Chapter 1
Introduction

Introduction

Many type of bone grafts material that available for oral and maxillofacial surgery are the autogenous , allogenous , alloplastic and xenogenic bone graft. Autogenous bone graft is considered to be the gold standard of graft materials because of the high population of pluripotential cells in the particulate cancellous bone and marrow portion of the grafts. (Boyne, 1997 : 3-21; Burchardt, 1983 ; De Lacure, 1994; Garg ,1999 : 83-101; Khan, Tomin and Lane, 2000; Tomford, 1995) Autogenous bone graft are usually harvested from iliac crest , rib , or calvaria bone. The advantages of autogenous grafts include early revascularization , high population of pluripotential cells , resistance to infection and immune activation. However, autogenous bone grafts also have several disadvantages : limitation of graft volume available , required donor site surgery , prolongation of the operation time and donor site morbidity. (Boyne, 1997 : 3-21; Burchardt, 1983; De Lacure, 1994; Garg, 1999: 83-101) The amount of cancellous bone grafts volume that available from iliac crest are about 30 – 80 cm^3 and the single rib are about 10 –15 cm^3. (Boyne, 1997 : 3-21) The hematoma , pain ,paresthesia,hernia and gait disturbance were principle complication of iliac bone harvesting. Thus, the many of alternative bone substitute have been used ,included allogenous , alloplastic and xenogenic bone material. The ideal of bone substitute should be biocompatible for accepted by the host tissue, high porosity, large inner surface area and it should be gradually replaced by new bone and it should be have the osteoinductive or the osteoconductive properties. (Boyne, 1997 : 87-100; Garg, 1999 : 83-101) The osteoinduction is a healing process in which local stimulating factor cause messenchymal cells to migration , proliferation and differentiation to chondroblast or osteoblast cell. (Boyne, 1997 : 3-21; Garg, 1999 : 83-101) The

osteoconduction , the bone substitute material serve as a scaffold for ingrowth of new vessel and osteoprogenitor cells from the host bone. (Boyne, 1997 : 87-100; Garg, 1999 : 83-101) **Allogenic bone** materials are compossed of tissue taken from another individual of the same species. (Boyne, 1997 : 3-21; Burchardt, 1983; De Lacure, 1994; Garg, 1999 : 83-101; Khan, Tomin and Lane, 2000; Tomford, 1995) Allogenic bone material that available are demineralized freeze – dried bone (DFDBA), freeze dried bone allograft (FDBA). The allogenic bone materials were have osteoinductive and osteoconductive properties. In the osteoconductive properties ,the porous structure of allogenic bone grafts are a collagen matrix that supported the mesenchymal cell to facilitates the attachment , migration and differentiation to osteoblast cell. (Boyne, 1997 : 3-21; Burchardt, 1983; De Lacure, 1994; Garg, 1999 : 83-101) For the osteoinductive properties, allogenic bone grafts contains many growth factor, which are embedded in the bone matrix and can be liberated when the bone matrix were resorpted by osteoclast cell. These growth factors included insuline-like growth factor , transforming growth factor-beta , platelet derived growth factor, fibroblast growth factor and bone morphogenetic hormone (BMPs). (Boyne, 1997 : 3-21; Burchardt, 1983; De Lacure, 1994; Garg, 1999 : 83-101; Gordh, et al., 1999; Herndon, Nguyen and Glipin, 1993; Minamide, et al., 2001; Stewart, et al., 1999) Allogenic bone graft materials have several advantages over autogenous bone grafts : 1.the mobility and morbidity of harvesting autogenous bone is eliminated 2.allogenic bone grafts provide an essentially unlimited volume of graft material 3.allogenic bone grafts can be provided into a wide variety of physical forms which can be customized to specific application. However ,the disadvantages of allogenic bone grafts are : 1.the material are expensively 2.the material have risk of disease transmission especially hepatitis B and HIV still exists ,although this risk have been minimized by extensive donor screening and testing ,extensive washing to remove donor cells and cell debris and sterilization using high dose radiation. (Boyne, 1997 : 3-21; Garg, 1999 : 83-101; Khan, Tomin and Lane, 2000; Tomford, 1995) For this reasons, the researcher has been developed the biosynthetic bone material for substitute autogenous bone. **Alloplastic material** are synthetic substance used as substitute for bone grafting. The available of alloplastic bone

materials are hydroxyapatite , tricalcium phophate which have biocompatibility and osteoconductive properties. The advantage of alloplastic materials were eliminated limitation of the quantity of graft materials , eliminated donor site morbility , no risk of disease transmission and have many form for clinician used. However ,the alloplastic material such as HTR have higher stiffness and lower flexibility that made the stress at the interface between the graft material and host bone. These stress were made the microfractures between graft and host bone that induced fibrous encapsulation of synthetic bone materials. Moreover , alloplastic material such as HTR may have a migration of graft materials that made the distortion at the graft site. (Boyne, 1997 : 87-100; Khan, Tomin and Lane, 2000; Garg, 1999 : 83-101; Tomford, 1995) **Xenogenic bone grafts** are composed of tissue taken from another species such as from the animal. The most common used of xenogenic bone grafts was **Bio-Oss®** that prepared from bovine bone. Bio-Oss® (Geistlich Phamaceutical, Wolhusen, Switzerland) is a xenograft consisting of deproteinized natural bovine bone mineral that have biocompatible, osteoconductive and biodegradable. (Artzi and Nemcovsky, 1998; Artzi, Nemocovsky and Tal, 2001; Berglundh and Lindhe, 1997; Boyne, 1997 : 87-100; Camelo, et al., 1998; Haas, et al., 1998) Bio-Oss® does not make any allergic reaction or immunologic reaction after implantation in human because of Bio-Oss® were prepared by the process that removed any protein or organic component from bovine bone. The technical process used in producing the xenograft Bio-Oss® (Geistlich Phamaceutical, Wolhusen, Switzerland) from a bovine bone source makes possible the removal of all organic components of the bone product, leaving a pure, nonorganic bone matrix in unchanged inorganic form. The absence of any protein in the bone substrate material is important to be certain that no allergic or immunologic reaction occurs after implantation of the xenograft material in human patients. The complete removal of all organic materials is confirmed for each batch of Bio-Oss® material produced. (Boyne, 1997 : 87-100) In recent studies, Bio-Oss® have been used in many experimental and clinical studies, both in animal and human. Bio-Oss® was most frequently used as bone substitute in oral and maxillofacial surgery such as ridge

augmentation , sinus lift bone graft, bone grafting in implant surgery that showed the successive of treatment. However, Bio-Oss® is not an ideal bone graft material because of Bio-Oss® have only osteoconductive property. (Artzi and Nemcovsky, 1998; Artzi, Nemcovsky and Tal, 2001; Camelo, et al., 1998, 2001; Haas, et al., 1998; Hammerle, et al., 1997, 1998; Klinge, et al., 1992; Lundgren, et al., 1992; Richardson, et al., 1999; Rudkin and Miller, 1997; Ruhaimi, 2001; Schmitt, et al., 1997; Valentini and Abensur, 1997; Valentini, et al., 1998, 2000) The considerable attention has focused on the potential application of growth factors to enhance wound healing. Several polypeptide growth factors, e.g. fibroblast growth factor, insulin-like growth factor-I, platelet derived growth factor, transforming growth factor-β and bone morphogenetic proteins, alone or in combination have been demonstrated to be effective on cell proliferation, chemotaxis, differentiation and extracellular matrix synthesis and consequently facilitate bone regeneration in animal and human studies. (Ahn, et al., 2003; Arm, et al., 1996; De Obarrio, et al., 2000; Fleming, Cornell and Muschler, 2000; Garg, 1999 : 83-101; Gordh, et al., 1999; Khan, Bostrom and Lane, 2000; Lekovic, et al., 2002; Lundgren, et al.,1992; Wozney, 1999 : 103-123) During the last decade, several in vivo animal studies have used biologic mediators such as polypeptide growth factors to obtain soft and hard tissue regeneration. (Arm, et al.,1996; Fleming, Cornell and Muschler,2000; Gordh, et al.,1999; Herndon, Nguyen and Glipin, 1993; Khan, Bostrom and Lane, 2000; Lind, 1996; Mellonig, 2000; Miller, et al., 1991; Minamide, et al., 2001) In the our knowledge of wound healing and by extension bone graft regeneration has been greatly enhanced by the identification and understanding of growth factors and the technologic means to use them. However, the use of growth factors are limited to clinical research due to there are hard to purify, very limited available and very expensive. Moreover, the recent studies, PRP, autogenous growth factors obtained from platelet, was might be used as alternative other growth factors such as BMPs.

