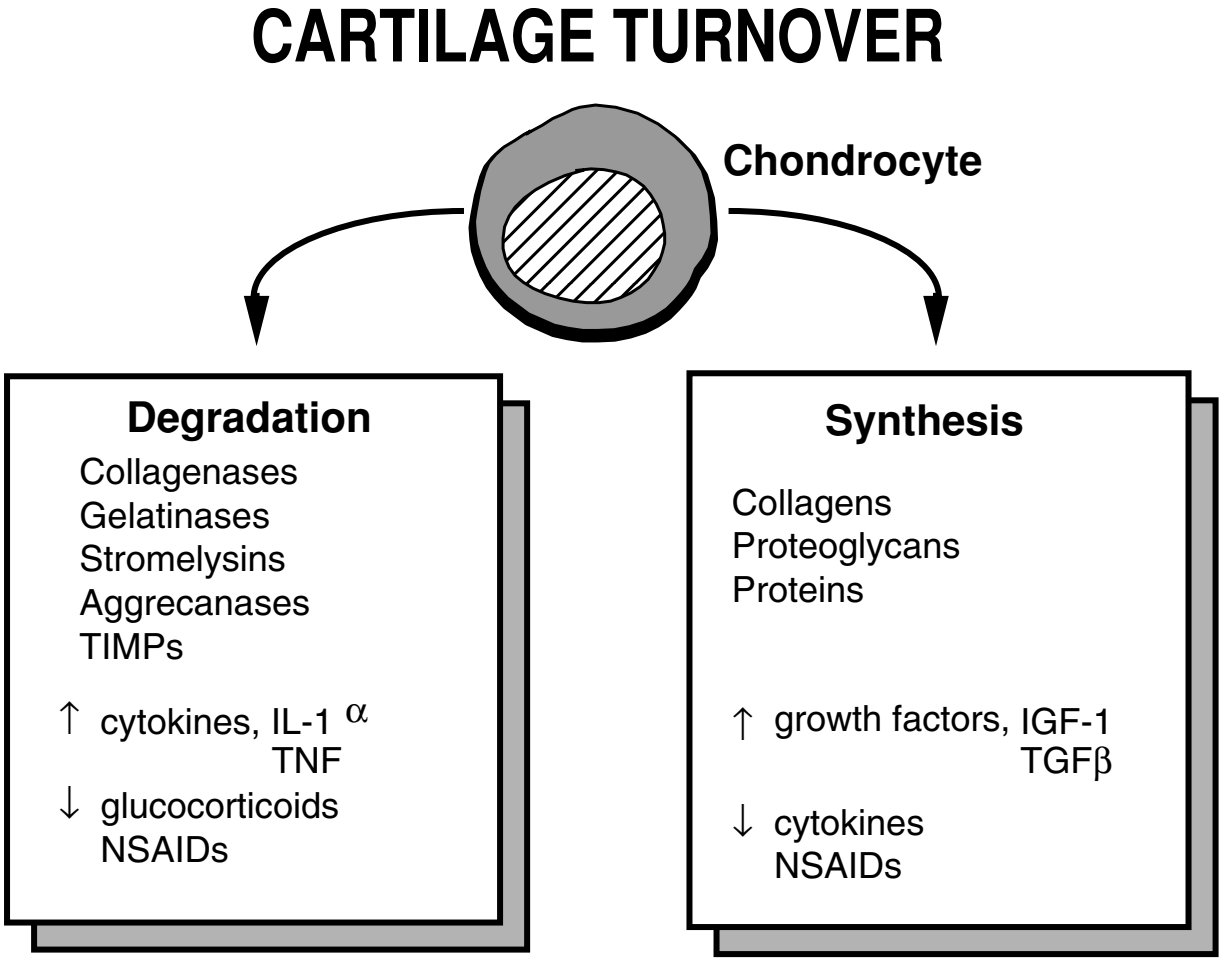
Nutrition and compression

Survival of the chondrocytes depends on adequate **nutrition**, and the passive diffusion of nutrients from the synovial fluid is aided by joint loading and motion. The aggregating proteoglycans, because of their high sulfatation, have a strong affinity for water and try to expand their molecular domain by drawing water into the tissue. As more water is drawn into the tissue the swelling potential of the proteoglycan decreases. Under normal circumstances an equilibrium is attained, whereby the outward swelling of the proteoglycan is resisted by stretching forces developed in the collagenous framework of the tissue. When a load is applied across the joint, tissue compression occurs immediately beneath the load, with water being displaced. Because of the large size of the proteoglycan aggregate and its entrapment by the collagen, the proteoglycan is not displaced and its concentration is focally increased. On removal of the load, the original equilibrium is restored by water being drawn back into the tissue. Along with the water comes nutrients. Such a mechanism may contribute to the beneficial effects of **CPM** (continuous passive motion) on the healing of cartilage lesions, and to the observed cartilage **atrophy** upon prolonged joint underuse or immobilization. The properties of the aggregating proteoglycans also provide the articular cartilage with its resilience to **compression**, as compressive forces are counterbalanced by the focal increase in proteoglycan swelling potential. Compression is minimized because of the high proteoglycan concentration, and this serves to protect the chondrocytes from adverse forces. This protection breaks down when the joint is subjected to excessive loads or overuse, as the chondrocytes release proteolytic enzymes which damage the proteoglycan and collagen so causing tissue **degeneration**. In general, dynamic (cyclic) loading is beneficial to matrix synthesis, whereas static loading is detrimental.



Matrix homeostasis

Both the production of the extracellular matrix and its physiological regulation throughout life are controlled by the **chondrocytes**. These cells not only make the structural macromolecules (**collagen** and **proteoglycan**) responsible for tissue formation, but also make a variety of secreted metalloproteinases (**collagenases, gelatinases, stromelysins** and **aggrecanases**) responsible for tissue turnover. During growth it is thought that synthesis of the structural macromolecules is promoted by growth factors, such as **IGF-I** (insulin-like growth factor-1) and **TGF**β (transforming growth factor-β) . In contrast, matrix degradation is promoted by a variety of cytokines, such as **IL-1** (interleukin-1) and **TNF**α (tumor necrosis factor-α). These cytokines stimulate the secretion of proteinases from the chondrocytes and inhibit the synthesis of the structural macromolecules. Under normal conditions, the destructive capacity of the proteinases is kept under control by the concomitant secretion of **TIMPs** (tissue inhibitors of metalloproteinases), which can inhibit their action. Drugs such as **NSAIDs** and **glucocorticoids** used to treat arthritic joints retard cartilage degeneration by affecting the synthesis/degradation balance. However, some NSAIDs have an undesirable inhibitory effect on matrix synthesis by chondrocytes.

