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# Bone Grafting and Bone Graft Substitutes

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**Abstract:** Restoring skeletal integrity and bone tissue regeneration is still a significant challenging issue. In this regard, bone grafting have been used to augment orthopedic repairs in human and veterinary surgery for several decades and still being under many investigation to hunt for new approaches to improve bone healing following incidences of bone complications. Bone graft is bone transplant and is categorized into autogenous and allogenic grafts as well as synthetic bone graft which are bone graft substitutes. Each of these classified grafts have some advantageous as well as a range of drawbacks, which researchers are still looking to remove those disadvantageous. Finding new instruments and new sites for graft harvests are the major concerns of researchers to diminish the morbidities of donor site in autografts. Looking for agents boosting inductivity of the allografts is the main worries of these kinds of grafting materials and finally new fabrication techniques by new pore sizes are the significant bothering for synthetic bone graft substitutes. This review would consider all grafting methods and materials that would open new windows to the bone grafting techniques.

**Key words:** Bone graft, graft incorporation, autograft, allograft, xenograft, synthetic bone grafts, bone graft substitutes

## INTRODUCTION

**Grafts and grafts history:** Tissue grafting attempts above all was made by Hunter (1728-1793). Much of Hunter's acquaintance might be attributed to his military practice and his experience with animals. He explained how to assess muscle power in a weak muscle. Facing joint disorders, he stated that voluntary movement shall not be allowed until inflammation has been resolved otherwise contracture is predicted. He believed that healing depends on the body's innate power and the surgeon's mission is to aid that power. Hunter believed that bone disease often require mechanical supports. He conducted researches on loose bodies in joints, pseudoarthroses and fracture healing. Later he described the transformation from fracture hematoma to fibrocartilagenous callus that goes toward deposition of new bone, trabeculation, reestablishment of the medullary canal and the resorption of excess bony tissue. Hunter wrote a dissertation on Blood, Inflammation and Gunshot Wounds in 1794 and also made heaps of attempts at tissue grafting (The history of orthopedics, 2009).

Bone grafting was followed later by Louis Xavier Eduard Leopold Ollier (1830-1900). Ollier was born in Vans in Ardeche and studied at Lyons and Ontpellier. Ollier performed pioneering bone grafts. Although he was successful, his methods and the theories behind them were in fierce opposition. In 1877, Ollier suggested that bone growth may be inhibited in order to correct certain deformities by resecting the epiphyseal plate. In 1899, Ollier for the first time described dyschondroplasia or Ollier's Disease. Ollier carried out researched on bone growth to an enormous extent and believed that it might be possible some day to treat patients by stimulating their cartilage to ossify (The history of orthopedics, 2009).

Sir William Macewen (1848-1924) acted upon many bone grafting after ward. In term of his orthopedic contributions, he completed many osteotomies and developed a one-piece osteotome. Macewen's key research interest was bone growth and in 1879 he performed the first of his pioneering bone grafts. Numerous of his grafts were done on patients who had had portion of their bones excised but who had otherwise normal function (The history of orthopedics, 2009).

Probably the most significant figures in bone grafting at turn of the century is Sir Robert Jones (1855-1933). Indeed many would argue that he was the greatest orthopedic surgeon that the world had ever seen. Jones was an advocate of tendon transplantation, bone grafting and other conservative, restorative procedures.

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Willis Campbell (1880-1941) was also a major figure in bone grafting and performed inlay full thickness grafts for non-union fixed with screws of beef bone (The history of orthopedics, 2009).

**Bone grafts:** Bone grafts have been used to augment orthopedic repairs in veterinary as well as human surgery for several decades and still being researched to look for new approaches to improve bone healing (Fox, 1984; Griffon, 2002). Encouragement of bone healing by bone grating application is used in significant numbers of orthopedic surgeries each year. Bone is the second most common transplantation in human and as estimation between 500,000-600,000 bone grafting procedures are performed annually in the United States (Bauer and Muschler, 2000; Betz, 2002). Laurencin *et al.* (2006) stated that in 1998, only 300,000 bone grafting procedures were performed in the United Stated and 9 of 10 was involoved in autograft. The bone grafting number had been reached to 500,000 in the US and 2.2. million worldwide in 2006 (Laurencin *et al*., 2006).

Common indications for bone graft utilizing in the orthopedic surgery include gaps at fracture sites, comminuted fractures delayed unions and non-unions, arthrodeses, corrective osteotomies and spinal fusion. Grafts also have been reported to be used to replace bone lost of bone cysts or neoplasia (Alexander, 1987; Brinker, 1997). Other bone graft using indication are limb lengthening procedures, enhancement of joint replacement prostheses and to fill the empty screws holes left after bone plate removal. Bone grafts are also being used in neurosurgeries, maxillofacial surgeries as well as dental procedures (Zamprogno, 2004).

Bone grafts are bone transplant from a site to another and are classified as autograft, allograft, xenograft, synthetic graft and combination graft (Bauer and Muschler, 2000). Autogenous bone graft is defines as the bone harvested from one site and transplanted in other site in the same individual and include cancellous, cortical, corticocancellous and vascularized bone grafts (Fox, 1984; Bauer and Muschler, 2000; Zamprogno, 2004). Allogenous bone graft is bone harvested from an individual and implanted into another in the same species. Allografts are categorized in different ways. They can be classified by anatomy (cortical, cancellous and corticocancellous), methods of processing (fresh, frozen, freeze-dried and demineralized) method of sterilization (sterile, irradiated, ethylene oxide) and handling process (powder, gel, particulate, chips, strips, blocks and massive). Allograft in the genetically related individuals is termed isogenous graft (Zamprogno, 2004). Xenogenous bone graft is described as the bone harvested from an individual and implanted into another from different species. Synthetic bone grafts such as ceramics, coral derives ceramics, ceramic combined with collagen, bio active glass (Ladd, 1999; Linovitz, 2002; Muschler, 1996) have different characteristics in structural strength, rate of resorption or replacement by host, mechanism of action, osteoinductive potential, osteoconductive properties and handling capability (Ladd, 1999). Some of disadvantages of these graft materials include cost, poor handling, poor resorbability and presence of animal tissue in composition of the grafts (Vaccaro, 2002). Due to these disadvantages, synthetic graft materials utilizing in orthopedic surgeries is only about 10% in the United States. As a demand to improve bone healing many of these synthetic bone grafts have been studied so far. Many still remain unproven to enhance bone healing and implantation of autogenous and allogenous graft materials stay the most common methods used to boost the bone healing and bone formation (Zamprogno, 2004).

