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Role Of Platelet Rich Fibrin (PRF) In Periodontal Regeneration: Review Article

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Abstract

The second generation of platelet concentrates, known as platelet-rich fibrin (PRF), has drawn more attention in recent years because to its potential applications in regenerative medicine. This biologic additive offers various benefits over conventionally manufactured platelet concentrates, including being fully autologous, being simple to make, cost-effective, and having sustained growth factor release. Since its debut, several preparation methods for platelets have been put forth, using varying concentrations of growth factors and other macromolecules essential for the healing of wounds. Reference information about the possible impact of some PRF components on the healing of both soft and hard tissues is still ambiguous. The goal of the current paper is to shed light on pertinent developments regarding the physiological roles of specific PRF components and to offer an understanding of the new developmental paradigm. The development of platelet concentrates and the biologic characteristics of various PRF technique modifications are also discussed in this review article.

Keywords: platelet-rich fibrin, platelets, growth factors, wound healing

Introduction

Periodontal tissue regeneration has always been a challenge for the periodontists owing to its structural complexity.

Periodontal regeneration, referred to as the formation of alveolar bone, cementum and a new functional periodontal ligament (PDL), requires an orchestrated sequence of biological events for its surgical outcome. Therapeutic modalities that attempt to enhance these biological events such as cell migration, adherence, growth and differentiation have the potential to increase the success and predictability of periodontal regenerative procedures.[1]

Common bone graft biomaterials include autografts (a patient's own bone), allografts (human cadaver bone), xenografts (animal bone) and synthetic biomaterials.[2] Of these, autografts are used as the current standard since they are osteogenic, osteoconductive and osteoinductive.[3] Although autografts produce satisfactory results, they carry the risk of donor site morbidity and are limited in availability. With autograft, allograft and xenografts, each having their own unique set of disadvantages, synthetic biomaterials are emerging as potentially viable substitutes for bone regeneration, considering that they satisfy requirements such as being biocompatible, biodegradable and bioactive. However, synthetic biomaterials often lack sites for cell adhesion thus making biocompatibility frequently questionable. Also, stem cell differentiation and rate of degradability is not clear, while immune reactions are also possible.

To overcome these limitations, search for a material that would serve as both healing and interpositional material ended with the introduction of Platelet rich fibrin (PRF) by Choukron et al. from France in 2001.

Platelet Rich Fibrin (PRF) has shown to bring with it a biological revolution in dental field. Enhancement of these regenerative process of human body by utilizing the patient's own blood is a unique concept to dentistry. Platelet Rich Fibrin (PRF) can be used alone or in combination with scaffolds and biomolecules as an alternative bone graft substitute.[4]

This review article attempts to cover all the aspects of platelet derivatives, in particular, platelet rich fibrin, and show how these biomolecules have helped to enhance the present treatment modalities and thus brought in a new wave of regeneration in the field of periodontology.

Historical perspective

The use of blood derived products to heal wounds started back in 1970 with the use of fibrin glues or fibrin sealants which were formed by polymerizing fibrinogen with thrombin and calcium. It was originally prepared using donor plasma; however, because of the low concentration of fibrinogen in plasma, the stability and quality of fibrin glue was low.[5]

Regenerative potential of platelets was introduced in 1974 when Ross et al. identified platelet derived growth factor as a serum growth factor for fibroblasts, smooth muscle cells and glial cells (Kohler and Lipton 1974; Ross et al. 1974; Westermark and Wasteson 1976). Now it has been well documented that platelets provide a rich pool of varied growth factors such as PDGF-AB (platelet derived growth factor A B), TGF-ß1 (transforming growth factor beta-1), VEGF (vascular endothelial growth factors), fibroblast growth factor, insulin like growth factor, epidermal growth factor, connective tissue growth factor etc.

Platelet rich plasma (PRP), the first generation platelet concentrates showed positive results. However, the complexity of PRP preparation protocol and the risk of cross-infection due to the use of bovine thrombin lead to development of a newer generation of completely autologous platelet concentrates- platelet rich fibrin also called as Choukroun's platelet rich fibrin named after its inventor.

