THE EFFECT OF AUTOGENOUS NONVASCULARISED PERIOSTEUM TRANSPLANTATION ON BONE HEALING -AN ANIMAL STUDY

UNIVERSITI SAINS MALAYSIA

BY

DR. AZUHAIRY AZID
M.D (USM)

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ABSTRAK

Tujuan kajian ini dijalankan adalah untuk menyelidik kesan keatas pemindahan alogeraf kepada ruang kosong pada tulang tibia subjek arnab apabila membran periosteum yang tiada bekalan darah menyelaputi permukaannya. Ini adalah kajian keatas model haiwan yang pertama dijalankan di PUSAT PENGAJIAN SAINS PERUBATAN, UNIVERSITI SAINS MALAYSIA, KUBANG KERIAN KELANTAN.

Sebanyak 12 ekor arnab dewasa jantan digunakan dalam kajian ini. 9 ekor dikumpulkan di dalam 3 kumpulan (A,B,C) and sebanyak 3 ekor lagi di jadikan model kawalan. 3 cm panjang daripada bahagian tengah tulang tibia sebelah kanan telah dikeluarkan dan kekosongan itu digantikan dengan tulang alogeraf tanpa tisu lembut yang sama panjang dan seterusnya distabilkan dengan dawai bersaiz 2 mm didalam rongga sum-sum. Kaki tersebut diperkukuhkan dengan pemakaian Plaster of Paris.

Didalam kumpulan A, B dan C tulang alogeraf tersebut dibaluti dengan membrane periosteum yang di keluarkan dari tulang tibia sebelah kiri arnab-arnab tersebut. Manakala arnab-arnab didalam kumpulan D tulang alogeraf tidak dibaluti dengan membrane periosteum.

Diakhir minggu yang ke 2, 4 dan 6, arnab daripada setiap kumpulan dijalankan pemeriksaan X ray. Diikuti dengan pemeriksaan C.T skan dengan penggunaan bahan

kontras. Pemeriksaan- pemeriksaan tersebut dijalankan selepas subjek-subjek dibius penuh.

Hasil kajian mendapati, arnab-arnab yang menerima membran periosteum yang menyelimuti tulang alogeraf, penyembuhan tulang berlaku pada kedua-dua tempat penyambungan tulang alogeraf dan tulang asli pada akhir minggu yang ke 4 dan ke 6. Diakhir minggu yang ke 2 kedua-dua bahagian penyambungan tulang alogeraf dan asli masih belum bersatu, tetapi terdapat sedikit pembentukkan kalus dibahagian tersebut. Walaubagaimana pun di akhir minggu yang ke 6, disamping terdapat proses penyatuan tulang yang baik, terdapat pembentukkan kalus yang banyak menyelaputi tulang alogeraf tersebut. Di sebalik nya bagi kumpulan kawalan, tulang alogeraf tanpa membrane periosteum, di dapati tiada pembentukkan kalus menyelaputi tulang alogeraf tersebut. Penyatuan tulang tidak berlaku pada bahagian penyambungan tulang alogeraf dan tulang asli di akhir minggu yang ke 2 dan 4. Di akhir minggu yang ke 6 tulang alogeraf menjadi pecah kepada beberapa bahagian dan teleskop ke dalam rongga sum-sum tulang yang asli.

Kesimpulannya, mendapati membran periosteum yang tidak mendapat bekalan darah membantu proses penyembuhan tulang alogeraf dan juga bertindak mengekalkan keteguhan tulang alogeraf.

ABSTRACT.

The purpose of this study is to evaluate the effect of Fresh Frozen allograft on alarge-sized tibia defect in a rabbit model when a non vascularised periosteum sleeve is wrapped around it. This is an animal experiment, a pilot study and descriptive in nature carried out in MEDICAL SCHOOL. UNIVERSITI SAINS MALAYSIA, KUBANG KERIAN KELANTAN.

A total of 12 matured male rabbits were used. Nine(9) rabbits were divided into three groups (A,B,C) and three (3) other rabbits were used as control group (group D). A 3 cm segment of the (R) tibia shaft was removed and the defect was then replaced with bare allograft and stabilized with Kirschner wire (K wire) size 2 mm. The leg was immobilised with Plaster of Paris.

In the groups A,B and C the allograft segments were wrapped circumferentially by the free non-vascularised periosteal flap that was harvested from the (L) tibia. The rabbit model in group D had allograft transplantation only without the incoorperation of free autogenous nonvascularised periosteal transplantation.

At the end of 2^{nd}, 4^{th} and 6^{th} weeks the rabbits from each of these groups were X rayed. The plain C.T scan of the (R) Tibia were taken and later with infusion of I/V radio opaque dye. These procedures were being done under general anaesthesia.

The results observed in the rabbits where there were periosteum used to wrap around the entire segment of allografts, there were union at both ends of the allografts seen at the end of 4^{th} and 6^{th} weeks. At the end of 2^{nd} week both ends of the allograft were still seen not united, but some callus was seen to form at the fracture ends. However at the end of 6^{th} weeks, beside a good union that was observed at the host-graft junctions, there were solid callus seen encasing the whole allograft segments. But in a control group where the allograft segments were left bare without periosteum transplant, there were no callus form surrounding the allograft segments. Union at the both ends of the allograft segment have not occurred at the end of 2^{nd}, 4^{th} weeks and at the end of 6^{th} week the allograft was seen to fragment and telescope into the medullary cavity of the host.

In conclusion, the free non – vascularised periosteum transplantation modified the healing of allograft and maintained the intergrity of the allograft.

INTRODUCTION

The periosteum is a specialized connective tissue that forms fibro-vascular membrane covering the entire surface of bone except the articular cartilage, ligament at site of tendon insertions and the surface of sesamoid bones. It consists of two histological layers; an outer fibrous layer containing blood vessels and nerves supplying the bone and the inner cambium layer adjacent to the bone in which the cambium (osteogenic) cells reside.