The combination of various regenerative biologic agents, such as growth factor, and techniques has recently attracted the interest of researchers in the field of reconstructive surgery. The ideal bone substitute should be has the osteoinductive

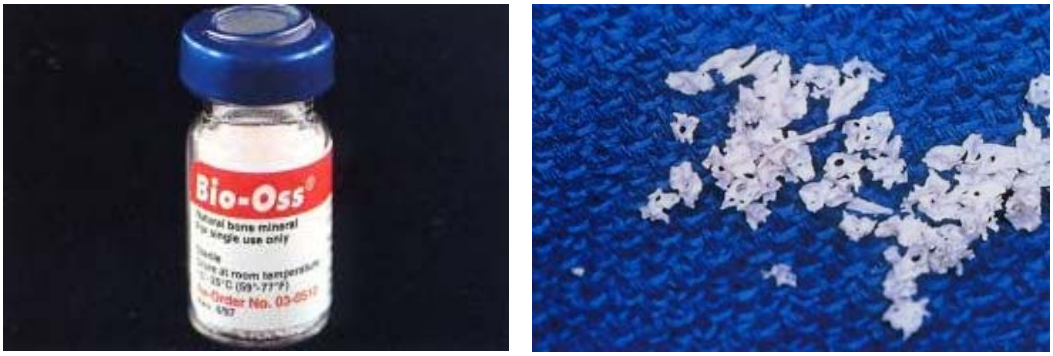
property to stimulate osteogenesis and the osteoconductive property to provide a scaffold for establishing optimal conditions for ingrowth of blood vessels and osteoblastic cells. (Garg, 1999: 83-101; Fleming, Cornell and Muschler, 2000; Herndon, Nguyen and Glipin, 1993; Khan, Bostrom and Lane, 2000; Lekovic, et al., 2002; Lynch, et al., 1991; Meraw, et al.,2000; Minamide, et al., 2001). Anorganic bovine bone is a hydroxyapatite bone substitute which is able to support osteoblastic cell attachment and growth, which are critical initial steps in process of osteogenesis. To enhance the osteogenic response, it may be possible to employ anorganic bovine bone as a matrix in combination with collagen and/ or growth factors. Anorganic bovine bone can provide the matrix support for the regulation of bone formation by three mechanisms: first, it acts as an insoluble space filling material, second, it provides a matrix which favors osteoblast attachment and proliferation, third, it may be able to provide a vehicle for the delivery of other factor which stimulate bone formation. (Stephan, et al.,1999) From the previous studies, Bio-Oss® has been used alone as an osteoconductive bone graft substitute in both experimental and clinical applications. (Artzi and Nemcovsky, 1998; Artzi, Nemcovesky and Tal, 2001; Boyne, 1997 : 87-100; Camelo, et al., 1998, 2001; Haas, et al.,1998; Hammerle, et al.,1998; Klinge, et al., 1992; Richardson, et al.,1999; Ruhaimi, 2001; Schmitt, et al., 1997) Moreover, these material may serve as carriers for bone inductive factors such as BMPs or other biological agent, such as PDGF, TGF-β and IGF-I may be harnessed for therapeutic intervention. (Ahn, et al., 2003; Gordh,et al., 1999; Hallman, et al., 2001; Jiang, et al., 1999; Kassolis, Rosen and Reynold, 2000; Kim and Lim, 2001; Lekovic, et al., 2002; Rodriguez, et al., 2003; Sculean, et al., 2002; Stephan, et al., 2000) The combination of these factors with bone mineral or organic matrix components of bone is potentially an effective method by which local osteoblastic cell modulation of bone formation could be induced. Jiang D and Stephan EB suggested that PDGF-BB did not effect cell attachment to the bovine mineral-collagen, so that the observed increased in proliferation on the PDGF-BB treated matrix can thus be attributed to growth factor stimulated proliferation of attached cells. (Jiang, et al., 1999; Stephan, et al., 2000) From these studies suggest a

possible role for the use of PRP growth factor with an anorganic bovine bone mineral matrix to stimulate bone regeneration.

Review of literatures

Characterization of Bio-Oss® (Boyne, 1997 : 87-100)

Bio-Oss® is a finely crystalline, carbonated apatite pratically identical to natural human bone mineral (fig.1). The chemical extraction process makes possible the desirable properties of this material. Bio-Oss® retains the natural mineral content of bone, which has a more complex composition than synthetic hydroxyapatites. Bio-Oss® bone consists of very tiny crystals similar to those of human bone. The spongiosa structure of Bio-Oss® shown in fig.2 demonstrates the wide interconnective pore system of natural bone mineral.



A. B.

Figure 1. A. Commercial package of Bio-Oss®. (from Garg AK. Grafting materials in repair and restoration. In : Lynch SE , Genco RJ , Marx RE, editors. Tissue engineering : applications in maxillofacial surgery and periodontics. Illinois : Quintessence publishing ,1999 ; 83-101)

B. Bio-Oss® particle. (from Boyne PJ. Characterization of xenogenic bone material. In : Boyne PJ, Peetz M , editors. Osseous reconstruction of the maxilla and mandible : surgical techniques using titanium mesh and bone mineral. Illinois : Quintessence publishing,1997;87-100.)

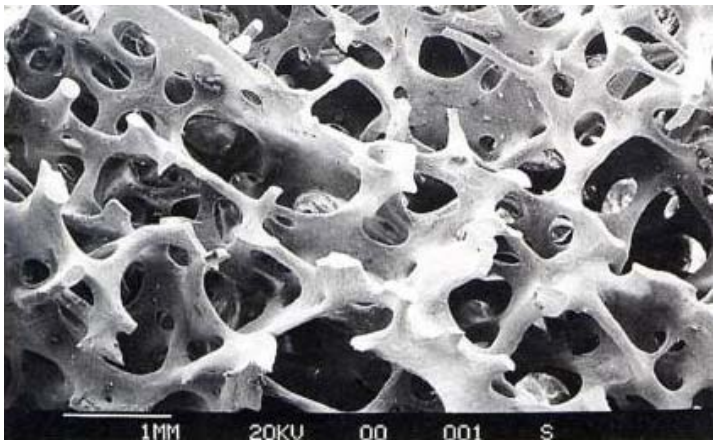


Figure 2. Scanning electron micrograph of Bio-Oss® cancellous structure that similar human concellous bone.(from Boyne PJ. Characterization of xenogenic bone material. In : Boyne PJ, Peetz M , editors. Osseous reconstruction of the maxilla and mandible : surgical techniques using titanium mesh and bone mineral. Illinois : Quintessence publishing,1997;87-100.)

Bio-Oss® consists of three pore systems: 1.macropores:pore in a visible range of 300-1500 μm 2.micropores: typical haversian canals and smaller vascular marrow channels in the bone structure 3.intercrystalline spaces:small pores in the range of 3-26 nm that have a overall of porosity of graft materials about 70-75%. Also these pore systems result in an overall porosity of 70% to 75%, when the material such as Bio-Oss® natural bone minerals is placed into a given defect, it usually occupies only 25% to 30% of the defect, leaving 75% of the space for regeneration of new bone tissue. The pore of porous hydroxyapatite bone substitute provide a passive scaffold for vascular ingrowth, which then leads to osteogenic cell ingrowth and bone apposition. The pore system of Bio-Oss® is architecturally structured to allow vascularization of new bone. The structure similarity of Bio-Oss® to cancellous bone implies that the pore size of this implant is well within this range and explain to a substantial extent they early and effective bone apposition observed in these implant. Growth penetration of bone tissue is only ensured if pore diameter is at least 100 μm and formation of osteo-like