Bone graft incorporation which defines as the rate of graft resorption and replacement by host bone depends on contact between the recipient bed and the donor tissue along with initiation of several independent processes such as osteogenesis, osteoinduction, osteoconduction and osteopromotion (Zamprogno, 2004).

## MATERIALS AND METHODS

**Osteogenesis:** Osteogenesis is defined as bone formation by living transplanted cell within the graft or on the other hand, a graft that supplies and supports bone forming cells is termed osteogenic (Attawia *et al*., 2003). As being defined the successful osteogenesis depends on the survival of the osteobasts and osteocytes of the graft materials (Alexander, 1987; Fitch, 1997; Keating and McQueen, 2001a; Ladd, 1999). These cells are preserved by diffusion from the surrounding host tissues until revascularization founding (Alexander, 1987). Bone formation requires the cellular machinery to fabricate its structural components. As such, no strategy of bone regeneration can neglect introduction of cells and the most efficacious strategies nurture an early cellular environment (Attawia *et al*., 2003).

Osteoinduction is process by which bone formation is being induced by active employment of bone forming cells or growth factors from within the transplanted tissue. Materials that have the capacity to induce bone formation, when placed into a site where no bone formation will occur are termed osteoinductive

(Attawia *et al*., 2003). These materials do not work alone but recruit bone forming cells or their progeny to infiltrate the material (chemoattraction and migration) then induce the multipotential cells to multiply and become cells that comprise the regenerating bony callus (proliferation and differentiation).

Osteoconduction is process in which the graft materials working as a suitable scaffold facilitating the bone positioning to its surface, improving attachments, migration and distribution of the cells involved in vascularization and bone healing (Fitch, 1997; Keating and McQueen, 2001b; Ladd, 1999) in easier word, the materials that provide a scaffold for bone forming cells and their progenies to migrate into and proliferate within is termed osteoconductive materials (Attawia *et al*., 2003). Osteoconduction vary greatly in different grafting materials and rely on graft's three dimensional structures, porosity, surface chemical properties and the rate and mechanism of degradation (Fleming *et al*., 2000).

Osteopromotion is promotion of bone healing and regeneration by encouraging the biologic and mechanical environment. On the other hand, materials or physical impetus that results in enhancement of regenerating bone is termed osteopromotive (Attawia *et al*., 2003). Osteopromotion can function at various stages during bone healing and provide different stimulatory signals to bone regenerating tissues. Osteopromotion differs from osteogenesis or osteoconduction as bone formation is enhanced without cells or a scaffold however, osteopromotive stimuli alone cannot induce bone formation. Osteopromotion can be achieved by introduction of substances or materials that enhance bone regeneration by physical or mechanical strategies that induce proliferation and differentiation of bone forming cells.

Eventually, successful bone graft incorporation requires a combination of osteogenesis, osteoinduction and osteoconduction. Although, these processes are important, there are other factor also can play role in physiology of graft such as mechanical load bearing graft surface texture, age and the level of health (Zamprogno, 2004).

**Bone graft physiology:** The incorporation of a bone graft is defined as the process of envelopment and interdigitation of the donor bone tissue with new bone deposited by the recipient (Morone *et al*., 1998; Gregory *et al*., 2009). This process pursues a typical multistep cascade the bone graft produces a response leading to the accumulation of inflammatory cells followed by the chemotaxis of host mesenchymal cells to the graft site. Thereafter, the host cells differentiate into chondroblasts and osteoblasts, a process under the influence of various osteoinductive factors. The additional process of bone graft revascularization and necrotic graft resorption occur concurrently. Finally, bone production from the osteoblasts onto the graft's three-dimensional framework occurs, followed by bone remodeling in response to mechanical stress (Goldberg and Stevenson, 1993).

An ideal bone graft would provide all elements required during these phases of graft incorporation and provide structural support during the process. This ideal graft would posses the following potentials: an osteoconductive matrix that provide a nonviable three-dimensional framework acquiescent to the ingrowth of blood vessels and osteoprogenitor cells requires for bone formation, osteoinductive factors that recruit the recipient's mesenchymal cells through chemotaxis and then induce or modulate bone formation, osteogenic cells that are osteoblast cells or graft cells with the potential to differentiate into osteoblasts and structural integrity that provides mechanical support and a porous, well-developed surface to let the bone forming cells and osteoinductive factors walk on that to lay down the new bone (Gazdag *et al*., 1995).

Many bone graft types are available today and each possesses some of the aforementioned properties. However, the best graft is one that carries all properties simulteneously.

The surgeon's choice of graft material depends greatly on which of the four elements are most crucial to the particular surgical application (more structural support or more osteogenic potentials). Between graft material the autogenous graft are pioneered in carrying the four properties and that is the reason it is the gold standard graft material and most common graft in use worldwide.

**Autogenous bone graft (bone autograft):** Fresh autogenous bone graft is deemed as the most efficient graft material since it provides the highest number of viable osteoprogenitor cells and contains noncollagenous matrix protein and growth factors as the osteoinduction property. It also carrying bone mineral and collagen which provide a scaffold for osteoconduction mean (Betz, 2002; Keating and McQueen, 2001a, b; Ladd, 1999; Linovitz, 2002). After transplantation autogenous bone graft become thoroughly incorporated into the grafted spot with neither initiation of immune reaction nor potential for disease transmission (Keating and McQueen, 2001a, b; Ladd, 1999; MacNeil, 1999).

