CHAPTER TWO

LITERATURE REVIEW

Macroscopic structure of the bone

Bone shape

Bone is a specialized connective tissue. Structurally, it is either spongy (cancellous) or compact. Both spongy and compact bone can be found in almost every bone. Cancellous, or trabecular bone, accommodates either hematopoietic or fatty marrow. It is normally found in most of the axial skeleton and in the ends of the long bones. Hard, compact cortical bone can be found in the shafts of the long bones. Individual bones are classified according to their shape as long, short, flat, or irregular (figure 1). Long bones are longer than they are wide. Most of the bones of the upper and the lower limbs are long bones. Short bones are about as broad as they are long. They are almost cube-shaped or round and are represented by the bones of the carpals and tarsals. Flat bones have a relatively thin, flattened shape and are normally curved. Examples of flat bones are certain skull bone, the ribs, the sternum, and the shoulder blades (scapulae). Irregular bones, such as the vertebrae and facial bones, have shapes that do not fit readily into the other three categories (Seeley et al, 2003).

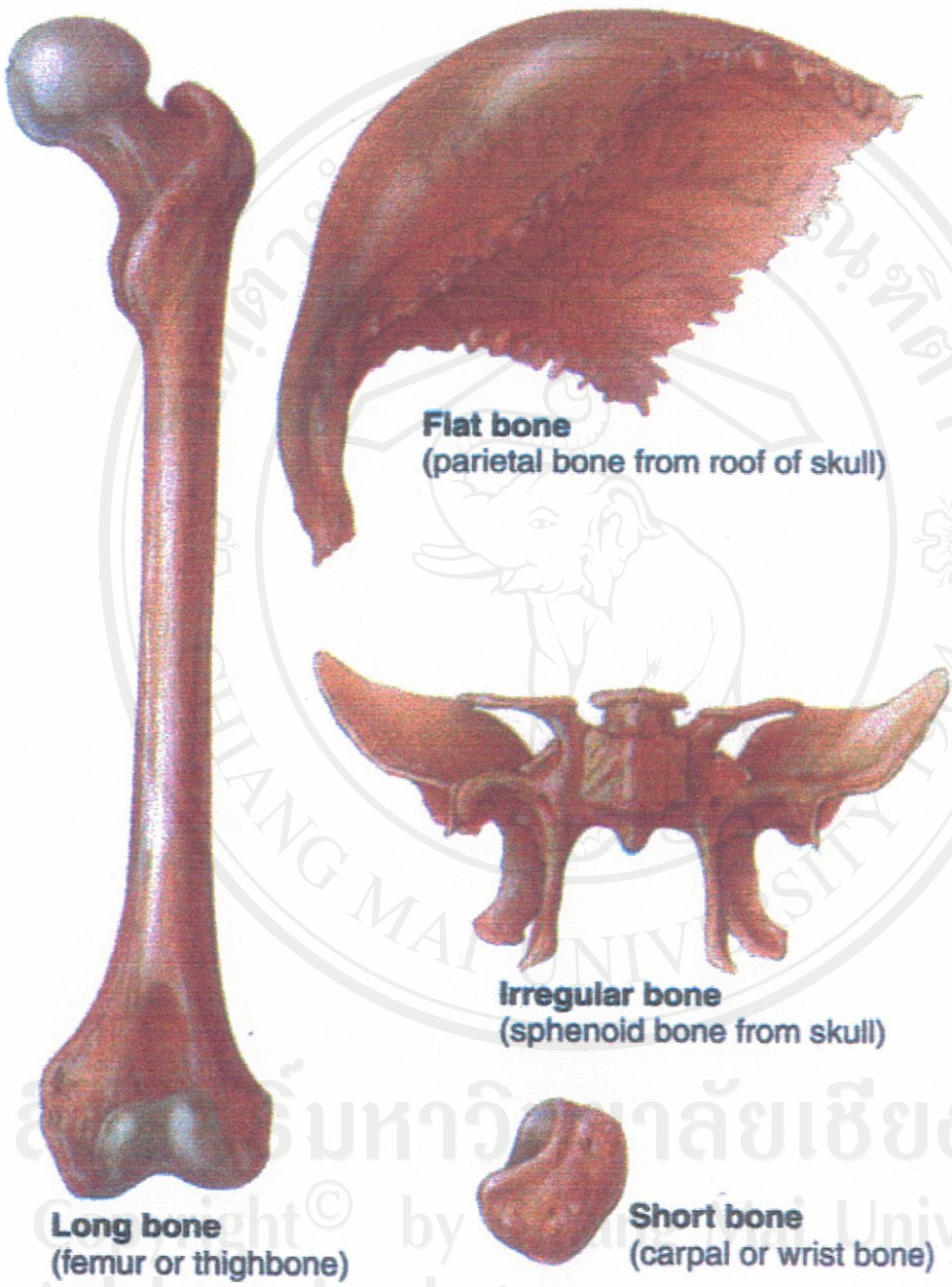

Figure 1 Bone Shape (Seeley et al, 2003)

Long bone structure

Bones of the appendicular skeleton are regularly long, cylindrical structures with narrow, predominantly cortical midportions. The expanded ends of long bones decrease the stresses that act on the articular surfaces by distributing loads over a larger cross sectional area (Markel, 1996a).

Each growing long bone has three major parts; a diaphysis, an epiphysis, and an epiphyseal plate (figure 2a and table 1). The diaphysis, or shaft, is composed mostly of compact bone, which is mostly bone matrix with a few small spaces. The epiphysis, or end of the bone, consists predominantly of cancellous, or spongy, bone, which is mainly small spaces or cavities surrounded by bone matrix. The outer surface of the epiphysis is a layer of compact bone, and within joints the epiphyses are protected by articular cartilage. The epiphyseal, or growth, plate is hyaline cartilage establish between the epiphysis and diaphysis. Growth in bone length occurs at the epiphyseal plate, but, when bone stops growing in length, the epiphyseal plate becomes ossified and is called the epiphyseal line (figure 2b) (Seeley et al, 2003). As an animal matures, the physis is obliterated and the entired expanded end of the bone is represented by the metaphysis, which is composed of trabecular (cancellous or spongy) bone surrounded by cortical and dense subchondral bone. The diaphysis is a hollow tube of cortical bone with a central cavity that contains the major arterial blood supply to the bone and fatty marrow. A portion of this marrow contains hematopoietic elements, but most of the hematopoiesis in the body takes place in the cancellous bone of the metaphyses and in the bones of the axial skeleton (Markel , 1996a).

