CHAPTER FIVE

DISCUSSION

This study evaluated the possibility of local use of autologous platelet-rich plasma at the mandibular bone. When a mandibular bone is lost or fractured, there is usually a gradual loss of bony tissue from the jaw bone at the area of the fracture site. This bone loss causes fragility that can extend and contribute to further bone fracture and tooth loss, and can make the use of dental implants less feasible. Autologous platelet-rich plasma may become a source of multiple bioactive growth factors for bone regeneration to strengthen this area. The therapeutic osteogenic effect of local platelet administration probably depends on the amount of growth factors delivered within (Dugrillon et al ., 2002).

Deficient or inappropriate healing of bone impacts clinical decision-making and treatment options in orthopedics, oral and maxillofacial surgery, plastic surgery and periodontics. While a number of regenerative techniques have been used to regenerate craniofacial defects caused by infective, neoplastic or trauma-induced bone loss, each method has significant limitations. This study showed concentration and centrifugation methods to increase and expand platelets obtained from animals' blood.

Clinicians in specialties such as dental, plastic & reconstructive, otolaryngology, orthopaedic, neurology, cardiovascular, vascular, general, non-healing wounds, and pediatric surgery routinely use platelet-rich plasma due to their ability to deliver multiple bioactive growth factors. Growth factors shown to promote the body's natural healing process include platelet derived growth factors (PDGF), transforming growth factor beta

(TGF-ß), epidermal growth factors (EGF), and vascular endothelial growth factors (VEGF). These growth factors play an important role on angiogenesis, stimulation of chemotaxis and mitosis of cells, proliferation of stem cells, cohesion between bone fragments, and enhancement of osteoconduction through fibrin network, thus promoting early regeneration of bone in the repair process of bone graft. Recent research in several laboratories suggests that growth factors may act locally to modulate new bone formation by stimulating osteoblast proliferation and activity (Mohand and Baylink., 1991)

Platelet-rich plasma is defined as plasma containing a concentrated number of platelets. This component contains platelets, coagulation factors, and plasma proteins. PRP may be stored at room temperature (25-26 degrees Celsius) up to 8 hours. PRP permits the body to take advantage of normal healing pathways at the greatly accelerated rate. During the healing process, the body rushes many cells and cell-types to the wound in order to initiate the healing process. One of those cell types is platelets. Platelets perform many functions, including formation of a blood clot and release of growth factors into the wound. These growth factors function to assist the body in repairing itself by stimulating stem cells to regenerate new tissue. The more growth factors released/sequestered into the wound, the more stem cells stimulated to produce new host tissue. Thus, one can easily see the PRP permits the body to heal faster and more efficiently. PRP has many clinical applications. Bone grafting for dental implants can be effectively performed in combination with PRP. This includes onlay and inlay grafts, sinus lift procedures, ridge augmentation procedures, and closure of cleft, lip and palate defects. Repair of bone defects created by removal of teeth or small cysts and repair of fistulas between the sinus cavity and mouth can also successfully performed when PRP is

added. PRP has many advantages. It is a by-product of the patient's own blood, therefore, disease transmission is not an issue. PRP can be generated in the doctor's office while the patient is undergoing an outpatient surgical procedure, such as placement of dental implants. PRP decreases the incidence of both intraoperative and postoperative bleeding at the donor and receptor sites because of its inherent hemostatic properties. In addition, when using tissue regenerative membranes, the following benefit also obtained—localized delivery of growth factors specific for the regeneration of the bone (Petrungaro, 2001).

However, Arpornmaeklong et al (2004) suggested that PRP inhibited osteogenic differentiation of marrow derived pre-osteoblasts in a dose dependent manner. PRP can not be a substitute for BMP-2 in osteogenic induction. Some researchers believe that, at this time, basic research does not strongly endorse PRP's ability to promote healing. Some of the basic science issues surrounding PRP should be clear in the hope that this will provide surgeons with more knowledge and insights surrounding its use.