Survival of the cells in the autograft is necessary for graft success and any damage to the graft cells, while

transplantation cause delayed bone healing (Burchardt and Enneking, 1978). To overcome this problem the autogenous bone graft is harvested right prior to the transplantation also completely aseptic techniques should be employed to thwart the graft contamination. Graft cells also should be taken care of to avoid any unwanted damage to do so once harvested, the autogenous bone graft should be wrapped in the blood soaked sponge and saline utilizing should be shunned since it is harmful and toxic to the osteoprogenitor cells. Another issue that should be considered is the temperature and sterilization technique. Temperature >42°C as well as cold sterilizer agents such as organic mercurials and bone waxes is fatal to the graft cells (Fox, 1984). Antibiotic application is contraindicated as well. Predominantly some antibiotics such as kanamycin and neomycin are not only bactericidal but also cellucidal and should be avoided in bone grafting procedures (Fox, 1984; Hulse, 1980; Zamprogno, 2004).

In addition, graft success is also depended to other factors, one of them is physical obstacles between the host bed and the graft material such as necrotic tissues and heamatomas. These obstructions will result in delayed angiogenesis and revascularization which should be debrided or lavaged preceding to transplantation. Capillary preservation at the recipient bed in the grafting procedure should not be neglected to avert hypoxia since, the mesenchymal stem cells differentiation is highly depended to the availability of oxygen and its level in addition to mechanical stimuli. At presence of sufficient amount of oxygen and under compression mesenchymal cells will differentiate into bone, while they can also be differentiated into cartilage at the insufficient level of oxygen. Fibrous tissue is produced when mesenchymal cells are positioned under tension with adequate level of oxygen (Zamprogno, 2004).

The potential sources of viable cells in autogenous bone graft are the periosteal cells, the endosteal cell, the bone marrow cells and the cells of the bone (Hulse, 1980). The importance of viable cells in bone graft was stated by Gray and Elves (1979). They showed with different amount of periosteum, endosteum and marrow. Endosteal and Haversian system osteoblast were responsible for 60% of new bone formation and periosteal osteoblast were responsible for only 30% of new bone formation (Enneking, 1957; Gray and Elves, 1979). Other studies showed that the osteocytes of the grafted bone were only responsible for only 10% of newly formed bone (Vasseur, 1987) and the heamatopoietic marrow cells had insignificant role in bone regeneration (Gray and Elves, 1979). Some studies suggested that the cells of the bone marrow are beneficial to new bone regeneration because it provides a great source of osteogenic factors contains osteoblastic progenitor cells and cytokines and also contains a biodegradable fibrin scaffold that rapidly would be revascularized (Fleming *et al*., 2000; Zamprogno, 2004).

**Cancellous bone autograft:** Autogenous bone graft (bone autograft) as stated earlier includes cancellous, cortical, corticocancellous and vascularized bone grafts. Cancellous bone autograft is the first constituent to be described in this study.

**Overall charactristics:** Cancellous bone autograft when appropriately handled and transplanted, offers the considerable amounts of viable cells that boost the osteogenesis, matrix protein that promotes the osteoinduction and bone matrix that encourage the osteoinduction. Hence, attributed to carrying these factors this graft is the most common graft material used in practice and is considered an ideal graft material (Alexander, 1987; Damien and Parsons, 1991; Fox, 1984; Griffon, 2002; Ladd, 1999; MacNeil, 1999; Vaccaro, 2002). It can be used for all indication that requires graft but it could be most effective in conditions where osteoblast cell population and as the consequent new bone formation is scarce such as long bone defects, pre-traumatized tissues, infection affected sites and highly vascular damaged bones (Fleming *et al*., 2000).

The most common harvesting site for autogenous cancellous bone graft is the iliac crest, tibial crest, humeral greater tubercle and greater trochanter of femur (Alexander, 1987; Damien and Parsons, 1991; Fox, 1984; Griffon, 2002). Place of bone graft harvesting depends on different factors including patient positioning, amount of graft required type of surgery to be performed instrumentation available and surgeon's preference.

**Incorporation procedure:** Incorporation process of cancellous autograft relies on different issues such as status of surrounding host tissue, general health level of the host graft cell survival percentage (wich greatly depend on the technique of harvesting and transplantation) size and location of the recipient bed, condition of vascularity and the age of the patient (Bauer and Muschler, 2000). Incorporation process is primarily achieved by a process termed creeping substitution (Burchardt and Enneking, 1978). Once the graft implanted, the donor cells of the graft are replace by the host mesenchymal cells which would be differentiated into osteoblasts. The osteoblasts are responsible for new bone production and formation. The creeping substitution continues to the point that all of the

graft cells are removed and replaced by new host bone (Alexander, 1987). The viable cells of bone marrow, endosteum and periosteum contribute to early stages of new bone production at the graft site and are stimulated by the oxygen tension, PH and cytokine environment (Abbott *et al*., 1947; Bauer and Muschler, 2000). Right away after graft implantation, heamatoma forms at the transplant-host site, attracting cytokines and growth factor to the area. Heamatoma formation indicates the first step of graft incorporation and also prevents blood loss (Abbott *et al*., 1947; Bauer and Muschler, 2000). Vascular granulation tissue arises from the heamatoma innitially at the margins of graft. The granulation tissue carries small blood vessels, neutrophils, monocytes, lymphocytes, plasma cell and edematous fibrous tissue enriched in cytokines and growth factor (Abbott *et al*., 1947; Bauer and Muschler, 2000). Capillaries from granulation tissue invaded from the host to the graft are oriented at an angle to the long axis of the graft (Enneking, 1957). These vessels increase in number and size to up to the point that the whole graft is vascularized. At this point, granulation tissue begins developed through the margins of the graft, dead bone would be replaced by new bone by resorption procedure (Abbot *et al*., 1947) and also periosteal proliferation begins at the transplant-host region (Zamprogno, 2004). Periosteal proliferation gradually becomes sturdier, stiffer and thicker that finally envelopes the host-graft region.

As soon as the granulation tissue becomes dominant, the graft become revascularized and the graft replaced by new host bone in which osteoclast activity starts and multiplies (Burchardt and Enneking, 1978). The graft-host site develops a layer of cartilage (Enneking, 1957) and later after that fibroblast influenced by growth factors and interleukins manufacture collagen (Bauer and Muschler, 2000; Zamprogno, 2004).

As vascular invasion of the graft progresses, mesenchymal cells differentiate into osteogenic cell. The mesenchymal cells originate from both graft and the recipient bed (Gray and Elves, 1979). The osteogenic cells differentiate into osteoblasts which initially line at the edge of dead trabeculae.