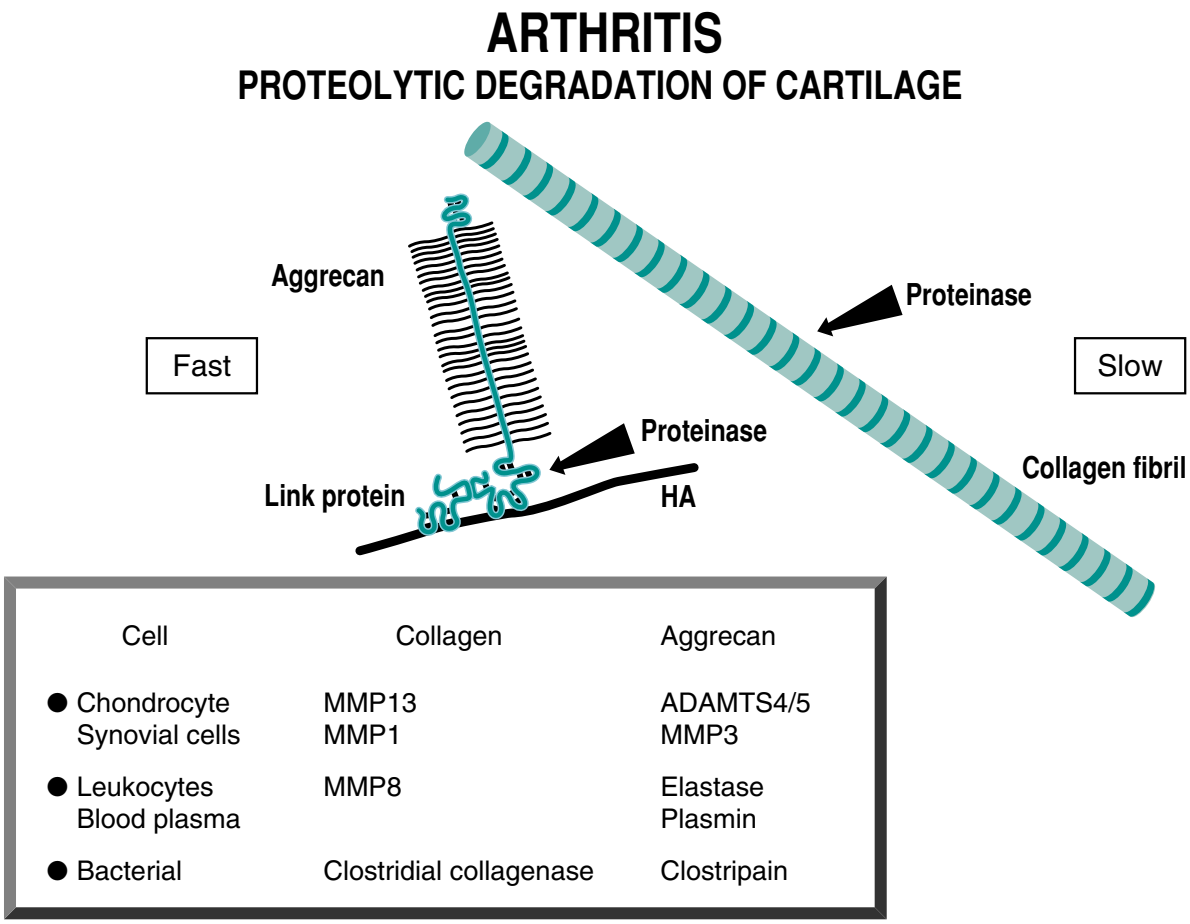


Arthritic destruction

Arthritic destruction of articular cartilage takes place mainly via the action of proteolytic enzymes. In the early stages of disease proteoglycan is lost, whereas at later stages the collagen framework is damaged resulting in tissue **fibrillation** and ultimate **erosion**. The most important enzymes are collagenases (MMP1 and MMP13) and aggrecanases (ADAMTS4 and ADAMTS5). Collagenases are the only enzymes able to degrade the collagen triple helix. Aggrecanases specifically degrade the core protein of the proteoglycan aggrecan, resulting in its diffusion from the cartilage matrix. In **osteoarthritis** (degenerative arthritis) these enzymes are released directly by the chondrocytes, due to abnormal forces acting on the cells. Under conditions of joint **inflammation** the same enzymes can also arise from the synovial cells. PMN leukocytes possess a distinct collagenase (MMP8) and contain other proteinases (elastase and cathepsin G) able to degrade proteoglycan, and may therefore help damage the cartilage matrix in inflammatory arthritides. In **rheumatoid arthritis** degradation products of cartilage matrix macromolecules are thought to initiate a T-cell mediated autoimmune response, so exacerbating inflammation. In **infectious arthritis**, bacterial collagenases may give rise to very rapid cartilage destruction, because of their multiple sites of action along the collagen molecules. The mammalian collagenases cleave at only a single site in the fibrillar collagen molecule and cleavage of the collagen fibril is a slow process. In contrast, loss of aggrecan is a rapid process. In general, it appears that if only proteoglycan loss occurs, the cartilage may regenerate a normal matrix, but once the collagen framework is damaged the degenerative process is irreversible.

ARTHRITIS

Type	Origin	Disorder	Cause of Increased Proteolysis
Degenerative	Mechanical	Osteoarthritis	• Normal matrix, abnormal load (malalignment, trauma, occupation) • Abnormal matrix, normal load (chondrodysplasia, drug, arthritis)
Inflammatory	Microorganism	Septic	• Microorganism within joint (infectious) (bacterial, viral, fungal)
		Reactive	• Bacterial infection at remote site (related antigen in joint)
	Autoimmune	Rheumatoid	• Recognition of cartilage degradation product? (type II collagen, aggrecan)
	Crystal	Gout Pseudogout	• Sodium urate • Calcium pyrophosphate (chondrocalcinosis)



COX and arthritis

Joint inflammation in all forms of arthritis is associated with increased production of **COX-2** (cyclooxygenase-2) in response to cytokine stimulation of synovial cells. Prostaglandins produced by the action of COX-2 mediate the features of inflammation. COX-2 is also a product of chondrocytes in the non-inflammatory OA joint, though its precise role is unclear. The anti-inflammatory role of **NSAIDs** is due to their inhibition of COX-2, but undesirable side effects are present due to the co-inhibition of the ubiquitous and constitutively produced **COX-1**. Specific inhibitors of COX-2 would be more desirable. Glucocorticoids are potent inhibitors of COX-2.

Markers of cartilage metabolism

The release of cartilage matrix components into the synovial fluid has been used to monitor disease status in the arthritic joint. Such markers include products derived from **type II collagen** (C-propeptide, telopeptide-derived cross-links and collagenase-derived neoepitopes) and **aggrecan** (KS and CS-derived neoepitopes). Care must be taken in interpreting the meaning of increase in marker levels, as some reflect increased degradation (collagen cross-links and neoepitopes), some reflect increased or altered synthesis (collagen C-propeptide and CS-neoepitopes), and others may reflect both (KS).

MARKERS OF CARTILAGE METABOLISM

Cartilage Formation Markers	Cartilage Degradation Markers
• Type II collagen C-propeptide • CS-neoepitope (aggrecan)	• KS (aggrecan) • COMP • Type II collagen telopeptide cross-links • Type II collagen/collagenase neoepitope

Cartilage repair

It is well accepted that lesions confined to the avascular articular cartilage have a very limited capacity for repair. However, when lesions penetrate the subchondral bone a wound healing response is observed, as cells derived from the bone marrow fill the lesion and differentiate into chondrocytes. This observation forms the basis of surgical repair techniques using **drilling** or **abrasion** to penetrate the subchondral bone. Periosteum is also a source of cells that can differentiate into chondrocytes, and **periosteal grafts** have been used to repair cartilage lesions in a more controlled manner. Other repair techniques have utilized chondrocytes or cartilage directly. **Chondrocytes** can be obtained directly from cartilage or by in vitro differentiation of **bone marrow stem cells**. In either case, the cells need to be embedded in an artificial matrix for implantation in a lesion. Various macromolecules have been used for such matrices, including collagen, hyaluronic acid, fibrin and some synthetic polymers. Various growth factors have been used in these cell repair systems to promote matrix synthesis and stabilize the chondrocyte phenotype. Repair systems using intact articular cartilage have commonly involved **osteochondral grafts**. A major problem in all repair systems is achieving integration between the repair cartilage and the surrounding normal cartilage.

CARTILAGE REPAIR

●**Blood Clot Formation:**	Abrasion or drilling subchondral bone
●**Cell Implantation:**	Chondrocytes/marrow stem cells Artificial matrix for support
●**Tissue Transplantation:**	Osteochondral grafts Periosteal grafts
●**Limitations:**	Cell/tissue availability - stem cells/allografts Phenotype stability - growth factors Chondral integration

Chondrodystrophies

Functionally abnormal cartilage may result when a gene for one of the matrix macromolecules or for one of the cellular components involved in chondrocyte metabolism is defective. Impairment in the function of the growth plate results in a **chondrodysplasia**, and impairment in the function of the articular cartilage results in a premature familial **osteoarthritis**. Defects in the **matrix** molecules include the genes for type II collagen (COL2A1), type IX collagen (COL9A2), type XI collagen (COL11A2), type X collagen (COL10A1), cartilage oligomeric protein (COMP) and perlecan. Defects in **cellular** components include the genes for a growth factor receptor (FGFR3), a hormone receptor (PTHrPR), a sulfate transporter (DTDST), transcription factors (SOX9 and SHOX) and enzymes involved in glycosaminoglycan metabolism (GAL, GLCN6S and EXT). In many cases defects in the same gene can give rise to different clinical phenotypes (COL2A1 and FGFR3), depending on the site and type of mutation. It is also possible that defects in different genes can give rise to the same clinical phenotype, if the different gene products interact with one another (COL9A2 and COMP in multiple epiphyseal dysplasia) or if they are involved in regulating the same biochemical event (GAL and GLCN6S in Morquio syndrome).

CHONDRODYSPLASIAS

Defects in Matrix Macromolecules

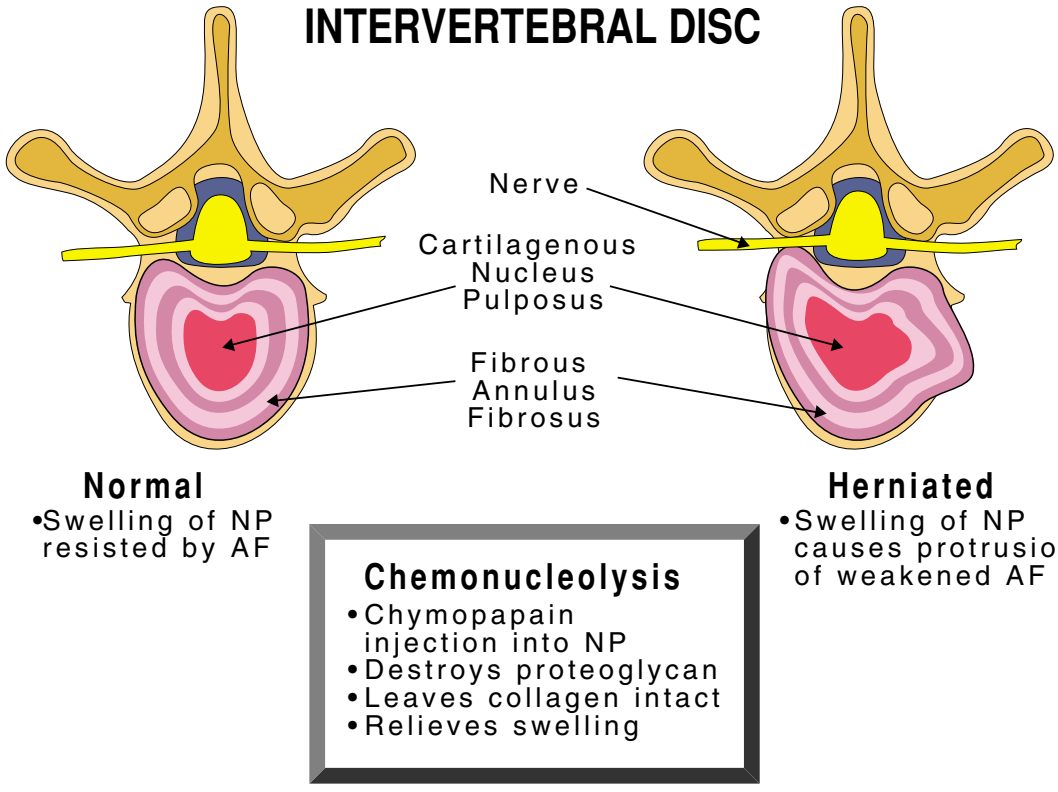
Gene	Disorder
COL2A1	Achondrogenesis type II Hypochondrogenesis Spondyloepiphyseal dysplasia Kniest dysplasia Stickler dysplasia Familial osteoarthritis
COL9A2	Multiple epiphyseal dysplasia type II
COL11A2	Stickler dysplasia (no eye)
COL10A1	Schmid metaphyseal dysplasia
COMP	Pseudoachondroplasia Multiple epiphyseal dysplasia type I
Perlecan	Dyssegmental dysplasia Schwartz-Jampel syndrome