PRF was developed in France by Joseph Choukroun et al. in 2001. They used PRF to improve bone healing in cases of implants. It is a fibrin matrix in which platelet cytokines, growth factors and cells are trapped and may be released after a certain time and that can serve as a resorbable membrane. Growth factors are released after activation from the platelets trapped within fibrin matrix, and have been shown to stimulate the mitogenic response in the periosteum for bone repair during normal wound healing.[6]

PRF Cytokines[7]

Transforming growth factor- β (TGF-β): Platelets are known to be a major source of TGF-β production. The role of TGF-β mediates tissue repair, immune modulation and extracellular matrix synthesis. Bone morphogenic proteins (BMPs) are also part of the TGF subfamily. TGF β1, the predominant isoform, is important in wound healing, with roles in inflammation, angiogenesis, re-epithelialization and connective tissue regeneration.

Platelet-derived growth factors (PDGF): (PDGFs) are essential regulators for the migration, proliferation, and survival of mesenchymal cell lineages and promotes collagen production for remodelling of ECM during wound healing. Platelets are the major source of PDGF with various groups divided into homo- (PDGF-AA, PDGF-BB, PDGF-CC, and PDGF-DD) and hetero-dimeric (PDGFAB) polypeptide dimers linked by disulphide bonds. They are present in large amounts in platelet granules.

Insulin-like growth factors (IGFs): IGF are positive regulators of proliferation and differentiation of most cell types, which act as cell-protective agents. This growth factor is released from platelets during their activation and degranulation and stimulates differentiation and mitogenesis of mesenchymal cells.

<u>Inflammatory cytokines</u>

Interleukin-1β (IL-1β)

IL-1β remains the prevalent isoform and is a key mediator of inflammation control. Its main activity is the stimulation of T helper lymphocytes. In combination with TNF-α, IL-1 would be implied in

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osteolysis, where it activates osteoclasts and inhibits bone formation.

Interleukin 6 (IL-6)

IL-6 is a multifunctional cytokine that was originally identified as a B cell differentiation factor which induced the final maturation of B cells into antibody producing cells. Within the B lymphocyte populations, IL-6 significantly stimulates the secretion of antibodies by 120-400 times. In addition, IL-6 is an essential accessory factor for T cell activation and proliferation. IL-6 induced not only proliferation but also differentiation of cytotoxic T cells (CTL) in the presence of IL-2 from murine as well as human thymocytes and splenic T cells.

Tumor necrosis factor α (TNF-α)- TNF derives its name from the ability to stimulate tumor necrosis and regression. TNF-α is one of the first cytokines released during the inflammatory response to bacterial endotoxin aggression. It activates monocytes and stimulates the remodeling capacities of fibroblasts. In addition, it increases phagocytosis, neutrophil cytotoxicity and modulates the expression of key mediators such as IL-1 and IL-6.

Healing cytokines[8]

Interleukin 4 (IL-4)- IL-4 induces differentiation of naive helper T cells into TH2 cells. This cytokine also supports proliferation and differentiation of the activated B cells. During inflammatory phenomenon, it supports healing by moderating inflammation.

Vascular endothelial growth factor (VEGF)- VEGF is considered as a master regulatory molecule for angiogenesis-related processes. Factors like IGF-I and IL-1β regulate angiogenesis by upregulating the expression of VEGF. It plays a direct role in the control of endothelial cell behaviors, such as proliferation, migration, specialization or just survival.

PRF processing[9]

Blood drawing

1. Blood is drawn from the patient using a sterile 10 ml vacutainer just before or during surgery (**picture 1**).
2. The tubes with collected blood samples are immediately (within 2 minutes after collecting the blood sample) placed in the centrifuge and processed using a single centrifugation step.
3. The clinical success of the PRF protocol is dependent on a quick collection of blood and its transfer to the centrifuge because blood will automatically start to coagulate after 1-2 minute and make it difficult to obtain the required clot quality.[25]
4. Failure to accomplish the quick preparation of PRF could cause a diffuse polymerization of fibrin, which is not ideal for tissue healing.