structures require a pore diameter of 200 μm. (Boyne, 1997 : 87-100; Garg, 1999; Pallesen, et al.,2002) Bio-Oss® has a complex surface ,which consist of biologic apatite as bone layer. The osteoblastic cell can recognize and used this surface for deposition of new bone. This surface condition enhances the penetration of host bone repair into the inner part of the graft material. Bio-Oss® reaches a surface of almost 100 m^2/g. This huge surface allows it to achieve intimate contact with osteoblasts and pluripotential osteogenic cells. (Boyne, 1997 : 87-100; Kim and Lim, 2001; Schmitt, et al., 1997) Bio-Oss® may easily be penetrated and invaded by host bone forming cells. Moreover Bio-Oss® has compressive strength and modulus of elasticity similar to the natural human bone, whereas the synthetic hydroxyapatite has higher compressive strength and modulus of elasticity. The higher stiffness and lower flexibility of synthetic hydroxyapatite was cause of the stress at interface of the graft materials under long-term function. The stress at interface of the synthetic hydroxyapatite can make the microfractures between graft and host bone , which lead to fibrous encapsulation around synthetic bone substitute. (Boyne, 1997 : 87-100; Kim and Lim, 2001) The investigations have shown Bio-Oss® to have a natural morphologic structure; a chemical composition identical to that of bone; a large inner surface and porosity comparable to that of bone; a crystalline structure identical to that of bone tissue and a composition that is purely anorganic. Many studies were shown, the physical and chemical properties of Bio-Oss® are host compatible, offering excellent conductive surfaces for the promotion of bone repair. (Artzi and Nemcovsky, 1998; Cohen, et al., 1994; Haas, et al., 1998; Hammerle, et al., 1997, 1998; Klinge, et al.,1992; Valentini, et al., 1997) In addition, bovine derived bone xenograft (Bio-Oss®) is safe because many studies have been reported that a systemic or local immune response does not develop following implantation with Bio-Oss® and the risk of disease transmission has been calculated to be $1X10^{-18}$. (Sogal and Tofe, 1999; Wenz, Oesch and Horst, 2001) Although, Bio-Oss® have been widely used and reported for the high successive rate, but Bio-Oss® has only the osteoconductive material. (Alberius, et al., 1990; Boyne,

1997 : 87-100; Garg,1999 : 83-101; Jensen, et al.,1996; Pripatnanont, Nuntanaranont and Chungpanich, 2002; Schwartz, et al., 2000)

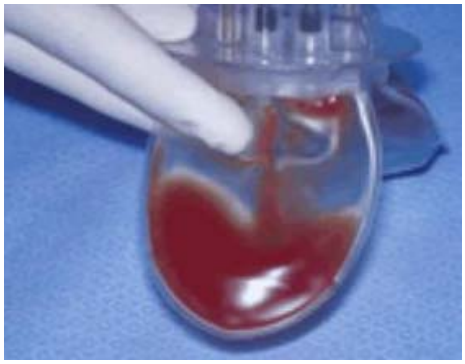
The growth factors are a class of natural biologic mediators that regulate key cellular events in tissue regeneration, including cell proliferation, chemotaxis, differantiation and matrix synthesis via binding to specific cell surface receptors. The growth factors that seem to play an important role in periodontal and bone healing are platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), Insulin-like growth factor (IGF-I), bone morphogenetic proteins (BMPs). (Herndon, Nguyen and Glipin, 1993; Lind,1996) In the cell culture studies support a significant role for PDGF, TGF-β, IGF-I in the wound healing process by demonstrating its ability to stimulate chemotaxis, cellular activation and proliferation in fibroblasts and osteoblast cells. Jiang D (Jiand, et al.,1999) were examined the interaction of this bone graft material with PDGF-BB and IGF-I and determine if the combination of growth factors with the matrix could stimulate osteoblastic cell proliferation. Adsorption of PDGF-BB or IGF-I was done using ^{125}I radiolabeled growth factors. The PDGF-BB or IGF-I was incubated with the anorganic bovine bone matrix and the amount of adsorbed growth factor was measured. In the desorption studies, radiolabeled growth factors were adsorbed to the matrix material. The samples were incubated in buffer for various time periods and the amount remaining on the matrix was measured to calculate the percentage of released growth factor. The biological activity was tested in an in vitro assay with primary culture neonatal rat osteoblastic cells. Porous bone matrix with known amounts of adsorbed PDGF-BB or IGF-I was produced. The osteoblastic cells were cultured on the bone mineral matrix, with and without adsorbed growth factor and proliferation was assessed by ^{3}H-thymidine incorporation. Both PDGF-BB or IGF-I adsorbed to bone mineral matrix in a concentration dependent fashion. The affinity of IGF-I for the material was 10 fold greater than PDGF-BB. In the experiments that measured the release of the initially adsorbed growth factors, approximately 50% of the PDGF-BB and 10% of IGF-I were released after 10 days. PDGF-BB adsorbed to the matrix material significantly enhanced the proliferation of cultured osteoblastic cells compared to the mineralized

matrix alone. However, IGF-I adsorbed to the matrix material did not significantly enhance cell proliferation. They concluded that PDGF-BB can be adsorbed to the anorganic bovine bone mineral matrix and that this growth factor subsequently enhance the osteogenic properties of this bone graft material. IGF-I also adsorbed to the graft material; however, it was not readily released and it did not produce significantly effects in the biologic assay. It appears that it may be clinically feasible to adsorb PDGF to anorganic bovine bone and that this combination of bone growth factor and mineral matrix has the potential for clinical applications. Stephan EB (Stephan, et al., 2000) were examined the interaction of this mineral-collagen matrix with PDGF-BB and determine if the adsorption of PDGF-BB to mineral-collagen matrix stimulates osteoblastic cell proliferation above that of the untreated matrix. Measurement of PDGF-BB adsorption and release was accomplished using ^{125}I radiolabeled growth factor. The PDGF-BB was incubated with the anorganic bovine bone-collagen matrix and the amount which adsorbed was determined. In the release studies, radiolabeled PDGF-BB was adsorbed to the matrix material, then the samples were incubated in buffer for various time periods. The amount of PDGF-BB retained on the matrix was measured and the percent of growth factor released calculated. The biological activity was tested in an in vitro assay with primary culture neonatal rat osteoblastic cells. Osteoblastic cells were cultured on bone mineral-collagen matrix with known amounts of adsorbed PDGF-BB. Proliferation of the cells was assessed by ^{3}H-thymidine incorporation and cell attachment measured by prelabelling cells with ^{3}H-leucine. PDGF-BB adsorbed to the mineralized-collagen matrix material in a rapid, concentration-dependent fashion. The growth factor was slowly released from the matrix such that approximately 30% of the adsorbed protein was liberated over 10 days. PDGF-BB treated mineralized-collagen matrix displayed significantly enhanced proliferation of cultured osteoblastic cells compared to the mineralized-collagen matrix alone. They concluded that PDGF-BB is rapidly adsorbed then slowly released from the anorgnic bovine bone-collagen matrix. PDGF-BB adsorbed to this material is able to stimulate proliferation of the attached osteoblastic cells. Despite their potential usefulness, animal derived or genetically engineered the growth factors are still not available for routine use in pratice because

