Part 2- Basic dentistry for implants

Chapter 1 The histology of bone

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The adult human body is composed of 206 bones that differ in size and shape, each with a structure to suits its function. Adult bones can largely be divided into the axial skeleton and the appendicular skeleton. The axial skeleton is composed of 23 cranial bones, 6 auditory ossicles, 26 vertebrae, a breastbone and 24 ribs, and the appendicular skeleton consists of the 64 bones of the upper limb and the 62 bones of the lower limb (Fig. 2-1-1-a).

Bones are differentiated into long bones, short bones, flat bones and irregular bones according to their structural form. Other unique bones include the pneumatic bones such as the maxilla, which makes up the maxillary sinus. The long bones constitute the majority of bones in the body as can be seen in both the upper and the lower limbs, and are comparatively long and thin (Fig. 2-1-1-d). They are hollow cylinders surrounded by thick cortical bone, with a medullary cavity in the interior. The short bones form the small bones of the hands and feet, the surfaces of which are covered by relatively thin cortical bone and the interiors of which consist of cancellous bone (Fig. 2-1-1-e). Flat bone forms the calvarium and the breastbone, which surfaces are covered by relatively thick cortical bone, with cancellous bone in the interior (Fig. 2-1-1-c). The irregular bones have the complex form seen in the vertebrae and the hip bones, in which the ratio of cancellous to cortical bone differs among the bone types (Fig. 2-1-1-b).

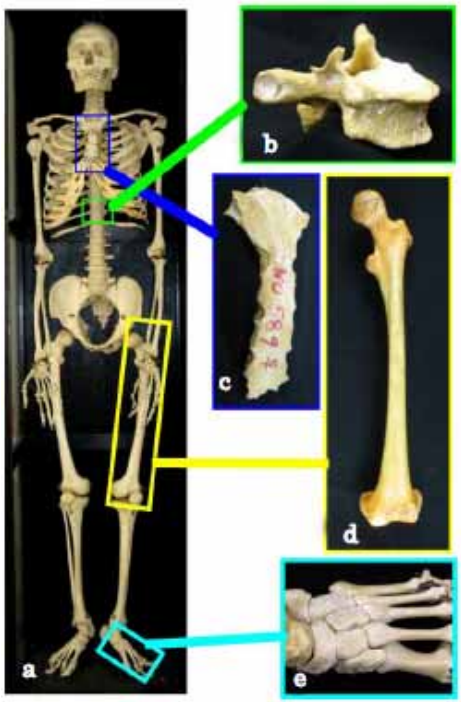

Fig. 2-1-1-a,b,c,d,e
The classification of bones according to their structural forms

a. Human skeleton
b. The irregular bone (the vertebra)
c. The flat bone (the breastbone)
d. The long bone (the thigh bone)
e. The short bone (the tarsal bone)

I. The function of osseous tissue

Osseous tissue makes up 18% of the body's weight, produces hardness and strength, and functions in the form of a skeleton to provide overall support. It is also essential for organization of the following bodily functions:

1) Support: The skeletal structure supports the body, providing points for the attachment of the skeletal muscles, and giving rise to the overall framework of the body.

2) Protection: Protects the internal organs, brain, spinal cord, heart and lungs.

3) Motor: Motor activities are the result of the collective action of bone and the skeletal muscles.

4) Homeostasis of calcium and phosphate: Osseous tissue stores minerals such as calcium and phosphate as inorganic compounds, which make the bones strong. In addition, it maintains homeostatic equilibrium by releasing minerals into the blood and storing extra minerals, deposited as calcium compounds.

5) Hemopoiesis: Hematocytes are generated in the red marrow, a hematogenous tissue present in the medullary cavity of the bone.

The osseous tissues are constructed of matrix that contains a high concentration of calcium salts, with osteocytes, osteoblasts and osteoclasts that are entrapped through their projections. The bones are continuously formed throughout the lifetime of the individual; even in adults several percent of the bone is undergoing active remodeling, so a bone is completely replaced in roughly five years.

II. The structure of osseous tissue

The inner surface of the long bone, referred to as the diaphysis, is composed of compact bones that are hard and thick. The epiphyses at both ends are cancellous (have spongy architecture). The diaphysis encloses a hollow medullary cavity that communicates freely with the intratrabecular spaces. The bone marrow, the site of hematopoiesis, also resides in this space. (Fig. 2-1-2 a,b,c).

The proximal and distal ends of bones are covered with articular cartilages that are made up of hyaline cartilage, forming a joint. During development of the long bone, epiphyseal cartilages (the epiphyseal plate) exist in between the diaphysis and epiphysis, and growth occurs in a longitudinal direction from this location. In fully-grown adults where growth has ceased, the epiphyseal cartilage becomes ossified and completely replaced by bone, though remnants of the communication with the marrow cavity of diaphyses can be seen, referred to as the epiphyseal line (Table 2-1-3).

The external structure of the cortical bone is surrounded by a fibrous layer (the periosteum), and in its interior resides the potential for osteogenesis. The periosteum consists of a rich supply of blood vessels and nerve fibers that enter into the bone structure via Volkmann's canals. The periosteum and cortical bones interact intimately by means of Sharpey's fibers that are well-developed bundles of collagen fibers. The periosteum of immature bone is thick, with a rich supply of blood vessels for efficient osteogenesis. However, in elderly individuals, this structure is thin with a poor blood supply, and reduced osteogenic ability. The interior surface of cortical bone is covered with the endosteum which consists of osteogenetic cells as with the endosteal layer.

There are red hematopoetic, and yellow adipose types of marrow, depending on the age and site of the marrow. The former type is the active site of hematopoiesis, whereas the latter loses its hematopoietic function and turns into fatty tissue. Hematopoiesis in the bone marrow starts at the 8th week of embryonic development, and after birth, becomes the only site of hematopoiesis.

The main types of bone are classified into cortical bone (substantia compacta) (Fig. 2-1-2-c), and cancellous bone (trabeculae) (Fig. 2-1-2-b). The quantitative ratios of these two bony tissues are dependent on the functions of the bone, and vary within the bone at different parts.

Cortical bone displays substantial hardness, is thickened particularly at sites of compression, and lamination is seen here in large mammals. Cancellous bone exists in the medullary cavity in its spongy form (trabeculae), forming a lattice network that connects each component. The surface area covered by cancellous bone is extremely large. The trabeculae of cancellous bones are ordered in such a way that they can resist external forces.

The intercellular matrix of bone is embedded with numerous collagenous fibers in the ground material. The spaces between these fibers are filled with non-collagenous, organic and inorganic mineral substances that include hydroxyapatite crystals as the main constituent, as well as a small quantity of carbonic salts.