Centrifugation

1. After collection of blood, it is immediately centrifuged on a table-top centrifuge at a rate of 3000 rpm for 10 minutes.
2. The tubes should always be balanced by opposing two tubes to equilibrate the centrifugation forces and to prevent vibrations **(picture 2).**
3. At the end of the centrifugation spin, the caps are removed and the tubes placed in a sterile tube holder.
4. The blood sample with clot is allowed to rest/mature for approximately 4-8 minutes before extracting the clot from the tube.
5. The centrifugation process activates the coagulation process and separates the blood sample into three different layers: the topmost layer consisting of acellular PPP (platelet poor plasma), PRF clot in the middle and RBCs at the bottom of the test tube **(picture 3).**

Preparation of PRF membrane

1. The PRF clot is removed from the tube with a sterile tweezer. The fibrin clot is separated from the red blood cell fragment, approximately 2 mm below the dividing line, using a scissor. The section of the blood clot attached to the fibrin clot contains the stem cells.
2. The PRF clots are placed in the PRF box (**picture 4**) and covered with the lid.
3. The PRF membranes are ready for use after 2 minutes.
4. A PRF membrane remains usable many hours after preparation, as long as the PRF is prepared correctly and conserved in physiologic conditions.
5. The use of the PRF Box is a user-friendly and inexpensive tool, allows for standardized preparation of homogeneous PRF membranes with a higher growth factor content, avoids the dehydration of the leukocytes living in the PRF

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clot, and also prevents the shrinkage of the fibrin matrix architecture.

Factors to consider

1. Always make sure that the centrifuge tubes are filled equally (1cm from the top).
2. Always balance the rotor properly. Every tube must have a balance or opposing tube.
3. Do not balance with a vacutube filled with water, the distribution of the densities will be incorrect and cause unnecessary vibration.
4. If properly balanced and used, the rotor should accelerate smoothly and with a constant change in the pitch of the motor sound.
5. Any vibrations, or unusual sounds should cause the cessation of operation immediately by the operator.

Handling of the PRF

PRF membranes are easy to drape over a surgical or augmented site. The elastic consistency of the PRF membrane also allows the clinician to punch a hole in the membrane to drape over a healing abutment before suturing the flap.[10]