The cambium cells are osteogenic progenitor cells of mesenchymal origin. In children the cambium layer contributes to the increase of the diameter of bone during growth. In adults, the cambium cells may be activated after mechanical stimulus, trauma, infection and by some tumourous growth. Under these circumstances, this layer is capable of inducing callus tissue formation and osteogenesis (Timothy et al 2003). This ability for the cambium cells to differentiate into chondrogenic or osteogenic cells has stimulated interest in their uses.

There are many technical precautions that should be observed during periosteal harvesting or transplantation. The surgical technique of harvesting the periosteum is critical to the success of transplantation.The cambium layer which contain the osteogenic cells and is delicately adhere to the fibrous layer and can be damaged or left behind on the bone when periosteum is harvested. Shawn and James (2000).

In Orthopaedic practice a large segmental bone loss or defect is a common problem encountered and several surgical methods are available to bridge such defects. A large segmental bone allograft has been used and become an accepted method of replacing any bone defects. But there are many complications which may arise when performing this procedure, such as non union, delayed union, fracture or resolution of allografts. In an attempt to improve host-graft union, cancellous bone grafts or autogenous vascularised periosteum overlying allografts are widely used to augment the fixation of large allografts in reconstructive surgery.(Keith et al. 1991).

However the effect of periosteal grafts transplanted onto a large segmental autografts or allografts bone are not well discussed. There are no available published articles on the autogenous free nonvascularised periosteum to augment the healing of allografts. This study was designed to observe the effect of the free autogenous nonvascularised periosteum transplantation on the segmental allografts.

2.0 LITERATURE REVIEW

2.1 INTRODUCTION

Periosteum is an envelope of fibrous connective tissue that wrapped around the bone in all places except at synovial joints (which are protected by cartilage). Periosteum is also absent from sesamoid bones (e.g.patella), which are formed within tendons and function to increase the mechanical advantage across a joint. This tissue is missing in dry bone. As opposed to bone itself, it has nociceptive nerve endings, making it very sensitive to manipulation.

It is composed of two layers, an outer fibrous layer, whose primary function is to distribute vascular and nerve supply to the bone, and an inner osteogenic (cambium) layer adjacent to the bone in which the cambium cells reside. The cambium cells are osteogenic progenitor cells of mesenchymal origin. In children the cambium layer contributes to increasing the diameter of the bone during growth. In adults, the cambium cells may be activated after mechanical stimulation, trauma, infection and by tumors. Under these circumstances, this layer is capable of inducing callus formation and osteogenesis.

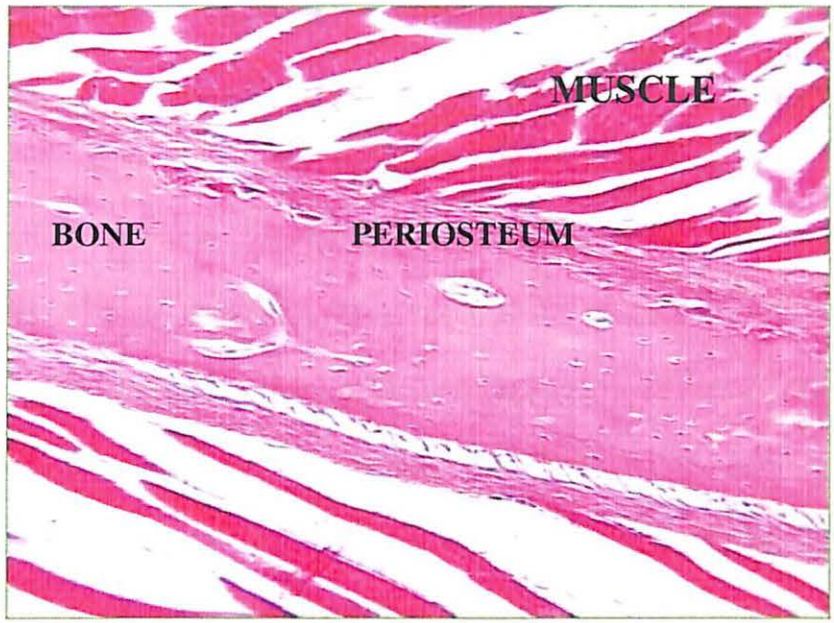

Figure 2.1 : Two layers of periosteum

The periosteum is a noncalcified, dense, irregular, collagenous connective tissue covering the bone on its external surface and inserting into it via strong collagenous fibres called Sharpey's Fibres, which extend to the outer circumferential and interstitial lamellae of bone. The periosteum contains a store of osteogenic osteoblasts, and thus plays a vital part in the healing of fractures. The site at which the fibers of tendons or ligaments blend with the periosteum is termed an enthesis. Bones require their own blood supply which travels through the periosteum to the inner bone marrow. It also provides nourishment in the form of blood supply to the bone.

The clinical experience using periosteum for biologic resurfacing of joint in humans has confirmed its feasibility from a technical standpoint. Periosteal explants of different sizes cultured in organ culture mimic the behaviour of periosteal grafts in vivo. A study

by Shawn (1999) has agreed to earlier report by Rubak et al (1982) that whole tissue explants of periosteum can survive, grow and differentiate to produce cartilaginous extracellular matrix when grown in organ culture. E Vogeline et al (2005), concluded that circumferential wrapping of the defect with a vascularised periosteal flap significantly enhanced bone formation. They speculated that the cambium cells layer of periosteum respond to osteogenic signal of Bone Morphogenic Protein (BMP) but do not recognized the osteoinductive signals generated by BMP. Its has been assumed that BMPs work by recapitulating the embryonic sequence of bone formation by enchondral ossification. However evidences are accumulating that suggest BMPs are also capable of inducing bone formation directly by intramembranous ossification. Their study supports this mechanism in that bone formation occurred mainly by intramembranous ossification within the BMPs constructs. However area of new bone formation by enchondral ossification could also be observed at the junction of the constructs and host bone.

