

Role of PRGF (Plasma Rich in Growth Factors) in Periodontal Regeneration

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Abstract

Platelet-rich-growth-factors (PRGF), a modification of fibrin glue made from autologous blood, is being used to deliver growth factors in high concentration to sites requiring osseous grafting. Growth factors released from the platelets include platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), platelet-derived epidermal growth factor (PDGF), platelet-derived angiogenesis factor, insulin-like growth factor 1 (IGF1), and platelet factor 4. These factors signal the local mesenchymal and epithelial cells to migrate, divide, and increase collagen and matrix synthesis. Anitua et al in 2001 introduced the concept of PRGF and since then it has been used for a variety of purposes in the field of dentistry and oral implantology for proper wound healing and regeneration of periodontal tissues. This review aims to give a brief idea about the various uses of PRGF and the possibility of utilization in the future taking into consideration many studies that have been done. The main advantage of using PRGF is that it lacks the leukocyte content thus preventing damage to the tissues during healing due to the acid hydrolases and proteases released from the neutrophils. It has been previously used for treating intrabony defects, to increase the implant-bone contact area, for recession coverage etc. In other fields of medicine, it has been used to cure osteoarthritis, to treat chronic ulcers as well. Therefore, PRGF is a very effective blood concentrate with a high concentration of growth factors and its use should be increased in the clinical practice.

Keywords: Healing, leucocytes, periodontal regeneration, plasma rich in growth factors

Introduction

The field of dentistry is evolving to provide techniques that are minimally invasive and biologically accelerated which reduces patient morbidity and results in improved healing. Healing in dentistry is broadly related to repair and regeneration, both of which are two different terminologies. Regeneration refers to the complete restitution of the injured tissues with their proper architecture and normal function whereas repair is the type of healing that occurs in response

to injury for normal functioning. The requirement of regeneration has given rise to many new biomaterials that have been used in dentistry. However, the disadvantage of the cost-effectiveness is persistent with these biomaterials. Recently the use of platelet-rich treatments has revolutionized the field of regenerative dentistry, specifically in the field of periodontics and oral implantology.¹ It has been able to do so due to the repair potency of the growth factors and the proteins released by the platelets.²

Megakaryocytes derived cell platelets have an intense tubular structure which helps in the eviction of the growth factors and the proteins. Thus they are a very good source for different types of growth factors and proteins that are required for regeneration. Platelets when activated, release various growth factors from the α granules. The justification for the utilization of

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platelets was to reinstate the clot with platelets which on stimulation or activation releases various growth factors like Platelet-Derived Growth Factor (PDGF), Transforming Growth Factor- β (TGF- β), Vascular endothelial Growth Factor(VEGF), Insulin-like Growth Factor(IGF), Thrombospondin, etc which help in the process of regeneration.² One advantage of using Plasma rich in growth factors is the exclusion of the RBC's and the WBC's resulting in accelerated wound healing with minimal post-operative complications. Furthermore, it can be easily be prepared from the patient's blood followed by its centrifugation thus it is cost-effective as well as saves a lot of time clinically.

PREPARATION OF PRGF

Anitua et al in 1999 affirmed the concept of PRGF.¹ It is completely autologous and biologically compatible and obtained by a single centrifugation process utilizing sodium citrate (anticoagulant) and calcium chloride (platelet activator). The beneficial effects of PRGF are dependent on the amount of growth factors, rate of release of the peptides and the centrifugation procedural considerations like the speed.³

PRGF Endoret technology for the collection of PRGF gel is the most effective techniques to obtain PRGF with maximum beneficial attributes and has provided clinical development in the field of Periodontics and Oral Implantology.⁴

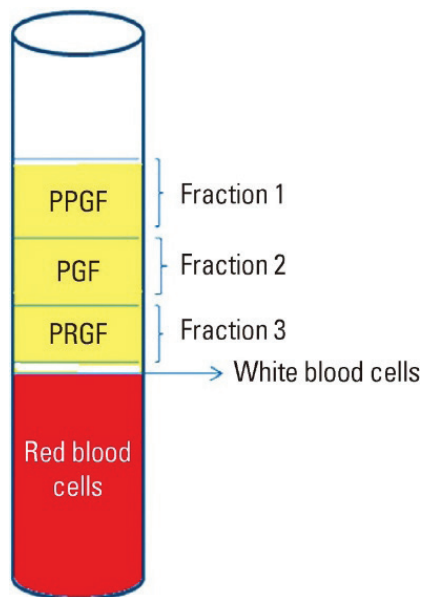


Figure 1 – Fractions obtained following centrifugation

Protocol includes –

- Venous blood should be collected from a healthy donor into 10 ml tubes that contains 3.8% sodium citrate
- Single centrifugation process should be performed in the PRGF Endoret system at a speed of 580g for around 8 minutes
- Fraction(F3) just above the buffy coat (collection of white blood cells) as shown in **Figure 1** should be pipetted out carefully and transferred to a separate disinfected tube or a petridish
- Addition of 10% CaCl₂ (Calcium Chloride) should be done for the stimulation of platelets to get activated (Around Fifty microlitre per ml of PRGF)
- Incubation for 60 minutes in the PRGF Endoret activator needs to be done at a temperature of 37°C to obtain the PRGF in a gel consistency.