Fig 1 Blood drawing

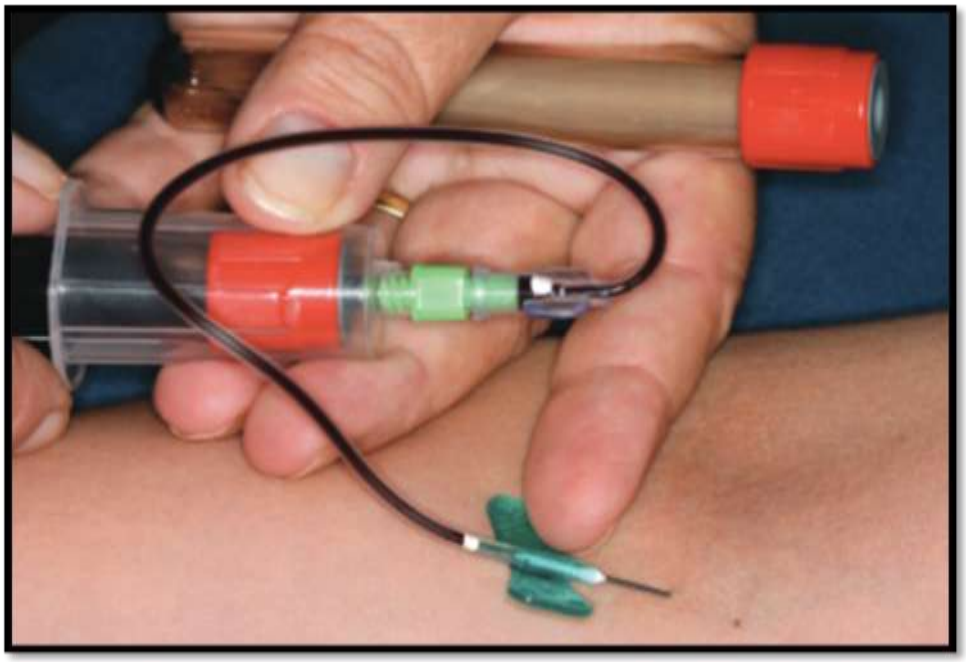

Fig 2 - Test tubes placement inside PRF centrigugation machine

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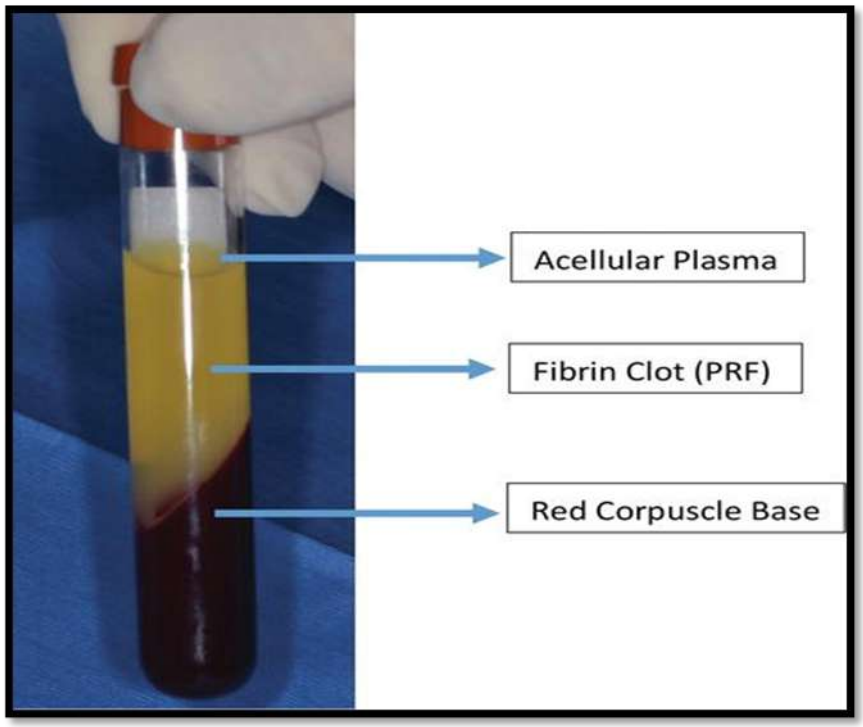
PICTURE 3- Formed PRF after centrifugation



PICTURE 4- Formation of PRF membrane in PRF box

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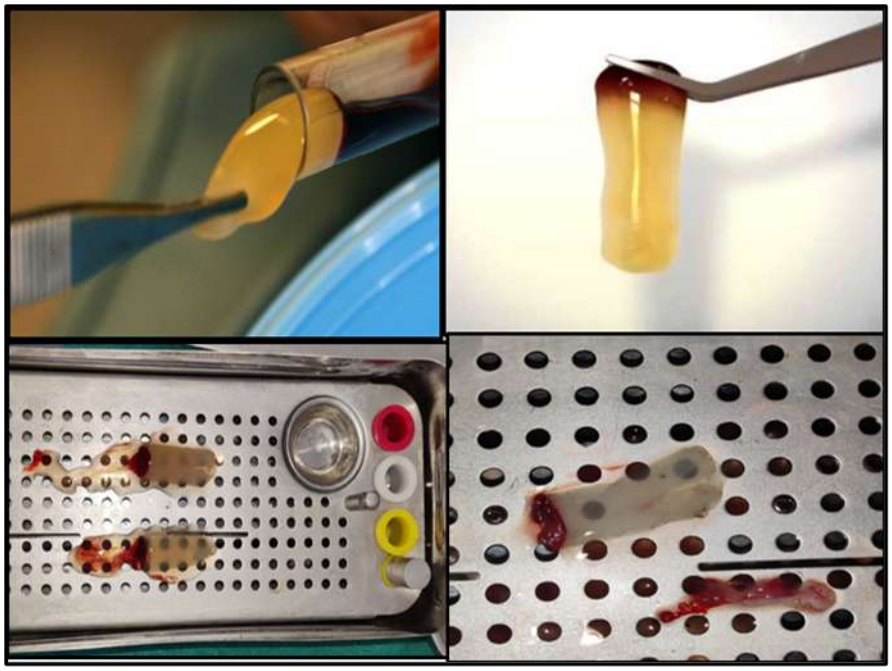

Biologic mechanisms of PRF membrane[10]

a) Bioactive barrier

A PRF membrane is a blood clot prepared in an optimized form that is rich in cells and growth factors, and acts as a natural bioactive barrier, allowing interaction with the tissues below and above it.