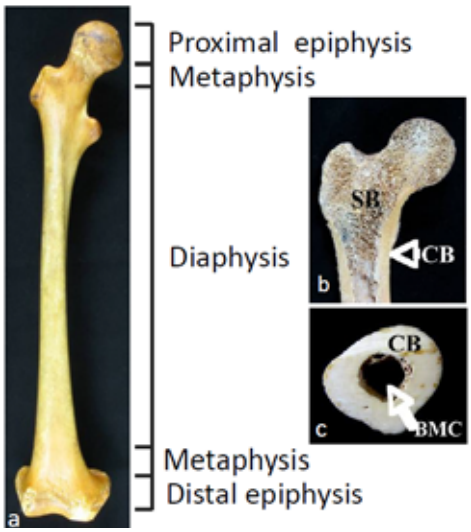

Fig.2-1-2-a,b,c
The structure of the long bone
CB: cortical bone (compact bone)
BMC: medullary cavity
SB: cancellous bone (sponge bone)

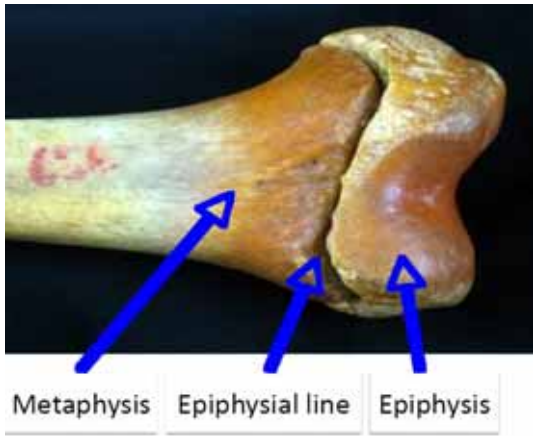

Fig.2-1-3
The epiphysial line which is seen during the growth period in the long bone (epiphysial cartilage is present in an organism).

A. Bone tissue structure

A large pore known as the nutrient foramen exists close to the center of the diaphysis of the long bone, for supply of nutrients to the osseous tissues. One or two main diaphyseal nutrient arteries enter the shaft obliquely through this pore of the compact bone after which they divide into branches in the intramedullary cavity. The blood vessels that are arranged parallel to the major axis of the bone are known as the Haversian canals, and the blood vessels arranged in a perpendicular direction to the Haversian canals, and supply the bone surface (periosteum), are known as Volkmann's canals. Haversian and Volkmann's canals are interconnected, together with the blood vessels of the bone interior (bone marrow). Therefore, it can be seen that there are two types of developed blood vessel systems that reside

in the compact bones (Fig. 2-1-4-a, b).

The Haversian canal is a vessel with a diameter of 20 to 100 μm, which consists of blood capillaries or fine arteries and veins in the center, and loose connective tissue in the surrounding area. The canals placed at the center, the lamellae of the bones, are 5 μm thickness, and are arranged in a concentric manner similar to annual rings of a tree, forming a Haversian system or osteon (bone unit) that is 200 μm in diameter (Fig. 2-1-5-a). The Haversian system is regarded as the fundamental functional unit of compact bone, and consists of lacunae situated between lamellae. Osteocytes make contact with the cytoplasmic processes of their counterparts via a network of small canals or canaliculi, and it is through this network that the cells are oxygenated and nutrients are supplied from the Haversian canal (Fig. 2-1-5-b, Fig. 2-1-6-a). Haversian canals run in the same direction as the blood vessels, and also branch and anastomose, or form a cul-de-sac in a similar manner. In contrast, the Volkmann's canal does not have a sheath of lamellae.

The boundary of a Haversian system or osteon is the cement line, and between adjoining osteons, angular intervals are present that are occupied by interstitial lamellae. An interstitial lamella is incomplete and does not contain a Haversian canal, the bone tissue of which is under continuous generation and absorption; the remnants of destroyed osteons therefore exist as interstitial lamellae in between newly generated osteons (Fig.2-1-5-a).

No Haversian system is present in either the outermost or innermost (marrow) layers of compact bones. Instead, these layers are covered by a few to ten parallel layers of lamellae, referred to as the external and internal basic lamellae, respectively (Fig. 2-1-4-a). These structures are formed by the accumulation of osteoblasts on both the periosteal and endosteal layers. The collagenous fibrils inside both lamellae run in parallel, though in the Haversian system the direction is arranged diagonally in relation to the major axis. The adjacent collagenous fibrils are orientated so that they cross each other at a 90º angle.

The fibrils that run parallel with the long axis contribute to the tensile and compressive strength of the osteon, whereas those that are horizontal contribute to elasticity.

The trabecular bones, in contrast, do not consist of blood vessels or Haversian systems, and therefore rely on canalicular diffusion of oxygen and nutrients directly from the adjacent marrow. The lamellae are orientated towards the direction of the applied force, and are continuously formed and arranged by the coordinated action of osteoclasts and osteoblasts in such way that the bone can withstand the application of external forces.

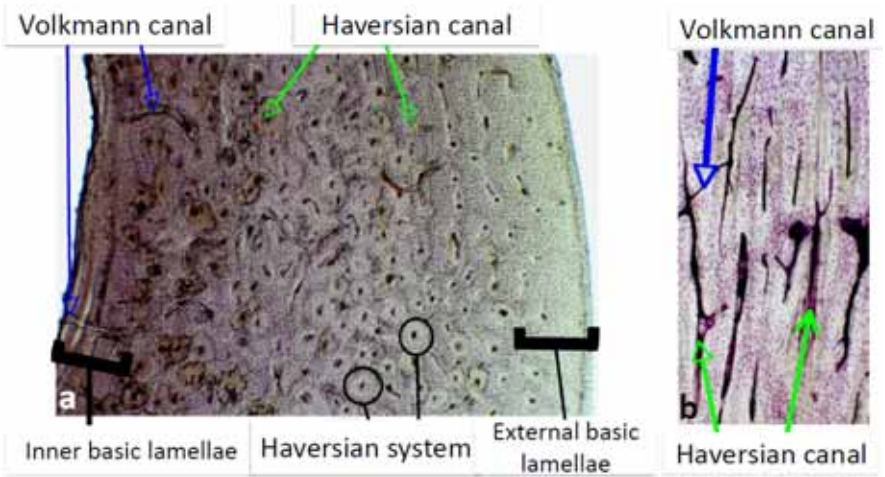

Fig.2-1-4-a,b

The ground section of the bone

a. A number of Haversian systems can be seen in the ground longitudinal section of the long bone.

b. Haversian canal runs longitudinally at the center of the Haversian system.