There are many techniques by which the platelets can be separated from the blood which includes gravitational platelet sequestration process, standard cell separators and plateletpheresis. However, the gravitational technique which utilizes the gravitational force to separate contents of blood is the most useful since it has a yield of 78% platelets with minimum RBC's and WBC's.⁵

ROLE OF GROWTH FACTORS IN TISSUE REPAIR & REGENERATION

The growth factor is a generalized term used for a group of proteins that function together in the body to promote the migration of the cells and carry out specific metabolic activities.⁶ Following tissue injury, there are a series of events that occur at the cellular and molecular level which includes several stages of inflammation.⁷⁻⁹ The events include proliferation and migration of various cells, the appearance of new blood vessels, formation of granulation tissue, etc. These events are initiated by the virtue of certain growth factors and cytokines that are released due to the process of platelet activation which occurs as a part of the healing process to injury. The primary clot is the most important since it acts as a scaffold to hold all types of growth factors and cells that would help repair the wound later on in the healing process.¹⁰

PLATELET DERIVED GROWTH FACTOR (PDGF) –

PDGF is the most important growth factor responsible for the regeneration of periodontal ligament tissues. It has three types of isomers PDGF-AA, PDGF-BB and PDGF-AB. The concentration of PDGF in blood serum is around 15-50 milligrams per milliliter. Lynch et al discovered the effectiveness of PDGF in the regeneration of components of periodontium that includes cementum, bone and PDL.¹¹ The sources of PDGF include degranulating platelets, macrophages, fibroblasts, endothelial cells and smooth muscles.

PDGF is a major factor in the healing of wounds since it promotes the division of cells and is responsible for the cell to enter into the division cycle from the G₀ quiescent phase. It also has a chemotactic function and also prevents apoptosis of cells.

Major functions include: Angiogenesis, neutrophil chemotaxis, improves the function of osteoblasts and fibroblasts, helps in secretion of other growth factors and mitogenesis.¹²

Platelet-derived growth factor also helps in the regeneration of periodontium as per many recently conducted studies. Nevins et al in 2012 conducted a study to check the effectiveness of using PDGF-BB along with tricalcium phosphate over 36 months in correcting a localized osseous defect and concluded that hr-PDGF-BB provides a long term results with stable clinical and radiographic parameters.¹³ In an in-vitro study Manoranjan et al in 2012 studied the effectiveness of PDGF-AB on PDL fibroblasts and concluded that PDGF-AB had maximum mitogenic response on PDL fibroblasts at 48hrs.¹⁴ In an animal study, Cho Moon et al in 1995 compared the efficacy of GTR therapy with PDGF and GTR alone and concluded that GTR with PDGF provided better results than GTR alone.¹⁵

INSULIN LIKE GROWTH FACTOR(IGF)-

A.K.A **erythropoietic factor**, they are a family of proteins with one chain and consists of two isomers IGF I and IGF II which are functionally similar but regulated differently. IGF is usually secreted from the liver due to the action of growth hormone. Therefore its release and

action are growth hormone-regulated which is secreted from the pituitary gland. It helps in the promotion of cell division.^{8,9,16}

Not only does it encourage cell division but also prevents cell apoptosis. In an in vitro study, Xie et al in 2017 studied the effects of insulin-like growth factor-1 on the periodontal ligament stem cells and concluded that IGF-1 promotes the proliferation of PDLSC by binding to its receptor and activating the AKT pathway and it also elevates the differentiation of osteoblasts and thus can be crucial in periodontal regeneration.¹⁷ Platelets have a high content of insulin-like growth factors and are released on their activation. It is also released by fibroblasts on activation by PDGF. In an animal study, Lynch et al in 1989 studied the combined effect of IGF and PDGF when applied after periodontal surgery and concluded that the combination accentuates the migration of fibroblasts and osteoblasts and also stimulates the activity of these cells to lay down collagen and new bone thus helping in regeneration of PDL cells.

TRANSFORMING GROWTH FACTOR(TGF)-

Transforming growth factor constitutes isomers i.e. **TGF- α** and **TGF- β** that are both configurationally and functionally different. They compete for the **epidermal growth factor** receptor and functionally activate epithelial and endothelial cells. TGF- β also has three isomers that are TGF β 1, TGF β 2 and TGF β 3. When TGF α helps in the proliferation of different types of cells, TGF β has a dual-action where it can accentuate or minimize the proliferative action of cells.

TGF β has the property of altering the secretion of other growth factors like PDGF, EGF, TGF α etc.¹⁸ It has the property for the proliferation of fibroblasts and collagen production and also downgrade the production of matrix metalloproteinases. In the process of wound healing, TGF β may produce fibrosis due to the excessive production of collagen.