Defects in Cellular Macromolecules

Gene	Disorder
FGFR3	Achondroplasia Hypochondroplasia Thanatophoric dysplasia type I Thanatophoric dysplasia type II
PTHrPR	Jansen metaphyseal dysplasia
SOX9	Campomelic dysplasia
SHOX	Chondrodysosteosis
DTDST	Diastrophic dysplasia Atelosteogenesis type II Achondrogenesis type IB
GAL GLCN6S	Morquio syndrome (MPS4)
EXT1/2	Hereditary multiple exostosis

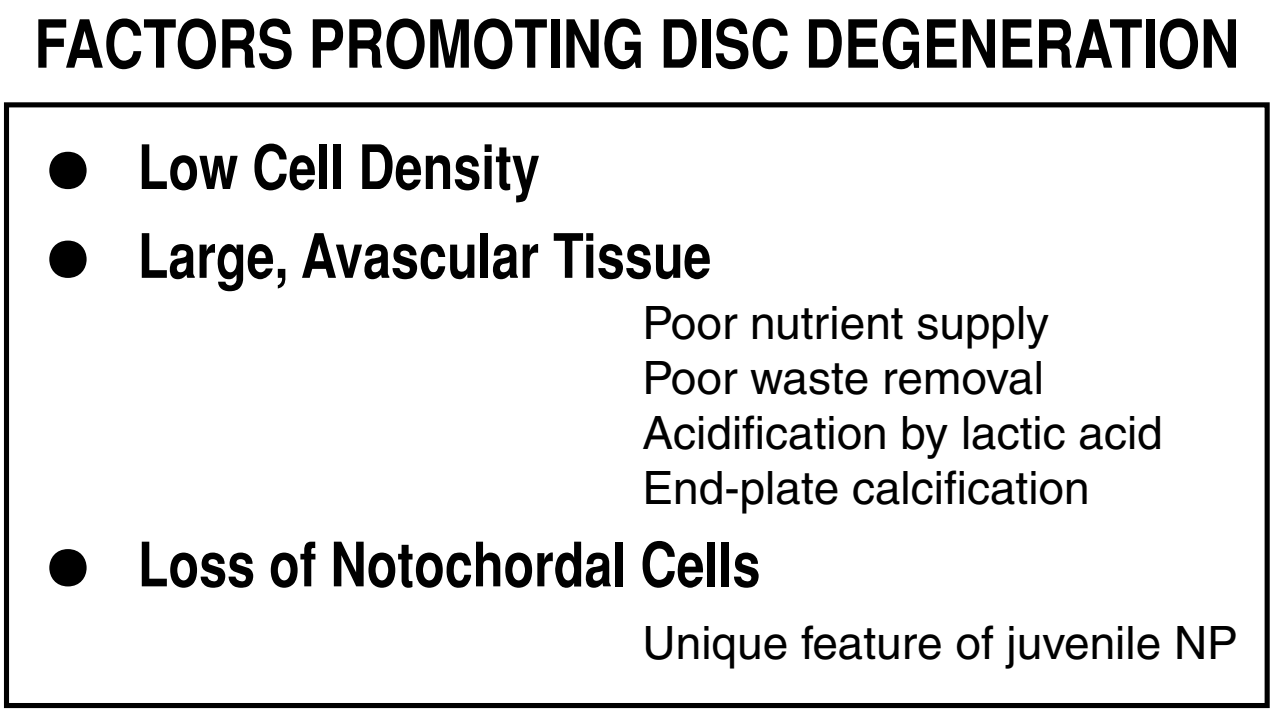
Intervertebral disc

The **nucleus pulposus** of the intervertebral disc has a composition similar to articular cartilage. In contrast, the **anulus fibrosus** is fibrocartilagenous, with a composition more akin to a ligament. The function of the proteoglycan in the healthy nucleus is to provide the potential for tissue expansion, which is resisted laterally by the collagenous lamellae of the anulus and vertically by weight on the axial skeleton. Under zero gravity or with bed rest, disc height will actually increase due to decrease in the resistance to swelling. Herniation of the anulus will also result in increased swelling of the nucleus at a focal point, which can cause nerve root entrapment (e.g. sciatica). One treatment used to relieve the problem in the lumbar region is **chemonucleolysis**, in which the proteinase chymopapain is injected into the nucleus. This enzyme can rapidly degrade the proteoglycan but not the collagen, and hence can prevent the swelling of the disc without causing extensive damage to its architecture.



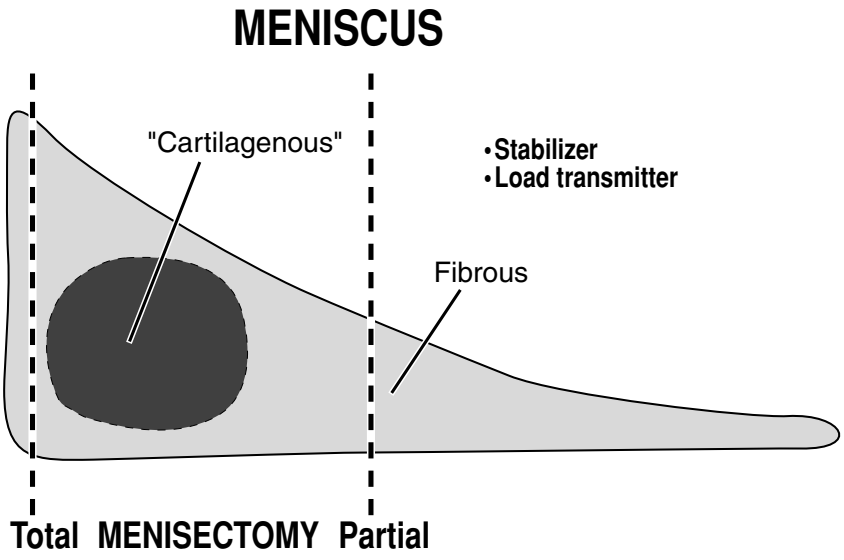
Disc degeneration

Degeneration of the intervertebral disc is a common consequence of aging, often beginning early in adult life in the lumbar region. Many factors are thought to contribute to this inability of the disc cells to maintain their surrounding matrix, including the low cell density of the tissue, its large avascular nature (resulting in poor nutrient supply and waste removal), and the loss of notochordal cells during juvenile development. Degeneration begins in the nucleus pulposus and progressively worsens and involves the surrounding annulus fibrous. Early changes involve loss of proteoglycan from the nucleus pulposus.



Meniscus

The meniscus of the knee joint is also a fibrocartilage, but in its central region, where it is subjected to greatest compressive forces, it does produce aggregating proteoglycans. These are most abundant in the adult, and may contribute to the weight-bearing characteristics of the joint. Such chondroid metaplasia is not unique to the meniscus, but is also observed in tendons where they are subjected to compression as they pass over bone. The chondroid characteristics of the central body of the meniscus may have some relevance in relation to the incidence of post-operative osteoarthritis following **menisectomy**. This is increased following total menisectomy, where the central region is removed, compared to partial menisectomy, where it is retained.



- Cartilagenous central body in adult meniscus
- Probably as a response to compression
- Similar phenomenon in tendons

BONE

Types

Bone may be divided into immature and mature types. **Immature bone** is termed woven, fibrous or spongy bone, and contains collagen fibrils running in various directions. **Mature bone** is formed from immature bone following its resorption and remodeling, and is often called **lamellar bone** as it is laid down in adjacent layers with the collagen fibrils in each layer having a parallel orientation. Two types of lamellar bone exist: **trabecular or cancellous bone** and **compact or cortical bone**. Trabecular bone has a loose architecture, with spaces occupied by marrow. Compact bone is much more solid in organization, with the only spaces being the narrow canals through which blood vessels pass. The compact bone is responsible for the strength and rigidity of the tissue, whereas trabecular bone confers lightness and some flexibility and is the major reservoir for calcium homeostasis. With age, the proportion of trabecular bone decreases.

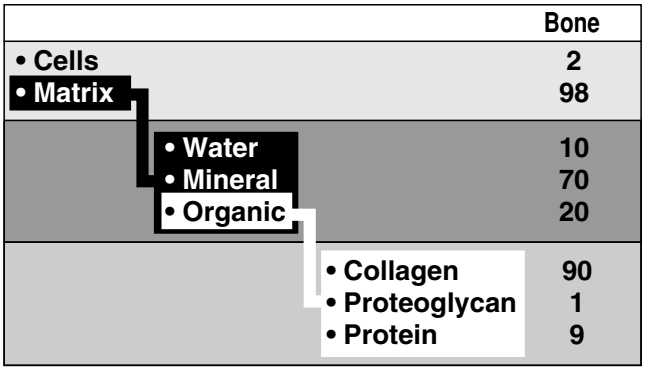
Composition

Mature compact bone contains about 2% of its volume as **cells**, the remainder being extracellular matrix. Of this matrix 70% is occupied by **mineral**, about 20% is organic, and the remainder is water. Of the organic material 90% is **collagen**, about 1% is proteoglycan and the rest is a series of matrix proteins. The collagen of bone is almost all type I. The proteoglycans of bone are all of the small non-aggregating type (decorin and biglycan) and contain only chondroitin sulfate. The **matrix proteins** are characterized by their anionic nature, being rich in phosphate (phosphoproteins, e.g. osteonectin), sialic acid (sialoproteins, e.g. osteopontin) or gamma-carboxyglutamic acid (Gla proteins, e.g. osteocalcin). Growth factors such as TGF-β and BMP (bone morphogenic protein) are also stored within the bone matrix.