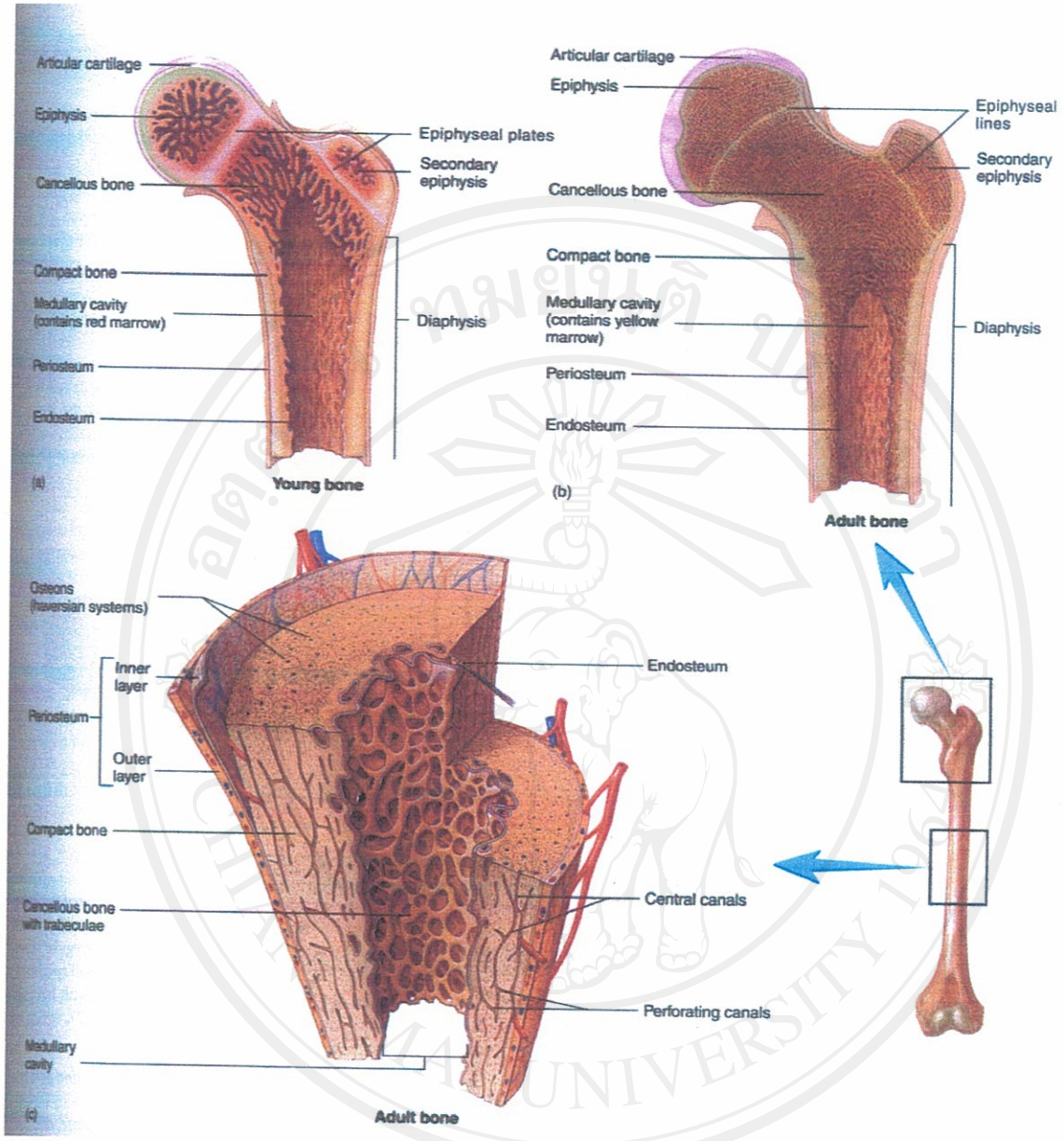

Figure 2 Long Bone

(a) Young long bone (the femur) showing epiphysis, epiphyseal plates, and diaphysis. *(b)* Adult long bone with epiphyseal lines. *(c)* Internal features of a portion of the diaphysis *(a)*. (Seeley et al, 2003)

Table 1 Gross Anatomy of a Long Bone(Seeley et al, 2003)

Part	Description	Part	Description
Diaphysis	Shaft of the bone	Epiphyseal plate	Area of hyaline cartilage between the diaphysis and epiphysis; cartilage growth followed by endochondral ossification results in bone growth in length
Epiphyses	Ends of the bone		
Periosteum	Double-layered connective tissue membrane covering the outer surface of bone except where articular cartilage exists; ligaments and tendons attach to bone through the periosteum; blood vessels and nerves from the periosteum supply the bone; the periosteum is the site of bone growth in diameter	Cancellous (spongy) bone	Bone having many small spaces; found mainly in the epiphysis; arranged into trabeculae
		Compact bone	Dense bone with few internal spaces organized into osteons; forms the diaphysis and covers the cancellous bone of the epiphyses
Endosteum	Thin connective tissue membrane lining the inner cavities of bone	Medullary cavity	Large cavity within the diaphysis
Articular cartilage	Thin layer of hyaline cartilage covering a bone where it forms a joint (articulation) with another bone	Red marrow	Connective tissue in the spaces of cancellous bone or in the medullary cavity; the site of blood cell production
		Yellow marrow	Fat stored within the medullary cavity or in the spaces of cancellous bone

In addition to the small spaces within cancellous bone and compact bone, the diaphysis of a long bone can have a large space called the medullary cavity. The cavities of cancellous bone and the medullary cavity are filled with marrow. Red marrow is the site of blood cell formation, and yellow marrow is mostly adipose tissue. In young animals, yellow marrow replaces the red marrow in their skull and limbs. In mature animals, the bones of the skull and limbs, except for the proximal epiphyses, have yellow marrow. The rest of the skeleton contains red marrow.

The periosteum is a connective tissue membrane that covers the outer surface of a bone (except in the joint) (figure 2c). The outer fibrous layer is dense, irregular collagenous connective tissue that contains blood vessels and nerves. It has the potential to form bone during growth periods and in fracture healing. The inner layer is a single layer of bone cells, which includes osteoblasts, osteoclasts, and osteochondral progenitor cells. Where tendons and ligaments attach to bone, the collagen fibers of the tendon or

ligament become continuous with those of the periosteum. In addition, some of the collagen fibers of the tendons or ligaments penetrate the periosteum into the outer part of the bone. These bundles of collagen fibers are called perforating, or Sharpey's fibers, and they strengthen the attachment of the tendons or ligaments to the bone (Seeley et al, 2003; Carola et al, 1990).