Prarop Tiyapatanaputi et al (2004) described a murine segmental femoral allograft model based on the healing cortical-cortical junctions in autogenous and allogenic grafts. They removed 4 mm mid-diaphyseal segment of the femur, and then replaced with fresh bone graft from the same animal without disturbing or devitalized bone graft from the same inbred strain of mice(isograft) or graft from a different outbred strain of mice (allograft). They found that the autografts were rapidly incorporated and underwent extensive remodeling in short period of time. In contrast, incorporation of frozen allografts depended upon host repair activity and occurred only at allograft-host junctions. The rest of the allografts remained inert with minimal evidence of periosteal

bone formation or remodeling. They concluded that preservation of the periosteum or use of a periosteum tube graft significantly improves cortical bone graft incorporation and remodeling.

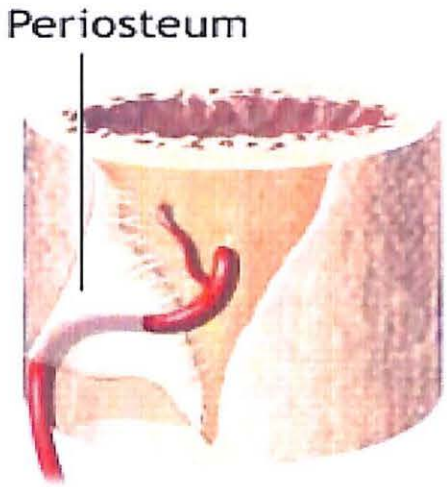

Figure 2.2 Periosteal blood supply to the long bone

2. BONE

2.1 Overview

Bone is a mineralized connective tissue, which is dynamic and well organized. It also has a remarkable compressive and tensile property due to the composition of its extracellular matrix. Bone is one of the hardest substances of the body, it is a dynamic tissue that constantly changes shape in relation to the stresses placed on it. It is the primary structural framework for support and protection of the organs of the body. Bone also serve as levers for the muscles attached to them. Bone is a reservoir for several minerals of the body, its stores about 99% of body's calcium

All of the bones in adult skeleton have two basic structural components; **compact** and **spongy** bone. The solid, dense bone that is found in the walls of bone shafts and on external bone surfaces is called compact or cortical bone. The second kind of bone has a more spongy, porous, lightweight, honeycomb structure. This bone is found where tendons are attached, in the vertebral bodies, in the ends of long bones, in short bones and within flat bones. This **cancellous,** or **trabecular** bone is named after the thin bony spicules (trabeculae) that form it. The molecular and cellular compositions of compact and trabecular bone tissue are identical; it is only difference in porosity that separates these gross anatomical bone types. There are two histological types of bone, immature and mature. Immature bone is the first kind of bone to develop in prenatal life. Its existence is usually temporary, as it is replaced with mature bone as growth continues.

Immature bone is usually formed rapidly and characterizes the embryonic skeleton, sites of fracture repair and variety of bone tumours.

Mature or lamellar bone composed of parallel or concentric lamellae, 3 – 7 μm thick. Osteocytes in their lacunae are dispersed at regular intervals berween or within lamella. Canaliculi, connect neighbouring lacunae with each other, forming a network of intercommunicating channels that facilitate the flow of nutrients, hormones and waste products to a from osteocytes.Additionally, osteocytes processes within these canaliculi contact similar processes of neighbouring osteocytes and form gap junctions, permitting these cells to communicate with each other

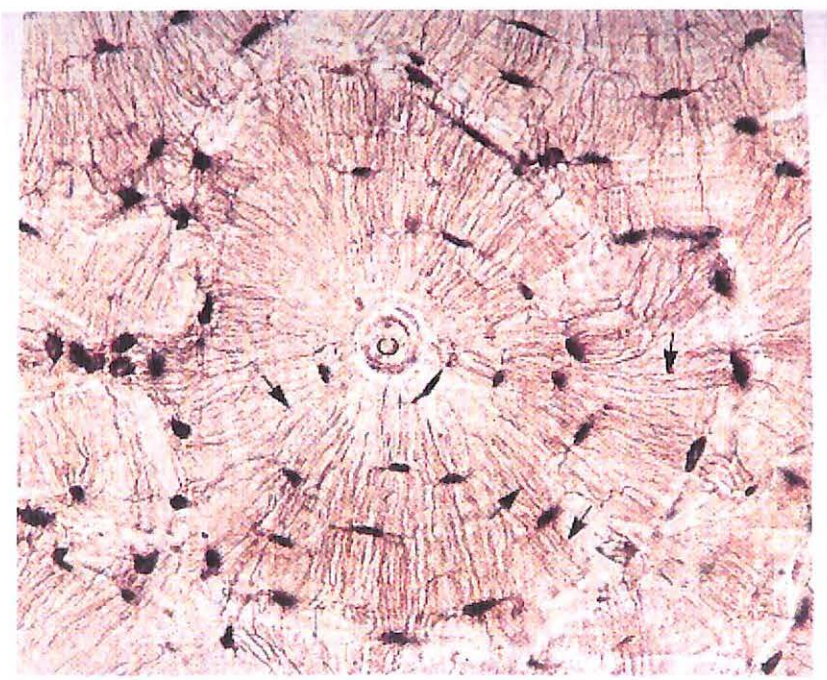

Figure 2.2 Compact bone

The bulk of compact bone is composed of an abundance of haversian canal systems (osteons), each composed of cyclinders of lamellae, concentrically arranged around a vascular space known as the **Harvesian Canal.** Each harvesian canal, lined by a layer of osteoblasts houses a neurovascular bundle with its associated connective tissue. Haversian canals of adjacent osteons are connected to each other by **Volkmann Canal.** These spaces are orientated oblique to or perpendicular to haversian canals.