BONE

Immature		
	Woven **Fibrous**	**Newly formed:** metaphysis fracture Paget
Mature Lamellar		
	Cortical **Compact**	**Outer core:** rigidity strength
	Trabecular **Cancellous**	**Adjacent to marrow:** light weight Ca homeostasis

TISSUE COMPOSITION



Structure

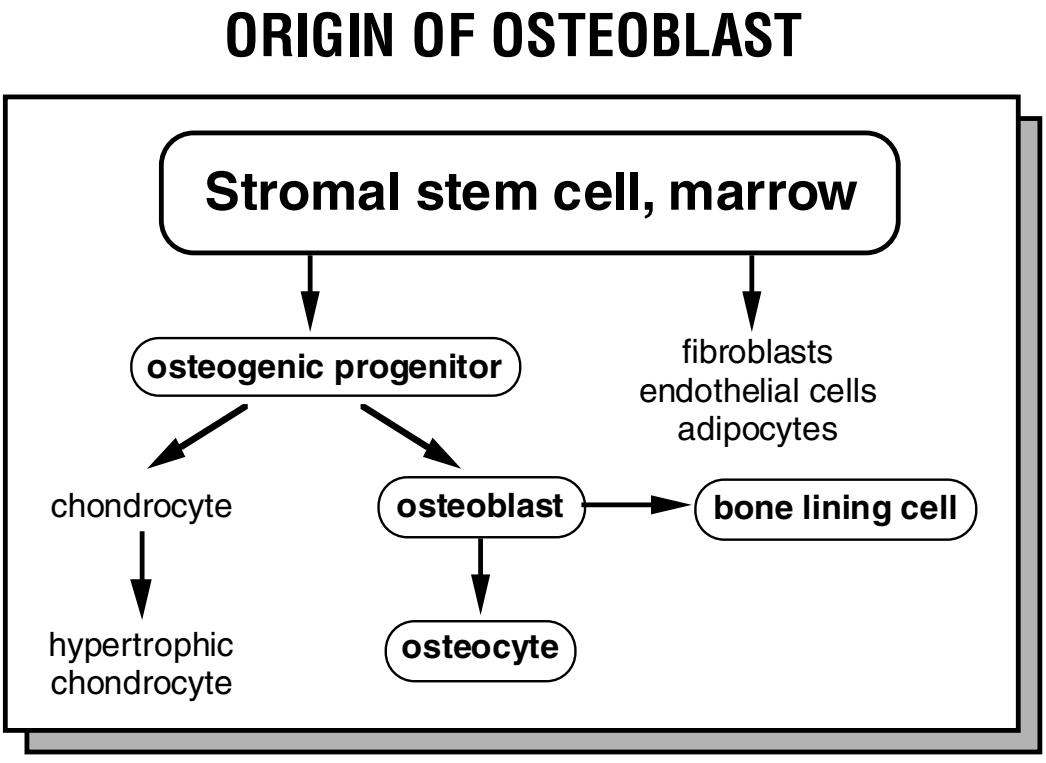
Bones are heterogeneous in their organization and may be divided into distinct structural regions. At the outer surface is the **periosteum**, which is the source of the cells responsible for growth in bone width. Adjacent to this is **cortical bone**, and on the inner surface of the bone, adjacent to the marrow cavity, is **trabecular bone**. The inner surfaces of the bone are covered by cells that form the **endosteum**. Both trabecular and cortical bone are made of calcified lamellae in which **osteocytes** are entombed. The osteocytes are linked by a network of uncalcified channels termed **canaliculi**. In cortical bone, the canaliculi are linked to the **Haversian canals** in which the bone vasculature resides. Surrounding the blood vessels are concentric rings of lamellar bone, which form a unit termed an **osteon** or **Haversian system**. The Haversian canals run parallel to the axis of the bone and arise through bone remodeling. The vessels penetrate the bone from the periosteum to the marrow cavity through transverse **Volkmanns canals**. The bone surfaces are covered with cells, which include dormant **bone lining cells, osteoblasts** responsible for new bone formation, and **osteoclasts** responsible for bone resorption.

Nutrition

Bone essentially receives all its nutrients via its extensive vascular system, and when blood flow ceases the **osteocytes** entombed in their calcified matrix rapidly die. Nutrients move to the osteocytes through the **canaliculi** by diffusion from the Haversian canals. Fluid flow through the canaliculi is not very efficient and osteocytes must be within 0.1-0.2 mm of a blood vessel for adequate nutrition. When a bone **fracture** occurs, blood supply to the severed vessels ceases, and the osteocytes in the immediate vicinity of these vessels die. This accounts for the fact that a cortical bone **graft** is essentially a dead tissue. Dead bone grafts are useful not only for the support that they provide, but also for the ability of their matrix growth factors to promote resorption and subsequent new bone formation. Impairment of blood supply to a region of bone due to vessel trauma or occlusion results in **osteonecrosis**.

Bone forming cells

Osteoblasts are derived from **stromal stem cells** (fibroblast colony forming units, F-CFU) that differentiate into **osteoprogenitor cells**. The same stem cells can also give rise to **fibroblasts**, **endothelial cells** or **adipocytes**. In more mature bone, osteoprogenitor cells are present at the periosteum and stem cells are present in bone marrow. The number of stromal stem cells in the bone marrow decreases with age and the number of adipocytes increases. The osteoprogenitor cells may differentiate into **chondrocytes** or osteoblasts depending to a large degree on the environmental conditions. In regions with a good blood supply, via the presence of capillaries, the development of osteoblasts is promoted, whereas in regions of inadequate blood supply differentiation into chondrocytes occurs. Such a relationship between osteoblasts and chondrocytes explains why the healing **fracture callus** often contains regions of osseous and chondroid tissue, and why **periosteal grafts** can be used to resurface cartilage defects. The osteoblast is responsible for producing the organic extracellular matrix of bone, termed **osteoid**, which subsequently becomes calcified. In the adult, osteoid is present on the surface of remodeling trabecular bone and on the inner lining of the osteons of cortical bone. In the course of osteoid production some osteoblasts become embedded within their secreted matrix, and once this matrix becomes mineralized these cells differentiate into the relatively dormant **osteocytes**. When osteoid production is complete the mature osteoblasts become dormant and remain on the bone surface as **bone lining cells**. Mature bone lining cells and osteocytes are not involved in further bone formation. The osteocytes in cortical bone may act as mechanotransducers, recognizing abnormal stresses and signalling bone remodeling.

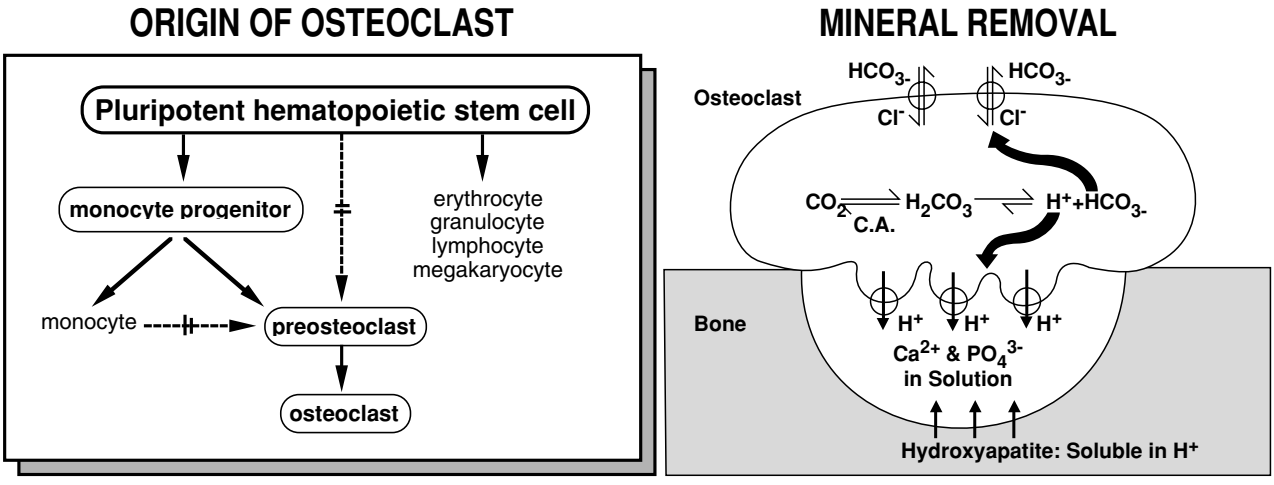


Bone resorbing cells

Osteoclasts are not derived from stromal stem cells, but instead are of hematopoietic origin. They are derived from a pluripotent **hematopoietic stem cell** (granulocyte/macrophage colony forming unit, GM-CFU), which can differentiate into a **monocyte progenitor cell** or into erythrocytes, granulocytes, lymphocytes and megakaryocytes. The monocyte progenitor cell may differentiate into either **preosteoclasts** or premonocytes. The preosteoclasts can travel directly through the marrow to the trabecular bone surfaces, or via the vascular system to the cortical bone. The mature osteoclasts then arise by fusion of preosteoclasts to form a multinucleated giant cell. Commonly five to ten preosteoclasts are involved in the fusion process. The osteoclast is responsible for bone resorption associated with bone modeling, remodeling and pathology.

Resorption

Resorption takes place at the surface of the osteoclast where it interacts with the calcified matrix. The region adjacent to the bone surface develops a characteristic appearance due to the formation of pleats in the plasma membrane of the cell. This pleated region is termed the **ruffled border** or **brush border**. The mineral beneath the ruffled border is dissolved by the secretion of **acid**. The acid is formed intracellularly from carbon dioxide by the action of **carbonic anhydrase**, and hydrogen ions are then pumped through the ruffled border. Solubilization of the uncalcified bone matrix then occurs via the action of secreted lysosomal enzymes (e.g. **cathepsin K**), whose activity is optimal at acid pH. The region of the osteoclast in contact with the mineralized bone matrix is termed the **clear zone** or **sealing zone**, as it is devoid of the cellular processes involved in acid and lysosomal enzyme secretion. Attachment of the osteoclast to the bone matrix occurs via the interaction of cell surface **integrins**.