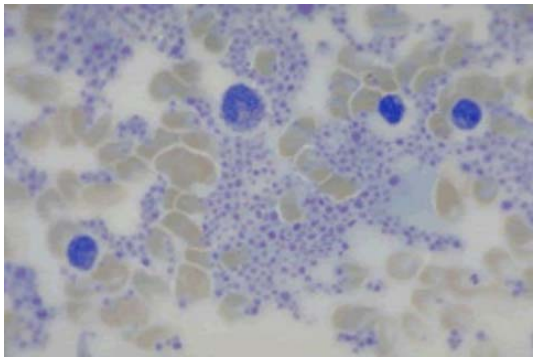
their safety and effectiveness have not been completely confirmed. (Herndon, Nguyen and Glipin,1993; Lind,1996) Moreover, the growth factors are hard to purify and therefore very expensive to buy. At this moment the use of growth factors is limited to clinical research and they are not commercially available for clinical use. While looking for an available and affordable treatment modality that provided the benefits of growth factors, Marx was used the autologous growth factors obtained from platelets as an alternation to the use of either native or recombinant human growth factors. (Marx, et al.,1998) Platelets produce and release multiple growth factors that are critical for stimulation and regulation of wound healing , including PDGF, TGF-β, IGF-I. Platelet rich plasma (PRP) is rich in the platelet, which in release growth factors such as PDGF, TGF-β_1 , TGF-β_2 and IGF-I. (Garg, 2001; Marx, et al.,1998; Rudkin and Miller, 1997) In the recent publications conclude that PDGF and TGF-β, alone or in combination, increase tissue vascularity, promote fibroblast proliferation, increase the rate of collagen and granulation tissue production and enhance osteogenesis. (Arm, et al.,1996; Lind, 1996; Marx, et al.,1998) The possibility of bone graft enhancement has recent interested researchers and clinicians in exploring the potential of PRP for use in implant, periodontal and oral and maxillofacial surgery. (Garg, 2000; Marx, 1999: 71-82; Marx and Garg, 1999: 183-189) The strategy is to accelerate and enhance the effect of growth factors contained in platelets to wound healing. This can be done today by use of autologous platelet rich plasma (PRP) which is both nontoxic and nonimmunoreactive. Therefore, the amplification of the influence of PDGF and TGF-β through the technique of platelet sequestration and concentration into a PRP is seen as an available and practical tool to enhance the rate of bone formation and the quantity of bone formed. (Anitua, 1999; Garg, 2000; Gonshor, 2002; Landesberg, Roy and Glickman, 2000; Lozada, et al., 2001; Rosenberg and Torosian, 2000; Weibrich, et al., 2001, 2002) The proposed value of this product in dental implantology and in bone augmentation procedures lies in the ability to incorporate high concentrations of the growth factors such as PDGF, TGF-β_1, TGF-β_2 and IGF-I, as well as fibrin, into the bone graft material.

Platelet rich plasma (PRP):

PRP is an autologous source of growth factor that is obtained by sequestering and concentrating platelets by gradient density centrifugation (fig.3). PRP is procured from the centrifuge of autogenous whole blood and then combined with thrombin and calcium chloride to procedure a viscous coagulum gel capable of being introduced as a surgical graft material. The documented evidence demonstrate the release of a cascade of growth factors through the activation of the platelets with calcium chloride and thrombin. (Marx, et al., 1998; Man, Plosker and Winland-Brown, 2001) In addition, PRP was prepared from the patient's own blood, the risk of disease transmission is virtually eliminated, making it a safe treatment modality. (Garg, 2001; Landesberg, Moses and Karpatkin, 1998; Marx, et al., 1998; Whitman, Berry and Green, 1997)



A.



B.

Figure 3. A. Platelet rich plasma (PRP) is a source of growth factor to support bone and soft tissue healing. (from Marx RE. Platelet-rich plasma : a source of multiple autogenous growth factors for bone grafts. In : Lynch SE , Genco RJ , Marx RE , editors. Tissue engineering : applications in maxillofacial surgery and periodontics. Illinois : Quintessence publishing ,1999;71-82.)

B. PRP smear was showed the greatly increased platelet density. (from Marx RE, Carlson ER, Eichstaedt RN, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85:638-646.)

The autogenous growth factors in PRP. (Marx, et al., 1998; Marx, 1999: 71-82)

The growth factors released from the platelets are included Platelet-derived growth factor, transforming growth factor β and insulin-like growth factor. These growth factors are a diverse group of polypeptides that have important roles in the soft tissue and bone regeneration.

Platelet-derived growth factor (PDGF). (Marx, et al., 1998; Marx, 1999: 71-82)

PDGF is a glycoprotein with a molecular mass of approximately 30 kd. It exists mostly as a heterodimer of two chains, termed A and B chains, of about equal size and molecular mass (approximately 14 to 17 kd). Homodimers of A-A and B-B chains are also present in human platelets and have the same effects on bone regenration. Although it is the primary growth factor in platelets, it is also synthesized and secreted by other cells, such as macrophages and endothelial cells. PDGF appears to be the first growth factor present in a wound and initiates connective tissue healing, including bone regeneration and repair. The most important specific activities of PDGF include mitogenesis, increase in the cell populations of healing cells, angiogenesis, endothelial mitoses into functioning capillaries, up-regulation of other growth factors and cells, resulting in promotion of fibroblastic and osteoblastic functions, cellular differentiation and acceleration of the effects of growth factors on other cells such as activated macrophages activities for debridement of the wound site and act as a second-phase source of growth factors for continued repair and bone regeneration. There are approximately 0.06 ng of PDGF per 1 million platelets, or about 1200 molecules of PDGF per individual platelet, underscoring this molecule's great potency. It is theorized that this enhanced quantity of PDGF initiates the osteocomponent cellular activity more completely than what will inherently occur in the graft and clot milieu alone. In addition, the enhanced fibrin network created by PRP is believed to enhance osteoconduction throughout the graft supporting consolidation.

Transforming growth factor beta (TGF-β). (Marx, et al., 1998; Marx, 1999: 71-82)

TGF-β is a term applied to a superfamily of growth and differentiating factors, of which the13 described BMPs are members. The TGF-βs proven to exist in PRP are

the TGF-β_1 and TGF-β_2 proteins.The TGF-β_1 and TGF-β_2 proteins are the more protein and generic growth factors, involved with general connective tissue repair and bone regeneration. Both TGF-β_1 and TGF-β_2 are proteins with molecular masses of approximately 25 kd. They are synthesized and found in platelets. They are also synthesized and found in macrophages, osteoblasts and some other cell types. These TGF-β proteins represent a mechanism to sustain a long term healing and bone regeneration module and even evolve into a bone remodeling factor over time. When released by platelet degranulation, or actively secreted by macrophages, they act as a paracrine growth factor such as fibroblasts, marrow stem cells and preosteoblasts. However, each of these target cells also has the ability to synthesize and secrete its own TGF-β proteins to act on adjacent cells as a paracrine growth factor, and to act on its own cell membrane, as an autocrine growth factor, to direct, alter, or maintain a certain activity. Therefore, TGF-β represents a growth factor mechanism that not only can initiate bone regeneration but also can sustain long term healing and bone regeneration, including bone remodeling of a maturing bone graft. The most important functions of TGF-β_1 and TGF-β_2 appear to be the chemotaxis and mitogenesis of osteoblast precursors and the ability to stimulate their deposition of collagen matrix of wound healing and of bone. In addition, the two TGF-β proteins inhibit osteoclast formation and bone resorption, thereby favoring bone formation over resorption by two different mechanisms.

Insulin-like growth factor-I and Insulin-like growth factor-II (IGF-I , IGF-II). (Marx, et al., 1998; Marx, 1999: 71-82)

IGF-I and IGF-II are usually thought of as growth factors secreted by osteoblasts during bone formation to increase numbers of osteoblasts and thereby accelerate bone deposition. IGFs are also deposited in bone matrix, when the bone matrix is resorbed, IGFs are released to couple new bone formation to bone resorption. The presence of IGF in platelets would be expected to act on precursors of osteoblasts and on endosteal osteoblasts, which are the cells that produce the initial phase 1 bone in bone grafts. IGFs are therefore mitogenic to osteoblast lineage cells and are also

stimulators of bone formation from existing differentiated osteblasts. It is not likely that IGFs can actually guide differentiation toward bone formation as members of the TGF-β family, particularly the BMPs, are known to do. Both IGF-I and IGF-II are relatively small proteins with molecular masses of 7.7 kd and 7.5 kd, respectively. They each bind to a specific IGF cell membrane receptor that excites kinase activity to a cytoplasmic signal protein. The end result of IGF binding is mitogenesis of bone forming cells.

