

Applications of Platelet Rich Plasma for Regenerative Therapy in Periodontics

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Introduction

Regeneration of tooth-supporting structures destroyed by periodontitis is a major goal of periodontal therapy. Periodontal regeneration is perhaps one of the most complex to occur in the body since at least six tissues are involved: the gingival epithelium, gingival connective tissue, periodontal ligament, tooth root surface cementum, alveolar bone and corresponding vasculature. All these mineralized and nonmineralized components must be restored to their original position and architecture for regeneration of the periodontium to occur. Growth factors are a class of naturally occurring proteins involved in three key cellular events in tissue repair: mitogenesis, migration and matrix synthesis and remodeling¹. A combination of growth factors may more effectively stimulate formation of mineralized as well as nonmineralized tissues¹. Platelets are rich in growth factors that may contribute to an accelerated tissue regeneration process.

Sequence of Bone Regeneration

Platelets are responsible for initiation of regeneration of tissue from trauma. During repair platelets become entrapped in a fibrin clot and degranulate releasing two primary growth factors: PDGF and TGF- β . PDGF binds to endothelial cells to initiate capillary ingrowth; and TGF- β binds to osteoblasts and stem cells to initiate mitosis and stimulate osteoid production².

The lifespan of platelets in a wound is less than five days. Macrophages are attracted into the graft

site through an oxygen gradient of 30-40 mm Hg and drive the remaining bone regeneration process. By day 14, complete revascularization of the graft is seen. Stem cells differentiated into osteoblasts - osteoid is being laid down. Early bone formation is occurring. By four to six weeks, random cellular bone, called woven bone, is formed which is immature and disorganized. In phase two remodeling lamellar bone is formed, representing a more organized bone.

What is Platelet Rich Plasma?

A recent innovation in dentistry is the preparation and use of platelet-rich plasma (PRP), which is a component of blood in which the platelets are concentrated in a limited volume of plasma. Autologous platelet gel was first developed as a byproduct of multicomponent pheresis. The platelet count in PRP can exceed 2 million platelets per micro liter. A natural blood clot contains 95% red blood cells, 5% platelets, less than 1% white blood cells, and numerous amounts of fibrin strands. A PRP blood clot contains 4% red blood cells, 95% platelets, and 1% white blood cells. It can be considered that PRP "jump starts" the cascade of regenerative events leading to the formation of a mature graft site². The PRP obtained offers up to a 2.16-times increase in the maturation rate and substantially greater density of a bone graft procedure³.

Components of PRP,

1. Growth Factors
 2. WBC & phagocytic cells
 3. Native fibrogen concentration
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4. Vasoactive and chemotactic agents
5. High concentration of platelets

Dental applications of PRP include, sinus lift procedures, onlay grafts, particulate grafts, alveolar cleft palate repair, oral/nasal fistula repair, post-operative hemostasis of bone graft donor sites, continuity defects of the mandible and hemophiliacs undergoing surgery. In periodontal surgery it has been used in gingival grafting, crown lengthening and periodontal regeneration.

Contraindications for PRP usage are few and include platelet dysfunction syndrome, critical thrombocytopenia, hemodynamically unstable patients and pregnancy.

Role of Growth Factors In PRP

PRP is an autologous source of concentrated suspension of the growth factors found in platelets. Activated PRP release growth factors, enhancing wound healing and wound strength.

Growth factors derived from platelets initiate connective tissue healing, bone regeneration and repair, promote development of new blood vessels, and stimulate the wound healing process.

Periodontal wound healing involves gingival fibroblasts, gingival epithelial cells, periodontal ligament fibroblasts and osteoblasts, all of which are important for tissue repair and hard-tissue regeneration. A series of well-orchestrated cell-cell interactions is initiated after injury. Disruption of the vasculature as a result of injury leads to fibrin formation and platelet aggregation. Several growth factors are then released into the tissue from the platelets and from the adjacent cells after injury

Platelet Derived Growth Factor (PDGF) - PDGF is a very powerful regulatory growth factor and a sentinel growth factor that begins nearly all wound healing. PDGF's main function is to stimulate cell replication (mitogenesis) of healing capable stems and premitotic partially differentiated osteoprogenitor cells, which are part of the connective tissue-bone healing cellular make-up. PDGF also causes replication of endothelial cells, causing budding of new capillaries (angiogenesis). PDGF exists in three forms: PDGF_{aa}, PDGF_{bb}, PDGF_{ab}. All isoforms

of PDGF are released after adhesion of platelets to an injured site. The AA and BB isoforms enhance proliferation of bone cells, increasing the production of PDGF-AA in osteoblast cultures by an autocrine process⁴ in reconstructive periodontal studies in rats, in vivo application of PDGF increased bone regeneration in calvarial defects when a resorbable membrane was used as a carrier⁵.