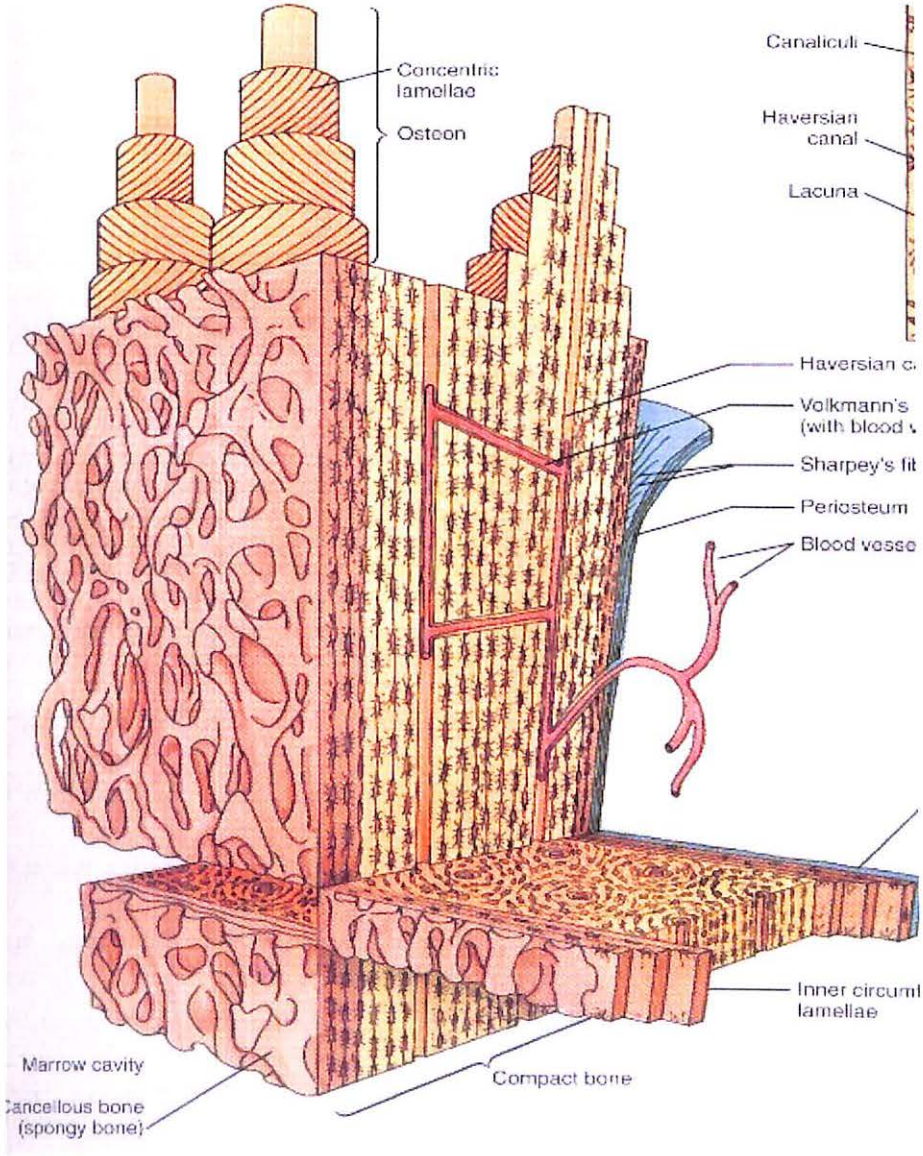

Figure 2.3 : Vascular channel system in the bone

2.2.2 Cellular Biology

Bone consists of living cells embedded in a highly vascular, mineralized matrix. These cells are essential for bone formation, bone resorption, bone repair, maintenance and mineral homeostasis. The predominant bone cells are osteoblasts, osteocytes and osteoclasts.

2.2.2.1 Osteoblasts

Osteoblasts, derived from osteoprogenitor cells, are responsible for the synthesis of the organic components of the bone matrix, including collagen, proteoglycans and glycoproteins. Osteoblast are located on the surface of the bone in a sheet-like arrangement of cuboidal to columnar cells. The organelles of osteoblasts are polarized so that the nucleus is located away from the region of secretory activity. These cells are histologically basophilic and contain abundant amounts of rough endoplasmic reticulum (for protein synthesis) golgi apparatus (for secretion), mitochondria and cytoskeleton elements. During active bone formation, osteoblasts secrete high levels of alkaline phosphatase, which elevates the levels of this enzyme in the blood. Osteoblasts have parathyroid hormone receptors on their cell membranes. When parathyroid hormone bind to these receptors, it stimulates osteoblasts to secrete osteoclast-stimulating factor, which activates osteoclasts to resorb bone. Osteoblasts also secrete enzymes responsible for removing osteid, so that the osteoclasts can contact the bone.