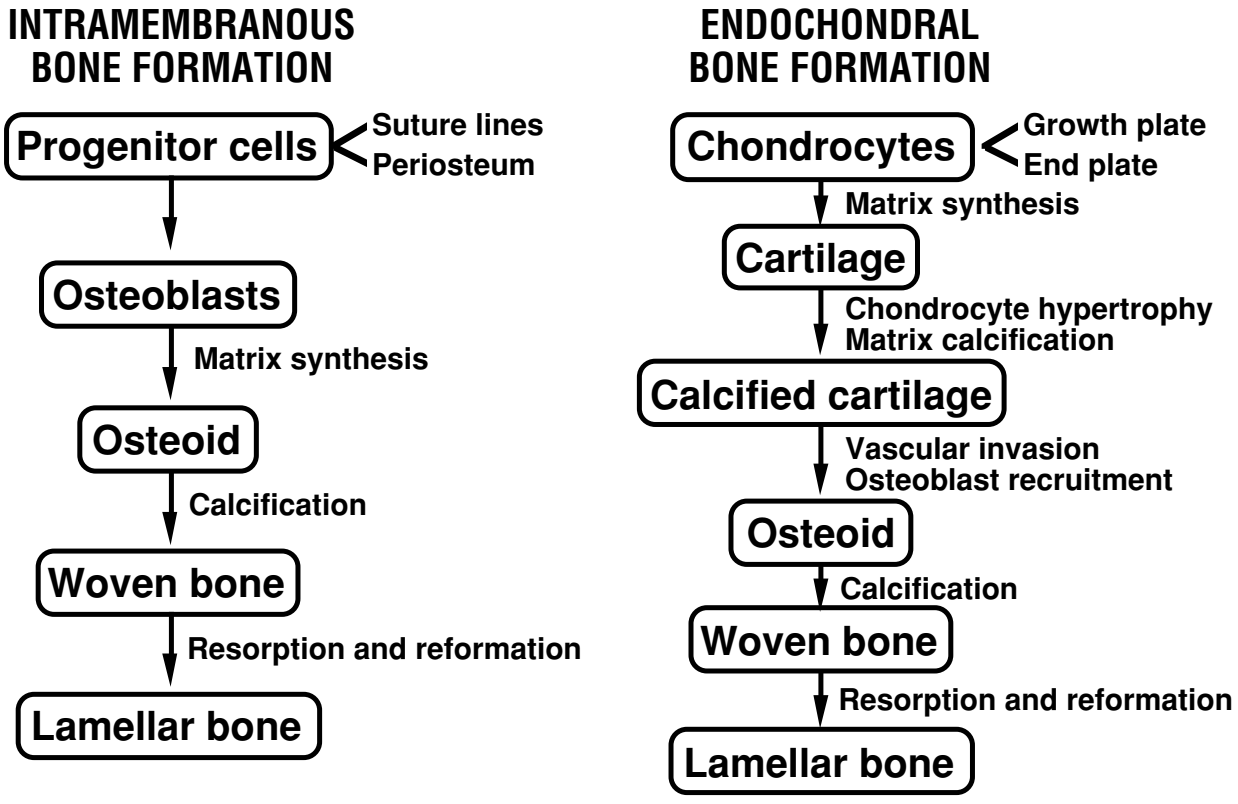


Intramembranous bone formation

In the embryo, the flat bones of the **skull** are formed by intramembranous bone formation, which takes place in regions of well vascularized mesenchymal tissue. Intramembranous bone formation also takes place during juvenile growth, at the **suture lines** of the skull and at the **periosteal surfaces** of all bones. It is characterized by the direct differentiation of osteoprogenitor cells into osteoblasts, which lay down non-mineralized osteoid that subsequently calcifies to yield a woven bone. This will then undergo resorption and reformation to yield a mature lamellar bone.

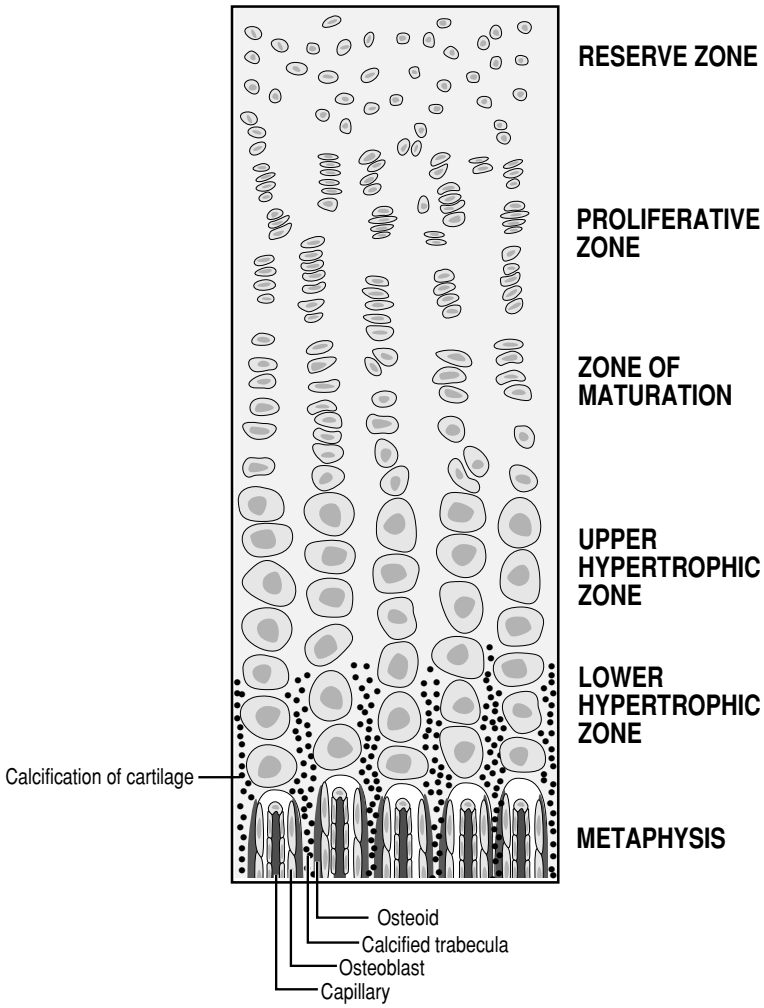
Endochondral bone formation

In the embryo, the bones of the **limbs** and **spine** develop via endochondral bone formation, which takes place within regions of mesenchyme low in vascularity. Endochondral bone formation also takes place during juvenile growth, within the **growth plates** of the long bones and the **end plates** of the vertebrae. It is characterized by the initial formation of cartilage, which subsequently calcifies and acts as a support for osteoid deposition and bone formation. Ultimately the mixture of calcified cartilage and woven bone is replaced by lamellar bone.



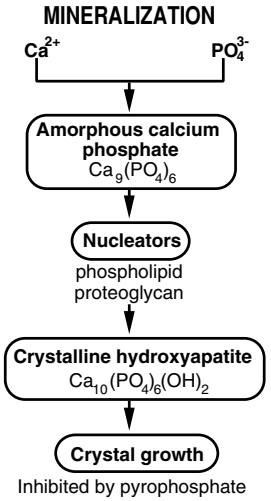
Growth Plate

Growth plates are composed of areas of different cellular appearance. Adjacent to the epiphysis is the **reserve or resting zone** composed of small round chondrocytes. Beneath this lies the **proliferative zone**, composed of columns of flattened cells lying parallel to the axis of the long bone. At the base of these columns, the cells mature and enlarge to form the **hypertrophic zone**. It is in the lowermost portion of the hypertrophic zone that matrix **calcification** begins in the **longitudinal septa** separating the columns of cells. Calcification does not occur in the **transverse septa** between cells in the same column. Under the calcified cartilage of the lower hypertrophic zone lies the newly formed spongy woven bone of the metaphysis. Increase in bone length occurs within the cartilage in the proliferative zone. The growth plate itself does not, however, increase in width, as the cells in the lower portion are continually maturing into hypertrophic chondrocytes, which initiate the matrix calcification. The cells within the deepest region of the calcified cartilage die and their lacunae are invaded by capillaries from the metaphysis. Migrating osteoblasts from the metaphysis settle on the spicules of calcified cartilage and produce a layer of osteoid on its surface, which subsequently calcifies to form a woven bone. As growth continues, the woven bone of the metaphysis is modeled by osteoclasts and osteoblasts into the mature trabecular and later compact bone of the diaphysis.



Mineralization

All mineralization, whether it be of osteoid or hypertrophic cartilage, is due to the precipitation of **calcium phosphate** and the growth of crystals of **hydroxyapatite**. Calcium phosphate is extremely insoluble and its precipitation will occur spontaneously when elevated levels of calcium or phosphate ions are present. Mineralizing tissues possess mechanisms for focally increasing calcium and/or phosphate concentration. In non-mineralizing tissues, calcium phosphate precipitation is prevented in part by the presence of agents such as **pyrophosphate** that inhibit mineral growth, and in part by the storage of phosphate in an organic rather than inorganic form. In the mineralizing tissues, pyrophosphate is destroyed by the action of **alkaline phosphatase,** which also releases free phosphate from phospholipids. In woven bone and hypertrophic cartilage, mineralization is mediated by matrix vesicles, and occurs in the matrix between collagen fibrils. In mature remodeling bone, mineral formation first takes place within the holes that separate the collagen molecules within the collagen fibrils. Mineralization then continues between adjacent collagen molecules within the fibril and finally extends to the space between adjacent fibrils so that the whole matrix is entombed in mineral.