The role of platelet growth factors in bone regeneration. (Marx, et al., 1998; Marx, 1999: 71-82)

Within the graft, whether for a mandibular continuity defect, a sinus augmentation surgery or dental implant, is placed in a dead space filled with clotted blood. The platelets entrapped in the clot degranulate within hours of graft placement, releasing PDGF, TGF-β_1 , TGF-β_2 . Both of these factors begin the bone regenerative process. PDGF binds to endothelial cells to initiate the ingrowth of capillaries, while TGF-β_1 and TGF-β_2 bind to the endosteal osteoblasts and marrow mesenchymal stem cells to initiate mitosis to increase their numbers as well as stimulate their production of osteoid. This continues during the first 3 days of the graft, at which time capillaries are already seen to be entering the graft. However, by this time, the platelets have degranulated and are no longer a primary source of growth factors to drive the bone regenerative process. At this time, macrophages take over this role. Macrophages were initially attracted to the graft as circulating monocytes of free tissue cells by the inherent oxygen gradient in the graft. The hypoxia in the graft of 0 to 5 mmHg oxygen tension, compared to the normoxia of the adjacent tissue, which is 45 to 55 mmHg oxygen tension, creates an oxygen differential greater than the 20 mmHg oxygen tension to which the macrophage is programmed to respond. Thus, macrophages, which are very prolific and efficient synthesizers of growth factor, drive the remaining bone regeneration of the graft and healing. Essentially, macrophages secrete the same PDGF and TGF-β proteins, but also secrete basic fibroblast growth factor and vascular endothelial growth factors, among others. Thus, the inherent properties of the wound, particularly the oxygen gradient, PDGF and TGF-β, initiate early angiogenesis from

surrounding capillaries and mitogenesis of the transferred osteocomponent cells. The complete revascularization of the graft is seen by day 14. By this time, the endosteal osteoblasts have already laid down osteoid on the original bone trabeculae and the marrow stem cells have dramatically increased in number and have begun differentiating into osteoblasts. As capillaries revacularize the graft, they effectively reduce the oxygen gradient, thus creating a shut-off mechanism to the macrophage, which prevents excessive angiogenesis. During the first 3 to 7 days, the stem cell population and endosteal osteoblasts produce only a small amount of osteoid. The initial formation of osteoid develops on the surface of the mineralized cancellous trabeculae from the endosteal osteoblasts. Shortly thereafter, individual osteoid islands develop between the cancellous bone trabeculae, presumably from the stem cells transferred to the graft. A third source of osteoid production developed from circulating stem cells, which are also attracted to the biochemical environment of the wound. It is believed that these stem cells seed into the graft, where they proliferate and contribute to the osteoid production.

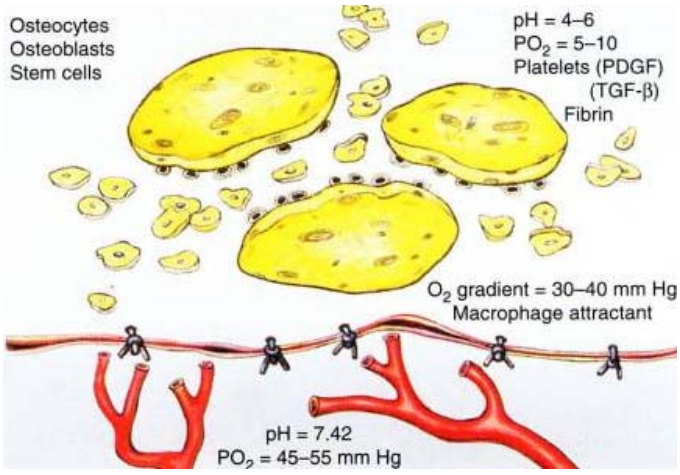


Figure 4. Platelet entrapped in the blood clot degranulate and released growth factors such as PDGF, TGF-β, IGF-I. These growth factors begin the bone regenerative process. (from Marx RE. Platelet-rich plasma : a source of multiple autogenous growth factors for bone grafts. In : Lynch SE , Genco RJ , Marx RE , editors. Tissue engineering :

applications in maxillofacial surgery and periodontics. Illinois : Quintessence publishing ,1999;71-82.)

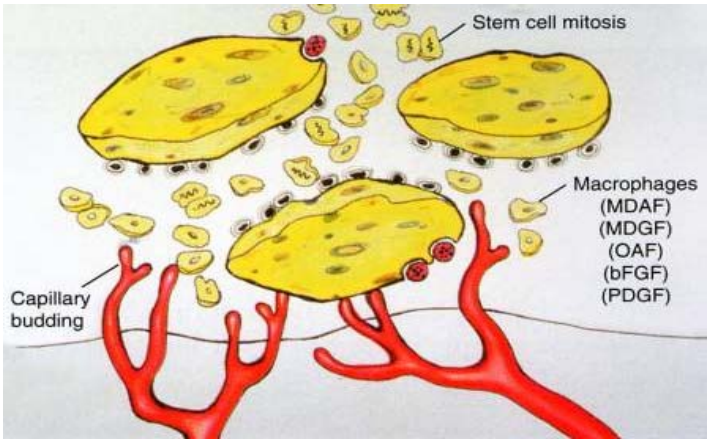


Figure 5. During the first 3 days of graft placement, capillaries begin entering the graft. Macrophage release other growth factors such as macrophage derived angiogenic factor (MDAF), macrophage derived growth factor (MDGF), osteoclast activating factor (OAF), basic fibroblast growth factor (bFGF), platelet derived growth factor (PDGF). (from Marx RE. Platelet-rich plasma : a source of multiple autogenous growth factors for bone grafts. In : Lynch SE , Genco RJ , Marx RE , editors. Tissue engineering : applications in maxillofacial surgery and periodontics. Illinois : Quintessence publishing ,1999;71-82.)

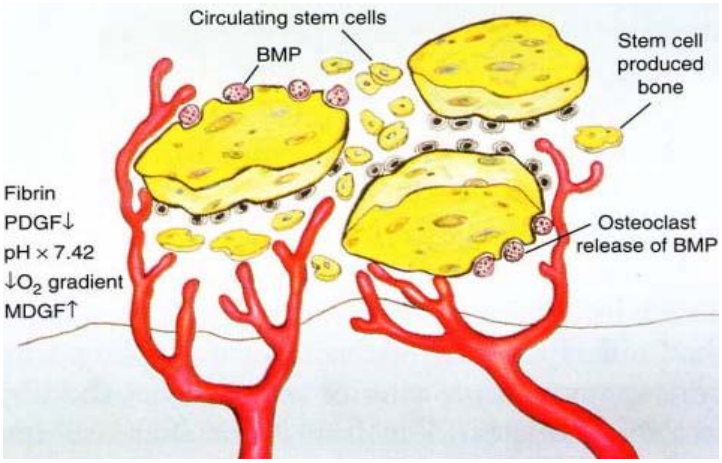


Figure 6. Completed revascularized of the graft is seen by day 14. The osteoblast cells are now sustaining themselves with their own autocrine production of TGF-β.(from Marx RE. Platelet-rich plasma : a source of multiple autogenous growth factors for bone grafts. In : Lynch SE , Genco RJ , Marx RE , editors. Tissue engineering : applications in maxillofacial surgery and periodontics. Illinois : Quintessence publishing ,1999;71-82.)

<u>The bone regeneration process</u>:

Phase 1 bone regeneration. (Marx and Garg, 1999: 183-189)

During the first 3 to 4 weeks, this biochemical and cellular phase of bone regeneration proceeds to clinically consolidate the graft by coalescing individual osteoid islands, surface osteoid on cancellous trabeculae and host bone. This process is essentially transplanted osteogenesis. However, it uses the fibrin network of the grafts as a framework. This is referred to as osteoconduction, which provides a scaffold for what has been call “creeping substitution”. That is, the normally nonmotile osteoblasts can be somewhat motile via the process of endocytosis along a scaffold like fibrin. The process of endocytosis is merely the transfer of cell membrane from the retreating edge of the cell, through the cytoplasm as a vesicle, to the advancing edge to reform a cell membrane and thus increase the cellular surface area at the advancing edge. This mechanism slowly advances the cell and allows it to secrete its product

in the process. In this case, the product is osteoid on the fibrin network. This cellular regeneration is often referred to as phase 1 bone regeneration or woven bone phase. By the time it is nearly complete (4 to 6 weeks), sufficient osteoid production and mineralization have occurred to permit graft function. At this stage, the bone has formed without going through a chondroblastic phase and histologically appears as random cellular bone that a pathologist would refer to as woven bone.

Because the amount of bone formed during phase 1 will depend on osteocomponent cell density, donor sites with the highest cancellous trabecular bone are chosen. In rank order, the posterior and anterior iliac and mandibular symphysis have been found to be potential donor sites with greater cancellous bone than the calvarium, rib. In addition, enhanced phase 1 bone yields are achieved by compacting the graft material. Technically, this is often accomplished with a bone mill, followed by syringe compaction, and then by further compaction into the graft site with bone packing instruments.