They ultimately envelop the core of the dead bone. Simultaneously, marrow cells accumulate within the grafted bone. Over time, cancellous bone graft is repaired with the necrotic and dead bone entirely replaced by new bone (Zamprogno, 2004). Strength of the grafted site would be gradually increase as the old bone is resorpted new bone is laid down and cancellous bone is replaced by cortical bone (Burchardt and Enneking, 1978). It is reported that after 100 days grafted site is indistinguishable from the normal bone (Abbott *et al*., 1947; Zamprogno, 2004).

**Cancellous autogenous bone graft weaknesses and techniques to trounce:** Although, cancellous autogenous bone graft is considered an ideal graft material, but there are some weaknesses in this graft material as well. Cancellous bone grafts lack biomechanical strength (Griffon, 2002) and do not supply structural support. However, this type of graft is used in occasions in which there is osteogenesis enhancement is required. Another weakness is donor site morbidity (Fleming *et al*., 2000) in which the main concern is the donor site pain. Another weak spot of autogenous cancellous grafts is limited graft material obtained from the patients (Betz, 2002; Fleming *et al*., 2000; Griffon, 2002; Linovitz, 2002; Vaccaro, 2002). However, autogenous cancellous grafts are good enough to fill up to 6 cm of bone defect. Also he affirmed that up to 30 cc of graft could be attained from anterior iliac crest and posterior iliac crest is even more capable to supply more and has abundant supply of cancellous bone autograft. There is large supply of cancellous bone autograft at the posterior aspect of iliac crest which could be harvested very easily. It is also believed that cancellous bone autograft harvesting increases the time and cost of surgery (Betz, 2002; Fleming *et al*., 2000; Griffon, 2002; Linovitz, 2002; Vaccaro, 2002) however, the patient prognosis should be considered. Most importantly as Greenwald *et al*. (2001) stated either cancellous bone autograft or an osteoinductive agent is necessary for critical sized defects.

The advantages of the cancellous bone autograft are carrying all characteristics required for osteogenesis, osteoinduction and osteoconduction properties simultaneously, excellent success rate in which only 2% of the patients require the second operation for donor site problem no risk of disease transmission and histocompatibility.

**Cortical bone autograft:** Other member of autogenous bone graft family is cortical bone autograft.

**General characteristics:** This type of bone autograft supply structural support at the transplanted site and is sufficiently competent to fill large defects (Bauer and Muschler, 2000; Burchardt and Enneking, 1978; Fleming *et al*., 2000).

The cortical bone autograft is proficient of filling of up to 12 cm defects. The graft has osteoinduction and osteoconduction properties but unlike cancellous bone autograft is short of the osteogenic properties. Since they are harvested and implanted in the same individual graft materials do not stimulate and initiate immune reaction but is prone to infection (Abbott *et al*., 1947; Zamprogno, 2004). When transplanting the autogenous

cancellous bone graft the incorporation process initiates with osteoblastic activity. In this occasion, the incorporation process launches with osteoclastic activity. As such graft materials become progressively porous as bone mass and density are reduced (Burchardt and Enneking, 1978). Studies has demonstrated that from 6 week to 6 months the grafted bone is 40-50% weaker than normal bone and only after 1-2 years, the mechanical strength and density of the graft become equal to normal bone (Burchardt and Enneking, 1978).

Given that the harvesting procedures of this graft material is more invasive than that of cancellous bone autograft and carry excessive donor site morbidity and because studies has shown that there is no significant difference between this graft material and allogenous cortical bone graft (Bauer and Muschler, 2000), the clinical use of cortical bone autograft is such scarce. The sites of graft material harvesting are fibula, ribs, distal ulna and iliac wing (Fleming *et al*., 2000).

**Incorporation process:** Even though the overall incorporation mechanism is similar for cancellous and cortical bone autografts, the status of revascularization, creeping substitution and complete graft repair are entirely different. Bone production is highly depended on environmental conditions such as nutrition and chemical stimulation (Hulse, 1980). The early stage of graft incorporation behaves similar to that of cancellous autograft (Abbott *et al*., 1947). However, in later phases the graft is almost enveloped by new bone trabeculae that originated from the graft bed. Host trabecular activated bone is united to the mature graft reactive bone by a thin layer of cartilage which would be replaced by new bone cells soon after (Enneking, 1957). By this time, there is increased revascularization, new bone formation is traceable on the marginal areas of Haversian system (Abbott *et al*., 1947) and blood vessel have invaded into the canals of host Haversian system.

Incorporation of this class of graft material takes place slowly. The new bone primarily develops from the graft bed en route for the graft via granulation tissue (Abbott *et al*., 1947). At the time, when the cancellous bone autograft is completely incorporated, the cortical bone autograft is completely attached to its bed but the replacement of bone graft with the host new bone is in process (Abbott *et al*., 1947) and this is only because of the least osteogenic properties of the graft.

Vascularization of graft is initially via peripheral bone resorption and vascular infiltration into volkmann's and Haversian canal (Boonen *et al*., 2003). The complete revascularization process usually accomplishes in one to two months. This could be well described by the type of structure of this graft material which is dense, lacks porosity and the surface to volume ratio is low (Abbott *et al*., 1947; Bauer and Muschler, 2000; Fleming *et al*., 2000). As soon as the vascular condition reaches that of normal bone and function same as a normal bone, remodeling of the graft begins (Albrektsson, 1980). When the resorption procedure completes the union occurs between the reactive host bone and the graft (Enneking, 1957). Healing in occasion of utilizing this type of graft material starts within the cortical interior at the periphery, near the graft-host joint and the process gradually moves toward the centre of the bone (Burchardt and Enneking, 1978). The resorption process gradually declines after a year to the normal level (Enneking, 1957) and dissimilar to cancellous bone autograft which will be completely healed and repaired over the time, the cortical bone autograft would be remain unaltered once the catabolic and anabolic stages of repair have been completed and they are remain as a mixture necrotic and viable cell (Burchardt and Enneking, 1978).

Vascularized autogenous bone grafts have been utilized in both human and animals. Since they are transplanted with the blood supply (vascularized graft), all concerns about the revascularization will be omitted. This graft material is capable to fill the defects >12 cm.