Transforming Growth Factor (TGF) - TGF regulates proliferation and differentiation of multiple cell types. TGF found in platelets is subdivided into TGF β 1 and TGF β 2, which are the more generic connective tissue growing factors involved with matrix formation influencing osteoblasts to lay down bone matrix through the process of osteogenesis. Also cells activated by TGF β 1 and TGF β 2 include fibroblasts, endothelial and osteoprogenitor cells, chondroprogenitor cells and mesenchymal stem cells. A condoroprogenitor cell will further differentiate and produce the matrix for cartilage. A mesenchymal stem cell stimulated to mitosis provides wound-healing cells. In vitro, TGF- β has been observed to promote extracellular matrix production in many cell types, such as periodontal ligament fibroblasts. TGF- β 1, used alone or in combination with PDGF-BB, stimulates the proliferative activity of periodontal ligament fibroblasts. In a recent study involving a canine model, the application of rhTGF- β 1 in conjunction with nonresorbable barrier membrane greatly enhanced bone regeneration in oral osseous defects (after 2 months)⁶.

Insulin Growth Factor(IGF) - IGF is also important in wound healing, and stimulates both proliferation and differentiated function in osteoblasts. IGF has 2 forms, II, and I each of which has 2 single chain peptides. IGF binds to the same receptors as insulin and is involved in the development of many tissues, including the teeth. In the area of periodontal regeneration, more research has been done on IGF-I. This form of IGF is chemotactic for periodontal ligament cells, and it has strong effects on periodontal ligament fibroblasts and protein synthesis. IGF-I stimulates bone formation by proliferation and differentiation, and it is synthesized and secreted by osteoblasts⁷. Human patients treated with a combination of 150 mg/ml each of recombinant human platelet-

derived growth factor-BB (rhPDGF-BB) and rhIGF-I in a methylcellulose vehicle experienced 43.2% osseous defect fill, whereas the control group (vehicle only) had 18.5% osseous fill⁶.

Epidermal Growth Factor (EGF) - EGF is responsible for cell differentiation and stimulates re-epitheliation, angiogenesis and collagenase activity. **Vascular Endothelial Growth Factors (VEGF)** have potent angiogenic, mitogenic, and vascular permeability-enhancing activities specific for endothelial cells.

The short shelf life and inefficient delivery to target cells are major concerns associated with local administration of recombinant human growth factors. The growth factors are expensive, and many doses may be required to achieve any therapeutic effect. An easy, cost-effective way to obtain high concentrations of growth factors for tissue healing and regeneration may be autologous platelet storage via PRP.

Major Benefits of PRP

“Jump-starts” osteogenesis by releasing growth factors at the local site. Osteoblasts can be enticed to move across a greater distance by creating a scaffold system (fibrin) that will assist their movement.

Early consolidation of the graft: the increased amount of PDGF in the graft initiates osteocompetent cell activity at an enhanced molecular rate.

Speeds up mineralization: because mineralization on a graft site is a coupled phenomenon, osteogenesis must proceed in such a way that activation and bone formation is greater than resorption. Mineralization of the collagen matrix is speeded up due to PDGF being added right from the start in the mineralized bone segment of the graft, instead of being released from collagen. Use of PRP has shown to improve the rate of bone formation by 1.62 to 2.18 times that of the controls

Improves trabecular bone density: there has been reported in the literature a 15% to 30% improvement in trabecular bone density when platelet rich plasma factor is added to the graft.

Allows placement of implants into the grafted site at an earlier time by enhancing osteoconduction : osteoblasts are normally non-motile cells in

that they will not normally move across a distance greater than 0.4mm (400 microns).

PRP also accelerates endothelial, epithelial, and epidermal regeneration. They stimulate angiogenesis, enhance collagen synthesis, promote enhanced soft tissue wound healing, decreased dermal scarring, enhanced hemostatic response and reverse the inhibition of wound healing caused by glucocorticoids. High leukocyte concentration in PRP has an added antimicrobial effect. It provides a watertight seal (necessary for dural closures) and augmented rate of extracellular matrix deposition resulting in earlier wound closure. As it is an autologous blood product there is no risk of infectious disease transmission or clerical errors, thus making it a safe product with no time consuming visits to the blood bank for pre-donation. It provides an immediate surgical hemostatic agent that is biocompatible, effective and safe. The native fibrinogen concentration imparts a gelatinous adhesive consistency, for ease of surgical application and results in reduction of pain and infection.

PRP-Related Studies

In vitro, platelet membranes have been shown to stimulate the mitogenic activity of human trabecular bone cells, thus contributing to the regeneration of mineralized tissues.