The endosteum is a connective tissue membrane that lines the internal surfaces of all cavities within bones, such as the medullary cavity of the diaphysis and the smaller cavities in cancellous and compact bone (see figure 2). The endosteum is a single layer of cells, which includes osteoblasts, osteoclasts, and osteochondral progenitor cells (Seeley et al, 2003).

Microscopic bone structure

Microscopic examination of bone shows 2 varieties; primary, immature, or woven bone and secondary, mature, or lamellar bone. Primary bone is the first bone tissue to appear in embryonic development and in fracture repair and other repair processes. It is characterized by random position of the fine collagen fiber, in contrast to the organized lamellar disposition of collagen in secondary bone (Junqueira and Carneiro, 2003).

Immature Bone

Bone tissue initially formed in the skeleton of a developing fetus called immature bone. It differs from mature bone in some respects (figure 3). Immature bone does not exhibit an organized lamellated appearance. On the basis of its collagen fiber arrangement, such bone is designated nonlamellar. Nonlamellar bone is also referred to as bundle or woven bone because of the interlacing arrangement of the collagen fibers. Immature bone contains relatively more cells per unit area than does mature bone. The

cells in immature bone tend to be randomly arranged, whereas cells in mature bone tend to be arranged with their long axes in the same direction as the lamellae. The matrix of immature bone has more ground substance than does the matrix of mature bone. The matrix in immature bone stains more intensely with hematoxylin, whereas the matrix of mature bone stains more intensely with eosin (Ross et al, 2003).

Primary bone tissue is usually temporary and is replaced in adults by secondary bone tissue except in a very few places in the body e.g. near the sutures of the flat bones of the skull, in tooth sockets, and in the insertions of some tendons. In embryonic development and in the repair of fractures, woven bone is the first bone to appear and is gradually replaced by lamellar bone as healing or growth progresses (Markel , 1996a).

In addition to the irregular array of collagen fibers, other feature of primary bone tissue are a lower mineral content (it is more easily penetrated by x-rays) and a higher proportion of osteocytes than that in secondary bone tissue (Junqueira and Carneiro, 2003).

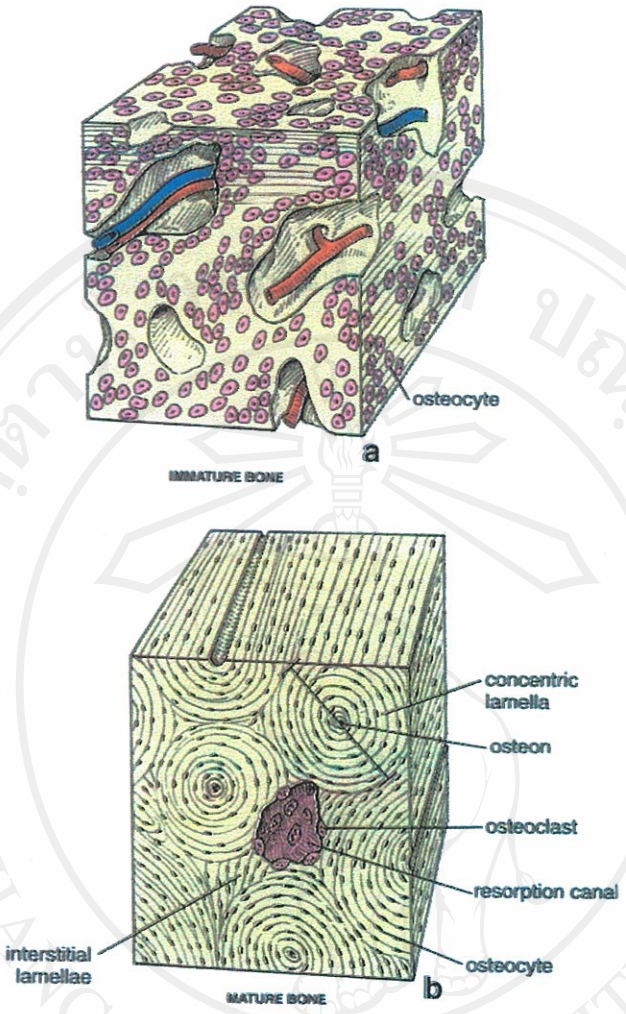

Figure 3

Diagram of immature and mature bone. Immature bone does not display an organized lamellar appearance because of the interlacing arrangement of the collagen fibers. The cells tend to be randomly arranged, whereas the cells in mature bone are organized in a circular fashion that reflects the lamellar structure of the Haversian system. Resorption canals in mature bone have their long axes in the same direction as the Haversian canals (Ross et al, 2003).

Mature bone

Mature bone is composed of structural units called osteons or haversian systems (figure 4). The osteons consist of concentric lamellae of bone matrix, surrounding a central canal, the osteonal (Haversian) canal, which contains the vascular and nerve supply of the osteon. Canaliculi containing the processes of osteocytes are regulary arranged in a radial pattern with respect to the canal. The system of canaliculi that opens to the osteonal canal also serves for the passage of substances between the osteocytes and blood vessels. Between the osteons are remnants of previous concentric lamellae, called interstitial lamellae (figure 4). Because of this arrangement, mature bone is also called lamellar bone (Ross et al., 2003).

In compact bone (e.g. the diaphysis of long bones), the lamellae show a regular organization consisting of haversian systems, outer circumferential lamellae, inner circumferential lamellae, and interstitial lamellae.

Inner circumferential lamellae are located around the marrow cavity, and outer circumferential lamellae are located immediately beneath the periosteum. There are more outer than inner lamellae.

Between the two circumferential systems are numerous haversian systems, including triangular or irregularly shaped groups of parallel lamellae called interstitial (or intermediate) lamellae. These structures are lamellae left by haversian systems destroyed during growth and remodeling of bone (Junqueira and Carneiro, 2003).