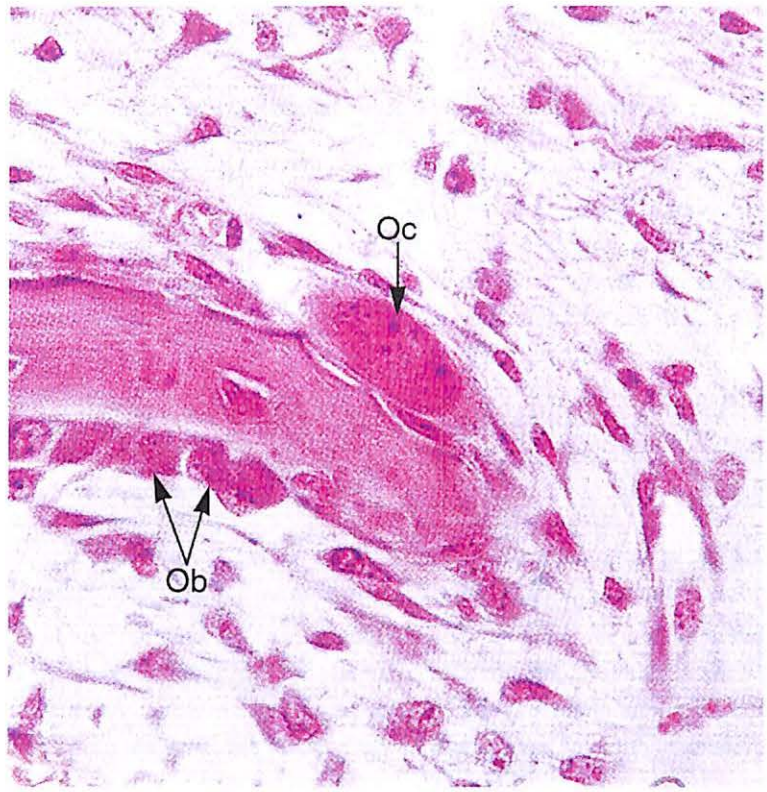

Figure 2.4: Light micrograph showing osteoblast(Ob) line the bone spicule and osteoclast(Oc) housed in How- ship lacunae.

2.2.2.2 Osteocytes

Osteocytes are mature bone cells, derived from osteoblasts, that housed in lacunae within the calcified bony matrix. They are connected through canaliculi to each other and to the surfaces of the bone. The total surface area of the canaliculi and osteocytes accounts almost 90% of the total internal and external surface area of the bone. Osteocytes conform to the shape of their lacunae. Their nucleus is flattened and their

cytoplasm is poor in organelles, displaying scant RER and greatly reduced Golgi apparatus. Though osteocytes appear to be inactive cells and secete substances necessary for bone maintenance.

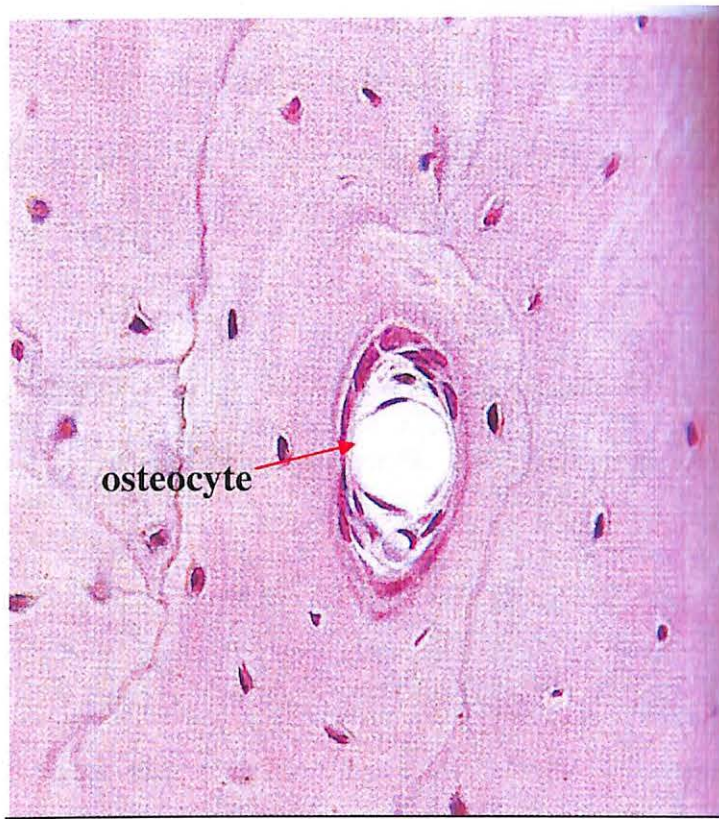

Figure 2.5 : Photomicrograph, showing the osteocyte in lacunae

2.2.2.3 Osteoclasts

Osteoclasts control bone resorption. These cells are derived from multiple macrophages that consolidate and bind to the surface of the bone. The precursor of the

osteoclasts originates in the bone marrow. Osteoclasts have receptors for osteoclast-stimulating factor and for calcitonin. Morphologically, the osteoclasts are large, motile, multinucleated cells and they contain up to 50 nuclei. Osteoclasts occupy shallow depressions called **How-ship's Lacunae**, which identify regions of bone resorption. Osteoclasts synthesise tartrate-resistant acid phosphatase and they are attached to bone surface via specialized protein called integrins. Resorption of bone occurs via production of hydrogen ions (via carbonic anhydrase) leading to steady decrease of pH from 7 to 4, which then increases the solubility of hydroxyapatite crystals and organic materials are removed by proteolytic digestion.

The bone resorbing activity of osteoclasts is regulated by two hormones; parathyroid hormone and calcitonin, produced by the parathyroid and thyroid glands, respectively.

2.2.2.4 Other Bone Cells

Osteoprogenitor cells are located in the inner cellular layer of the periosteum, lining haversian canals, and in the endosteum. These cells derived from embryonic mesenchyme and have potential of differentiating into osteoblasts. Under certain conditions of low oxygen tension, these cells may differentiate into chondrogenic cells. Osteoprogenitor cells are spindle in shaped and process a pale staining cytoplasm and

poorly developed Golgi apparatus but an abundance of free ribosomes. These cells are most are most active during the period of intense bone growth.