Another study assessed the efficacy of demineralized bone powder alone or combined with PRP in enhancing the osseointegration of dental implants in a dog model. Standard histomorphometric methods at 6 and 12 weeks after surgery revealed a higher percentage of bone contact with bone powder and PRP than with bone powder alone. The authors

concluded that bone defects around titanium implants could be treated successfully with bone powder and that PRP may improve bone formation⁹. Human studies have also shown that PRP can be advantageously and easily applied in surgery. PRP was used in 20 patients undergoing cosmetic surgery, including face-lifts, breast augmentations, breast reductions and neck lifts. The application of PRP yielded adequate hemostasis if platelet-poor plasma (PPP) was also applied to create a seal to halt bleeding. It has the advantage of minimizing use of

electrocautery so as to minimize the chance of damage to the adjacent nerves¹⁰.

The first clinical dental results with PRP were reported by Marx and others in 1998, who used PRP to improve graft incorporation in mandibular reconstructions in patients who had received cancellous bone marrow grafts after tumor removal³. Their data strongly suggested that adding PRP to bone grafts accelerated the rate and degree of bone formation. When patients contemplating subsequent implant placement after extraction received a mixture of autologous bone and PRP, much better epithelization and compact mature bone with well-organized trabeculae was demonstrated compared to the control group¹¹.

Combination of PRP and freeze-dried bone graft has been suggested as an alternative therapeutic method for implants placements. PRP in combination with bone allograft and guided tissue regeneration as periodontal therapy for intrabony defects in humans resulted in significant gain in clinical attachment and filling of the treated defects, as revealed by 2-year follow-up.

Procurement

PRP can be obtained in various ways in the dental office. Techniques for PRP preparation vary from using 10 cc of a patient's blood and spinning it in a lab centrifuge, to using a unit of blood that is put through a cell separator, that sequesters and concentrates the platelets¹².

Systems for procurement,

* *One touch automated PRP systems (Eg: Harvest SmartPREP)*

It provides simplicity in operation and may provide a good platelet count before plasma re-suspension. This system requires 50ml blood for procurement and do not sense the Plasma/Blood Interface and hence may yield low platelet count

* *Plasmapheresis*

Requires approx. 450ml of blood from which 20-60cc of PRP obtained. This method of cell separation is used only when large quantities of PRP are required. The use of platelet concentrates obtained from blood banks by the

discontinuous plasmapheresis method is limited because of high cardiovascular stress to the recipient, known health risks and high production costs¹³. The processed blood can be autotransfused to the patient.

* *Manual PRP systems*

End User can manually recover the maximum amount of platelets. Clearly the best way to produce a true PRP product. up to Counts of 4 to 10 times the patient's baseline can be achieved using a manual system. Since there is no re-suspending (dilution) of platelets, the final product has a high concentration of platelets.

Eg: *Curasan* (Pharma GmbH AG, Germany), 3I PCCS (3i Implant Innovations, Florida), Medtronic Sequestra, Haemonetics Cell Saver

A simple chairside technique for PRP procurement

Recent publications have indicated that PRP prepared from 8 to 10 ml of whole blood is sufficient for periodontal regenerative therapies¹⁴. We are procuring PRP with the help of a general purpose tabletop laboratory centrifuge by the following method. It is simple and cost-effective method for producing PRP in an in-office environment. Patients are selected based on the absence of any blood abnormalities or use of anti-coagulants. 10 ml blood is withdrawn from the antecubital region with a 10ml syringe and transferred to a container containing 1.4ml anticoagulant (Citrate phosphate dextrose solution). It is then centrifuged for 10 mins at 1300 rpm. The result is a separation of whole blood into a lower red blood cell (RBC) region and upper straw-colored plasma region as shown in fig1B. There is relatively high concentration of platelets found in the boundary layer between these two regions. The upper straw colored plasma layer (platelet poor plasma;PPP) and 1-2 mm of the top part of the RBC layer is aspirated and transferred into another container and again centrifuged for 10 mins at 2000 rpm. This results in an upper portion of clear yellow supernatant serum and the bottom red tinged layer consisting of highly concentrated PRP as shown in fig1C. The upper clear layer is aspirated until 1.5ml of serum is left. The contents of the tube is mixed well and transferred into a sterile container.