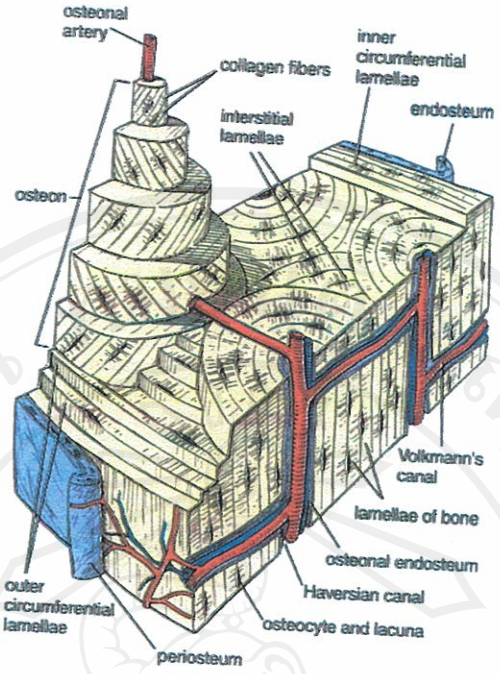

Figure 4

Diagram of a section of compact bone removed from the shaft of a long bone. The concentric lamellae and the Haversian canal that they surround constitute an osteon (Haversian system). One of the Haversian systems in this diagram is drawn as an elongated cylindrical structure rising above the plane of the bone section. It consists of several concentric lamellae that have been partially removed to show the perpendicular orientation of collagen fibers in adjacent layers. Interstitial lamellae result from bone remodeling and formation of new Haversian systems. The inner and outer surfaces of the compact bone in this diagram show additional lamellae—the outer and inner circumferential lamella is covered by a thin layer of endosteum that faces the marrow cavity, similar to the outer surface of the bone, which is covered by periosteum. Branches of nutritional arteries accompanied by small veins are shown within the Haversian and Volkmann's canals. These arteries also supply the periosteum, endosteum, and bone marrow (Ross et al., 2003).

The long axis of an osteon is generally parallel to the long axis of the bone. The collagen fibers in the concentric lamellae in an osteon are laid down parallel to one another in any given lamella but in different directions in adjacent lamellae. This organization exhibits the cut surface of lamellar bone, the appearance of plywood and imparts great strength to the osteon.

Lamellar bone is also found at sites other than the osteon. Circumferential lamellae follow the entire inner and outer circumferences of the shaft of a long bone, appearing much like the growth rings of a tree (see Figure 4). Perforating canals (Volkmann's canals) are channels in lamellar bone through which blood vessels and nerves travel from the periosteal surfaces to reach the osteonal canal; they also connect osteonal canals to one another. They normally run at approximately right angles to the long axis of the osteons and of the bone (Figure 4). Volkmann's canals are not surrounded by concentric lamellae, a key feature in their histologic identification (Ross et al, 2003).

Bone formation

Bone can be formed in 2 ways; by direct mineralization of matrix secreted by osteoblast (intramembranous ossification) or by deposition of bone matrix on a preexisting cartilage matrix (endochondral ossification). In both processes, the bone tissue that appears first is primary, or woven bone. Primary bone is a temporary tissue and is soon replaced by the complete lamellar, or secondary, bone. During bone growth, areas of primary bone, areas of resorption, and areas of secondary bone appear side by side. This combination of bone synthesis and removal (remodeling) occurs not only in

growing bones but also throughout adult life, although its rate of change in adults is remarkably slower (Junqueira and Carneiro, 2003).

Intramembranous ossification

In intramembranous ossification, bone is formed by differentiation of mesenchymal cells into osteoblasts. The first evidence of intramembranous ossification takes place around the eight week of gestation in humans. Some of the pale-staining, elongate mesenchymal cells within the mesenchyme migrate and aggregate in specific areas, the regions where bone is destined to form. This condensation of cells within the mesenchymal tissue is the membrane referred to intramembranous ossification. As the process continues, the newly organized tissue at the presumptive bone site becomes more vascularized, and the aggregated mesenchymal cells become larger and rounded. The cytoplasm of these cells changes from eosinophilic to basophilic, and a clear Golgi area becomes obvious. These cytology changes result in the differentiated osteoblast, which then secretes the collagen and other components of the bone matrix (osteoid). The osteoblasts within the bone matrix become increasingly separated from one another as the matrix is produced, but they remain attached by thin cytoplasmic processes. Because of the abundant collagen content, the bone matrix appears denser than the surrounding mesenchyme, in which the intercellular spaces exhibit only delicate connective tissue fibers (Ross et al, 2003).

In the mesenchymal condensation layer, the starting point for ossification is called a primary ossification center. The process begins when groups of cells differentiate into osteoblasts. Osteoblasts produce bone matrix and calcification follows, resulting in the encapsulation of some osteoblasts, which then become ostecytes (figure 5). These islands

of developing bone form walls that delineate elongated cavities containing capillaries, bone marrow cells, and undifferentiated cells. Several such groups arise almost simultaneously at the ossification center, so that the fusion of the walls gives the bone a spongy structure. The connective tissue that remains among the bone walls is penetrated by growing blood vessels and additional undifferentiated mesenchymal cells, giving rise to the bone marrow cells.

The ossification centers of a bone grow radially and definitively fuse together, replacing the original connective tissue. The frontanelles of newborn infants, for example, are soft areas in the skull that correspond to parts of the connective tissue that are not yet ossified.

In cranial flat bones there is a marked predominance of bone formation over bone resorption at both the internal and external surfaces. Thus, 2 layers of compact bone (internal and external plates) arise, whereas the central portion (diploë) maintains its spongy nature.

The portion of connective tissue layer that does not undergo ossification give rise to the endosteum and the periosteum of intramembranous bone (Junqueira and Carneiro, 2003).