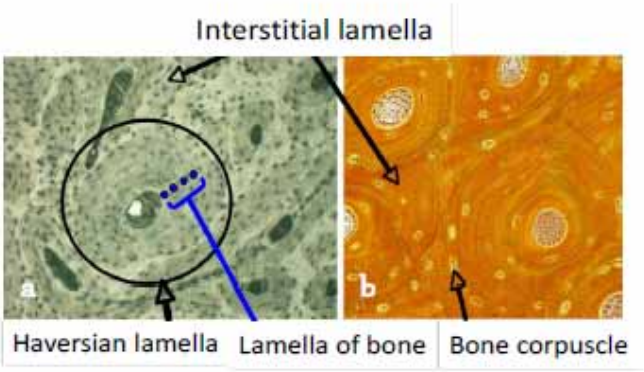

Fig.2-1-5-a.b
The structures of the Haversian system
a. A ground section featuring the lamella of bone in the Haversian system.
b. Bone corpuscles exist between the lamellas of the bone (Schmorl's stain)

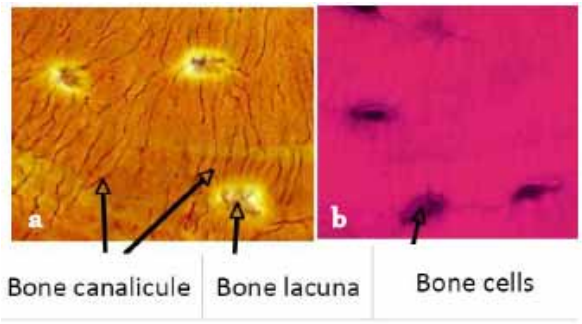

Fig.2-1-6-a,b
The organized structure of the Haversian system
a. Bone corpuscle consists of the bone lacuna and the bone canalicule (high-powered image of the Schmorl's stain).
b. Bony tissues exists inside of the bone corpuscle [high-powered image of the hematoxylin and eosin (HE) stain]

B. The cellular components of osseous tissues

1. Osteocytes

Osteocytes occupy a space called a lacuna which can be found distributed in bony tissue. Typically, the lacuna originates from the two layers of epiphyseal plates, however, it can also be seen within one layer (Fig. 2-1-6-b). Osteocytes form as the osteoblasts become embedded in the matrix that they have secreted, within the lacunae. Osteocytes have a structure that is characterized by numerous dendritic projections, and communicate with the adjacent cells or osteoblasts via gap junctions, forming a cellular network within the osseous tissues. This network system allows efficient communication, transmitting information from the osteoblasts on the bone surface, and relaying nutrients and oxygen into the depths of the bone. Furthermore, this system also has an essential role in the rapid mobilization of calcium that has been released from cellular osteolysis resulting from the coordinated actions of osteocytes and osteoblasts.

The morphological characteristics of osteocytes vary with their distance from the exterior of the bone, and they are classified depending on their morphological properties into: 1) generative osteocytes, 2) resorbable osteocytes, and 3) degenerative osteocytes.

The generative osteocytes are also known as juvenile osteocytes and are localized close to the surface of the bone where osteogenesis is at its most active; these cells have the ability to generate matrix. They occupy a large volume within the lacunae, react to mechanical stimuli and transmit this information to the osteoblasts and osteoclasts. The resorbable osteocytes, found in the depths of the bone matrices, are small, and have little ability to develop organelles. The degenerative osteocytes are found deeper in the bone matrices and thought to be the cells in which processes such as pyknosis, accumulation of lipid droplets, vacuolization or clasmatosis occur, resulting in denaturation and necrosis of the cells.

2. Osteoblasts

These large cells, 20 to 30 μm in diameter, proliferate and differentiate to become osteocytes and are arranged on the surfaces of bone matrices. The greater part of the organic constituents of the bone matrix is synthesized and secreted by the osteoblasts. In addition, these cells also play essential roles in calcification, formation of matrix vesicles, and induction of differentiation of osteoclasts. Osteoblasts mature to become osteocytes, embedded in the matrix that they have secreted; a fraction of the cells remain as quiescent osteoblasts — flattened epithelioid cells on the surface (Fig. 2-1-7-a,b).

The active osteoblasts that undergo osteogenesis often assume a cubic or ovoid shape, and the presence of rough endoplasmic reticulum (rER), a Golgi apparatus and mitochondria can be observed. The rER of osteoblasts that are undergoing active proliferation shows an expanded structure, and the Golgi complex consists of the layers of the Golgi body, alongside the vesicles, and the Golgi vacuole, forming a developed area of Golgi (Fig. 2-1-7-c,d).

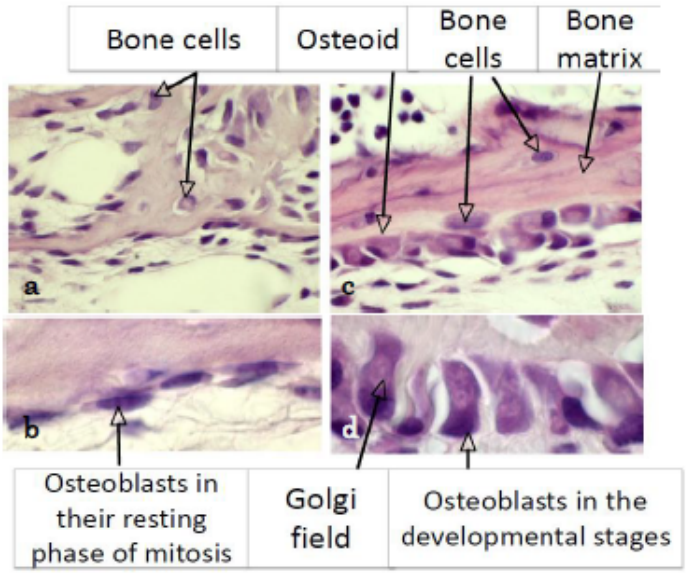

Fig.2-1-7-a,b,c,d

The histology of osteoblasts (HE staining)

a. Osteoblasts in their resting phase of mitosis
b. Osteoblasts in their resting phase of mitosis (high powered image). It exists on the surface of the bone in a uniform layer, without the presence of a large number of osteoids.
c. Osteoblasts in the developmental stages
d. Osteoblasts in the developmental stages (high powered image). Exists in either the rectangular or egg shape, undergoing active secretion, with the presence of developed golgi apparatus. The osteoblasts exist in contact with the thick osteoids.

The bone matrix does not calcify immediately after it is secreted by osteoblasts, but exists as an uncalcified structure called osteoid. The osteoblasts are in constant interaction with the osteoid, and determine the thickness of the layers of the osteoid depending on the activation state of the osteoblast. During the active stages, it can be 5 to 10 μm thick, however, it can be scarce or non-existent in areas adjacent to quiescent osteoblasts.

Osteoblasts synthesize and secrete organic matrix, i.e. type I collagen, or glycoproteins such as osteocalcin, bone sialoproteins, osteopontin and osteonectin. The majority of the collagenous proteins and glycoproteins show affinity for calcium and are thought to control the calcification process. Osteoblasts also secrete cytokines such as the IGF-I, IGF-II, TGF-ß and BMP and store them in the matrix.