Phase 2 bone regeneration. (Marx and Garg, 1999: 183-189)

The cellular bone regeneration that has occurred in phase 1 produces this disorganized woven bone that is structurally sound but not to the degree of mature bone. The random organization and hypercellular nature of this bone is similar to that seen in a fracture callus. This bone will undergo an obligatory resorption and replacement type of remodeling. Eventually, it is replaced by phase 2 bone, which is less cellular, more mineralized,and structurally more organized into lamellar bone.

The replacement of phase 1 bone by phase 2 bone (woven bone by lamellar bone), like all bone remodeling, is initiated by osteoclasts. Osteoclasts are fused mononuclear cells that arrive at the graft site through the newly developed vascular network. It is theorized that these osteoclasts resorb phase 1 bone in a normal remodeling-replacement cycle. As both the phase 1 bone and the nonviable original cancellous bone trabeculae are resorbed, bone morphogenetic protein and IGF-I and IGF-II are releases. As with normal bone turnover, BMPs,IGF-I and IGF-II act as the link between bone resorption and new bone apposition. Such growth and differentiation

factors are deposited into the mineral matrix of bone by osteoblasts during osteoid production. Stem cells in the graft from the original transplantation and newly arrived stem cells from local tissues and the circulation respond to the released BMPs, IGF-I and IGF-II by osteoblast differentiation and new bone formation. This new phase 2 bone forms as the jaw and graft are in function. It responds to the demands placed on it and develops mature haversian systems and lamellar bone capable of withstanding the normal shear forces placed on the jaw through opening and closing functions. The bone is capable of tolerating impact compressive forces typical of denture and implant-borne prosthetic functions. Histologically, such grafts enter a long-term remodeling consistent with normal skeletal turnover. A periosteum and endosteum develop as part of this long-term remodeling cycle. The graft cortex never becomes as thick as a normal jaw cortex, and the graft itself retains a dense, cancellous trabecular pattern. This pattern is advantageous in promoting osseointegration and is adaptable to a variety of functional stresses. Over several years, the graft takes on the radiographic morphology and cortical outlines of a mandible or maxilla.

From above mention, PRP has been clinically applied to enhance wound healing and improve the osseous wound healing of autogenous bone grafts in both quality and quantity. (Anitua, 1999; Garg, 2000; Kassolis, Rosen and Reynold, 2000; Marx, et al., 1998; Whitman, Berry and Green, 1997) Autologous PRP has been used widely in thoracic and cardiac surgery in the past and has only recently come into use in oral and maxillofacial surgery. (Anitua, 1999; Fennis, Stoelinga and Jansen, 2002; Kim, et al., 2002; Marx, et al., 1998; Robiony, et al., 2002; Zechner, et al., 2003) In the recent reports suggest that PRP may enhance the formation of new bone when used in combination with autogenous bone. The previous work and new knowledge of growth factor influences, Marx et al model for the bone regeneration observed in cancellous cellular marrow grafts has been developed. **Marx RE** (Marx, et al., 1998) were proposed a model of bone regeneration that is observed in cancellous bone grafts. 88 cancellous cellular marrow bone graft reconstructions of 5 cm or greater mandibular continuity defects; 44 received grafts with PRP added, with 44 additional cases serving as a control. The PRP was prepared by the cell separator (Medtronics), 400 to 450 ml of

autologous whole blood was drawn into blood collection bag contain citrate phosphate dextrose (CPD) at a ratio of 1 ml of CPD to 5 ml of blood for anticoagulation. Two steps of centrifugation was used at speed of 5,600 rpm and 2,400 rpm, then the blood was separated into three basic component following gradient density; RBC at the lowest level, PRP in the middle, PPP on the top. The PRP was collected and the upper 1 mm of RBC was included in the PRP product. The PPP and RBC were returned to the patient from their collection bags. The PRP and autogenous bone graft were stained with PDGF and TGF-β monoclonal antibody stains. A mixture of 10 ml of 10% calcium chloride mixed with 10,000 units of bovine thrombin. 6ml of PRP, 1 ml of calcium chloride/thrombin and 1 ml of air, act as a mixing bubble, were mixed in 10 syringe. The syringe was agitated for 6 to 10 seconds to initiate clotting. The PRP, now a gel, was added to the graft and placed into defect. The bone graft was allowed to consolidate and mature for 6 months. Monoclonal antibody staining demonstrated uptake of PDGF and TGF-β in platelets of PRP preparations. The cancellous bone graft was demonstrated positive results when tested with monoclonal antibodies for PDGF receptors and TGF-β receptors. The mean whole blood platelet count of 232,000 with a range of 111,000 to 523,000 while mean PRP platelet count of 785,000 with a range of 595,000 to 1,100,000. The mean platelet count of PRP was increase of 338% from platelet count of whole blood. The grafts with PRP added showed a radiographic maturation rate that was 1.62 to 2.16 times that of the grafts without PRP. Histomorphometry demonstrated, a greater bone density in the PRP added sited (74% $\pm$ 11%) than in the sites where PRP was not added (55.1% $\pm$ 8%). They concluded that the amplification of PDGF and TGF-β through the PRP preparation technique was available and pratical tool for enhancing the rate of bone formation and the final quantity of bone formed. **Kassolis JD** (Kassolis, Rosen and Reynold, 2000) reported the clinical and radiographic results from 15 consecutively treated patients using PRP with freeze-dried bone allografts for sinus elevation and ridge augmentations. PRP gel formed by mixing autologous thrombin-rich plasma with PRP (1:4 ratio) was used to cover the graft material. Total of 36 implants were surgically placed in this study. The

grafts were mixed at a ratio of 0.5 g : 2 ml PRP prior to insertion into the sinus. 32 of 36 implants (89%) implants were clinically successful and histologic sections revealed numerous areas of osteoid and bone formation around the freeze-dried bone allograft particles, with no evidence of inflammatory cell infiltrate. **De Obarrio JJ** (De Obarrio, et al., 2000) presented case reports that PRP gel was used in combination with demineralized freeze-dried bone allograft (DFDBA) for treatment periodontal osseous defect. 400 ml of whole blood was drawn from patient, mixed with acid citrate-dextrose (ACD-A) for anticoagulant and centrifuged by ELMD-500 (Medtronic) at 5,600 rpm and 2,400 rpm. The Blood was separated into three basic components, PPP and RBC were return to patient, but PRP was collected. 10 ml of 10% calcium gluconate was injected into 5,000 unit of bovine thrombin. 7ml of PRP, 2ml of air and 1 ml of thrombin/calcium gluconate were mixed in 10 ml syringe, within 10 seconds the contents was gel-like consistency that resulting from formation of fibrin, and cause the platelets degranulated and released PDGF and TGF-β contained in their granules. The platelet gel was mixed with DFDBA in a sterile cup and applied into the periodontal osseous defect. Results showed a significant bone fill of the periodontal osseous defects and radiographically showed a significant amounts of new bone as early as 2 months post-operative. **Fennis JPM** (Fennis, Stoeling and Jansen, 2002) were proposed a reconstruction method in goat mandible. 28 goats were divided in two groups of 14 animals each. In the first group of 14 animals, the bone marrow of the resected segment was removed. The cortical tray was filled with an autogenous bone graft taken from anterior iliac crest. The trays were placed back in their original position and fixed with two plate. The 14 goats in the second group underwent the same treatment, except that the bone graft was mixed with PRP. 250 ml of whole blood was drawn 2 days before surgery. The blood was treated by centrifugation at the laboratory in various cycles. PRP was obtained (paltelet count exceeding 800 X 10^9), PRP was activated at the time of surgery with 10% calcium chloride solution and 300 i.u. of bovine thrombin to form gel, while the erythrocytes were given back. After 3, 6 and 12 weeks respectively, the animals were sacrificed. The mandibles were retrieved for radiographic and histologic evaluation. All

specimens of both the first and second groups showed the same overall picture in that abundant bone formation. At the 6 and 12 weeks interval, however, the obliteration of bone gaps and cortical perforations appeared to indicated bone healing. Bone healing, as manifested by the obliteration of the bone gaps at the osteomies sites, was particularly present in the PRP group at 6 weeks. This appeared to be statistically significant. This is well illustrated by the presence of radio-opaque areas at the osteotomy gaps exclusively seen in PRP group at 6 and 12 weeks. The histologic and histomorphometry would be presented in the next report.