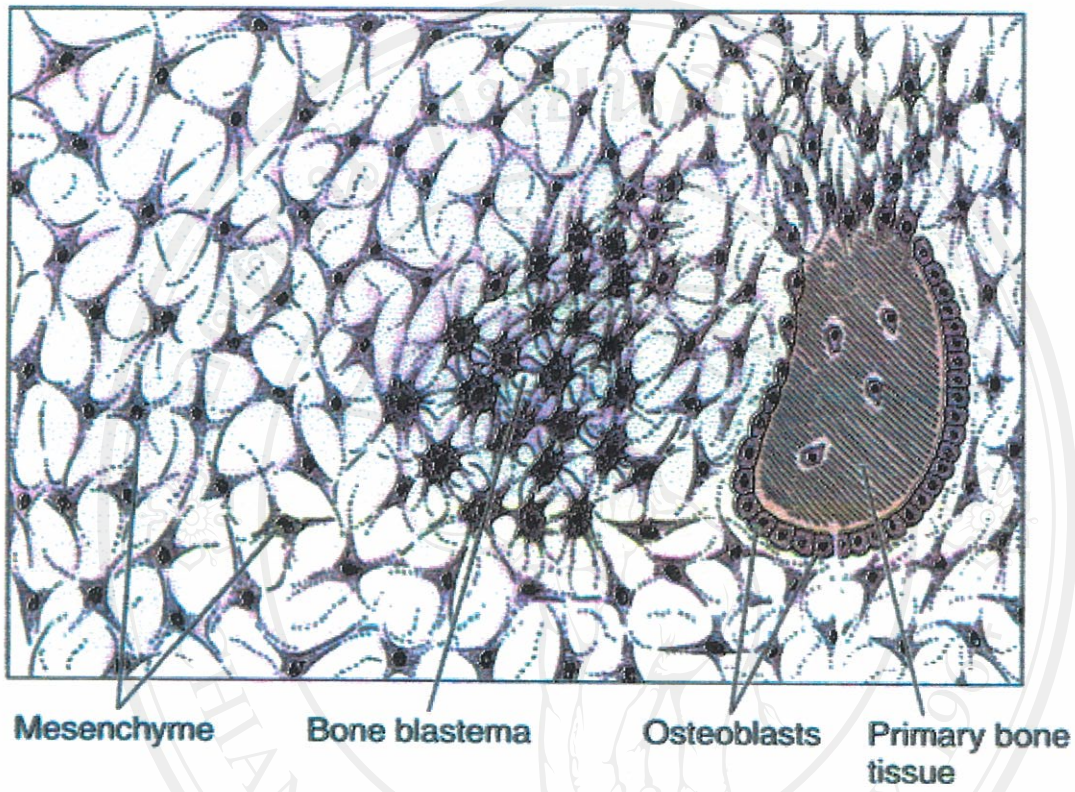

Figure 5 The beginning of intramembranous ossification.

Mesenchymal cells round up and form a blastema, from which osteoblasts differentiate, producing primary bone tissue (Junqueira and Carneiro, 2003).

Endochondral ossification

Endochondral ossification also begins with the proliferation and aggregation of mesenchymal cells at the site of the future bone. However, the mesenchymal cells differentiate into chondroblasts that, in turn, produce cartilage matrix (Ross et al, 2003). The chondroblasts produce matrix, transforming the model into hyaline cartilage, and a peripheral perichondrium develops. Interstitial and appositional growth of the cartilage ensue. Lengthening of the model is mainly the result of continuing chondrocyte division with further matrix production by daughter cells. Widening is primarily due to the peripheral addition of matrix by new chondroblasts differentiating from the perichondrium.

In the midregion of the model, chondrocytes enlarge (hypertrophy) and mature, and hydroxyapatite becomes deposited in the matrix partitions between their lacunae. Following this stage, the chondrocytes die, probably as a consequence of activation of their apoptotic pathway. Many of the large lacunae become vacant, and the thin partitions between them begin to break down. At this stage, numerous capillaries vascularize the perichondrium. Differentiation of the osteoprogenitor cell progeny then occurs in a vascular environment. The surrounding membrane becomes a periosteum, producing osteoblasts that lay down a thin shell of bone matrix around the midregion. A strong collar of subperiosteal bone gradually builds up under the periosteum around the weakened midsection of the model (Cormack , 2001). Next, osteoblasts adhere to the calcified cartilage matrix and produce continuous layers of primary bone that surround the cartilaginous matrix remnants. At this stage, the calcified cartilage appears basophilic, and the primary bone is eosinophilic. In this way the primary ossification

center is produced. Then, secondary ossification centers appear at the swellings in the extremities of the cartilage model (epiphyses). During their expansion and remodeling, the primary and secondary ossification centers produce cavities that are continuously filled with bone marrow.

In the secondary ossification centers, cartilage persists in 2 regions: the articular cartilage, which remains throughout adult life and does not contribute to bone growth in length, and the epiphyseal cartilage, also called epiphyseal plate, which connects the two epiphyses to the diaphysis. The epiphyseal cartilage is responsible for the growth in length of the bone, and it obliterates in adults, which is why bone growth ceases in adulthood (Junqueira and Carneiro, 2003).

Bone cells

Bones contain five types of cells that are capable of changing their roles as the needs of the body change in the growing and adult skeletons (Carola et al,1990).

1. Osteoprogenitor cells

The osteoprogenitor cell is a resting cell that can convert into an osteoblast and secrete bone matrix. Osteoprogenitor cells are found on the external and internal surfaces of bones. They comprise the periosteal cells that form the intermost layer of the periosteum and the endosteal cells that line the marrow cavities, the osteonal (Haversian) canals, and the perforating (Volkmann's) canals. Osteoprogenitor cells can divide and proliferate. In growing bones, osteoprogenitor cells appear as flattened cells with lightly staining, elongate or ovoid nuclei and inconspicuous acidophilic or slightly basophilic cytoplasm. Electron micrographs exhibit profiles of rough endoplasmic reticulum (rER) and free ribosomes as well as a small golgi apparatus and other organelles. The

morphology of the osteoprogenitor cell is consistent with the finding that its stimulation leads to differentiation into a more active secretory cell, the osteoblast (Ross et al., 2003).

2. Osteoblasts

Osteoblasts are responsible for the synthesis of the organic components of bone matrix (type I collagen, proteoglycans, and glycoproteins). They can also secrete copious growth factors such as bone morphogenetic proteins (BMPs), transforming growth factor beta (TGF-β), colony stimulating factor 1, granulocyte colony-stimulating factor, basic fibroblast growth factor (bFGF), and insulin-like growth factor (IGF) (Garant, 2003). Deposition of the inorganic components of bone also depends on the presence of viable osteoblasts. Osteoblasts are exclusively located at the surfaces of bone tissue, side by side, in a way that resembles simple epithelium (figure 6). When they are actively engaged in matrix synthesis, osteoblasts have a cuboidal to columnar shape and basophilic cytoplasm. When their synthesizing activity declines, they flatten, and cytoplasmic basophilia declines.

Some osteoblasts are gradually surrounded by newly formed matrix and become osteocytes. During this process a space called a lacuna is formed. Lacunae are occupied by osteocytes and their extensions, along with a small amount of extracellular noncalcified matrix.

During matrix synthesis, osteoblasts have the ultrastructure of cells actively synthesizing proteins for export. Osteoblasts are polarized cells. Matrix components are secreted at the cell surface, which is in contact with older bone matrix, producing a layer of new (but not yet calcified) matrix, called osteoid, between the osteoblast layer and the previously formed bone (figure 6). This process, bone apposition, is completed by

subsequent deposition of calcium salts into the newly formed matrix (Junqueira and Carneiro, 2003).