In the surrounding structures and the circulating blood near active osteocytes that are undergoing matrix secretion, a raised concentration of alkaline phosphatase can be detected.

Matrix calcification occurs once matrix formation has finished. At the initial stages of calcification, matrix vesicles of 0.03 to 1 μm in diameter surrounded by unit membrane can be detected in the extracellular

matrix (ECM). It is in these vesicles that initiation of apatite crystal formation occurs. Matrix vesicles that show enzymatic activity with the presence of alkaline phosphatase and ATPase, and have a high lipid concentration, bud off from the osteoblast cell surface into the newly formed osteoid. The alkaline phosphatase acts to remove the pyrophosphoric acid that is an inhibitor of calcification, and increases the phosphoric acid concentration locally. The phospholipid contained in the vesicle, which shows strong affinity for calcium, is highly adapted to concentrating the calcium in the vesicle as the source of calcification. The apatite crystals grow, and eventually extend externally, and playing a role in the induction of calcification of collagenous fibrils.

3. Osteoclasts

These are large (over 50 μm in diameter), polymorphic cells that consist of up to 100 different nuclei. The presence of projections results in an irregular structure, and the osteoclast crawls around, the way a slug crawls, causing resorption and thus modeling and remodeling bone. It is now known that the osteoclasts are derived from fusion of the precursor forms of monocytes or macrophages. They can be found lying on the bone surface inside resorption bays called Howship's lacunae or found lying free, away from the bone surface, or wandering with their pseudopodia extended. Their size and structure is extremely diverse (Fig. 2-1-8-a).

The cytoplasm of the osteoclasts is typically acidophilic and stains red with hematoxylin and eosin, with a brush-like structure adjacent to the bone (Fig. 2-1-8-1). Microscopically, the height of the cells adjacent to bone is often 1.5 to 2 μm, with complex invaginations on the plasma membrane that sometimes contain calcareous structures. A vast number of vesicles of various sizes fill the cytoplasm, alongside large numbers of mitochondria, rER, and free ribosomes, as well as lysosomes that are involved in protein degradation, and the Golgi apparatus located in the area surrounding the nucleus.

In terms of enzymatic histochemistry, osteoclasts display a strong tartrate-resistant acid phosphatase (TRAP) activity, which has been adopted as a marker of osteoclast activity (Fig. 2-1-8-c).

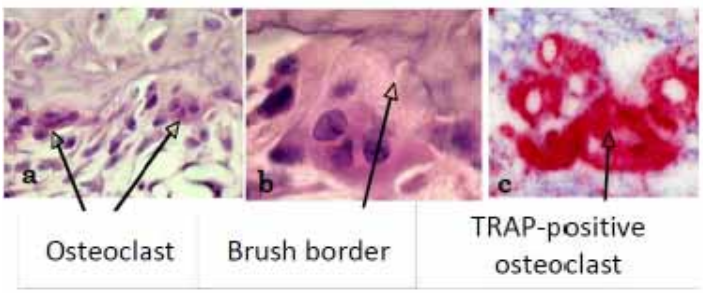

Fig.2-1-8-a,b,c

The histology of osteoclasts

a. Osteoclasts with multiple nuclei exist in the resorption pit, present on the bone surface (HE staining).

b. The high-powered image of the osteoclast. Brush border exists next to the bone.

c. Osteoclasts that display high tartrate-resistant acid phosphatase (TRAP) activity

The osteoclast forms a belt-like isotropic band when they adhere to the cytoplasm, and it is through this structure that it interacts with bone. A ruffled border exists in the interior where the active bone

resorption takes place.

Resorption by the osteoclast can be considered to occur by two different means: the absorption of the minerals that constitute the matrix, and the decomposition of the organic components. For mineral dissolution, an acidic condition is required in the resorption bay. Mechanistically, protons produced by carbonic anhydrase (Type II) located in the cytoplasm are actively transported into the resorption bay by proton ATPase (a proton pump) localized on the ruffled ridge of the plasma membrane. This maintains strong acidity with a pH of around 3–4, necessary for the dissolution of hydroxyapatite (Fig. 2-1-9).

Dissolution of organic material is promoted by lysozymes, mainly cathepsin K and matrix metalloprotease 9 (MMP-9), both of which digest Type I collagen, the main constituent of the bone matrix in the resorption cavity.

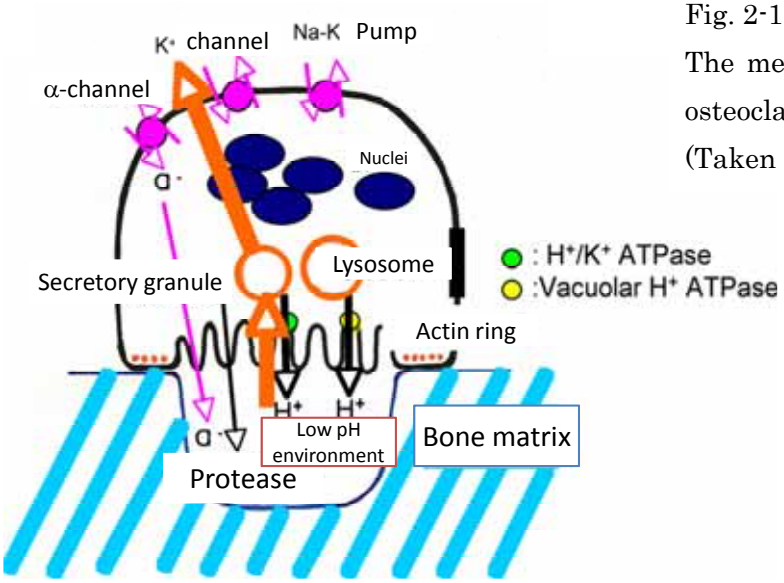

Fig. 2-1-9
The mechanism of the bone resorption by the osteoclast
(Taken from Biology of the bone - in Japanese).

III. Growth of bone

There are intramembranous and endochondral processes of ossification. Intramembranous ossification is the formation of bony tissues within the primitive mesenchyme. This process is applied to the rudimentary formation of cranial bones (except in some parts of the cranial base) and clavicles. In endochondral ossification, small cartilages are formed, then osseous tissues grow and replace the cartilages absorbed and eliminated as they grow. This type of issification is seen with the bones of other parts of the body, including the bones of the cranial base, the torso and the limbs.

A. Intramembranous ossification

The mesenchymal progenitor cells, which are in contact with each other through their projections, differentiate into osteoblasts upon invasion of blood vessels into the center of ossification. Osteogenesis is initiated with the secretion of collagenous fibrils and bone matrix by the osteoblast in the proximal regions of the bone. Calcification of the matrix occurs in association with the extracellular matrix vesicles, and the embedded osteoblast is converted to an osteocyte. The cancellous bone that was formed first is replaced by bone tissue that proliferates from the center out to the surroundings (Fig. 2-1-10-b). The point

where ossification starts is referred to as the primary center of ossification, and the bones formed in this manner are called membranous bones.

