

Platelet-rich Fibrin Potential Role in Periodontal Regeneration: A Review Study

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Abstract

Periodontitis is a persistent bacterial-causing disease which damages the supporting periodontium of the teeth. The complexity of supporting tissue structure makes the regeneration a challenge for periodontists. Early investigations were focused on discovering therapeutic substitutes that are biocompatible, simple to prepare and economic. This might cause a local release of growth factors that accelerate the healing process of the soft and hard tissue. Recently, platelet-rich fibrin (PRF) has received a wide attention as a biocompatible regenerative material in both dental and medical fields. PRF is a natural fibrin-derived biomaterial, and it is easy to obtain. It can be gotten from individual blood without the use of any external anticoagulant. The principle of PRF technology use in the regeneration process is to use patients' blood to extract all the growth factors which promote regeneration of tissue by accelerating the healing process. The main objective of this review is to concisely illustrate PRF and its contribution in periodontal regeneration.

Keywords: Growth factors, periodontal regeneration, platelet concentrates, PRF

INTRODUCTION

Periodontal diseases are complicated and multifactorial diseases by which attachment loss and destruction of supporting bone and cementum are associated. The aims of periodontal clinical therapy are eradication of inflammation, inhibit disease progression and regeneration of the lost periodontium. Periodontal regeneration is a complex multi-connected biological event such as cells adhesion, proliferation, differentiation and migration. One of utilized materials in periodontal regeneration is graft which is either autogenous or alloplastic.^[1] Even though, various biomaterials applied in the autogenous and allogenic bone grafts as well as periodontal regeneration application, no specific graft material is deemed the best for the intra-bony defect's treatment. Several drawbacks associated with graft application. First, it requires second surgical procedure and result in gingival recession. Second, may provoke antibodies host reaction. Third, it is expensive and long post-operative time and discomfort. Therefore, utilization of PRF in periodontal regeneration field are advantageous for the patient and the practitioner.^[2]

PRF consists of natural fibrin matrix containing a high concentration of both platelets and growth factors.^[3,4] PRF exhibits a complex architecture with distinctive mechanical characteristics. The structure is obtained from a patient's blood with no anticoagulant or biochemical agents. PRF acts locally by quickly stimulating different cells recruitment, proliferation, and/or differentiation. PRF exerts its effect on gingival fibroblasts, endothelial cells, osteoblasts, and chondrocytes. Thus, PRF affects tissue repair and regeneration.^[5,6] PRF is promising regenerative biomaterials in periodontics field for several reasons. First, PRF can heal wound in very fast pace.^[7,8] Second, it can be gotten using simple and inexpensive preparation method. Third, exogenous compounds (e.g., calcium chloride or bovine thrombin) are not required. Moreover, PRF is collected from patient's own blood in PRF preparation. Thereby, blood-transmitted diseases is

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reduced or eliminated. It is important to determine how the released growth factors from PRF may be equivalent to the food and drug administration (FDA)-approved recombinant BMP2 (bone morphogenetic protein2) and PDGF (platelet derived growth factor) proteins.^[9-12] The FDA approved- proteins may have several drawbacks regarding expenses and unfavorable effects among potential swelling, lower stability, and biocompatibility. Thereby, the use of autogenous PRF is preferred.^[13,14] Platelet-rich fibrin (PRF) was the key in utilization of maxillofacial and oral surgery.^[2]

PERIODONTAL WOUND HEALING AND TISSUE REGENERATION

Periodontal wound healing process is an order of complex events that involves interaction between epithelial cells, osteoblasts and fibroblasts. While the wound is healing, the disruption of vasculature triggers platelet aggregation and fibrin formation. Several growth factors are released from platelets into tissues, through molecular signals by which cytokines and growth factors mediate.^[15] The presence of cytokines and growth factors in platelets perform vital roles in wound healing and inflammation.^[16] Furthermore, platelets release fibronectin, fibrin, and vitronectin which act as a matrix as well as adhesion molecules for well-organized cell migration.^[17] The use of platelets as therapeutic agent enhances tissue repair particularly in periodontal wound healing.