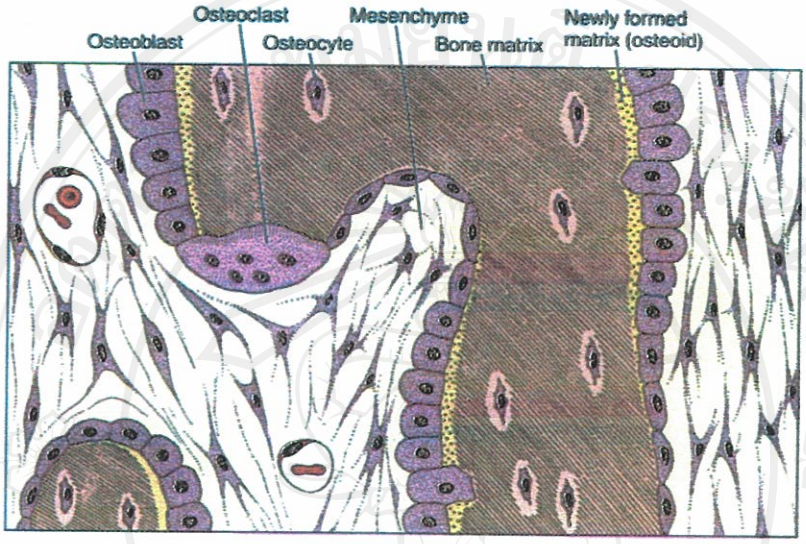

Figure 6 Events that occur during intramembranous ossification.

Osteoblasts are synthesizing collagen, which forms a strand of matrix that traps cells. As this occurs, the osteoblasts gradually differentiate to become osteocytes. The lower part of the drawing shows an osteoblast being trapped in newly formed bone matrix (Junqueira and Carneiro, 2003).

3. Osteocytes

Osteocytes are less basophilic and somewhat smaller than osteoblasts. Their numerous interconnecting cytoplasmic processes are generally indistinguishable in H&E-stained sections, but the canaliculi they create are obvious in ground bone sections. Osteocyte lacunae normally retain a thin, unmineralized lining layer of osteoid tissue.

The osteocytes within them are nondividing, so cell nests such as those seen in cartilage are not found in bone. Most osteocytes have a minimal amount of rER and a relatively small Golgi apparatus and are believed to maintain the bone matrix in good repair. They represent the final stage of maturation of the bone cell lineage (Cormack , 2001).

Osteocytes play an active role in homeostasis by helping to release calcium from bone tissue into the blood, thereby regulating the concentration of calcium in the body fluids. Osteocytes also appear to keep the matrix in a stable and healthy state by secreting enzymes and maintaining its mineral content (Carola et al, 1990).

4. Osteoclasts

Osteoclasts are very large, branched motile cells. Dilated portions of the cell body contain from 5 to 50 (or more) nuclei. In areas of bone undergoing resorption, osteoclasts lie within enzymatically etched depressions in the matrix known as Howship's lacunae. Osteoclasts are derived from the fusion of bone marrow-derived cells.

In active osteoclasts, the surface-facing bone matrix is folded into irregular, often subdivided projections, forming a ruffled border. Surrounding the ruffled border is a cytoplasmic zone—the clear zone—that is devoid of organelles, yet rich in actin filaments. This zone is a site of adhesion of the osteoclast to the bone matrix and creates a microenvironment in which bone resorption occurs.

The osteoclast secretes collagenase and other enymes and pumps protons into a subcellular pocket (the microenvironment referred to above), promoting the localized digestion of collagen and dissolving calcium salt crystals. Osteoclast activity is controlled by cytokines (small signaling proteins that act as local mediators) and

hormones. Osteoclasts have receptors for calcitonin, a thyroid hormone, but not for parathyroid hormone.

However, osteoblasts have receptors for parathyroid hormone and, when stimulate by this hormone, produce a cytokine called osteoclast stimulating factor (Junqueira and Carneiro, 2003).

5. Bone-lining cells

Bone-lining cells are found on the surface of most bones in the adult skeleton. These cells are believed to be originated from osteoblasts that discontinue their physiologic activity and flatten out on the bone surface. These cells may have numerous functions. They may serve as osteogenic cells that can divide and differentiate into osteoblasts. Most probably, they serve as an ion barrier around bone tissue. This barrier contributes to mineral homeostasis by controlling the movement of calcium and phosphate into and out of the bone matrix, which in turn helps control the deposition of hydroxyapatite in the bone tissue (Carola et al, 1990).

Healing process of the bone

Fracture repair follows the principles that govern embryonic and fetal development of the skeleton. Bone has the unique ability to heal completely after a fracture, thereby returning to its original tissue structure and associated mechanical properties. The mechanisms behind such a distinguished response involve bone growth, modeling and remodeling. Fracture healing is a specialized form of wound repair in which there is regeneration of the injured tissue without scar formation. Skin, muscle, and tendon tissues are unable to fully regenerate after injury but, rather, heal with permanent scar tissue. Both local and systemic factors influence fracture healing.

Systemic factors include age and nutritional status of the patient, hormone levels, functional activity, and nerve function. Local factors include degree of trauma, presence of vascular injury, type of bone affected, degree of bone loss, degree of immobilization, presence of infection, local pathologic conditions and biological growth factors (Skerry , 1998 and Markel, 1996b).

Fracture healing can be considered a series of processes that occur in sequence but are often overlapping. The healing process can be divided into at least three distinct phases: inflammation, reparation, and remodeling. Bone reacts to fracture within a few hours by uniform periosteal cell activity. This initial cellular reaction, a basic response of bone to any injury, is called the primary callus response.

Inflammatory Phase

The inflammatory phase is the most important prerequisite for the reparative phase of fracture healing, similar to that in soft tissue wounds, and normally occurs over the first 2 to 3 weeks after injury. The initial stage of fracture repair is characterized by hematoma formation and inflammatory exudates from ruptured blood vessels of the bone marrow and cortex, the periosteum, and the surrounding tissue. During the inflammatory phase, the cellular mechanisms necessary for repair and the processes protecting the healing tissue from infection are activated. In brief, injury is translated to cells by waves of chemical messengers, such as kinins, complement factors, histamine, serotonin, prostaglandins, and leukotriens. The coagulation cascade contributes fibrin and fibrinopeptides. Together, these elements mediate the inflammatory reaction by causing vasodilation, migration of leukocytes, and chemotaxis. Platelets also contribute growth factors, which initiates angiogenesis and mesenchymal cell proliferation. On reaching

the injured tissue, granulocytes ingest and destroy bacteria but do not contribute to repair. Macrophages and, to a lesser extent, lymphocytes aid in the destruction of bacteria and also stimulate repair by releasing angiogenic factors and other cell growth factors (Markel , 1996b ; Brand and Rubin , 1987)

Reparative Phase

The reparative phase overlaps and follows the inflammatory phase. During the reparative stage, the pattern of fracture healing is highly susceptible to mechanical factors, predominantly the amount of interfragmentary motion. The reparative phase can take 2 to 12 months to be completed. The natural histologic course of fracture healing (without immobilization) begins with interfragmentary stabilization through periosteal and endosteal callus formation. This process restores continuity, and bone union occurs by intramembranous and endochondral ossification(Markel , 1996b).