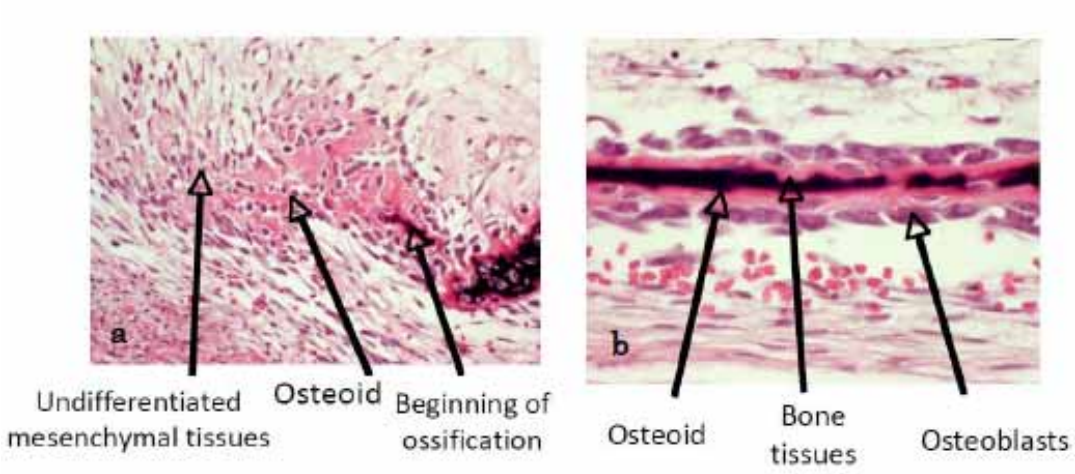

Fig. 2-1-10-a,b

The histology of the intramembranous ossification (HE staining)

a. The initiation source of osteogenesis

The osteoblast differentiates in the undifferentiated mesenchymal tissue, calcification occurs in the bone matrix, and initiating the osteogenesis process.

b. The new bone matrix is formed around the formed bone substance and the bone tissues spread in such manner.

B. Endochondral ossification

Bone can sometimes be referred to as replacement bone as it is completed by the replacement of cartilage, formed at the period of fetal development, by osseous tissue. Hyaline cartilage, which is covered by perichondrium, forms a miniature template. The differentiation of osteoblasts close to the center of the enchondrium of the cartilage follows alongside the development of sheathing bone that surrounds the diaphysis (Fig. 2-1-11-a). Simultaneously, changes occur in the middle of the diaphysis (i.e. the primary center of ossification). Hypertrophy (substantial enlargement) of chondrocytes, compression of nuclei, cytoplasmic vacuolation and calcification of cartilage matrix all occur. The blood vessels invade the calcified cartilage from the periosteum via the connective tissue (Fig. 2-1-11-b). The chondroclasts and macrophages that are transported from the invading blood vessels remove the chondrocytes and calcified matrix, forming a large cavity — the primary bone marrow (primitive medullary cavity) (Fig. 2-1-11-c). At the same time, cancellous bone is formed from the differentiated osteoblasts.

In the cartilaginous regions of the epiphysis, the differentiated and generated chondrocytes migrate towards the diaphysis, and through longitudinal arrangement of the chondrocytes, cartilage columns are constructed. Next, the chondrocytes situated on the diaphyseal side of this ordered arrangement metamorphose, and matrix becomes calcified (Fig. 2-1-11-d). Chondroclasts and macrophages infiltrate this area, remove the denatured chondrocytes and digest the matrix, and form a tunnel in the area between the rows of chondrocytes. The sides of this tunnel are lined with osteoblasts that initiate the construction of skeletal tissue (Fig. 2-1-12-a). The columns of chondrocytes become denatured, are then

resorbed, and gradually become replaced by osseous tissue. Newly differentiated chondrocytes are continuously added to the line of chondrocytes, but in order to achieve progressive longitudinal growth, the epiphyseal plate migrates in a mesiodistal direction for effective longitudinal extension (Fig.2-1-12-b). This process only ceases upon termination of growth of the bone (i.e. the person reaches their maximum height); until then the cartilaginous column exists as the epiphyseal cartilage.

A center of ossification also becomes apparent in the epiphyseal region, and the cartilage in this area becomes denatured and resorbed, at the same time becoming increasingly bony. The layers of articular cartilage involve in the formation of the articular cartilage.

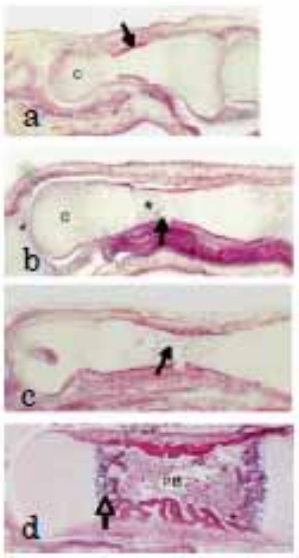

Fig. 2-1-11-a,b,c,d

The histology of the enchondral ossification (HE stain)

a. The vaginate bone which surrounds the diaphysis of the cartilage is formed close to the central part of the cartilage (arrow).
b. The cartilage at the center of the diaphysis becomes thicker, the calcification (★) of the cartilage occurs, and the blood vessel with connective tissue invades the calcified cartilage (arrow).
c. The chondrocyte and the calcified cartilage matrix are absorbed and the primitive bone marrow is formed in the diaphysis (arrow).
d. Longitudinal columnar (palisades) layer is formed in the cartilage situated closely to the epiphysis, followed by the cartilage becoming denatured on the diaphysis side, and the matrix becomes calcified (indicated by the arrow).
PB: the primitive bone marrow

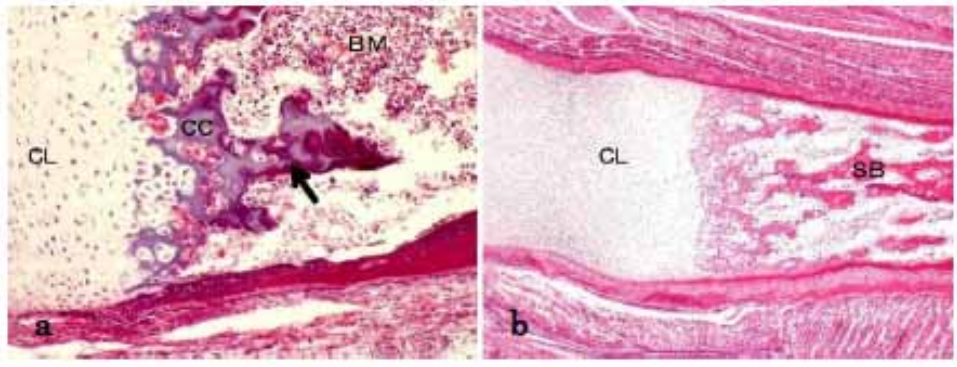

Fig. 2-1-12-a,b

The osteogenesis at the epiphysial cartilage (HE stain)

a. In the epiphysial cartilage (CL), the osteoclast becomes aligned on the surface of calcified cartilage matrix that becomes absorbed, forming bone tissues (arrow).