Osteoinduction, differentiation of mesenchymal tissue into wove bone, has been found to occur under the influence of several bioactive growth factors which are stored in PRP (Lamerigts et al, 1999). Specific studies of PRP have identified at least three important growth factors in the alpha granules of the sequestered platelets: platelet-derived growth factor (PDGF), transforming growth factor-ß1 (TGF- ß1), and transforming growth factor- ß2 (TGF- ß2). In addition, other studies have documented the presence of insulin-like growth factor-I (IGF-I) in platelets (Arpornmaeklong, 2004: Marx, 1999 and Weibrich, 2002).

Platelet-derived growth factor (PDGF) is involved in nearly all wound healing by virtue of platelets' dual role as a growth factor reservoir and a factor in hemostasis. PDGF in particular seems to have numerous positive effects on wound healing, including mitogenesis (causing an increase in the number of healing cells), angiogenesis (generating development of new capillaries), and up-regulation of other growth factors and cells (resulting in promotion of fibroblastic and osteoblastic functions, promotion of cellular differentiation, and acceleration of the effects of growth factors on other cells, such as macrophages) (Marx, 1999). Interest in the role of PDGF in wound repair has centered on the fact that platelets, monocyte/macrophages, and possibly injured endothelial cells can secrete PDGF together with other growth factors. Platelet-derived PDGF could be important in the initiation of the repair process because of its chemotatic properties for both leukocytes and fibroblasts, while PDGF from macrophages could play the major role in the continuing process of fibrogenesis (Ross et al, 1986).

Transforming growth factor-ß (TGF- ß) is a term applied to a superfamily of growth and differentiating factors, of which the bone morphogenetic proteins (MBPs) are members. TGF-ß act as a paracrine growth factor (ie, a growth factor, secreted by one cell, that exerts its effect on an adjacent second cell), 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-ß 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-ß 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 (Marx, 1999).

Insulin-like growth factor-I (IGF-I) and insulin-like growth factor-II (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. IGF-I, also known as somatomedin C, stimulates bone cell replication and differentiaton in a dose-dependent manner. It helps modulate the effects of systemic hormones on local bone formation. For example, both parathormone and estradiol 17ß stimulate the production and release of IGF-I. In most mammals, IGF-I is considerably less abundant than IGF-II, in terms of both its synthesis by bone cells and its serum level. IGF-II, previously termed skeletal growth factor, has a 40 % sequence homology with insuin. IGF-II is produced by bone cells derived from a number of species and exists in a protein-bound form in serum. IGF-II is believed to be intimately involved in modulating the effects of systemic hormones on local bone formation and lysis (Kirker-Head, 1996 and Marx, 1999).

A subfamily of TGF-ß, is bone morphogenetic protein (BMP). BMP has been shown to induce the formation of new bone in research studies in animals and humans. This is of great significance to the surgeon who places dental implants. By adding PRP, and thus BMP, to the implant site with bone substitute particles, the implant surgeon can now grow bone more predictably and faster than ever before. The recombinant human proteins are regcognized by use of prefix rh (eg, rhMBP-2). RhBMP-2 through rhBMP-9 are members of the transforming growth factor-ß (TGF-ß) superfamily. Some

investigators, however, refer to rhBMP-7 as human osteogenic protein-1 (hOP-1). BMPs initiate chondroblastic differentiation in pluripotent mesenchymal progenitor cells, followed by the synthesis of new bone by endochondral ossification. However addition of rhBMP-2, 4, 5, or 7 results in osteogenesis. Of the individual proteins, rhBMP-2 is one of the most potent. It could induce bone formation in a dose dependent manner. By comparison, rhBMP-4 is less potent, requiring twice the dose to generate the same amount of bone as the rhBMP-2. Both rhBMP-5 and rhBMP-7 are even less osteoinductive. The focus of most clinical investigations has therefore been on rhBMP-2, although rhBMP-7 is also being prepared for use (Kirker-Head, 1996). Kirker-Head et al (1995) reported that a recombinant human bone morphogenetic protein 2/ inactive demineralized bone matrix (rhBMP-2/IDBM) implant provided effective long term healing of a large critical sized bone defect in the weightbearing femora of 5 adult sheep.