**Corticocancellous bone autograft:** This graft type is also a constituent of autogenous bone graft material.

**Overall characteristics:** This class of graft material does not offer structural support, but boost the new bone formation by osteogenesis, osteoinduction and osteoconduction potentials (McLaughlin and Roush, 1998). The most common site for harvesting corticocancellous bone autograft are ribs and the craniodorsal iliac wing (Brinker, 1997).

**Incorporation mechanism:** The incorporation of cancellous bone graft is comparable to corticocancellous bone graft (Millis and Martinez, 1993). The incorporation procedure is similar to autogenous cortical bone graft after transplantation. It is initially revascularized and then replaced by host bone (Abbott *et al*., 1947). This graft material is revascularized more quickly than cortical autograft since it is less dense. Studies have demonstrated that corticocancellous autograft collected from iliac wing did not formed significantly more new bone than cancellous autograft alone harvested from same site meaning from same side of harvesting, corticocancellous and cancellous bone graft have same osteogenesis capability (Abbott *et al*., 1947). In corticocancellous bone autograft, while the medulary

bone is exposed, the cortical portion of the graft showed evidence of new bone formation from its endosteum (Abbott *et al*., 1947). This could be due to the high amount of endosteal cell of the graft materials within each layer of trabeculae. This explanation make the graft material qualify for more osteogenesis potential than cortical bone graft alone (Abbott *et al*., 1947). Consequently, the osteogenic potential of this type of graft is more or less similar to cancellous bone graft but it does not require long time for resorption and degredation like what is being seen in cortical bone graft.

The bone graft incorporation and bone induction cascade has often been separated into three prominent phases. The initial phase involves mesenchymal cell chemotaxis and proliferation. This is a growth factor stimulated accumulation of primary mesenchymal cells and is critical to the ensuing phases of bone induction. The second phase includes the differentiation of the stem cells into chondroblasts and chondrocytes with the subsequent production of cartilaginous matrix. This second phase concludes when blood vessels invade the newly formed cartilage carrying primitive mesenchymal cells along to populate the cartilage with osteogenic precursors. The third and final phase is the mesenchymal cell differentiation into osteoblasts and osteocytes, followed by bone and bone marrow production (Prolo and Rodrigo, 1985; Gregory *et al*., 2009).

The corticocancellous bone autograft that induced this cascade is a repository of various osteoinductive factors such as BMPs, TGFs and insulin-like growth factors. These factors have been shown to have a high influence on induction of osteoblast to proliferation and differentiation *in vitro* and also to stimulate bone formation when administered *in vivo* (Linkhart *et al*., 1996). Some of these factors have been implicated not only in the initiation of induction but also in the promotion, maintenance and termination of bone formation (Elima, 1993; Gregory *et al*., 2009).

**Phase I:** Mesenchymal Cell Chemotaxis and Proliferation (days 0-4). During the first few minutes following corticocancellous bone autogenous graft implantation, a blood clot forms producing a fibrin network. Platelet aggregations release multiple growth factors such as Transforming Growth Factor (TGF) and Platelet-Derived Growth Factor (PDGF) and also there is plasma fibronectin binding to the implanted matrix. The TGF has various effects that promotes osteoinduction including stimulating the production of other bone inducing growth factors, encouragimg the production of various bone and cartilage components (Linkhart *et al*., 1996) and causing osteoblast chemotaxis (Oikarinen, 1982) and proliferation. The PDGF is an important mitogen for connective tissue cells (Ross *et al*., 1986). It also may play a significant role in the regulation of cell growth and differentiation (Muller *et al*., 1984). Finally, fibronectin peptides have potent chemotactic and mitogenic effects as well (Prolo and Rodrigo, 1985). Consequently, within the first few minute of implantation, the bone induction cascade would be initiated through the release of various chemotactic and mitogenic factors (Gregory *et al*., 2009).

During the next 18 h, there is a chemotactic-driven factor arrival and accumulation of inflammatory cells such as PMNLs. There is also a release of collagenase and elastase, producing well-known chemotactic factors such as collagenous and fibronectin peptides. The chemotactic stimulus that initiated in the first few minutes following implantation has therefore undergone a considerable amplification during the consequent hours post implantation (Prolo and Rodrigo, 1985).

After blood clot has formed and inflammation cascade has begaun, there is a 2-day period of fibroblast like mesenchymal cell chemotaxis, a process mainly driven by the aforementioned proteolytic peptides and growth factors. The mesenchymal cells arrive and subsequently attach to the implanted matrix. This process is mediated by fibronectin and other cell-adhesive proteins. As the chemotactic process is nears completion, two activities have been observed: protein and nucleic acid synthesis is launched to prepare for the following cellular proliferation and further amplification of the osteoinduction cascade occurs through the release of additional growth factors (Prolo and Rodrigo, 1985; Gregory *et al*., 2009).

The fibroblast-like mesenchymal stem cells then proliferate during the 3rd and 4th days post implantation. Again, this proliferation is largely driven by the mitogenic effects of the previously released growth factors.

## RESULTS AND DISCUSSION

Graft bone induction cascade has many parallels with settings such as fracture healing and bone graft incorporation. With regard to fracture healing, many researhces have noted the initial development of a hematoma followed by the release of various chemoattractants, angiogenic factors and growth factors from the aggregating platelets and local damaged tissue. A prominent invasion of cells such as granulocytes and mesenchymal cells soon follows (Grotendorst and Martin, 1986; Hulth, 1989; Reddi *et al*., 1987; Seeley *et al*., 1989; Sporn *et al*., 1987). Nearly identical descriptions of these early cellular steps with some differences actually have

been noted in the literature concerning other types of autogenous bone grafts as well (Boden *et al*., 1995; Burchardt and Enneking, 1978; Holmes *et al*., 1984). Burchardt and Enneking (1978) compared cancellous and cortical bone graft incorporation and noted an identical early inflammatory process followed by fibroblast-like cell accumulation in both. It thus appears that this initial inflammatory phase and subsequent mesenchymal cell accumulation is a universal initiating step in the bone induction cascade, bone healing process and graft incorporation procedure (Burchardt and Enneking, 1978; Gregory *et al*., 2009).