PLATELETS AND PLATELET CONCENTRATES

Platelets are non-nucleated blood cells derived from bone marrow precursor (megakaryocytes) with lifetime 8-10 days in blood.^[15] Platelets which are specialized blood fragments have essential tasks in hemostasis, inflammation, and wound healing. Adherence and aggregation of platelets in a severed vessel form a plug and initiate the coagulation cascade. Activation of the alpha granules of platelets upon contact with connective tissue results in numerous growth factors releasing, for instance, (Vascular endothelial, Platelet Derived and transforming- β) growth factors. These growth factors are critical to wound healing in initial stages and results in a gene sequence expression which guides collagen synthesis, cellular proliferation, and osteoid production

CLASSIFICATION OF PLATELET CONCENTRATES

- Platelet-rich plasma (PRP): is the first-generation platelet concentrates. Even though it revealed relatively positive results,^[18] the risk of bovine thrombin-cross infection and complex preparation protocol were drawbacks.
- Platelet-rich fibrin (PRF) named as its inventor who was Choukroun's platelet-rich fibrin.

PRF Vs. PRP

During the last years while the discovery of PRF, the utilization of platelet concentrates has markedly raised.^[19] However, it is essential to mention that blood growth factors used in medical field for more than two decades.^[20] These earliest efforts to use concentrated growth factors of platelet were based on the fact that gain extra amounts of platelets promote wound healing.^[21] These principles were later recognized as "platelet rich plasma" (PRP). PRP, an autologous thrombocyte concentration, was developed in 1990s as an improvement factor for periodontal tissue healing.^[22] The main purpose of PRP use was to collect great amounts of platelets and their associated growth factors in highest quantity and re-use them throughout surgery.

Comparison between PRF and PRP can be illustrated as follows.

STANDARD PROTOCOLS

- PRF:** One centrifugation cycle with less time is required. Using a centrifugation machine with 3,000 revolution per 60 seconds, PRF was immediately centrifuged for 10 minutes.^[23] The following are the main difference points between PRF and PRP.
 - PRP:** Two centrifugation cycles are needed. After PRP preparation, around 45 minutes were needed to place PRP in the surgical defect.^[24] Ten minutes at 1,200 rpm (160 g) were recommended in the first spin in which the red blood cells were separated from the rest of the blood. In the second spin (i.e., concentration spin), it was recommended to spin 3,200 rpm (1,200 g) for 10 minutes after aspirated fluid was added. The big role of the second spin is to separate and compact white blood cells, the platelets, and a few number of remaining red blood cells from the plasma, which is clear straw yellow in color (platelet poor plasma [PPP]).^[25,26]
- The incorporation of leukocytes in PRF is one of the key difference between PRF and previously utilized PRP.^[27]
 - The lack of anti-coagulants resulting in a fibrin matrix is a major difference between PRF and PRP.
 - Bone grafting material is needed in PRP to maintain volume.
 - PRF provides a reduction in the amount of spent time and it is considered less technique sensitive.^[23]

The aforementioned limitations of the use of PRP have been reported even though the huge amount of attention PRP attained since it was first used in periodontitis. Due to the lengthy technique, extra anti-coagulant factors were required to prevent clotting. The inhibitors of wound healing used were bovine thrombin or CaCl_2 or both.^[28] These drawbacks limited the use of PRP in complex maxillofacial surgeries.^[29]

PRF DEFINITION AND MECHANISMS OF ACTION

PRF is a second generation of platelet concentrate with three-dimensional natural biological structure due to its natural concentrate without the use of any anticoagulants.^[16,17] PRF contains a condensed fibrin network which has cytokines, leukocytes and structural glycoproteins.^[30] The fibrin network also contains growth factors such as vascular endothelial growth factor (VEGF), transforming growth factor β 1 (TGF- β 1) glycoproteins (e.g. thrombospondin-1), and platelet-derived growth factor (PDGF).^[31] PRF Leukocytes release growth factors,^[31] regulate the immune system,^[4] and have anti-infectious properties.^[32] PRF Leukocytes play a part in a matrix remodeling during wound healing. The slow polymerization of PRF result in a physiologic architecture encouraging for wound healing.^[31]