2.2.3 Bone Matrix

Bone matrix has inorganic and organic constituents. The inorganic component of the bone, which constitutes about 65% of its dry weight. It composed mainly of calcium and phosphorus, along with other components including bicarbonate, citrate, magnesium, sodium and potassium. Calcium and phosphorus exist primarily in the form of hydroxyapatite crystals. Calcium phosphate is also present in an amorphous form. Hydroxyapatite crystals are arranged in an orderly fashion along with the type 1 collagen fibres. They are deposited into the gap regions of the collagen and also present along the overlap region. The free surface of the crystals is surrounded by amorphous ground substances. The hardness and strength of the bones are due to the association of hydroxyapatite crystals with the collagens. If the organic components is extracted from the bone, the mineralized skeleton still retains its original shape, but it becomes extremely brittle and can be fractured with ease.

The organic components of the bone matrix, constituting approximately 35% of the dry weight of bone, includes fibres that are Type 1 collagen. Collagen most of which type 1, makes up about 90% of the organic component of the bone. Type 1 collagen in the bones is highly cross linked, preventing it from being easily extracted. Several glycoproteins are also present in the bone matrix. These appear to be restricted to bone, including osteocalcin, which binds to hydroxyapatite and osteopontin which also binds to

hydroxyapatite but has additional binding sites for other components as well as for intergrins present on osteoblasts and osteoclasts. Vitamin D stimulates the synthesis of these glycoproteins. Bone sialoprotein, another matrix protein, has binding sites for matrix components and intergrins of osteoblasts and osteocytes, suggesting its involvement in the adherence of these cells to the bone matrix.

2.2.4 Blood Supply of the Bone

Bone receives about 5-10% of the cardiac output. The long bones receive blood from three sources: 1. Nutrient artery; 2. metaphyseal- epiphyseal arterial system and 3. Periosteal system. They may anastomose among themselves.

a. The nutrient arteries originate as branches from major arteries of the systemic circulation. The nutrient arteries enter the diaphyseal cortex through the nutrient foramen to enter the medullary canal. Once in the medullary canal, the nutrient artery branches into ascending and descending small arteries. They penetrate the endosteal cortex to supply at least the inner two third of the cortex via the vessels that traverse the haversian system. The nutrient artery system is a high pressure system.

b. The metaphyseal-epiphyseal arterial system supplies chiefly the cancellous bone of the proximal and distal metaphyses and anastomoses with the medullary system

c. The periosteal blood supply derives from vessels in the periosteum, especially at the regions of fascial and tendinous attachments. These vessels penetrate and supply the

outer third or less of the cortex. Wherever the surface of the bone is covered by the articular cartilage, a periosteal supply is absent.

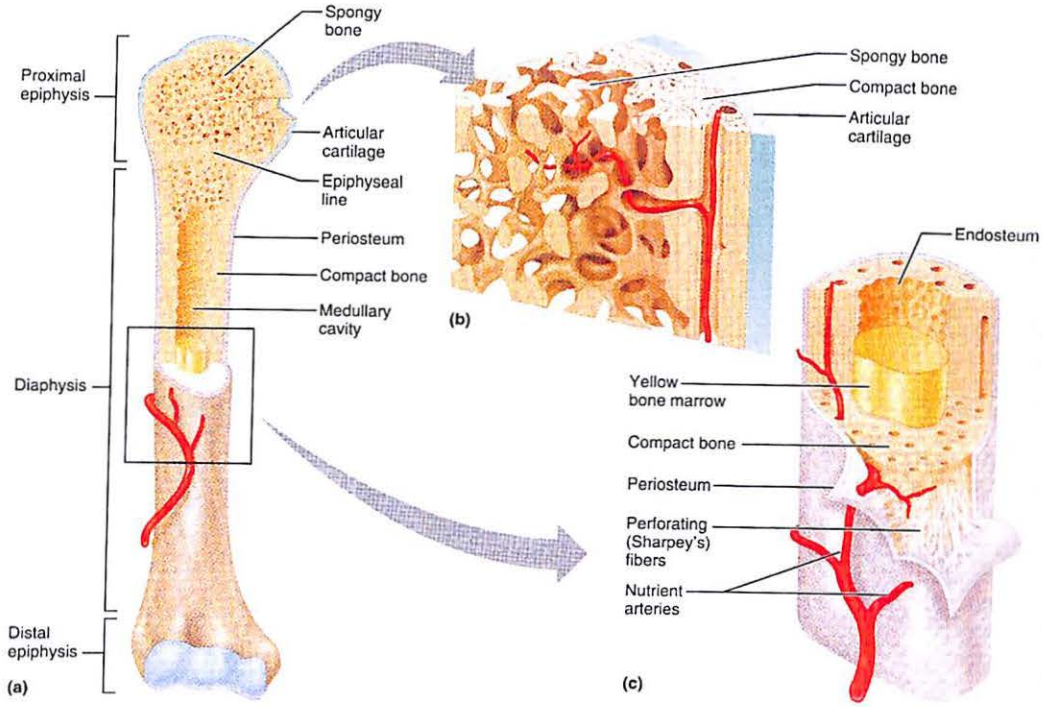

Figure 2.7: Metaphyseal and periosteal arterial blood supply to long

The principle direction of blood flow is centrifugal, from endosteum to the periosteum. The effect of fracture on the blood supply to a bone depends on the nature and severity of the fracture. In a minimal displaced fracture, the small vessels in cortical bone are disrupted, resulting in ischaemic death of the osteocytes near the fracture line. The major medullary and periosteal vessels may remain intact. With greater displacement and disruption of the medullary vascular system, the metaphyseal or periosteal vessels may play greater role in vascularisation of the callus. Internal fixation with plates and screws may cause periosteal ischaemia directly beneath the plate, but the procedure otherwise does not interfere with regional revascularization. Placement of a reamed intramedullary

nail obliterates the medullary blood supply, however shifting the source of vessel ingrowth to the metaphyseal. periosteal and soft tissue systems.