As there is overlap of the inflammatory and reparative phases, so there is overlap of the reparative and remodeling stages. The arbitrary end of the reparative phase is that point at which the fracture is healed enough to allow normal function but not necessarily to the extent of excessive activity such as athletics. However, the fracture will continue to stengthen during the final remodeling phase of healing (Brand and Rubin , 1987).

Remodeling Phase

Remodeling phase occurs during and following the reparative phase. Avascular and necrotic areas of bone are replaced by haversian remodeling. Malalignment of fragments may be collected to a certain extent by remodeling of the fracture site and by functional adaptation, particularly in young animals with remaining bone growth potential. On loading, convex surfaces carry a positive charge and attract osteoclasts,

whereas concave surfaces carry a negative charge and attract osteoblasts. Therefore, bone is removed from convex surfaces and laid down on concave surfaces. This process tends to realign the bone after malunion. Rotational deformities are affected very little by the remodeling process (Markel , 1996b).

Radiographically, the callus diminishes in size as the cortex reforms and regains its structural integrity. Angular deformities may slowly decrease or obliterate as bone is laid down on the concave surface and removed from the convex surface (Brand and Rubin , 1987).

Platelet-Rich Plasma and Growth Factors Involved in bone regeneration

Platelet-Rich Plsma(PRP)

The focal points of wound –healing knowledge are the identification, understanding, and now the ways to use growth factors to promote wound healing. The use of platelet-rich plasma (PRP), is one strategy available today that can regulate and promote wound healing. The processing of PRP primarily involves the sequestration and concentration of platelets and , therefore, the multiple growth factors they contain. The oversimplified strategy is to increase and accelerate the effects of growth factors contained in platelets, which are the universal initiators of almost all wound healing (Marx et al, 1999).

Soft tissue healing is also gradually improved through the use of PRP, by increasing collagen content, enhancing angiogenesis and increasing early wound strength. The growth factors found in PRP regulate key cellular processes, such as mitogenesis, chemotaxis, and cell differentiation and metabolism(Tischler, 2002).

Clinically, PRP was used in many areas of medicine especially in orthopedics, oral and maxillofacial surgery, and implantology. Marx et al. (1998) had shown that the addition of PRP to bone grafts evidenced a radiographic maturation rate 1.62 to 2.16 times that of grafts without PRP. As assessed by histomorphometry, there was also a greater bone density in grafts in which PRP was added. Anitua (1999) reported that reinforcing growth factor concentration through the application of PRP in the wound improved soft tissue repair and bone regeneration (Park et al, 1995). Kassolis et al (2000) suggested that ridge augmentation and sinus grafting with freeze-dried bone allograft in combination with PRP provide a viable therapeutic alternative for dental implant placement. The addition of PRP to the surgical sites of experimental animals appeared to enhance bone healing considerably(Fennis et al, 2001).

However the cases reported from Shanaman et al (2001) showed that the addition of PRP did not appear to enhance the quality or quantity of new bone formation over that reported in comparable guided bone regeneration (GBR) studies without PRP.

Recently, a study from Zechner et al (2001) has shown that when used dental implants with PRP, the histomorphometrical evaluation showed a significant higher percentage of bone-implant contact during the first 6 weeks. Okazaki et al (2001) has also shown that sinus floor augmentation using a combination of β-Tricalciumphosphate (β-TCP) and PRP cause a more rapid resorption of β-TCP in a maxillary sinus. PRP would have a potential to enhance bone formation and improve the healing of the graft in a maxillary sinus. Robiony et al.(2002) reported that the combination of osteogenesis distraction and PRP seemed to be effective in restoring the severe atrophic mandible.

Platelet rich plasma (PRP) has been investigated as an abundant source of PDGF and TGF-β. With autologous blood, PRP obtained by sequestering and concentrating platelets by gradient density centrifugation. This technique produced a concentration of human platelets of 338% and identified PDGF and TGF-β within them (Marx et al,1998).

Besides aggregation function, platelets produce and release multiple growth and differentiation factors that play the important role in stimulation and regulation the wound healing (Wartiovaara et al,1998). These factors are platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), and vascular endothelial growth factor (VEGF). The two important growth factors which are involved in wound healing both soft and hard tissues are PDGF and TGF-β.

Platelet-Derived Growth Factor (PDGF)

PDGF was first found in platelet. It is stored in the alpha granules (Antoniades, 1981), it can also be found in other cells such as macrophages (Rappolee et al, 1998), endothelial cells (Sitaras et al, 1987), monocytes and fibroblasts (Antoniades et al, 1991), as well as in bone matrix (Hauschka et al, 1986). PDGF is a polypeptide with 30,000 Daltons molecular weight, it remains stable under heat up to 100 ^{o}C and has a cationic nature. It has a dimetric structure formed by 2 amino acid chains named A and B (Antoniades et al, 1991).

Nash and coworkers (1994) and Vikjaer (1997) showed that the application of exogenous PDGF enhance osteogenic differentiation and bone repair in fracture models as well as critical size of calvaria defects. PDGF also enhances the periodontal repair and regeneration (Park et al, 1995). In an animal model study, it is shown that PDGF promote the periodontal regeneration without significant ankylosis or root resorption (Lynch et

al1991). Howell et al. (1997) showed that the application of PDGF resulted in a significant promotion in bone regeneration. PDGF is a polypeptide growth factor considered to have a role in the proliferation and migration of fibroblasts at a wound healing site. Wang et al (1994) showed that PDGF enhanced fibroblast proliferation when compared to the groups without PDGF.