**Phase II:** Mesenchymal Cell Differentiation into Cartilage (Days 5-9). Five days following graft implantation, the first cells and molecular markers indicative of cartilage differentiation of MSCs are detectable. Histologically, chondroblasts are noted between days 4-5, luanching the beginning of the differentiation phase. By day 7, chondrocytes are apparent and there is further synthesis and secretion of cartilaginous intercellular matrix. By day 9, the typical pattern of cartilage maturation is observed (initial phase of endochondral ossification). There is hypertrophy of chondrocytes, erosion of the intervening cartilaginous matrix and mineralization of the matrix trabeculae. The appropriate molecular markers of increased calcium incorporation and increased alkaline phosphatase activity are concurrently seen. Lastly, vascular invasion of the newly formed cartilage occurs. This is seen histologically and is also accompanied by the detection of Type-IV collagen, laminin and factor VIII (all common in blood vessel components) (Prolo and Rodrigo, 1985). The vascular invasion indicates the transition from the cartilage differentiation phase to the final phase of bone induction osteogenic precursor differentiation into bone (Gregory *et al*., 2009).

It has been generally accepted that endochondral ossification with the production of a cartilaginous intermediary is at least a component of the natural healing of fractures. On the other hand, the degree to which endochondral bone formation compared with intramembranous bone formation contributed to fracture healing has produced some debate. McKibbin (1978) described a two-callus response in a natural fracture healing experimental model. The primary callus involved the direct production of bone through membranous ossification, whereas the inductive callus involved indirect bone production through endochondral bone formation. Two separate sources of osteoprogenitor cells leading to this two-callus response were proposed.

Confirmatory evidence of these observations however, is lacking (McKibbin, 1978). Other researchers also described the two-callus responces that includes direct followed by indirect bone formation (Ham, 1974).

Evidences show that cartilage has unique potentials that provide themselves well for the clinical procedure of fracture healing and bone grafting incorporation. One of the first goals of bone healing in any setting is the restoration of stability and fibrocartilage has unique swelling properties that give it considerable stabilizing capabilities. The cartilage-specific proteoglycans have long glycosaminoglycan chains with negatively charged chondroitin sulfate and keratan sulfate. These result in large aqueous domains that provide mechanical stability to a developing callus by increasing its intrinsic pressure (Ross *et al*., 1986). It therefore appears that this cartilage is not only an intermediary in the process of nwe bone formation on bone healing but also supplies an important structural function to the ongoing fusion mass (Gregory *et al*., 2009).

**Phase III:** Mesenchymal Cell Differentiation into Bone (Days 10-21). Eight to ten days after graft implantation, the osteoblastic differentiations are noted and new bone formation is observed on the surface of the remaining calcified cartilage matrix. These cellular events are associated with cellular processes consistent with bone formation including Type I collagen synthesis (the major fibrillar collagen of bone) (Ross *et al*., 1986), bone-specific proteoglycan synthesis and a peak in Ca incorporation and alkaline phosphatase activity. By days 12 through 18, multinucleated osteoclasts are observed histologically that implicate the initiation of process of bone remodeling. The osteoclasts and osteoblasts work in tandem to replace remaining calcified cartilage with immature bone trabeculae. The expected molecular processes of increased lysosomal enzyme activity and release of collagenases and proteases is concurrently detected. By day 21, bone marrow differentiation occurs and the appearance of erythrocytic, granulocytic and megakaryocytic lineages is noted (Gregory *et al*., 2009).

Bone graft incorporation however, is considerably more complex with placing two processes including necrotic graft resorption and graft revascularization occurring concurrently with the bone induction cascade (Burchardt and Enneking, 1978). In this preocess, the chronology of the bone induction cascade is quite similar to aforementioned cascade but the temporal profile can vary greatly depending on the graft type used.

Burchardt and Enneking (1978) have elegantly demonstrated such a temporal difference, when they compared the graft incorporation characteristics of cancellous and cortical bone (Burchardt and Enneking, 1978). They first described the universal initiating steps of

bone induction in each graft type. This includes the formation of a blood clot, inflammatory cell accumulation and fibroblast chemotaxis (Week 1). An identical 2nd week of osteoclast resorptive activity and osteocyte autolysis is seen in each graft type as well. It is at this moment however, that differences occurred between the cancellous and cortical grafts. Cancellous bone typically revascularized within 2 weeks of implantation, whereas cortical grafts required 2 months for complete revascularizatio. Cancellous bone is initially strengthened by new bone formation and progressively gains more strength as the bone induction cascade proceeds. Cortical bone, on other hand, loses much of its initial structural integrity during the first 6 months because of the aforementioned osteoclastic resorptive process. As bone formation continues, the strength is gradually regained during the second 6-month period. Finally, cancellous grafts typically become completely remodeled (the entire graft is resorbed and replaced by new bone), whereas cortical grafts are often incompletely remodeled for many months (various pockets of necrotic graft remain). These observations thus illustrate a general temporal lag in the typical bone graft incorporation cascade when a more compact, cortical graft is used (Gregory *et al*., 2009; Burchardt and Enneking, 1978).

**Allogenous bone graft:** Use of allogenous bone graft or bone allograft is becoming more common in human as well as veterinary medicine since it does not carry the weaknesses (Fleming *et al*., 2000; Griffon, 2002; Griffon *et al*., 1996) of bone autograft and could be provided in an unlimited quantity. This graft material can be utilized alone or as an extender for autogenous bone graft (Betz, 2002). Allogenous bone graft materials have osteoinduction potential owing to the presence of growth factor in the graft material which include insulin-like growth factor type II, transforming growth factor-β, platelet derived growth factor, fibroblast growth factor and bone morphogenic proteins. These growth factors are in the matrix and are being released by osteoclastic resorption (Bauer and Muschler, 2000; Fleming *et al*., 2000; Ladd, 1999). Also the graft material possesses the osteoconduction properties on account of porous structure of the graft, the cross-linked collagen matrix and the available surface for osteoprogenitor and endosteal cell attachment (Bauer and Muschler, 2000; Fleming *et al*., 2000; Ladd, 1999). The efficacy of the osteoinduction and osteoconduction potential of the allogenous bone grafts completely relies on the graft stable fixation and the close contact between the graft and the recipient bed (Sinibaldi, 1989). Veterinary allogenous bone graft materials are commercially available in different forms including gel, powder, pastes, blocks and fibers (Fleming *et al*., 2000; Keating and McQueen, 2001a, b; Toombs and Wallace, 1985). Cortical bone allograft material is the most frequent type of graft material in this class used in patients for structural support in cases of multi fragmentary fractures and bone losses as a result of neoplasia (Zamprogno, 2004). The allogenous bone graft could be prepared in different manners such as radiation, freez-drying, freezing and ethylene oxide sterilization. Fresh allograft is not longer consumed owing to its potential to stimulate the severe immune response as well as transmit the infection (Keating and McQueen, 2001a, b).