BM: bone marrow

b. The cartilage (CL) lined in columnar form becomes denatured and absorbed, and becomes rearranged as the bone tissues.

SB: Bone marrow

IV. Growth of the bone

A. Longitudinal growth

The longitudinal growth of the long bones is mediated by the epiphyseal cartilage. The chondrocytes that make up the epiphyseal chondrocyte columns exhibit characteristic longitudinal structures, sequentially termed the resting zone, the proliferative zone, the hypertrophic zone and the zone of calcified cartilage (Fig. 2-1-13-a).

The chondrocytes in the resting layer constitute a set of undifferentiated cells, with reduced ability to develop organelles and a thin layer of intercellular matrix. The cells in the palisade layer are flattened and are packed very closely, and often undergo cell division. The cellular organelles show gradual development, accompanied by accumulation of glycogen granules, and the appearance of mitochondrial granules. The cartilaginous lacuna is filled with a proteoglycan-rich matrix that shows metachromasia, and in the mediastinal matrix, both fibers and proteoglycan accumulate simultaneously. The cells in the hypertrophic zone gradually increase in size, with the development of cellular organelles. Although cell division does not occur, the cells differentiate and swell. The cells in the zone of calcified cartilage are replaced by means of enchondral ossification, initiated by calcification of matrix vesicles. The cancellous bone that is formed by the addition of bone matrix by the osteoblast to the cartilaginous trabeculae is called “primary cancellous bone”. This bone forms the metaphysis (Fig. 2-1-13-b).

Remodeling replaces the primary cancellous bone with mature, secondary cancellous bone. In a manner similar to longitudinal extension of bone, the diaphyseal side of the trabecula is resorbed by osteoclasts in a manner that is proportional to the speed of osteogenesis. As a consequence, the width of the cancellous bone in the metaphysis is kept constant.

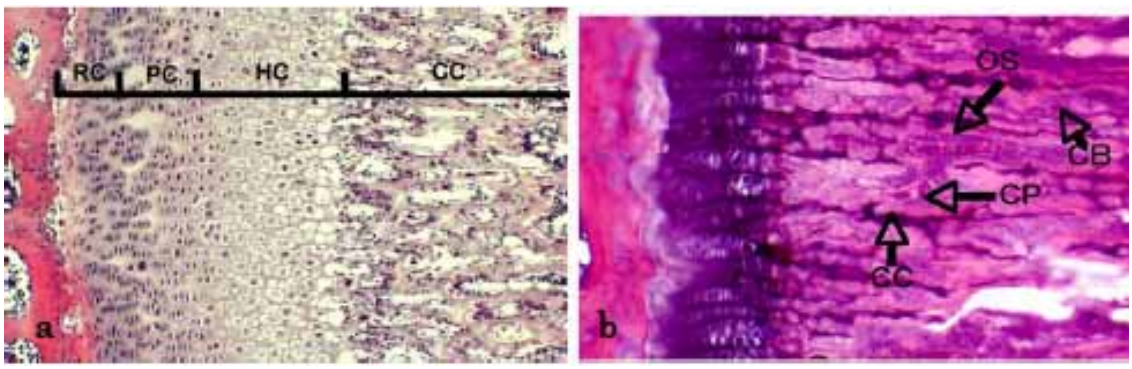

Fig. 2-1-13-a,b

The structure of the epiphysisal cartilage

a. Epiphyseal chondrocyte columns consist of layers of resting cells (RC), proliferating cells (PC), hypertrophic cells (HC) and calcified cells (CC).

b: In the layer of calcified cells, the calcification occurs to the cartilages and the blood capillary invasion (CP), and the primary cancellous bone is formed by the addition of the bone matrix (OS) by the osteoblast (CB) on the surface of the cartilaginous trabecula (CC).

B. Increase in the diameter

The diaphysis of the long bone increases in thickness due to subperiosteal ossification. Thickening of the periosteal collar occurs at the center of the diaphysis, and the trabecular meshwork increases as woven

bone forms at the endosteal side. Once the diaphysis reaches a certain thickness, concurrent actions of osteoclasts and osteoblasts define the thickness of the long bone. The coordinated actions of osteclast-mediated resorption of the trabeculae in the endosteum, and addition of bone by osteoblasts result in an increase in the volume of the bone marrow, retaining the distance between the exterior and the endosteum as the width of the long bone increases.

V. Vascular and nervous supply of bone

A. Blood vessels

Two main vascular systems are distributed within bone. One of the systems arises when numerous thin arteries invade and occupy the Haversian canals from the arterial plexus of the periosteum through Volkmann's canals. The other nourishes the bone marrow via nutrient foramina that enter the medullary cavity, also supplying oxygen and nutrition to the trabeculae of the cancellous bone on the surface. The thin vessels that feed parts of the compact bony interior are sourced from the inner medulla, passing via the Volkmann's canals (Fig. 2-1-14).

The artery that supplies the bone marrow of the epiphysis is an arterial branch that arises from the systemic circulation in a manner that is independent of the vessels of the diaphysis and metaphysis, and is referred to as the epiphyseal artery. This artery pierces the periosteum of the bone where cartilage is absent, and forms a capillary network. This network has no connections with those of metaphysis, and the blood vessels are underdeveloped in comparison to those of the metaphysis.

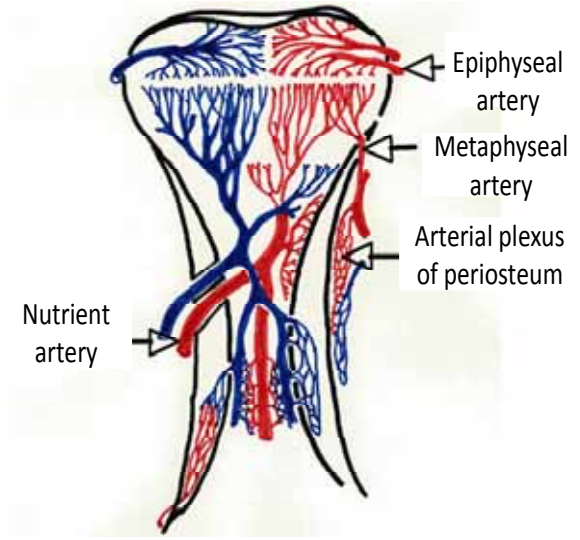

Fig.2-1-14
The vasculature of the bone
(Taken from "Cell and Tissue Biology")

B. Nerves

The abundant, predominantly medullated sensory nerves (some are non-medullated) are distributed in the periosteum and the joint capsule, and participate in detection of pain and proprioception. They also penetrate the bone marrow through the nutrient foramina.