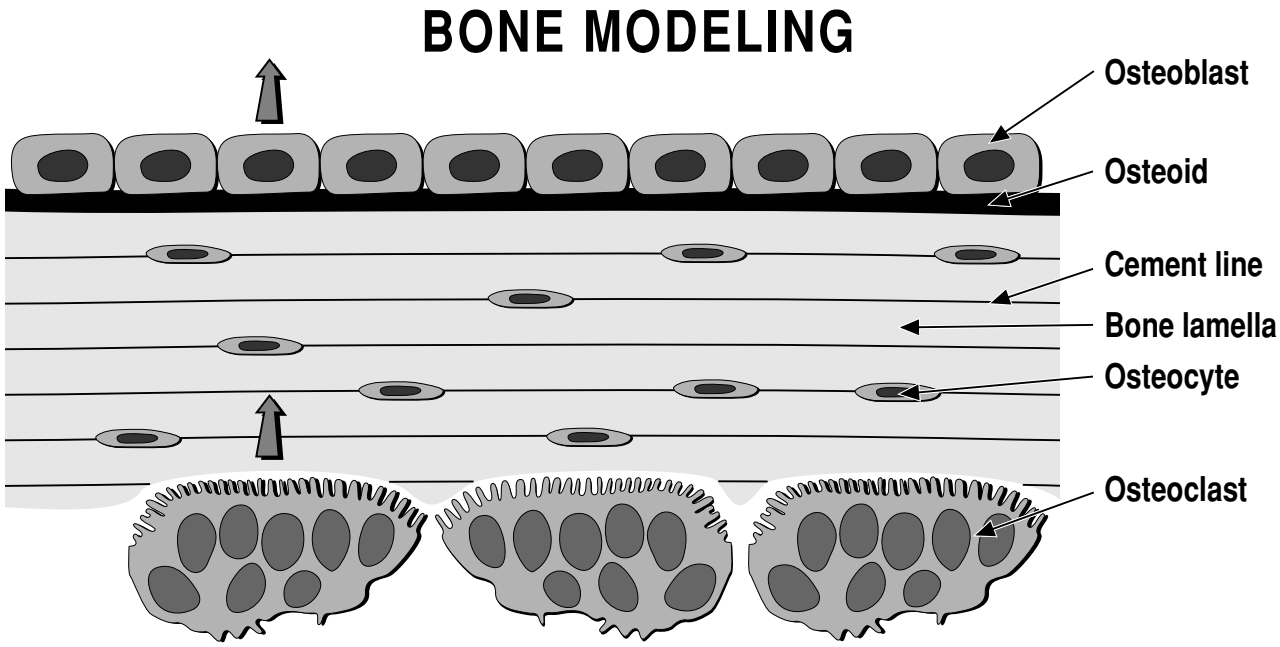


Matrix vesicles

Matrix vesicles are formed from hypertrophic chondrocytes and osteoblasts by budding off from the plasma membrane. They are thought to be sources of stored **calcium** (derived from the mitochondria of the parent cells) and **phosphate** (present in the phospholipids of the plasma membrane). They also contain an **alkaline phosphatase** which is responsible for converting the organic phosphate into inorganic phosphate ions, and which destroys matrix pyrophosphate and so facilitates mineral deposition. It is unclear whether all mineralization begins in the matrix vesicles or whether they can also act as sources of calcium and phosphate ions for mineralization beginning in the matrix itself. Initial theories of mineralization contended that hydroxyapatite crystals were formed within the matrix vesicle, where they grew and eventually burst free from the vesicle membrane. Phospholipids in the inner surface of the vesicle membrane act as nucleation sites for crystal growth. However, in the growth plate sites of mineral deposition have been observed in the matrix remote from matrix vesicles, with proteoglycan appearing to act as nucleation sites for crystal growth.

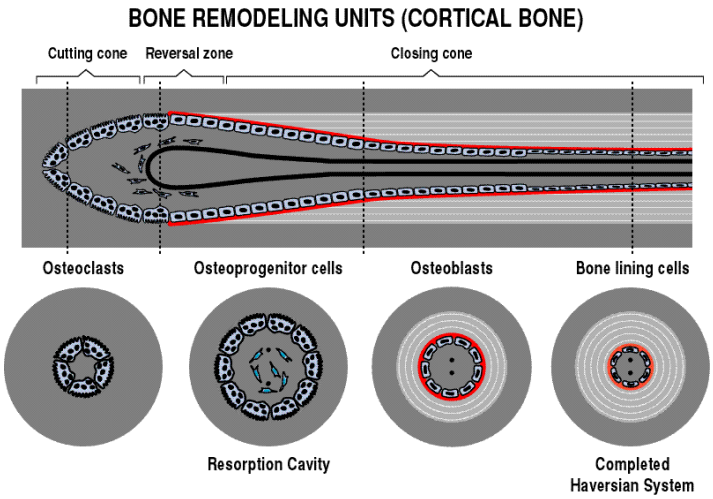
Modeling

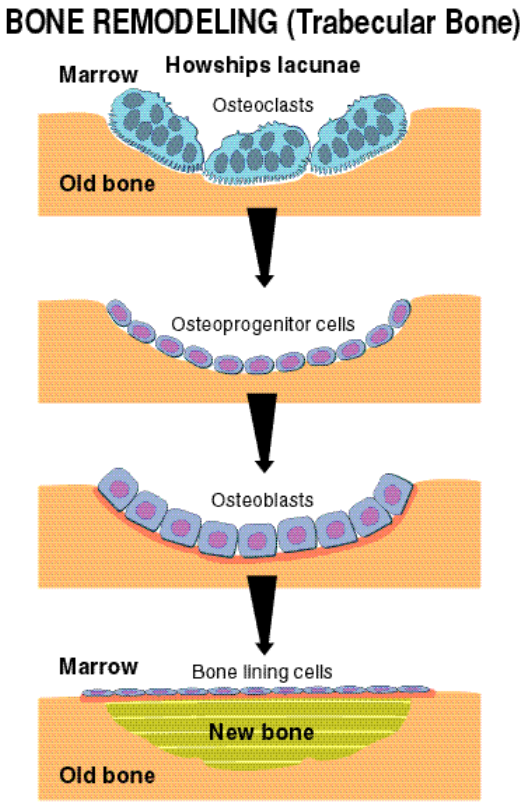
The alteration in the size and shape of bones during growth by the processes of bone formation and resorption is termed **modeling**. Modeling accounts for a) growth in width of the diaphyseal long bone cylinder and expansion of the interior marrow cavity, b) shaping of the ends of the long bones to convert the broad metaphyseal funnel into a narrow diaphyseal cylinder, and c) enlargement of the cranial vault curvature. In each case it is due to the processes of bone formation and resorption occuring independently at different sites. The two processes are not balanced, and modeling results in both a change in bone shape and a net increase in bone mass during growth. Bone **growth** can only take place by an **appositional** mechanism, in which new bone is added to an existing bone surface. Intersitial growth, where the tissue matrix itself expands, as occurs in cartilage, is prevented by the rigid calcified matrix.



Remodeling

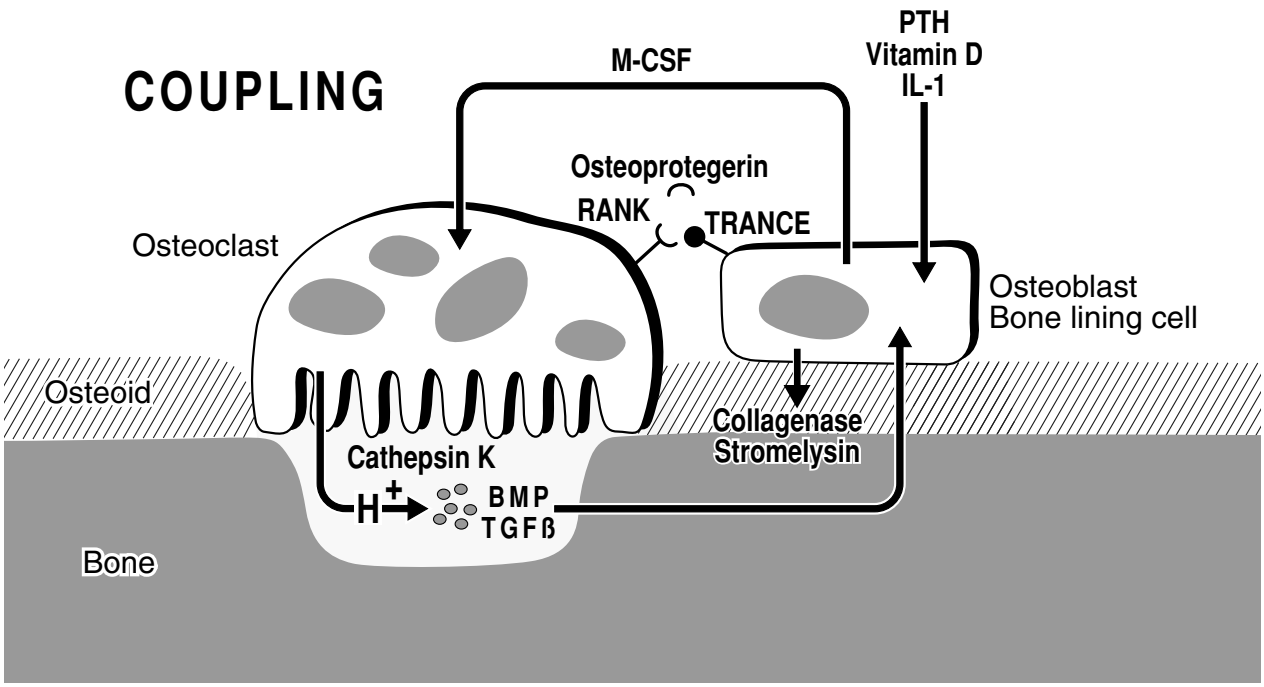
Bone **remodeling** occurs throughout life. In new bone it gives rise to the conversion of woven bone into mature lamellar bone, and in mature bone it accounts for the continuous turnover of bone that is necessary for maintenance of its structure and calcium homeostasis. In the normal adult about 3% of the cortical bone is remodeled per year, whereas about 25% of trabecular bone is remodeled. Remodeling of both cortical and trabecular bone occurs via the concerted action of osteoclasts and osteoblasts acting in unison as a **bone remodeling unit (BRU)**, which is also known as a **bone multicellular unit (BMU)**. In cortical bone, the BRU has osteoclasts at its leading edge **(cutting cone)** tunneling through the calcified matrix, and osteoblasts at its trailing edge **(closing cone)** filling in the tunnel with concentric bone lamellae. The center of the tunnel is occupied by the penetrating blood vessel, which is the source of the bone remodeling cells. In trabecular bone, the BRU resides in a **Howships lacuna** on the trabecular surface, and the osteoblasts and osteoclasts arise from the progenitor cells in the marrow. Remodeling gives rise to lamellar bone, as filling of the resorption cavities takes place in layers by the surface osteoblasts. In the cortical bone this process gives rise to the **osteon** structure. In the normal young adult bone remodeling does not result in a net change in bone shape or mass, as the processes of formation and resorption are balanced and occur sequentially at the same sites.