**Disadvantages of allogenous bone graft:** This class of bone graft has many disadvantages. Allogenous bone graft suffers from lack of osteogenesis potential in addition to weaker structure in comparison to autogenous graft materials (Kerwin *et al*., 1996; Ladd, 1999; McLaughlin and Roush, 1998) which caould lead to fatigue fracture (Burchardt and Enneking, 1978). The price of providing allograft is high because of the procedures required for its collection and processing. One of the most important disadvantages of the graft materials is high risk of infectious disease transmission from the donor to the recipient (Betz, 2002; Fleming *et al*., 2000; Kerwin *et al*., 1996; Mclaughlin and Roush, 1998; Muschler, 1996; Vaccaro, 2002). A study indicated that chondrocalcinosis, avascular necrosis, osteoma, malignant tumor, metabolic disease and inflammatory arthritis were found in ossteoartritic femoral head which is considered as a suitable bone donation site. Graft contamination, presence of toxins and the most important disadvantages, the potential for immunological rejection are the other allograft weaknesses (Dueland *et al*., 1989; Fleming *et al*., 2000). The most important diseases transmitted by allografts are hepatitis and AIDS. The technique of processing the allograft does affect the mechanical properties and its effectiveness as a graft material. Freez dried and irradiated bone grafts are weaker than frozen grafts (Ladd, 1999). The broad procedure involved in declining (but not eliminatimg) the immune rejection of allograft as well as making them ready to be stored, makes them very expensive make them mechanically weaker and eliminate the osteogenesis properties (Betz, 2002; Kerwin *et al*., 1996; Muschler, 1996; Vaccaro, 2002) however, after these preparation procedures still there are high percentage of disease transmission.

**Cancellous bone allografts:** This type of graft material is not used as commonly compared to cortical allografts. Their use in veterinary surgery is also limited (Dueland *et al*., 1989; Kerwin *et al*., 1996; Rose *et al*., 1986). Since this graft material is highly cellular, it has the

higher potential to stimulate and initiate the immune response and would result to the immune rejection by the host (Kerwin *et al*., 1996).

To avoid the rejection, it should undergo a procedure to remove the cellular component. While this procedure is taking place the osteogenesis potential would be limited but it would keep its osteoinduction and osteoconduction characteristics (Kerwin *et al*., 1996). A study indicated that at 12th week post implantation, osteoblastic and osteoclastic activity would be significantly increased and the graft becomes incorporated (Wilson *et al*., 1985). This study also showed that the incorporation process of cancellous allograft is much slower than cancellous autograft (Wilson *et al*., 1985).

**Cortical bone allograft:** This graft material is to be used when the mechanical support is required at the grafting site. Their common use in veterinary and human surgery is in cases of multifragmantary fractures and in bone losses because of tumors or cysts (Kerwin *et al*., 1996). As their osteogenic potential is very low, they are considered to be depended very little on grafted cell survival for their success (Alexander, 1987; Kerwin *et al*., 1996).

**Incorporation process:** Cortical allograft incorporation procedure differs completely from that of cortical autograft (Burchardt and Enneking, 1978). Bone formation and revascularizarion are significantly slower and less extensive in this graft material (Bauer and Muschler, 2000; Burchardt and Enneking, 1978). Incorporation process here is known by osteoclastic avtivity which increases the porosity and deteriorates the graft, though the chance of graft failure in the first 6-24 months after grafting is greater (Burchardt and Enneking, 1978; Fleming *et al*., 2000; Johnson *et al*., 1992). In autograft the immune response that is very obvious in allograft is not observed (Bauer and Muschler, 2000).

Bonfiglio and Jeter (1972) showed that a lymphocytic, eosinophilic and macrophages exudates develop between the graft and the surrounding soft tissue of the host, while cortical allograft implanted. The inflammatory reaction is more severe at the earlier stages. The study indicated that the second week is the peak of host inflammatory response and graft rejection. Vascular connective tissue then envelopes the allograft and resorption is increased in rate which is the indication of transplant rejection response by the host (Bonfiglio and Jeter 1972).

Elves and Pratt (1975) reported that at the second week post implantation, cortical allograft consists primarily of dead bone. By the 3rd week, the woven bone surfaces are surrounded by a layer of osteoblast and bone marrow is to be form (Elves and Pratt, 1975). In the following 5 weeks, the graft is becoming smaller and the woven bone is progressively replaced by lamellar bone. The smaller the allograft the more rapidly replaced by host, while larger pieces take longer time for replacement or they may never been resorbed and replaced (Sinibaldi, 1989).

Allograft even might not be incorporated or get separated after eight years. Wilson and Hoefle (1990) showed that in a dog the femoral allograft was enveloped by a layer of host new bone but there was no evidence of graft resorption.

Burchardt and Enneking (1978) stated there are two different ways for allografts repair procedure. The first method, involved in rapid and utter resorption of the allograft materials. This process takes place when there is significant genetic difference between the host and the donor. This increases the allograft rejection risk and occurs by continuous resorbtion at the periphery site of the graft material without any sign of replacement of the graft by the host bone (Burchardt and Enneking, 1978).

The second pathway of repair procedure occurs when there are fewer genetic differences between the host and the donor. In this process bridging callus is obvious but mostly leads to delay union, fatigue fracture less new bone growth in comparison with cortical autograft and reduction in graft size (Burchardt and Enneking, 1978).