b) Competitive interposition barrier

GTR membranes are cell-proof barriers against soft tissue invagination, whereas PRF membranes allow cells to migrate through it, thus allowing new blood vessel formation that will facilitate regenerative and healing interactions between the tissues below and above the PRF membrane. The PRF membrane is a highly stimulating matrix, attracting cell migration and differentiation preferentially, and also reinforcing the natural periosteal barrier. The hard and soft tissues migrate and interact within the PRF matrix.

c) Protective barrier and healing booster

PRF membranes are frequently used for the protection of the grafted area and as a healing booster for the soft tissues above the grafted defects or augmented sites. The purpose of the PRF membrane is not only to protect the blood clot and/or the graft material, like in the GTR concept, but also to promote the induction of a strong and thick periosteum and gingiva. This boosted periosteum functions as a true barrier between the soft tissue and bone compartments, and constitutes probably the best protection and regenerative barrier for the intrabony defects.

Potential benefits of using PRF in periodontal regeneration[11]

Platelet-rich fibrin is a second generation platelet concentrate which can enhance both soft and hard tissue healing. Its advantages over platelet-rich plasma include ease of preparation, ease of application, minimal expense, and lack of biochemical modification (no bovine thrombin or anticoagulant is required). This considerably reduces the biochemical handling of blood as well as risks associated with the use of bovine-derived thrombin. PRF also contains physiologically available thrombin that results in slow polymerization of fibrinogen into fibrin which results in a physiologic architecture that is favorable to wound healing. The cytokines which are present in platelet concentrates play an important role in wound healing. The structural configuration of PRF with respect to cytokine incorporation in fibrin meshes is different from that present in PRP. The natural polymerization in PRF results in increased incorporation of the circulating cytokines in the fibrin meshes (intrinsic cytokines). These intrinsic cytokines will be having an increased lifespan and they will be released and used only at the time of

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initial cicatricial matrix remodeling which creates a long term effect.

The three dimensional organization of a fibrin network in PRF and PRP affects the biologic and mechanical properties of these platelet concentrates. During gelling of these fibrin structures, the fibrin fibrillae can be assembled in 2 ways, bilateral junctions or equilateral junctions.

Added advantage of PRF is the presence of natural fibrin network in PRF which protects the growth factors from proteolysis. PRF also favors the development of microvascularization leading to a more efficient cell migration.

Clinical applications of PRF in dental surgery[12]

1. In the field of oral and maxillofacial surgery

2. In the field of cosmetic and plastic surgery

3. Bone grafting for dental implants that includes:

- Inlay and onlay grafts.
- Sinus lift procedures
- Ridge augmentation procedures
- Closure of cleft lip and palate defects
- Repair of bone defects created by removal of teeth or small cysts
- Repair of fistulas between the sinus cavity and mouth.

Clinical applications of PRF in periodontics

1. ln root coverage procedures and gingival surgery: Soft tissue periodontal surgery is indeed very technical, the purpose often being to improve the quality of the gingival tissue, or to cover gingival dehiscence/recessions around teeth through the promotion of a tissue attachment on the bare dental roots. The idea of using platelet for a better soft tissue attachment on the root surface is quite old, and the use of fibrin derivatives was also suggested in periodontal research as a potential matrix for gingival healing and attachment.

2. In periodontal defects along with guided tissue regeneration: The growth and differentiation factors are increasingly being used for improvement and enhancement of healing of periodontal tissues. Increased level of growth factors also lead to an improvement in bone regeneration. Also, they accelerate the healing of soft tissue around bone.

3. In the treatment of human intrabony defects: PRF is combined with bone grafts in the treatment of intrabony defects. Since the space maintenance of the defect is a crucial factor in periodontal regeneration, PRF's gel-like consistency may complicate the healing leading to flap collapse. In a study the clinical and radiographic results of PRF alone in the treatment of intrabony defects were evaluated.

4. In the field of implant dentistry; The most frequently encountered hindrances at the implantation site are lack of adequate bone available and proximity to anatomic structures, such as the maxillary sinus and the inferior alveolar nerve canal. Advanced surgical procedures that act as an adjunct in dental implants consist of sinus grafting and guided bone regeneration. With the application of PRF in addition to autogenous grafts used for these procedures like sinus lifts, ridge augmentations, etc., will promote and accelerate Osseointegration process.