The average platelet count of the PRP in this study is 518,900 or 356.16 % compared to the average baseline of platelet count in fresh blood. The platelet counts of PRP from 8 experimental dogs are reached at least 300 % when compared to the fresh blood, which was enough growth factors according to the suggestion of Marx et al (1998). But the platelet counts of PRP from two experimental dogs are not reached 300%. It may be because of the technical errors.

This study also confirmed that the PRP preparation method from Gernot et al (2001) can reach more than 300 % platelet compared to the baseline of fresh blood platelet. This method is practical and easy to performed and less cost by using the commercial PRP preparation machine. Normally, the clinical application of PRP requires initiating the coagulation process by using a mixture of 10 ml of 10 % calcium chloride

mixed with 10,000 units of topical bovine thrombin (Gentrac) to make PRP be easy to handle and actually improves the ease of application of bone substitute materials and bone grafting products by making them more gel-like. Fibrin gel (as formed by activation of PRP/PPP with thrombin and calcium chloride) provides a matrix for cell growth and differentiation by enhancing close intercellular contacts, which are important to growth and differentiation of osteoblasts to form bone-like tissue. The embedding of the cells in a gel like matrix contributes to a different microenvironment, which favours differentiation and reduces proliferation (Arpornmaeklong et al, 2004). However, PRP prepared from this study is a liquid form. Thus, collagen is mixed and added to the artificial defect to hold the PRP in the defect securely.

Collagen was filled in both experimental and control defects to ensure that blood clot and PRP would stay in the defects. But it seemed to retard the growth of the bone as less bone regeneration at different periods is measured. Collagen is a physiologic substance, and while it is abundantly available as cadaveric raw material, careful preparation is required for its medical use. Medical grade collagen for implantation comes in a variety of forms—solution, powder, membrane film, thread, sponge, and tubing. Little information is available regarding the means by which collagen retains and subsequently releases BMPs at the implant site, but it does so in a controlled fashion. At 8 weeks after implantation, solid bone (woven bone) should span the defect and no remnants of the collagen carrier should be apparent (Kirker-Head, 1996). In this study, remnants of the collagen are still obvious at 8 weeks period defect. It is possible that collagen may retard the bone growth. Study of the effects of collagen on bone regeneration is an interesting topic. New bone formation in the defects that are near and

slant to the periosteum show slightly more woven bone than the defects that are in the middle of mandibular bone because of the periosteal activity. Woven bone was normally found under the periosteum that was elevated during surgery. Elevation of periosteal flap can activate the osteoblastic osteoprogenitor cells.The results showed that at the 2 week healing period and 4 week healing period the woven bone found in PRP group quite prominently when compared with control group. However, at 8 and 12 week healing period, it may be no significant different finding in the bone healing of both group. It seems that, at the beginning of the bone healing period in this study, PRP may accelerate new bone formation than those without. However, histomorphometric analysis is more reliable when compared with a qualitative evaluation of the histological section.

Radiographic evaluation showed a significant increase in bone density of the artificial defects with PRP and artificial defects alone (control) at different periods (2, 4, 6, 8, and 12 weeks).

Histomorphometrical analysis has shown the result that PRP did not accelerate the healing process and new bone formation when used as a local factor. However, there are no artificial markers to indicate the exact frontier of the defects. If the frontier of the defect is exactly regcognized, the histomorphometrical result would be more reliable.

This study is focusing on local effects of PRP on new bone formation without the additional effect of systemic factors. Bone regeneration of PRP and control group as measured from this study has shown no statistical significant differences. Although PRP provides multiple bioactive growth factors to the wound, enhancing effects of PRP on new bone formation as reported in some clinical studies are not supported by this study. It may be necessary, that before using PRP as a local accelerator for bone formation, the

essential growth factors such as PDGF, TGF-ß should be counted or analysed to ensure that those growth factors are existing both in quanlity and quantity. As a reliable factor in bone formation, the use of BMP-2 and rhBMP-7 (rhOP-1) is a very interesting issue to research for being use in animal and human.