VI. Remodeling and modeling of bone

The skeleton of the human enlarges as growth and development occur. The bone tissues continuously undergo processes of resorption and deposition throughout life, becoming replaced with new bony tissue.

These processes are referred to as modeling and remodeling, and describe the restructuring of the bone due to the resorptive actions of osteoclasts, and deposition by osteoblasts. The restructuring of cortical bone and cancellous trabeculae that accompanies growth and development is called modeling, whereas the restructuring that does not involve alterations in size or form is known as remodeling.

In remodeling, the woven bone that is primarily constructed is converted gradually into cortical bone by enlargement of the trabeculae, and deposition of bone within the perivascular cavities. This process of osteogenesis that proceeds concentrically, with blood vessels at the center, forms the primary osteon. As the cortical bone constructed by this process is resorbed by osteoclasts that differentiate in the perivascular regions, a *secondary* osteon forms. Primary osteons thus become fully replaced by secondary osteons; the osteons of cortical bones are continually replaced by this remodeling process. This same process is observed in the trabeculae of cancellous bone, and after osteoclasts absorb the bone matrix, bone is formed by osteoblasts. Through this remodeling mechanism, degenerating parts of bone are renewed, permitting retention of the ability to withstand external forces applied to the body, as well as preserving the ability to automatically regulate the calcium concentration in bone and blood. By using these mechanisms to replace older parts with new tissues, bone provides the ability to adapt to new environments.

The details of how bony tissue remodels and bones develop, the factors involved in the remodeling process, including the osteoblast and osteoclast, as well as osteocyte cell division and the functional control of this process are all described below.

A. Reconstruction of the Haversian system by remodeling

The renewal of compact bones is conducted by reconstruction of Haversian systems. The osteoclast appears in the connective tissue of the Haversian canal, and causes bone resorption that is independent of the structure of the previous Haversian system, forming new cavities (Fig.2-1-15-a). These cavities are characteristically cylindrical, with the osteoclast at the front, and as bone is resorbed the cavity deepens due to the position of the osteoclast. Osteoblasts differentiate on peripheral surfaces of the cavity wall, and add further layers through osteogenesis, progressively narrowing the vascular channels (Fig. 2-1-15-b). This is how a new Haversian system forms, replacing the old, and restructuring the bone (Fig. 2-1-15-c). The remnants of old Haversian systems can be seen in the form of interstitial lamellae.

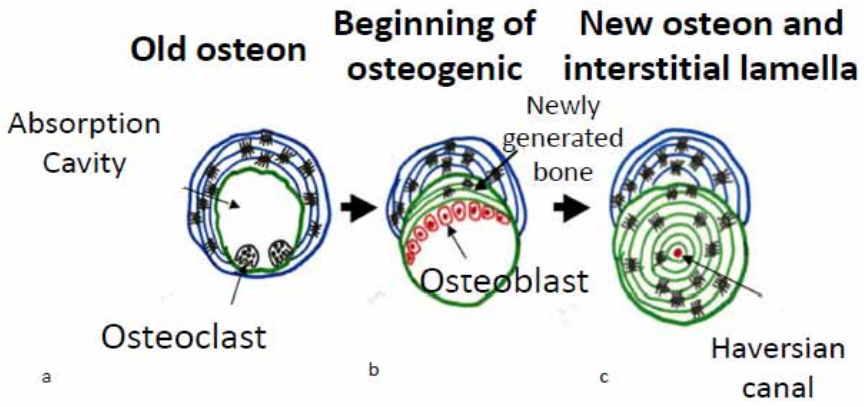

Fig.2-1-15-a,b,c

The reconstruction of the Haversian system by the remodeling of the bone

a. Old osteon

   The osteoclast appears in the Haversian system, and the bone resorption occurs independently of the structure of the Haversian system and forming new cavity.

b. The start of the osteogenesis

   The osteogenesis is initiated by the differentiation of the osteoblast on the surface of the resorption pit, lamella is added, narrowing down the internal circumference.

c. The new osteon and the interstitial lamella

   The new Haversian system is generated, and the bone reconstructed.

   (Taken from "The science of new bones").

The remodeling of the skeleton of a grown man occurs in the following order: activation, resorption, reversal, formation and quiescence; it is however thought that the majority of cells on the surface (approximately 80%) usually exist in a quiescent state (Fig. 2-1-16).

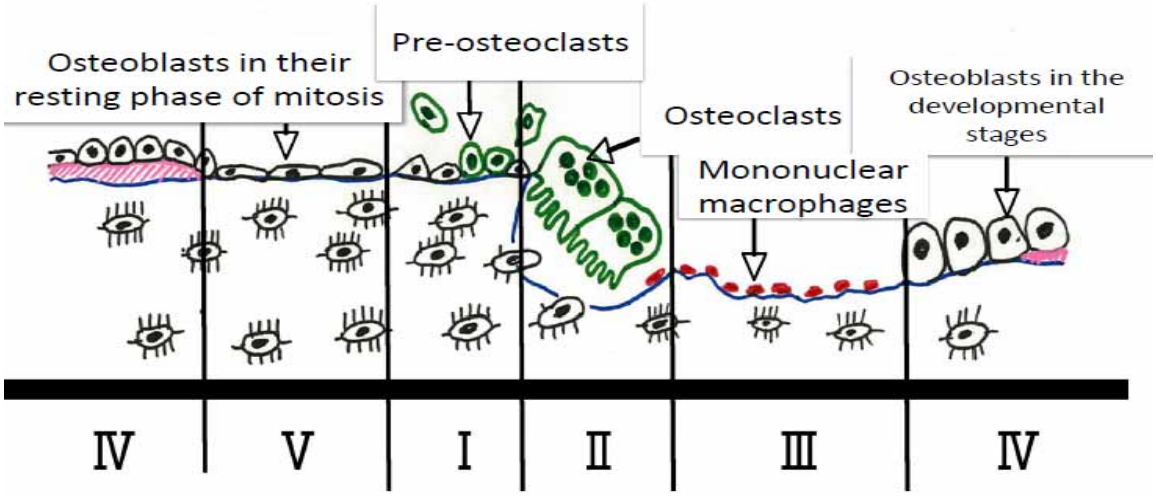

Fig.2-1-16

The remodeling cycle

The process of the osteogenesis occurs in the order of the activation phase (I), the resorption phase (II), the reversal phase (III), the formation phase (IV) and quiescent phase (V).