Transforming growth factor-β (TGF-β)

TGF-β was first isolated from transformed tissues (sarcomas) (Burgers,1989). There are 2 types: alpha and beta. TGF-β has molecular weight of 25,000 Daltons, formed by two 12,500 Daltons subunits linked together by disulphur bridges (Assoian et al,1983;Gentrella et al,1986). This factor has 3 different structures TGF-β1, TGF-β2, and TGF-β3. The β1 is found abundantly in platelets, lymphocytes, and neutrophils, while β2 is found mainly in bone extracts, platelets, lymphocytes, and neutrophils. Type β1 and β2 are 72% similar. Type β3 is a heterodimer formed of a single chain of TGF-β1 and a single chain of TGF-β2. These factors favor bone formation by enlarging the rate of stem cell proliferation. Another suggested role is the inhibition of osteoclast formation and these bone formation (Anitua,1999).

Fibronectin (FN)

Fibronectin is a large multidomain glycoprotein found in connective tissue, on cell surfaces, and in plasma and other body fluids. It plays a major role in wound healing and is associated with the attachment of cells to other cells and to extracellular matrix (Caffesse and Quinones, 1993). It interacts with a variety of macromolecules including components of the cytoskeleton and the extracellular matrix, circulating components of

the blood clotting, fibrinolytic, acute phase and complement systems, and with cell-surface receptors on a variety of cells including fibroblasts, neurons, phagocytes and bacteria.

Recently, a study from Wijelath et al (2004) has shown that combinations of vascular endothelial growth factor(VEGF) and fibronectin(FN) may be useful in promoting differentiation of circulating endothelial progenitors into endothelial cells for tissue engineering.

Fibroblast Growth Factors (FGFs)

FGFs have been originally identified as an activity in extracts of pituitary and brain that stimulated the growth of 3T3 cells. They are potent mitogens and chemoattractants for endothelial cells as well as for a variety of mesenchymal cells, including fibroblasts, osteoblasts, chondrocytes, smooth muscle cells and skeletal myoblasts. These factors have also been shown to stimulate the formation of new blood vessels in vivo. It has also shown that basic FGF stimulates proliferation and differentiated function of chondrocytes in vitro and promote cartilage repair in vivo. The activity is caused by two proteins, acidic and basic FGF. Both factors were initially isolated from neural tissue but have been subsequently found in numerous other tissues.(Caffesse and Quinones, 1993 ; Mohan and Baylink, 1991)

Insulin-like growth factors (IGFs)

IGFs are family of single-chain serum proteins that share 49% homology in sequence with proinsulin (Caffesse and Quinones, 1993). IGF-I and IGF-II are usually thought of as growth factors secreted by osteoblasts during bone formation to increase

numbers of osteoblasts and thereby accelerate bone deposition. IGFs are also deposited in bone matrix; when the bone matrix is resorbed, IGFs are released to couple new bone formation to bone resorption (Marx, 1999). IGF-I and IGF-II have 62% homology witheach other. They are synthesized by multiple tissues, including liver, smooth muscle and placenta, and are carried in plasma as complex with specific binding protein. IGF-I and IGF-II are similar to the somatomedins C and B, respectively (Caffesse and Quinones, 1993). IGFs stimulate osteogenic cell proliferation and increase the synthesis of collagen, alkaline phosphatase, osteocalcin, and integrins in osteogenic cells. They have also been shown to increase osteoclastic activity in vitro. Osteogenic cells mediate the osteoclast-stimulating effect of IGF. Because of this dual action, IGFs are thougt to be regulators of bone remodeling (Garant, 2003).

Epidermal growth factor (EGF)

EGF is a single-chain, 53 amino acid protein with a broad spectrum of activity. The major sources of EGF are urine and salivary glands, although it has also been isolated from Brunner's glands and platelets as well as from cerebrospinal and amniotic fluids (Caffesse and Quinones, 1993). EGF, like all growth factors, binds to specific high-affinity, low-capacity receptors on the surface of responsive cells. Intrinsic to the EGF receptor is tyrosine kinase activity, which is activated in response to EGF binding. The kinase domain of the EGF receptor phosphorylates the EGF receptor itself (autophosphorylation) as well as other proteins, in signal transduction cascades, that associate with the receptor following activation. EGF has proliferative effects on cells of both mesodermal and ectodermal origin, particularly keratinocytes and fibroblasts (King, 2004). EGF, however, stimulates prostaglandin production and induces bone resorption

in cultures of neonatal mouse calvaria. Investigation using different animal models have reported that the topical application of EGF to abraded corneas, partial-thickness wound, full-thickness wounds and superficial burns significantly enhances re-epithelialization and wound healing (Caffesse and Quinones, 1993).

Bone morphogenetic proteins (BMPs)

In 1965, Urist observed heterotopic bone formation after implantation of demineralized bone matrix at intramuscular sites in mice, rats, guinea pigs, and rabbits. The sequence of events that he recorded mirrored those of endochondral ossification and fracture healing. Undifferentiated mesenchymal cells migrate to the implantation site and proliferate. Chondroblasts, derived from the mesenchymal cells, secrete extracellular matrix components and form a cartilaginous template. At 10 to 14 days, the cartilage hypertrophies, and extracellular matrix is vascularized by hematopoietic and endothelial cells. Osteoblasts and osteoclasts also appear locally, and the cartilage is resorbed and replaced by bone. By 21 days, an ossicle of bone, complete with marrow, has been formed (Kirker-Head, 1996). The term bone morphoenetic protein was used to describe the substances in the demineralized bone matrix responsible for inducing this phenomenon (Kirker-Head et al, 1995).

Bone morphogenetic proteins (BMPs) are a subset of the transforming growth factor beta (TGF-β) superfamily of dimeric, disulfide crosslinked growth and differentiation factors (Wozney, 2001).

The foregoing studies were haunted by the possibility that, in a relatively crude extract of bone matrix proteins, certain co-factors may be present which are required for BMP's osteoinductivity. Absolutely pure BMP extracts are difficult, but not impossible,

to obtain. This question was answered with the cloning purification of human BMPs in sufficient quantity and purity to provide amino acid sequence data, cDNAs were isolated, cloned and expressed in host cells. To date, seven potentially bone morphogenetic proteins have been generated in this fashion, and four have shown bone morphogenetic activity in animals: BMP-2 (BMP2a), BMP-4 (BMP-2b), BMP-5, and BMP-7 (OP-1). Currently, there are two recombinantly-produced bone morphogenetic proteins nearing FDA approval in the US: recombinant human bone morphogenetic protein-2 (rhBMP-2) and recombinant human osteogenic protein-1 (rhOP-1). The latter is a trade name for rhBMP-7. Both have been reported to induced healing of bone defects in animal models, and are in various stages of human trials (Wolfe et al, 2001).