Coupling

Mature bone is a continuously remodeling tissue in which resorption and formation take place sequentially throughout life. The process whereby this sequential coordination between osteoclast-mediated resorption and osteoblast-mediated formation is maintained is termed **coupling**. The coupling of osteoblast and osteoclast function is brought about by a number of mechanisms. Possibly the most important is the fact that the major hormones (**PTH** and **vitamin D**) and local factors (**IL-1**) that stimulate bone resorption do not have their receptors on the osteoclasts but instead on the bone lining cells. Thus the signal for bone resorption by the osteoclast is mediated by the osteoblast cell lineage. This signalling involves the release of soluble factors produced by the bone lining cells (**M-CSF** and **osteoprotegerin**) and the interaction of cell surface molecules on the bone lining cells (**TRANCE**) and recruited osteoclasts (**RANK**). In addition, the osteoclasts will not bind to bone surfaces covered by unmineralized osteoid, but first require the bone lining cells to degrade the osteoid to expose underlying mineral. Demineralization of bone by the osteoclast releases matrix constituents (such as **BMP** and **TGFβ**) that can stimulate the differentiation and proliferation of osteoblast progenitors or stimulate matrix production by the osteoblasts, and so initiate new bone formation. Osteoclast action is terminated by apoptosis (programmed cell death) and the prevention of new recruitment by osteoprotegerin production by the osteoblasts. Thus each cycle of bone remodeling involves the sequential steps of activation-resorption-formation (**ARF**), carried out by the bone lining cells, osteoclasts and osteoblasts, respectively. In the young healthy adult resorption and formation are **balanced** such that bone mass remains constant. Pathological states develop when resorption and formation are **unbalanced**, as either excessive bone loss or excessive formation can occur.



Bone formation disorders

Osteoporosis may result as a consequence of insufficient bone formation by the osteoblasts. Insufficient bone formation occurs in the elderly (type II osteoporosis) because of a deficit in osteoblast stem cells (F-CFU) in the bone marrow, or in situations where the bone is not subjected to stress, such as prolonged bed rest and paralysis. Absence of stress limits bone formation and to a lesser extent increases resorption. So called stress shielding is thought to initiate loss of bone mass around the stem of joint prostheses. Bone loss in type II osteoporosis occurs at 0.3-0.5% per year, whereas in type I osteoporosis the rate of loss may increase 10 fold. A reduced bone mass can also result if osteoid synthesis is abnormal, such as in osteogenesis imperfecta or scurvy. **Osteogenesis imperfecta** (OI, brittle bone disease) is associated with mutations in the type I collagen genes. This may give rise to the underproduction of normal collagen as seen in the mild forms of OI (type I), or to the underproduction of a structurally abnormal collagen fibril architecture as seen in the more severe forms of OI (types II, III and IV). **Scurvy** is associated with under-hydroxylation of the collagen molecule. The enzymic conversion of proline to hydroxyproline is essential for stability of the collagen triple helix, and the enzyme responsible, prolyl hydroxylase, requires vitamin C as a cofactor. Vitamin C deficiency therefore results in unstable collagen molecules that are readily degraded and so gives rise to the underproduction of osteoid. Another disorder related to the osteoblast cell lineage is **fibrous dysplasia**. This mosaic disorder is associated with sporadic mutations in a G protein gene in osteoblast precursor cells, and results in abnormal differentiation and the excessive proliferation of fibrous tissue in the marrow cavity. Increased osteoblast differentiation, often due to a local stimulus, gives rise to **osteosclerosis**.

FACTORS PROMOTING DISC DEGENERATION

- **Low Cell Density**
- **Large, Avascular Tissue**
  - Poor nutrient supply
  - Poor waste removal
  - Acidification by lactic acid
- **Loss of Notochordal Cells**
  - Unique feature of juvenile NP

Bone resorption disorders

Pathological disorders of bone may develop when osteoclast production or function is impaired. A deficiency in osteoclast production or action results in deficient bone resorption and the development of **osteopetrosis**. Osteopetrosis is a relatively rare inherited disorder that may be due to either reduced numbers of osteoclasts or their decreased functional ability. The inability of the osteoclast precursor cells to differentiate and the inability to form a ruffled border have been associated with defects in the transcription factors fos and src, respectively. Another disorder associated with deficient osteoclast action is **pycnodysostosis**. In this case the cathepsin K involed in dissolution of the organic bone matrix following demineralization is defective. In contrast to the above, excessive bone resorption results in **osteoporosis**. Excessive resorption can be due to a decrease in the sex hormone levels, as occurs in post-menopausal women (type I osteoporosis) or castrated males. Excessive bone resorption also occurs in hyperparathyroidism due to the increased release of PTH, and with the prolonged use of glucocorticoid drugs. Uncontrolled bone remodeling prevents the production of a normal lamellar bone architecture and gives the bone an haphazard mosaic appearance in **Paget disease**. In normal cortical bone about 4 months is required for each BRU cycle to form a new Haversian system, but in Paget disease such cycles do not procede to completion before a neighboring BRU encroaches at the site. The disorder has been associated with possible viral infection of the osteoclasts. **Familial expansile osteolysis (FEO)** also shows uncontrolled bone resorption and is due to an activating mutation in the RANK gene, and **hyperostosis corticalis deformans juvenilis** shows high bone turnover and bone loss due to a mutation in the osteoprotegerin gene.

BONE RESORPTION DISORDERS

Disorder	Cause	Resorption
•Osteopetrosis	Defective precursor differentiation (fos)	↓
	Defective osteoclast maturation (src)	↓
	Defective carbonic anhydrase	↓
	Defective chloride channels (ClC-7)	↓
•Pycnodysostosis	Defective cathepsin K	↓
•Osteoporosis	Decrease in sex hormone (type I)	↑
	Glucocorticoids	↑ ?
	Hyperparathyroidism	↑
•Paget	Viral infection?	haphazard
•FEO	RANK mutation	↑
•Hyperostosis	Osteoprotegerin mutation	↑

Markers of bone metabolism

Various components of bone released into the circulation or urine during bone remodeling have been used as markers of bone formation and resorption. Markers of bone **formation** include **alkaline phosphatase**, the **C and N propeptides of type I collagen**, and **osteocalcin**. All are measured in serum. In each case there are potential limitations in interpreting results. Alkaline phosphatase and type I collagen are produced by tissues other than bone, and while osteocalcin is bone specific, its release may indicate new osteoid synthesis or resorption of old bone. Markers of bone **resorption** include **acid phosphatase**, **hydroxyproline**, the **C and N cross-linked telopeptides of type I collagen**, and the **pyridinoline cross-links** themselves. All are measured in urine except acid phosphatase. Again, there are potential limitations with some of these assays, as acid phosphatase is unstable and hydroxyproline is present in the collagens of all tissues. The collagen crosslink assays are specific for tissue resorption, and those involving immunological analysis of the telopeptides are specific for bone.

MARKERS OF BONE METABOLISM

- **Bone Formation Markers**
  - Alkaline phosphatase
  - Type I collagen propeptides
  - Osteocalcin
- **Bone Resorption Markers**
  - Acid phosphatase
  - Hydroxyproline
  - Pyridinoline cross-links
  - Type I collagen telopeptide cross-links

Calcium homeostasis

The bone of the skeleton serves two major functions: in providing a supporting structure for the other tissues and organs, and in providing a reservoir whereby calcium homeostasis can be maintained. Maintenance of calcium homeostasis is essential, as both hypocalcemia and hypercalcemia are physiologically deleterious. For example, **hypocalcemia** can result in tetany and **hypercalcemia** can result in calcification in tissues that do not normally calcify. Plasma calcium levels can be affected by the action of parathyroid hormone, vitamin D and calcitonin.

CALCIUM HOMEOSTASIS

■ **Parathyroid hormone (parathyroid)**	Released by low plasma calcium Stimulates bone resorption Prevents calcium excretion by kidneys Stimulates calcitriol synthesis
■ **Calcitriol (1,25-diOH-Vit. D) (Vit. D in diet)**	25-hydroxylation in liver 1-hydroxylation in kidney Stimulates bone resorption Stimulates intestinal calcium absorption
■ **Calcitonin (thyroid)**	Released by high plasma calcium Inhibits bone resorption

Parathyroid hormone

Plasma calcium levels are regulated by **parathyroid hormone (PTH)**, with increased production of the hormone being stimulated by low calcium levels and decreased production by high calcium levels. The major function of parathyroid hormone is to increase serum calcium. It does this 1) by stimulating osteoclastic bone resorption, 2) by preventing calcium excretion by the kidneys, and 3) by stimulating the conversion of vitamin D to the active metabolite responsible for enhancing calcium absorption through the intestines. PTH stimulates bone resorption by promoting osteoclast formation, promoting ruffled border formation, and promoting lysosomal enzyme production and carbonic anhydrase. This stimulation is, however, indirect as the receptors for PTH reside on the osteoblast not on the osteoclast. A related protein, PTHrP, is produced by some tumors. It can interact with the PTH receptors and produce the hypercalcemia associated with malignancy.

Vitamin D

Vitamin D is not itself hormonally active, but requires hydroxylation to **1,25-dihydroxy-vitamin D (calcitriol)**. The first metabolic modification is 25-hydroxylation occurring in the **liver**, followed subsequently by 1-hydroxylation in the **kidney**. Both steps are catalyzed by specific hydroxylases. Activity of the 1-hydroxylase in the kidney is enhanced by the action of PTH and low calcium levels. The active metabolite can both promote bone mineral formation through stimulating calcium and phosphate availability, and participate directly in bone resorption through osteoclast activation. As with PTH, the calcitriol receptors are possessed by the osteoblast not the osteoclast.