(Taken from "Cell and Tissue Biology")

The activation phase (I) begins when an osteoclast recognizes and adheres to the surface of the bone matrix. In this phase, the resting osteoblasts surround the surface of the bone matrix, calcification reaches the bone surface, and osteoid can barely be seen. The bone surface becomes exposed in the activation phase by degradation of the sparse collagen fibers that lie beneath a layer of quiescence osteoblasts, as MMP-13 (matrix metalloproteinase 13) is secreted by these osteoblasts. The exposure of the surface caused by removal of the degraded fibers promotes the adhesion of pre-osteoclasts to the bone matrix surface.

In the resorption phase (II), once the pre-osteoclasts adhere to the bone surface, several pre-osteoclasts fuse to form multinucleated osteoclasts that actively resorb bone.
In the reversal phase (III), upon termination of resorption, the osteoclasts retreat, and become replaced by mononuclear macrophages that absorb remaining components such as the matrix. Prior to a process called coupling, the transition between the reversal and the formation phases, the mesenchymal stem cells that occupy the pit differentiate into osteoblasts and proliferate.
In the formation phase (IV), the osteoblasts secrete bone matrix and form osteoid. The osteoid becomes calcified after a lag of few days, and this process progresses with hydroxyapatite deposition. The interface between the old and the newly formed bone is separated by the cement line. A small fraction of the osteoblasts becomes integrated into the bone matrix, while the majority remains on the surface as flat, quiescent osteoblasts.
The quiescent phase (V) describes a reduction in the bone-forming activity of osteoblasts. A flat, quiescent layer of osteoblasts forms, with bone formation ceasing as calcification spreads to the surface of the bone.

B. Differentiation of the osteoclast

It has been proved that the osteoclasts originate from hematopoietic cells of the monocyte-macrophage cell line. In studies that used myeloma cell cultures, active Vitamin D3 and PTH (parathyroid hormone) promoted the formation of osteoclast-like cells; calcitonin inhibits the formation process that is promoted by Vitamin D_3, and prostaglandin E2 does not promote formation of osteoclast-like cells. The multinucleated cells derived in this study had numerous calcitonin receptors, showed strong tartrate-resistant acid phosphatase activity, and had a very distinct ruffled border that formed resorption pits in the underlying bone, confirmatory morphologic characteristics of osteoclasts.
Such osteoclast-like cells were found to occupy regions adjacent to alkaline phosphatase-positive cells; it was therefore concluded that direct contact between osteoblasts and mesenchymal stem cells was essential for the formation of osteoclast-like cells; these osteoblasts are thought to act as feeder cells by producing inducers of osteoclast differentiation.
The inducing factors for osteoclast differentiation were identified by the following means. In a strain of osteopetrotic mice with a mutation in the MSF-gene (op/op mouse), the observed lack of osteoclasts in their bony tissues was hypothesized to be due to an abnormality in the process of osteoclast differentiation. When M-CSF was administered to op/op mice, generation of osteoclasts was induced, with subsequent formation of a bone marrow cavity. This indicates that M-CSF plays a role in osteoclast differentiation.
In addition, from previous observations that confirmed the importance for osteoclast differentiation of direct interaction between the osteoblast and mesenchymal stem cells, it was hypothesized that an osteoclast differentiation factor (ODF) exists. An inhibitor of osteoclast differentiation, osteoprotegerin (OPG), was then discovered - this completely inhibited osteoclast differentiation, and was also an effective inhibitor of physiological bone resorption.
Next a ligand for OPG called RANKL was identified, which acted as “ODF”. The specific interaction between OPG and RANKL, inhibition of binding of RANKL to its receptor, RANK, resulted in inhibition of osteoclast differentiation. Therefore, it is now believed that the RANKL - RANK interaction has an essential role in the physiological differentiation of osteoclasts.

Resorption of bone tissue by osteoclasts is conducted by secretion of acids and lysosomal enzymes into a space sealed by an isotropic band. The factors that control osteoclast functions were found to include Type II carbonic anhydrase, for acid production; and vacuolar ATPase, for acid secretion. Degradation of proteins in the bone matrix is mediated by cathepsin K, which digests collagen fibers. Activation of osteoclasts is induced by their adherence to bony tissue, and it is thought that integrins play an important role in both recognition and subsequent adherence. Current research in this area aims to uncover the intracellular signal transduction pathways, and the whole picture is becoming clearer.

C. Differentiation of the osteoblast

The osteoblast becomes flattened and the number of organelles becomes reduced once it has passed its activation phase. The majority of these cells in direct contact with the calcified bone surface have reduced ability to synthesize bone matrix. The extracellular matrix protein, osteopontin, localizes in some cellular borders. The quiescent cells secrete MMP-1 that digests and removes the collagen fibers in the space beneath the bone surface, and also plays a role in conditioning the surface microenvironment of the bone, preceding osteoclast induction and adhesion. The critical importance of these cells in the differentiation and activation of osteoclasts has thus become clear. Further, it is now known that osteoblasts in the resting phase are reactivated by various stimuli, and are converted into an activated form that readily synthesizes bone matrix.

Osteoblasts and osteoclasts are in contact with each other through projections. It has been suggested that their functions are synchronized and linked by gap junctions that play essential roles in intercellular signal conduction from osteoblasts, and in bone formation. The adhesive components that mediate the interaction between osteoblasts and bone matrix are thought to be collagen, fibronectin in the matrix, and integrinß-1 on the osteoblast. These types of cell-to-matrix adhesion have been indicated to be essential for the signal transduction pathways, and the signal transduction mechanisms that are transmitted through the cytoskeleton are essential for osteoblast differentiation and functional expression. These findings have led to further clarification of cellular communication, broadening our understanding of the signal transduction pathways.

The osteoblast, which plays a crucial role in the formation of bone, is derived from a common precursor of several cells including chondrocytes, myoblasts and adipocytes. Osteoblast differentiation was thought to be mediated by bone morphogenic proteins (BMP) and Wnt signals. However, recent findings have indicated involvement of a transcription factor, Runx2, and that this is the critical factor required for the differentiation of osteoblasts. Furthermore, another transcription factor, osterix (Osx), has been identified that induces both osteoblast differentiation and osteocalcin expression. Extensive investigations into this process have found that osteoblasts express genes for type I collagen, alkaline phosphatase and osteocalcin. It has also been found that expression of these genes differs, depending on various phases of differentiation that have been identified. Regarding the genes that control osteoblast differentiation, there are however still unknown factors that are required for us to establish a complete picture.

References

1) Fujita T, Fujita H. Standard histology Generalities, Igaku-Shoin Ltd. (in Japanese)
2) Ohno T, Kurosawa M, Takahashi K, Hosoya Y. Tortra Principles of anatomy and physiology. Maruzen Co., Ltd. (in Japanese)
3) Cell and Tissue Biology, A Textbook of Histology. Leon Weiss U&S
4) Suda T, Kurosawa M, Takahashi E, Tanaka S, Nakamura H, Mori S. Bone biology. Ishiyaku Pub, Inc. (in Japanese)
5) Noda M. Biology of the bone. Yodosha Co., Ltd. (in Japanese)