In fresh cortical allograft the endosteal cells are the major osteoprogenitor cell contributors and responsible for early stages osteogenesis. Bone marrow does not play an significant role in this regards and its removal does not carry any deleterious effect on the healing. Fresh allograft also considered to have osteogenesis potential (Elves and Pratt, 1975). However, fresh cortical allograft is not frequently used in practice owing to its high percentage of disease transmission and graft rejection. Mostly preserved and processed one is being utilized.

In processed allograft during the first two weeks, formation of small amount of cartilage has been detected. Very small amount of new bone formation was seen at the third week and extensive bone development was observed only after eighth week.

This shows relative lack of early osteogenic potential of cortical allograft (Elves and Pratt, 1975). The early osteogenic potential is related to the osteogenesity properties of the graft material which is only available in fresh allograft materials which are not frequently being used due to its risk of immune rejection. Later phase of incorporation process relies more on host cells and this is the phenomena happening in cortical allograft implantation (Sinibaldi, 1989; Zamprogno, 2004).

**Corticocancellous bone allograft:** Although this kind of graft material works almost similar to cortical allograft use

Table 1: Comparative properties of bone grafts (Greenwald *et al*., 2001; McLaughlin and Roush, 1998)

| Bone graft | Structural strength | Osteogenesis | Osteoinduction | Osteoconduction |
|---|---|---|---|---|
| Cancellous autograft | No | +++ | +++ | +++ |
| Cortical autograft | Yes | ++ | ++ | ++ |
| Corticocancellous autograft | No | +++ | +++ | +++ |
| Frozan cancellous allograft | No | No | + | ++ |
| Freez-deried cancellous allograft | No | No | + | ++ |
| Frozen cortical allograft | Yes | No | No | + |
| Freez-dried cortical allograft | Yes | No | No | + |

Table 2: Summary of bone graft substitutes (Moore *et al*., 2001)

| Substance | Bioactive glass | Glass inomers | Aluminium oxide | Calcium sulphte |
|---|---|---|---|---|
| Form | Granules, blocks, rod | Powder | Granules, blocks | Powder. pellets |
| Reabsorption | Non-resorbable to resorbable | Non-resorbable | Non-resorbable | Dissolves in 5-7 weeks |
| Incorporation of antibiotics or bone-promoting substances | Not possible | Yes | Not possible | Yes |
| Mechanical properties | Stronger than HA implants | Compressive strength and elasticity comparable to cortical bone | Stronger than HA implants, does not osteointegrate | No structural properties |
| Uses | -Bone graft expander<br>-Vertebral body prosthesis<br>-Ossicular replacement<br>-Orbital implants<br>-Coating metal implants | -Dental maxillofacial ossicular replacement | -Bone graft expander<br>-Wedge, osteotomy<br>-Ossicular replacement<br>-Prosthetic joint lining | -Void filler<br>-Bone graft expander<br>-Osteomyelities |
| Products name | NovaBone | Fugi IX gp | Alumina ceramic | Osteoset |
| Comparative costing | 6+for 10 $mm^3$ granules | 1+for 10 $mm^3$ powder | - | 5+for 10 $mm^3$ pellets |
| Manufacturer | US Biomaterials | GC Corporation | Orthomed SA | Wright Med. Tech. Inc. |

of this type of graft material is nor such common in veterinary medicine. It is though that there are two steps of osteogenesis for this graft material. In the first phase new bone formation is evident after 7 days post implantation and granulation tissue and osteoid had invaded the intratrabecular spaces of the graft (Elves and Pratt, 1975). Osteogenic activity achieved its peak after three weeks post implantation and was only detectable in viable grafts which specify the significance of surviving graft cells in stimulation and launching of early phases of osteogenesis (Elves and Pratt, 1975). The second phase and step of osteogenesis occurs after the 8th week post transplantation and does not rely on the survival of the cells of the graft but only depends on the host and recipient osteogenic precursor cells (Elves and Pratt, 1975). Table 1 compares the characteristics of autograft and allograft (Greenwald *et al*., 2001; McLaughlin and Roush, 1998). They have also mentioned that in challenging critical size defects either autogenous bone graft or an osteoinductive material is completely necessary for healing.

**Xenogenous bone graft:** Xenogenous bone graft or xenograft, bone substitute has its origin from a species other than the graft recipient species, such as bovine. Xenografts are usually only distributed as a calcified matrix.

**Synthetic bone grafts:** Synthetic bone grafts are also termed as bone graft substitutes are other types of graft material used to fill the osseous defect in human and veterinary medicine. As to be suitable for *in vivo* implantation they should possess many requirements. They should be biocompatible and permit fast incorporation.

They must have mechanical properties to prevent graft deformation. They must also permit regulated osteoclastic resorption. Synthetic bone grafts also should possess porosity to allow transmission of changes in hypodynamic pressure and allow bone ingrowth. Should be easy to handle, inexpensive, have easy implantation technique and fast manufactured (Zamprogno, 2004).

Some of different synthetic bone grafts types are calcium sulphate (plaster of paris), calcium phosphate, Hydroxyapatite (HA), Calcium Phosphate Cement (CPC), Tricalcium Phosphate (TCP), beta-tricalcium phosphate, Octacalcium Phosphate (OCP), collagen composites (biologic-synthetic graft) and bioactive glass (bioglass) (Zamprogno, 2004). Table 2 shows summary of bone graft substitutes and their clinical use (Moore *et al*., 2001).

## CONCLUSION

An ideal graft is one which posses osteogenic, osteoinductive and osteoconductive potentials however, synthetic bone grafts only posses osteoconductive properties hence an oetseogenic or inductive like cencellous bone or bone morphogenic proteins and other osteogenic inductive material should be added to this graft to promote osteogenic potentials. By their own use

of these graft materials are useless. The only advantageous of bone graft substitutes are their availability in large quantities, shape and size but they suffer from lots of drawbacks including lack of osteogenic and osteoinductive potentials, low biodegradability rate, low incorporation rate, potentials to transmit diseases, in some implants expensive and long manufacturing time and instigating the inflammatory reaction (Zamprogno, 2004). Some of them also require difficult technique to be implanted.

Synthetic graft materials commonly act as a scaffold to carry osteogenic materials into the defect. They are also being used as the autogenous graft extenders, which in this instance require additional exposure to autograft harvesting site. The most common use of them is graft filler.

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