Calcitonin

A third hormone which can have a direct effect on bone resorption is **calcitonin**, though it is still uncertain in humans whether this agent exerts any normal physiological effect. Calcitonin is made by the **thyroid** and, unlike PTH and calcitriol, it acts directly on the osteoclasts which possess the relevant receptors. Also, unlike PTH and calcitriol, calcitonin inhibits bone resorption. It is able to decrease both ruffled border formation and osteoclast number. The major reasons for its uncertain role in human physiology is that in some pathological situations where calcitonin production is elevated (thyroid tumors) or decreased (thyroidectomy) bone resorption is unaffected. However, in pharmacological doses calcitonin can prevent pathological bone resorption and it has been used therapeutically in the treatment of Paget disease and post-menopausal osteoporosis.

Mineralization disorders

Bone disorders may also result due to impaired mineralization of osteoid because of a lack of calcium or phosphate. This disorder is termed **osteomalacia**. In growing children, the same mineral deficiency will also affect calcification of cartilage within the growth plate and is termed **rickets**. Osteomalacia or rickets may be of the low turnover or high turnover type. Calcium deficiency results in high bone turnover as bone resorption is stimulated via PTH production. Phosphate deficiency does not stimulate PTH secretion and results in low bone turnover. In addition to dietary deficiency in calcium or phosphate, rickets is commonly associated with a deficiency in vitamin D. Calcium absorption via the intestines may be deplete due to a **dietary deficiency** in vitamin D (vitamin D deficient rickets, VDDR), or to a defect in the conversion of **vitamin D** to its active metabolite (vitamin D dependency type 1, VDD1; pseudovitamin D deficient rickets). A defect in the vitamin D receptor gives rise to a form of rickets that is refractory to the administration of vitamin D or its active metabolite (vitamin D dependency type 2, VDD2; hypocalcemic vitamin D resistant rickets). **Hypophosphatemic rickets**, which is due to impaired phosphate reabsorption by the kidneys, represents another form of vitamin D resistant rickets (VDRR). In the autosomal dominant form it is due to a mutation in FGF-23, whereas in the X-linked form it is due to a mutation in the proteinase PEX. Rickets also develops in **hypophosphatasia**, where there is impaired mineralization due to a defect in alkaline phosphatase.

RICKETS

Problem	Cause	Disorder
↓ Calcium (high turnover)	Ca deficiency in diet	Dietary rickets
	Vitamin D deficiency in diet	VDDR
	1,25 diOH Vitamin D deficiency (1 hydroxylase defect)	VDD-1, pseudo VDDR (PDDR)
	Organ resistance to Vitamin D (receptor defect)	VDD-2, VDRR (hypocalcemic)
↓ Phosphate (low turnover)	PO_4 deficiency in diet	Dietary rickets
	Impaired kidney reabsorption (FGF-23 defect)	Autosomal dominant hypophosphatemia
	Impaired kidney reabsorption (PEX defect)	X-linked hypophosphatemia
	Organ availability (alkaline phosphatase defect)	Hypophosphatasia

REFERENCES

Cartilage

1. Caplan, A.I. (1984) Cartilage. Scientific American, **251**, 84-94.
2. Kuettner, K.E. (1992) Biochemistry of articular cartilage in health and disease. Clin Biochem., **25**, 155-163.
3. Buckwalter, J.A. and Mankin, H.J. (1997) Articular cartilage. Tissue design and chondrocyte-matrix interactions. J. Bone Joint Surg., **79A**, 600-611.
4. Bruckner, P. and van der Rest, M. (1994) Structure and function of cartilage collagens. Microscop. Res. Technique, **28**, 378-384.
5. Thomas, J.T., Ayad, S. and Grant, M.E. (1994) Cartilage collagens: Strategies for the study of their organization and expression in the extracellular matrix. Ann. Rheum. Dis., **53**, 488-496.
6. Heinegard, D. and Oldberg, A. (1989) Structure and biology of cartilage and bone matrix non-collagenous macromolecules. FASEB J., **3**, 2042-2051.
7. Carney, S.L. and Muir, H. (1988) The structure and function of cartilage proteoglycans. Physiol. Rev., **68**, 858-910.
8. Roughley, P.J. and Lee, E.R. (1994) Cartilage proteoglycans: structure and potential functions. Microscop. Res. Technique, **28**, 385-397.
9. Hamerman, D. (1993) Aging and osteoarthritis: basic mechanisms. J. Amer. Geriatr. Soc., **41**, 760-770.
10. Jones, A.C. and Doherty, M. (1992) The treatment of osteoarthritis. Brit. J. Clin. Pharmacol., **33**, 357-363.
11. Lohmander, L.S. (1994) Articular cartilage and osteoarthrosis. The role of molecular markers to monitor breakdown, repair and disease. J. Anat., **184**, 477-492.
12. Ghosh, P. (1993) Nonsteroidal anti-inflammatory drugs and chondroprotection. Drugs, **46**, 834-846.
13. Toivanen, A. (1994) Infection and arthritis. Ann. Med., **26**, 245-248.
14. Sewell,K.L. and Trentham,D,E.. (1993) Pathogenesis of rheumatoid arthritis. Lancet, **341**, 283-286.
15. Brooks, P.M. (1993) Clinical management of rheumatoid arthritis. Lancet, **341**, 286-290.
16. Buckwalter, J.A. and Mankin, H.J. (1988) Articular cartilage repair and transplantation. Arthritis Rheum., **41**, 1331-1342.
17. Oegema, T.R.. (1993) Biocemistry of the intervertebral disc. Clinics Sports Med., **12**, 419-439.
18. Buckwalter, J.A. (1995) Aging and degeneration of human intervertebral disc. Spine, **20**, 1307-1314.
19. Benjamin, M. and Evans, E.J. (1990) Fibrocartilage. J. Anat., **171**, 1-15.
20. Horton, W.A. (1996) Progress in human chondrodysplasias. Molecular genetics. Ann. NY Acad. Sci., **785**, 150-159.
21. Spranger, J., Winterpacht, A. and Zabel, B. (1994) The type II collagenopathies: a spectrum of chodrodysplasias. Eur. J. Pediatr., **153**, 56-65.
22. Hopwood, J.J. and Morris, C.P. (1990) The mucopolysaccharidoses; diagnosis, molecular genetics and treatment. Mol. Biol. Med., **7**, 381-404.

Bone

1. Freemont, A.J. (1993) Basic bone cell biology Int. J. Exp. Pathol., **74**, 411-416.
2. Buckwalter, J.A., Glimcher, M.J., Cooper, R.R. and Recker, R. (1995) Bone biology. J. Bone Joint Surg., **77A**, 1256-1289.
3. Aarden, E.M., Burger, E.H. and Nijweide, P.J. (1994) Function of osteocytes in bone. J. Cell. Biochem., **55**, 287-299.
4. Burger, E.H., Klein-Nulend, J. van der Plas, A. and Nijweide,P.J. (1995) Function of osteocytes in bone. Their role in mechanotransduction. J. Nutr., **125 Suppl**., 2020-2023.
5. Hall, T.J. and Chambers, T.J. (1996) Molecular aspects of osteoclast function. Inflamm. Res., **45**, 1-9.
6. Teitelbaum, S.L., Tondravi, M.M. and Ross, F.P. (1997) Osteoclasts, macrophages, and the molecular mechanisms of bone resorption. Leuk. Biol., **61**, 381-388.
7. Roodman, G.D. (1996) Advances in bone biology: the osteoclast. Endocrine Rev., **17**, 308-327.
8. Roodman, G.D. (1999) Cell biology of the osteoclast. Exp. Hematol., **27,** 1229-1241.
9. Anderson, H.C. (1989) Mechanism of mineral formation in bone. Lab. Invest., **60**, 320-330.
10. Weiner,S. and Traub, W. (1992) Bone structure: from angstroms to microns. FASEB J., **6**, 879-885.
11. Poole, A.R. (1991) The growth plate: cellular physiology, cartilage assembly and mineralization. In 'Cartilage: molecular aspects,' (eds B.K. Hall and S.A. Newman), CRC Press, Boca Raton, pp 179-211.
12. Boskey, A.L. (1992) Mineral-matrix interactions in bone and cartilage. Clin. Orthop., **281**, 244-274.
13. Gallagher, J.C. (1990) The pathogenesis of osteoporosis. Bone Mineral, **9**, 215-224.
14. Mitlak, B.H., and Nussbaum, S.R. (1993) Diagnosis and treatment of osteoporosis. Annu. Rev. Med., **44**, 265-277.
15. Mankin, H.J. (1994) Metabolic bone disease J. Bone Joint Surg, **76A**, 760-788.
16. Thomas, D.W. and Shepherd, J.P. (1994) Paget's disease of bone: current concepts in pathogenesis and treatment. J. Oral Pathol. Med., **23**, 12-16.
17. Byers, P.H., Wallis, G.A. and Willing, M.C. (1991) Osteogenesis imperfecta: translation of mutation to phenotype. J. Med. Gen., **28**, 433-442.
18. Spiegel, A.M., Weinstein, L.S. and Shenker, A. (1993) Abnormalities in G protein-coupled signal transduction pathways in human diseases. J. Clin. Invest., **92**, 1119-1125.
19. Huffer, W.E. (1988) Morphology and biochemistry of bone remodeling: possible control by vitamin D, parathyroid hormone and other substances. Lab. Invest., **59**, 418-442.
20. Suda,T., Takahashi, N. and Abe,E. (1992) Role of vitamin D in bone resorption. J. Cell. Biochem., **49**, 53-58.
21. Calvo, M.S., Eyre, D.R. and Gundberg, C.M. (1996) Molecular basis and clinical application of biological markers of bone turnover. Endocrine Rev., **17**, 333-368.
22. Christenson, R.H. (1997) Biochemical markers of bone metabolism: an overview. Clin. Biochem., **30**, 573-593.