In an animal study with transgenic mice, when TGF β was introduced, they were declared dead due to an inflammatory syndrome. Thus proving that it regulates the immune system. TGF β also augment bone formation when injected near periosteal cells.¹⁹

EFFECT OF PRGF ON THE ALVEOLAR BONE

Bone regeneration has to be one of the challenging things to achieve as it involves a cascade of events that includes the release of various cytokines, chemokines, and growth factors that have to occur. **Anitua et al in 2013** conducted a study in which the primary osteoblasts were isolated and tested with PRGF-Endoret and concluded that it promotes osteoblast proliferation thus can be used to regenerate bone.²⁰ Some of the factors that are essential for regeneration of bone include VEGF, TGF β , PDGF-B, Insulin-like growth factor, etc. which are known to be secreted by the platelets on activation.²¹ Another growth factor known as Hepatocyte growth factor(HGF) has been proved to be involved in the coupling process and regulates both osteoclasts and osteoblasts in an in vitro study by **Grano et al in 1996**.²² The HGF released by the PRGF has also been shown to lower the expression of the pro-inflammatory Nuclear Factor of Kappa B thus allowing for a smooth coupling process.²³ The cytokines involved in bone resorption include TNF α , IL-6 IL beta are secreted by lymphocytes and macrophages when they are activated. However, due to the lack of leucocytes in PRGF, there is very little chance of release of these growth factors.²⁴ It was also observed that there was increased expression of osteocalcin and alkaline phosphatases which increased bone formation process as per the study done by **Lin et al in 2006**.²⁵ In an animal study done by **Eda et al in 2014** on rat cavalria proved that the use of PRGF in new bone formation was better than the use of PRP.²⁶ In another animal study done by **Rivera et al in 2013** proved that the combination of PRGF and Simvastatin produced better results in regenerating bone than the combination of PRP and Simvastatin although the results were not statistically significant.²⁷

PRGF has also been used for the correction of intrabony defects as well. As per a study done by **Khalifehzadeh et al in 2019** Metformin when used in combination with PRGF provided better radiographic and clinical changes than other groups in patients with chronic periodontitis and a localized intrabony defect. However, in a split-mouth study done by **Ravi et al in 2017**, evaluated the efficacy of the use of PRGF along with GTR and concluded that there was no additive effect of PRGF when used along with GTR membrane compared

to GTR alone in terms of clinical and radiographic outcomes.²⁸ Also in another study done by **Gamal et al in 2016** for treating intrabony defects, it was proved that although the level of growth factors was higher in the PRGF group but it was not statistically significant and the use of PRGF though showed improvement in clinical parameters in treating intrabony defect but the results were not statistically significant.²⁹ According to a systematic review done by **Panda et al 2016**, it was concluded that platelet-rich plasma when used along with bone grafts had an additive effect in treating intrabony defects but was not found to be effective when used along with GTR membranes.³⁰ Hence it can be established that PRGF definitely would have an additive effect in the treatment of bony defects and possesses an advantage due to the presence and release of various growth factors.

EFFECT OF PRGF ON SOFT TISSUE REGENERATION

PRGF, as already mentioned before is devoid of white blood cells and other pro-inflammatory products.³¹ They also have a rich source of platelet-derived growth factors that are usually released during the healing of a wound on the activation of platelets. In an in vitro study done by **Anitua et al in 2009** stated that the proliferation rate of fibroblasts was increased in the presence of PRGF. **Vahabi et al in 2015** compared the efficacy of PRF (platelet-rich fibrin) and PRGF and concluded that PRGF had a strong effect in terms of stimulation and proliferation of fibroblasts and epithelial cells.³² In a recently conducted human study done by **Jenabian et al in 2018** it was stated that when PRGF along with connective tissue graft for recession coverage using coronally advanced flap, the results showed significant differences in terms of Recession coverage, recession height and width and keratinized tissue width (KTW).³³ Following soft tissue injury, there is an increased expression of smooth muscle actin (α -SMA) in the fibroblasts resulting in increased production of myofibroblasts resulting in fibrosis, therefore weakening tissue characteristics.³⁴ Use of PRGF has shown to downregulate transforming growth factor-induced α -SMA expression thus reducing the possibility of healing by fibrosis and promoting regeneration.³⁵

EFFECT OF PRGF ON IMPLANTS AND PERI IMPLANT MUCOSA

The platelet-rich PRGF has shown remarkable results in soft tissue regeneration and bone regeneration in preparing sites for the placement of implants.¹ The protocol of implant placement has seemed to evolve with time with the use of platelet concentrates in it. **Anitua et al in 2006** stated that the platelet concentrate PRGF could be used to create new bone formation at the border of bone and implant, thus enhancing osseointegration by placing the implant covering its surface with PRGF.³⁶ In an animal study conducted by **Birang et al in 2012** stated that PRGF improved not only the healing process when placed along with an implant but also improved the percentage of Bone implant contact.³⁷ PRGF has also been used following extraction at the immediate implant placement site and has proved to be beneficial in post-extraction healing and prevent bone loss.³⁸

Conclusion

The main advantage of the use of PRGF is its healing property and the fact that it not only heals but allows regeneration. In this review, it has summarized the various usages of PRGF and their advantages in an abridged manner. However many studies are yet needed to be done to prove the exact mechanism of how it brings about regeneration. As mentioned in this review, PRGF can be used in various aspects of Periodontics and should be used in clinical practice daily.

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