2.2.5 Fracture Healing

The original descriptions of fracture healing were based upon histological observations and suggested discrete sequential phases including haematoma formation, acute inflammation, repair, remodelling and union (Miller 1996). With the introduction of cellular and molecular biology techniques it is now realized that there is a continuum of interrelated processes involving a variety of cell types, biochemical mediators and their cellular receptors . It is anticipated that accelerated fracture healing and improved treatment of delayed and non-union will be achieved by unlocking these genetic events.

Bone, when injured, can regenerate itself. Although the processes are similar to those employed by other tissue types, the latter are only able to repair themselves by the formation of scar tissue. Bone, however, has the ability to repair itself with bone. The construct may not appear identical on a radiograph but structurally a healed fracture, once it has undergone remodelling will have returned to its pre-injury state.

The fracture healing consist of three overlapping phases; an inflammatory phase, a reparative phase, and a remodeling phase. Fracture will initiates a biological cascade, a sequence of steps activated by and depending on the previous steps.

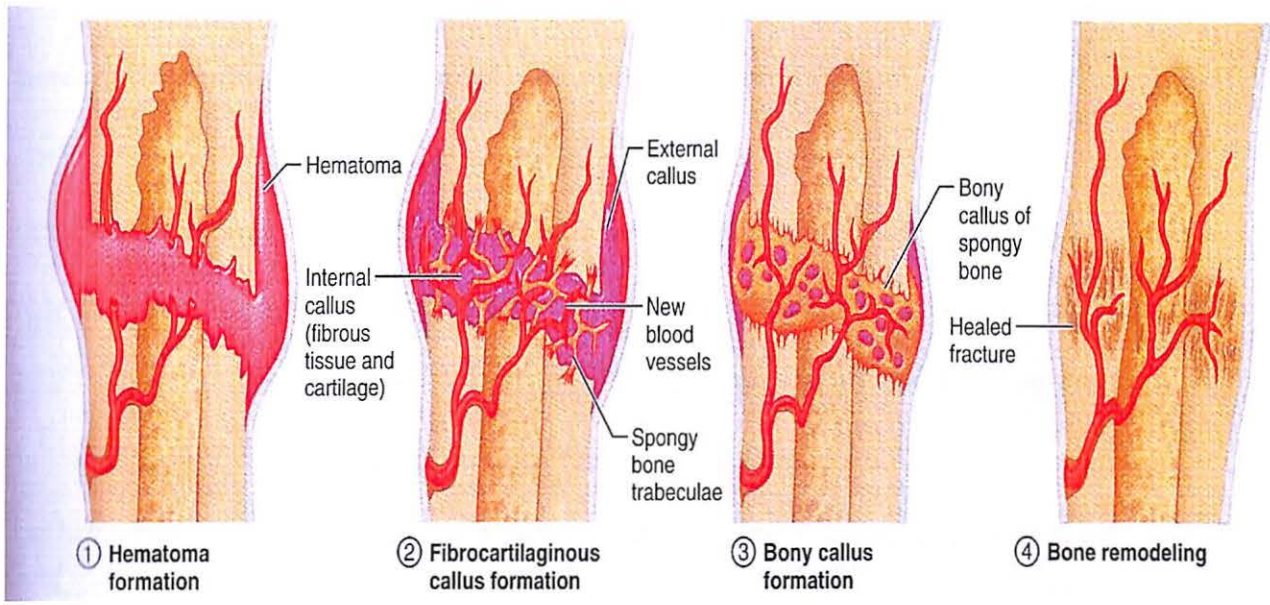

Figure 2.6 : Stages in the healing of a bone fracture

The inflammatory reaction immobilizes the fracture. Pain causes the patient to protect the injured part and swelling hydrostatically splints the fracture. At the tissue level, the inflammatory phase is identical to the typical inflammatory response of most tissues to traumatic. Vasodilatation and hyperemia, presumably mediated by histamines, prostaglandins and various cytokines accompany invasion of the injury site by neutrophils, basophils and phagocytes that participate in clearing away necrotic debris. The fracture haematoma becomes organized as a developing fibrin network that provides pathways for cells migration. It is also presumed that during the inflammatory phase, various noncollagenous protein growth factors that regulate cell migration and differentiation and that normally are trapped in the bone matrix are released into solution, where they become active. The inflammatory phase peaks within 48 hours and is quite diminished by1 week after fracture.

The reparative phase becomes activated within the first few days after fracture and persists for several months. Its chief feature is the development of a reparative callus tissue in and about the fracture site that gradually transformed into bone. The callus may consist of cartilage, fibrous tissue, osteiod, woven bone and vessels. The primary callus response is the direct response of bone to local inflammation, whether the inflammation is caused by fracture, infection, a foreign body or neoplastic process. If the primary callus is successful in connecting the fracture ends, healing progresses to the stage of bridging callus or hard callus. Calcification of callus may be by direct bone formation by osteoblasts or by endochondral ossification. Typically, growth of a large callus greatly outpaces the ingrowing vessels, and endochondral ossification predominates. The cellular components of the callus derive chiefly from the marrow and the periosteum. The number of osteoblasts and osteocytes present at the time of fracture is insufficient to sustain the high anabolic demands of the growing callus. The differentiation of pluripotential mesenchymal cells, fibroblasts and chondroblasts is primary source of callus cells. As the callus calcifies and becomes rigid the fracture becomes internally immobilized.