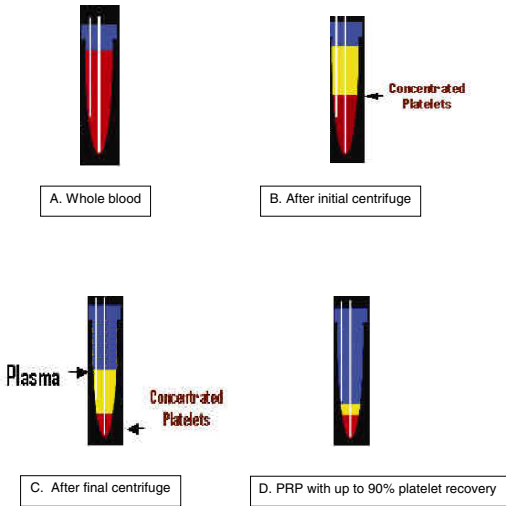


Figure 1 : Procurement of platelet rich plasma

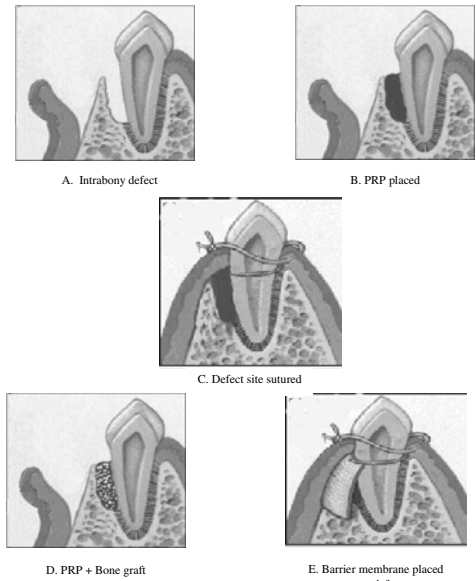


Figure 2 : Application of Platelet rich plasma

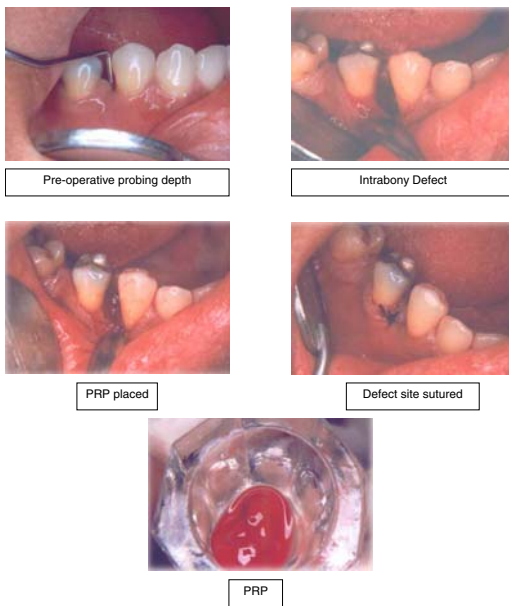


Figure 3

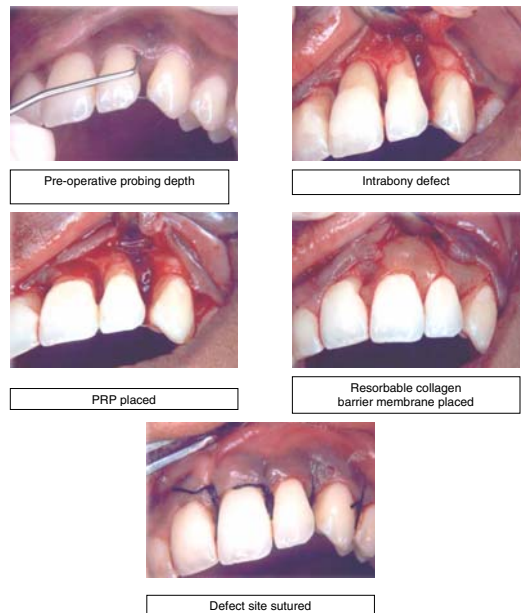


Figure 4

At the time of the application, the PRP is combined with an equal volume of a sterile saline solution containing 10% calcium chloride (a citrate inhibitor that allows the plasma to coagulate) and 100 U/ml of sterile bovine thrombin (an activator that allows polymerization

of the fibrin into an insoluble gel, which causes the platelets to degranulate and release the indicated mediators and cytokines). This results in formation of a sticky gel that is relatively easy to apply to the surgical defects.

Technique of application in periodontal intrabony defects

The periodontal defect site is opened and debrided. The PRP gel is placed into the defect site and sutured as shown in figure II(B,C). A barrier membrane can also be placed over the defect to augment the results (figure IIE). PRP can be mixed with a graft material and placed in the defect (figure IID). PRP results in early consolidation and take up of the graft. All these treatment modalities have shown good clinical results. Figure III presents a case report in which PRP was used alone; and figure IV presents a case where PRP was used in combination with a resorbable collagen barrier membrane.

Conclusion

PRP is a new application of tissue engineering and a developing area for clinicians and researchers. It is a storage vehicle for growth factors, especially PDGF and TGF- β , both of which influence bone regeneration. Although the growth factors and the mechanisms involved are still poorly understood, the ease of applying PRP in the dental clinic and its beneficial outcomes, including reduction of bleeding and rapid healing, hold promise for further procedures. Most important, this autologous product eliminates concerns about immunogenic reactions and disease transmission. PRP may become a routine treatment modality for periodontal regeneration in future.

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