In addition, the improved technology has allowed the blood draw required for production of the final product to be reduced from 450 ml to a much more manageable 50 ml. This negates the need for autotransfusion of the used fraction to maintain patient fluid volume. Also the PRP can be prepared for office procedures by obtaining small volumes of up to 50 ml of whole blood, drawn presurgically, with the use of a general purpose tabletop centrifuge and simplified separation techniques, which the sufficient amounts of PRP can be obtained for most surgical procedure.[37,59,63,102,114,115] As the growth factor source is autologous, there is no risk of disease transmission associated with use of this technology. **Anitua E** (Anitua, 1999) used PRP in the preparation of future implant sites. Extraction sockets in 20 patients were divided for treated with PRP or left untreated as controls. 10 to 20 ml of whole blood with 10% trisodium citrate solution as anticoagulant, in 5 ml tube, were centrifuged at 160 G for 6 minutes at room temperature. 1 ml of PPP was discarded, the remaining plasma was collected including the upper 1 to 2 mm of RBC layer (1.2 ml of PRP) and transferred to Eppendorf tube and 50 μl of 10% calcium chloride was added. After 15 to 20 minutes a PRP gel was formed and easy to manipulate to place in the socket. The time delay between the PRP gel formation and the filling of the defect was standardized to 5 to 10 minutes. Wound biopsies between 10 and 16 weeks depending on patient availability. The sockets with PRP added exhibited greater buccolingual/palatal bone width, greater bone density and faster soft tissue coverage than controls. In control group showed connective tissue filling the main part of the defect, in clear contrast with the patients treated with

PRP group that showed more mature bone with better organized trabeculae and greater bone regeneration. They were concluded that PRP enhanced and accelerated bone regeneration and soft tissue closure. **Lekovic V** (Lekovic, et al., 2002) were presented a comparison of clinical effectiveness of 2 regenerative techniques for treatment intrabony periodontal defect : a combination of PRP/bovine porous bone mineral (BPBM)/GTR versus a combination of PRP/BPBM. 21 patients with matched pairs of interproximal defects that grater than 6 mm were included in this study. 10 ml of whole blood, 10% trisodium citrate solution as an anticoagulant. The blood were centrifuged at 5,600 rpm for 6 minutes, which resulted in the separation of three basic fractions; PPP, PRP, RBC. PRP was collected in conjunction with the top 1 to 2 mm of RBC layer since the latter is also rich in newly synthesized platelets. Coagulation of PRP was achieved by it combination with an equal volume of a sterile saline solution containing 10% calcium chloride and 100 unit/ml of sterile bovine thrombin. Cancellous BPBM (Bio-Oss®), particle size 0.25 to 1 mm, were mixed with the coagulated PRP preparation at a proportion of 1:1. BPBM/PRP mixture was then packed in the experimental defects using amalgam condenser to the level of surrounding bony walls. Care was taken not to overfill defects. In the PRP/BPBM/GTR group, a porcine-derived collagen membrane was adapted over the grafted defect. 6 months after the initial surgery, all clinical measurements were repeated and surgical reentries performed. Site treated with PRP/BPBM/GTR or with PRP/BPBM healed uneventful. Reduction in probing depth was slightly grater in the PRP/BPBM/GTR group, but not statistically significant. Defect fill was similar for both treatment group; 4.96 $\pm$ 1.28 mm on buccal and 4.78 $\pm$ 1.32 mm on lingual sites for PRP/BPBM/GTR group and 4.82 $\pm$ 1.34 mm on buccal and 4.74 $\pm$ 1.30 mm on lingual sites for PRP/BPBM group. Results of the current study essentially suggested that GTR added no clinical benefit to a combination of PRP and BPBM. They suggested that the fibrin component of PRP not only work as a hemostasis agent, but also adhere to the root surface and so may impede the apical migration of epithelial cells and connective tissue cells from the flap. It is reasonable to speculate that PRP may exert a GTR effect in the treated defects. They proposed that

the further study are necessary to assess the individual role played by PRP in clinical outcome achieved with its combination with BPBM (Bio-Oss®). **Robiony M** (Robiony, et al., 2002) were purposed a new method of restoring severe atrophic mandible using PRP during distraction osteogenesis. 5 patients, with severe atrophy of a complete edentulous mandible have been treated with a novel distraction procedure. Two hours before surgery, 55 to 60 ml of whole blood was drawn into sterile test tubes containing sodium citrate as anticoagulant. The blood was centrifuged for 15 minutes at 180G and for 15 minutes at 560 G. At the end of such procedure, 8 ml of PRP were obtained. To form gel, a mixture is added to the PRP of equal volumes of 10% calcium chloride and patient's serum, as source of autologous thrombin. This mixture is quickly added to the autologous bone in a constant volume ratio (40% of PRP and 60% of bone). Within 120 to 180 seconds, the formation of the autologous bone platelet gel is completed, and filled the distraction gap. After a latency period of 15 days, a distraction run of 0.5 mm/day and a 60 days period of consolidation, the distraction device was removed and implants were placed simultaneously. In all treated patients, planned distraction height was achieved with a considerable enhancement of bony regeneration and in all case it was possible to place implants at a planned time. **Kim SG** (Kim, et al., 2002) were assessed the efficacy of demineralized bone powder (DBP) alone or combined in a mixture with PRP used to enhance osseointegration of dental implants in a dog model. 10 mongrel dogs were selected for the study. A total of 30 dental implants, 10 mm in length and 4 mm in diameter, were inserted in the animal. In each dog, the defects were treated with one of the following three treatment modalities; 1.no treatment (control) 2.grafting with DBP or 3. grafting with DBP and PRP. 10 ml of blood was drawn, from each dog, into tube that contained 10 % trisodium citrate solution as an anticoagulant. The blood was centrifuged at 1,000 rpm for 10 minutes and 1,500 rpm for 10 minutes at room temperature. The blood was separated into its three basic components, PPP, PRP, RBC. 50ml of 10% calcium chloride and 1,250 unit of thrombin were added to the DBP mixture and PRP to activate platelets in the PRP preparation, after 10 to 15 minutes, PRP gel was formed. The mean platelet count of whole blood

was 443,000/mm^3 with a range of 400,000 to 505,000/mm^3. The mean platelet count of PRP was 1,735,000/mm^3 with a range of 1,520,000 to 2,005,000/mm^3, a measured increased of 392%. At 6 and 12 weeks after implantation, the animals were sacrificed. Histologic analysis showed that all of the bone defects surrounding the implants that were treated with DBP, with and without PRP, were filled with new bone. The defects that were not treated (control) showed new bone formation only in the inferior threaded portion of the implants. Histomorphometric results revealed a higher percentage of bone contact with DBP and PRP compared with control and DBP. They suggested that bone defects around titanium implants can be treated successfully with DBP and that PRP may improve bone formation. **Aghaloo TL** (Aghaloo, Moy and Freymiller, 2002) were evaluated the effect of platelet rich plasma (PRP) on bone healing. In this study, the fifteen rabbits were used in the randomized, blinded, prospective pilot study. Four equal 8 mm diameter cranial bone defects were created and immediately grafted with autogenous bone, PRP alone, autogenous bone and PRP, and no treatment as a control. The defects were evaluated by digital subtraction radiography with step-wedge calibration, histology and histomorphometric analysis performed at 1,2 and 4 months. The results showed a significant increase in histomorphometric bone area and radiographic bone density in both bone and bone with PRP samples as compared with the control and PRP alone. No significant increase in bone formation was seen with the addition of PRP to autogenous bone. No significant difference in bone formation was seen between defects treated with PRP alone and control sites. They concluded, no significant improvement, radiographically or histomorphometrically, was seen with the additon of PRP in bone formation in noncritical sized defects in the rabbit cranial model. However, bone and bone with PRP showed a histomorphometric tendency toward increased bone formation at 1,2 and 4 months. **Maiorana C** (Maiorana, et al., 2003) presented the preliminary clinical and histologic evidence on the combination of a platelet rich plasma and anorganic bovine bone in maxillary sinus augmentation procedures. The clinical and histologic results from 10 patients underline the different clinical and biologic advantages offered by the combined use of a well-know