Once the fractures are united by bridging with cancellous bone, it is necessary to remodel the injury site by replacing the primary bone with secondary bone and resolving the callus. The first bone elaborated against injured bone develops by intramembranous bone formation and the new trabeculae become firmly cemented to the injured or dead bone. Matrices of the dead bone , located in the empty spaces between newly developing bony trabeculae are resorbed and the spaces are filled in by new bone. Eventually all of

the dead bone resorbed and replaced by new bone formed by the osteoblasts that invade the region. These events are concurrent, resulting in repair of the fracture with cancellous bone surrounded by a bone callus. Through the events of remodeling, the primary bone of intermembranous bone formation is replaced with secondary bone, further reinforcing the mended fracture zone and at tehsame time the callus is resorbed. It appears that the healing and remodeling processes at the fracture site are in direct response to the stresses placed on it. The repaired **zone** eventually restored to its original shape and strength. It is interesting that bone repair involves cartilage formation and both intermembranous and endochondral bone formation.

2.2.6 Augmentation of fracture healing

The potential of the bones for regeneration and repair is well known and documented. With the general knowledge on bone healing, we would expect most fractures to unite at a certain given time. However up to 10% will have some delay in healing and there is a need to treat these cases. There are many types of the augmentation of fracture healing and they can be classified broadly into biological, mechanical and biophysical heading.

2.2.6.1 Biological augmentation

This is the most ancient method to augment fracture healing. Modern medicine

had identified various proteins, which are potential to initiate and accelerate fracture healing. Local treatments are widely used via osteogenic, osteoconductive or osteoinductive measures. An osteogenic agents provides the necessary constituents for bone regeneration. Osteoconduction process presents as a framework that the indigenous bone healing process can build up within the skeleton. Whereas osteoinduction differs from osteoconduction by being able to support bone formation in extra skeletal sites. Prostaglandins have been shown to increase bone mass in animal models. Our knowledge of the molecular mediators of fracture healing has led to investigation of their potential role as osteoconductive agents. TGF ß accelerates wound healing and stimulates thickening of bone after local administration in rats. Rosier et al 1998 have shown increased matrix production, osteoblasts proliferation and ultimate healing strength in fractures injected with TGF ß in their animal study. There are evidences in animal models of enhanced union of fractures treated with implants containing recombinant BMPs. Einhorn TA et al have also shown a similar research progresses with PDGF and FGF. However all these treatments will require the administration of the active agents to the fracture site, either by injection or surgical implantation.

2.2.6.2 Mechanical enhancement

Stiffness is an important function of bone and it can be recreated by ensuring that each fracture is reduced, stabilized and allowed to heal in an optimal biological environment. Callus is a dynamic construct that requires some motion at the fracture site to develop. Therefore inadequate immobilization has been put forward as a cause of

delayed union. Excessive movements willl lead to a hypertrophic non-union. Bone response to the stresses acting on it and many studies have shown evidences of the effects of altering the mechanical forces on fracture healing. Goodship at el 1985 in their sheep study have shown the effect of cyclical dynamization lead to enhanced fracture healing. Kenwright et al 1991 have performed a randomized controlled trial on human tibial fractures and demonstrated that cyclic loading of 1mm for a 2 weeks period, speeded up fracture healing by 25%.

2.2.6.3 Biophysical methods

The knowledge that stress generates piezoelectric charges in bone, has led to investigation of the use of electricity in the healing of fractures. The modern application of electromagnetic stimuli to aid fracture healing arose from the discovery that dehydrated bone tissue generates an internal field when deformed by application of mechanical forces. When bone is mechanically loaded, stress generated potentials are produced by two mechanisms. Streaming potentials are produced when interstitial fluids are forced through the calcified matrix by dilatation of some regions and compression of others. Piezoelectric potentials are produced by deformation of the collagen molecules.

The physical mechanism of electric and magnetic field interaction and the biologic mechanism of transduction remain to be elusive. Recently, cellular studies had addressed the effects of electromagnetic on signal transduction pathways and growth factors. Fitzsimmons and Ryaby have postulated that magnetic fields stimulate

secretion of IGF-1 after short duration electromagnetic stimulus. Apart from improving of the fracture healing rate, many other researchers had also demonstrated that electromagnetic field had also prevented osteopenia in animal model (Brighton et al). Apart from electromagnetic stimulation, ultrasound and pulsed infrared radiation alsohave a positive effects on augmenting the fractures healing.

2. BONE GRAFTS

2.1 Overview

Bone grafting is an old surgical procedure. The first recorded bone grafting was performed in 1668. Bone grafts are used to treat various disorders, including delayed union and nonunion of fractures, congenital problems, and bone defects secondary to trauma, infection and tumors. Massive graft tissue are used in reconstructive surgery to help secure prosthetic devices and perform arthrodesis.

There are two types of bone grafts frequently used in Orthopaedic practice, the autografts and allografts. Autograft bone is transplanted from another part of the recipient's body. Allograft bone is transplanted from genetically non identical members of the same species. Both types of bone grafts are commonly used in spine, tumor and reconstructive surgery.

The ideal bone graft should have four important properties: 1) osteoinductive and osteoconductive; 2) biomechanically stable; 3) disease free; and 4) contain minimal antigenic factors. Bone grafts provide a latticework for ingrowth by host bone and supply living osteogenic cells to the host bed and growth factors induce bone formation by the host. Different from transplantation of other organs and tissues, bone transplantation does not seem to require the maintenance of viability as an indispensable condition to achieve its purpose. The bone graft or transplant is expected to be incorporated into the graft site after transplantation. Therefore, a transplant bone that does not obstruct or stimulates this incorporation is an excellent material.

Bone grafts may be cortical, cancellous or corticocancellous. Cortical bone grafts incorporate through slow remodeling of existing harversian systems via a process of