Advantages of using PRF [13,14]

1. Its preparation is a simplified and efficient technique, with centrifugation in a single step, free and openly accessible for all clinicians.
2. It is obtained by autologous blood sample.
3. Minimized blood manipulation.
4. It does not require the addition of external thrombin because polymerization is a completely natural process, without any risk of suffering from an immunological reaction.
5. It has a natural fibrin framework with growth factors within that may keep their activity for a relatively longer period and stimulate tissue regeneration effectively.

Disadvantages of using PRF[15]

1. The final amount available is low because it is autologous blood.
2. The success of the PRF protocol depends directly on the handling, mainly, related to blood collection time and its transference for the centrifuge.

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3. Need of using a glass-coated tube to achieve clot polymerization.
4. Possible refusal of treatment by the puncture required for blood collection.
5. Only needs a minimal experience of clinician for PRF manipulation.

Contraindications[16]

Absolute Contraindications

- Platelet dysfunction syndrome
- Critical thrombocytopenia-
- Hemodynamic instability-
- Septicemia

Relative Contraindications

1. Consistent use of NSAIDs within 48 hours of procedure
2. Corticosteroid injection at treatment site within 1 month,
3. Systemic use of corticosteroids within 2 weeks.
4. Hb < 10 g/dl.
5. Platelet count < 105/µl.

Recent advances in platelet concentrates[17]

After PRF, a concept of **"Concentrated Growth Factors (CGF)"** was introduced by Sacco in 2006. A special centrifuge called Medifuge (Italy), is used to prepare CGF, similar to PRF but with a different centrifugation speed which allows the separation of a fibrin matrix which is much denser, larger and richer in growth factors. CGF has been shown to have a greater versatility and better regenerative capacity, as reported for alveolar ridge and sinus augmentation. In a study, it demonstrated the presence of VEGF and TGF-β1 in RBC and CGF layers. This suggests that improved CGF procedure could enhance the quantity of growth factors in the CGF layer or, alternatively, a possible use of RBC layer in clinical applications.

In an attempt to incorporate the monocytes within the PRF, Choukroun introduced an **Advanced PRF** called **A PRF**. They have discovered earlier soft tissue growth, more release of BMPs, greater and faster vascularization and more cytokine release than conventional PRF.

A concept of fabricating growth factors-enriched bone graft matrix (also known as **"sticky bone"**) using Autologous fibrin glue (AFG) has been demonstrated since 2010. Sticky bone provides stabilization of bone graft in the defect, and therefore, accelerates tissue healing and minimizes bone loss during healing period. To obtain autologous fibrin glue, 20-60 CC of venous blood is centrifuged at 2400-2700 rpm) using a specific centrifuge (Medifuge, Silfradentsrl, Sofia, Italy) for 2 min.

Mourao et al. (2015) described a technique to obtain an Injectable form of PRF called **I-PRF**. In this technique a short centrifuge for 2 min at 3300 rpm gave an orange color fluid which can be injected or mixed with bone graft to give a well agglutinated "steak" for bone grafting.

Although successful procedures have been reported extensively using Choukroun's **L-PRF**, physicians such as O Connell had raised concern regarding possible health hazard with the particles of silica in the glass tubes.

However, Tunali et al in 2014, introduced a new product called Titanium prepared PRF (**T-PRF**). The use of titanium tubes for collection and centrifugation instead of glass tubes was established on the hypothesis that titanium may be a more efficient platelet activator than silica, for preparing L-PRF.

Limitation of using PRF

The rapid use of PRF without delay or short handling time may be a potential limitation. Lack of rigidity and fast degradation (biodegradability) may limit its application in GTR procedures.

Conclusion

PRF advocates promoting the procedure as an organically based therapy that enabled healing through the use of one's own natural growth factors in recent years, scientific research and technology has provided a new perspective on platelets. Studies suggest that platelets contain an abundance of growth factors and cytokines that can affect inflammation, postoperative blood loss, infection, osteogenesis, wound and soft tissue healing. Research now shows that platelets also release many bioactive proteins responsible for attracting macrophages, mesenchymal stem cells and osteoblasts that not only promote removal of degenerated and necrotic tissue, but also

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enhance tissue regeneration and healing. PRF is easy to obtain and safe for clinical use. Therefore, PRF might prove to be a valuable tissue healing strategy as a bone regeneration accelerator. More active use in reconstructive surgery is expected.

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