osteoconductive material, anorganic bovine bone (Bio-Oss®), and a new technique that consists of stimulating bone regeneration using the local increase of growth factors contained in autogenous PRP. **Rodriguez A** (Rodriguez, et al., 2003) investigated the clinical applicability of using deproteinated bovine bone (Bio-Oss®) mixed with autogenous platelet rich plasma (PRP) in human maxillary sinus augmentations in severely resorbed posterior maxillary alveolar processes with simultaneous insertion of endosseous dental implants. In this study, the fifteen patients with less than 5 mm of residual alveolar bone height in the posterior maxillary alveolus underwent a total of 24 maxillary sinus augmentations. Seventy endosseous implants were inserted simultaneously in the grafted sinuses. The implants were uncovered and loaded 4 months after insertion and the sinus augmentation. An osseous biopsy specimen was obtained from the augmented maxillary sinus in 1 patient. In 3 patients, computed tomography scans of the grafted maxillae were obtained and the bone density quantified and compared with native bone density using SIMPlant 7 (Columbia Scientific, Columbia, MD) software 4 months postoperatively. In the result, although a total of 5 implants in 4 patients were lost, this did not result in the loss of any of the restorations, for an overall success rate of 92.9%. Follow-up for patients in this study after insertion of the permanent restoration was between 6 and 36 months. The bone biopsy from the patients showed evidence of viable new bone formation in close approximation to the xenograft. The bone density of the grafted bone was similar or exceeded the bone density of the surrounding native maxillary bone. They believed that the use of platelet rich plasma in combination with deproteinated bovine bone is effective for maxillary sinus augmentation with simultaneous insertion of endosseous dental implants in severely resorbed posterior maxillae.

Although many studies were shown that PRP was supposed to increase proliferation of undifferentiated mesenchymal cells and to enhance angiogenesis. However, it has been controversy about the effect of PRP in promoted bone formation. Despite increasing application of PRP for a number of surgical procedures in oral and maxillofacial surgery, there are little scientific evidence about the efficacy of this

application. There not so many experimental studies on the use of PRP in reconstructive surgery, which could prove the benefit of using PRP. Moreover, the easy availability of this autogenous blood component has apparently prompted a lot of clinical approaches, which however, are rarely based on a controlled design but are mostly reported as clinical observations without a well defined set of success parameters or a control group. The studies which could provide more data on a more controlled scientific level were not available at this time. Clinical reports that exist are mostly limited to a few cases and describe improved and more rapid soft tissue healing after implant insertion. (Sanchez, Sheridan and Kupp, 2003; Schliephake, 2002) In **Kassolis** (Kassolis, Rosen and Reynold, 2000) study, although they clinical and histologic finding showed good result in sinus grafting and ridge augmentation with FDBA in combination with PRP, however, the histomorphometry was not performed and there were no controls ,therefore quantitative measurements could not be made. There is only one experimental goat study, **Fennis JPM** (Fennis, Stoelinga and Jansen, 2002), that provides provisional information on the use of PRP in mandibular reconstruction which indicates improvement in incorporation of particulate cancellous bone grafts from radiographic findings, but this was not a prospective randomized design. While the reports using autogenous bone with PRP are promising, other reports indicate that PRP may not be effective when used with bone substitutes. **Terheyden H** (Terheyden, 2000) compared rhOP-1 and PRP in bilateral sinus grafts that used 100% anorganic bovine bone as a grafting material. The PRP was not effective in producing bone regeneration, whereas in the contralateral sinus the rhOP-1 was effective. **Wironen JF** (Wironen, Jaw and Fox, 2000) were showed the experimental study to test osteoinductive property of PRP in 16 athymic nude rats. Human PRP were obtained less than 24 hours prior to surgery. The human PRP was combined with demineralized bone matrix and placed in pouches created in the recti abdimini muscle of athymic nude rats. Animals were sacrificed at 28 days post-implantation and the abdominal wall were excised. Bone ossicle implant were removed and processed for histologic evaluation. The PRP containing implants showed much more inflammation. The data showed that PRP is not osteoinductive when added to demineralized bone matrix placed in the recti abdomini

muscle of athymic nude rats. **Froum SJ** (Froum, et al., 2002) were reported the efficacy of PRP in three bilateral sinus graft cases with grafts of anorganic bovine bone that contained minimal or no autogenous bone. 3 patients, requiring bilateral sinus augmentation procedure prior to the placement of the implants, were selected. PRP was obtained from 350 to 450 ml whole blood, processed with a Sequestra 1,000 gradient density cell Separator (Medtronics). This yielded approximately 30 ml of PRP for clinical use, PPP and RBC were returned to patient. The Bio-Oss® graft material was hydrated with the PRP in a dappen dish. Bovine thrombin was added just prior to placement of the graft, result in the formation of an autologous platelet gel containing the particulate graft material. Membrane were placed over all six lateral windows. Placement of the permanent implants were performed at 7,7.5 and 11 months, respectively for case 1,2 and 3. At the time of implant placement, triphine core of 3 mm in diameter and 10 mm in length were harvested through the site of the former lateral window. Three test implants, in case 3, were evaluated for new bone formation and percentage of bone implant contact. The two test implants placed in PRP sinus had slightly higher percentage of implant-bone contact (37.6% and 38.8%) than the test implants placed in the contralateral non-PRP sinus (33.8%). Histomorphometry analysis indicated that the addition of PRP to the grafts did not make a significant difference either in vital bone production of in interfacial bone contact on the test implants. They concluded that; 1.PRP did not make a significant difference in the production of vital bone in sinuses grafted with Bio-Oss® 2.PRP did not make a significant difference in bone contact at the implant bone interface. 3.the use of PRP with grafts consisting of 100% Bio-Oss® should be considered only in respect to the improved handling quality (containment) of the particulate graft material that can be achieved through the activation of the PRP with thrombin. **Roldan JC** (Roldan, et al.,2004) augmented the mandible of Wistar rats on both sides with either anorganic bovine bone (Bio-Oss®) or autogenous rib bone. On the test side they applied either 20 μl of autologous PRP or 10 μl of rhBMP-7. In addition, bone induction was evaluated in an extraskeletal site. A polychrome sequential labeling was performed. The animals were sacrificed by intra-

vital perfusion on day 50. Undecalcified ground sections were evaluated by microradiography, digitized histomorphometry and under fluorscent light. The qualitative analysis of fluorochrome labels suggested that PRP and rhBMP-7 accelerated bone ingrowth. However, histomorphometric analysis revealed no significant differences in the area of newly mineralized bone under either the influence of PRP or rhBMP-7 on autologous bone graft. Likewise, the addition of PRP to anorganic bovine bone showed no statistical difference to the control group. The strongest bone stimulating effect was seen for the combination of rhBMP-7 with anorganic bovine bone. In the extraskeletal model, newly formed bone was evident in the presence of rhBMP-7, but not of PRP. They concluded that the addition of PRP failed to enhance bone formation on anorganic bovine bone and on autologous bone graft.

A. B.

Figure 7. A. The PRP has been mixed with Bio-Oss®. (from Garg AK. Grafting materials in repair and restoration. In : Lynch SE , Genco RJ , Marx RE, editors. Tissue engineering : applications in maxillofacial surgery and periodontics. Illinois : Quintessence publishing ,1999 ; 83-101.)

B. Thrombin and calcium chloride are then added to form a firm gelatinous mass in which the Bio-Oss® particles are embedded. (from Garg AK. Grafting materials in repair and restoration. In : Lynch SE , Genco RJ , Marx RE, editors. Tissue engineering : applications in maxillofacial surgery and periodontics. Illinois : Quintessence publishing ,1999 ; 83-101.)

From above mention, we wish to evaluate the effective of the combining between PRP and Bio-Oss® for promoting new bone formation. On the basis of available data, we hypothesized that treatment of rabbit calvarial bone defect with anorganic bovine bone such as Bio-Oss® could not be used to formulate the next generation of bone defect-filling materials by combination it with PRP.

Aim of the study:

To comparative study the new bone formation on rabbit calvarial bone defect between Bio-Oss® alone or combined with PRP.