The proposed mechanism of PRF during the initial stage of wound healing is that local delivery of platelet concentrate result in activation of platelet alpha granules. Platelet alpha granules release ample amounts of growth factors, for example, (transforming, insulin like, fibroblast and platelet derived) growth factors.^[31,33] All these growth factors act as a booster for healing events which include proliferation, migration, and differentiation of cell from nearby tissue.^[3,34]

EFFECT OF PRF ON PERIOSTEUM

PRF stimulates the periosteum blood supply. The direct contact of periosteum with PRF significantly improves the amount of the blood to the keratinized soft tissue included its thickness and blood supply to the underlying bone. This activity is PRF main benefit, by means of growth factors release stimulation over a long period.^[35]

The augmentation with PRF alone is associated with high success rate without major complications as well as the density of the regenerated bone in defects that demonstrated complete resolution was higher than that of defects that showed partial resolution.^[36]

MAJOR CELL TYPES IN PRF

Leukocytes, platelets, macrophages, granulocytes, and neutrophils are the major cells found within PRF. After the centrifugation cycle, most of the cells are entrapped within the fibrin matrix. The incorporation of leukocytes in PRF is the major difference between PRP and PRF. The autologous cells within PRF, particularly leukocytes, regard as a key of regeneration. major advantages of leukocytes can be addressed as follows:

- They show resistance to pathogen and function in immune regulation.^[37,38]
- They perform a substantial role during integration between host tissue and biomaterial.^[15]

Upon mentioned benefits of leukocytes, removal of third molars revealed to ten times reduction in osteomyelitis infections, swelling and postoperative pain. Furthermore, more wound healing after PRF placement into extraction sockets is noticed.^[39,40]

GROWTH FACTORS IN THE BLOOD

1) Transforming growth factor β (TGF- β)

Transforming growth factor β (TGF- β) is a superfamily of more than 30 members released by platelets. TGF- β 1, which facilitates immune regulation, tissue repair, and extracellular matrix production, is vital to inflammation, angiogenesis, wound healing, tissue regeneration, and re-epithelialization.^[41] TGF- β is essential in osteogenesis through influencing osteoblast precursors via chemotaxis, mitogenesis, and deposition of mineralized elements on the collagen matrix by stimulation of osteoblasts. The level of vascular endothelial growth factor can be increased by TGF- β , thereby, TGF- β encourages angiogenesis and recruitment of inflammatory cells.

2) Platelet-derived growth factors (PDGFs)

PDGFs induce the migration, survival of mesenchymal cell and proliferation, and stimulate collagen production which is required for extracellular matrix restoration throughout the wound healing.^[42] PDGF is gathered in large amounts in PRF matrix and released over time. Because of exceptionally short half-life of PDGF, PRF supports its gradual and slow release over time. Furthermore, PDGF is the main mitogen for fibroblasts and osteoblasts, smooth muscle cells, glial cells, and undifferentiated osteoprogenitor cells.

3) Vascular endothelial growth factor (VEGF)

VEGF is defined as the most powerful growth factor that triggers new blood vessel formation. Activated thrombocytes and macrophages release vascular endothelial growth factor to the injured sites for angiogenesis. Thus, providing nutrients supply and increasing blood flow to the injured site.^[43,44] Moreover, upregulation in osteogenesis can be noticed upon combination of recombinant human VEGF into different bone biomaterial agents.

4) Insulin-like growth factors (IGFs)

The IGFs stimulate proliferation and differentiation of most cells and act like a cell-protective agents.^[45] Platelets, during their activation and degranulation, are responsible for IGFs releasing which induce mesenchymal cells differentiation and mitogenesis. IGFs protect cells from different apoptotic stimuli by stimulates survival signals.^[45] Thus, it considered a major regulator of programmed cell apoptosis

PRF PREPARATION PROTOCOL

The main equipment used for PRF preparation are 24-gauge butterfly needles, 10ml vacuumed tube without any external anticoagulants, centrifuge, and PRF box.^[46] Centrifugation of 10ml intravenous blood in sterilized tube at 3000 rpm for 10 minutes is used in PRF preparation technique^[47] to form of a yellow gel like fibrin plug in the central part of tube has been established^[17] [Figure 1]. PRF continue release growth factors for 300 minutes afterwards its formation. So, it should be utilized immediately. The progressive release of leukocytes and cytokines lasts for 7–11 days, while the fibrin scaffold undergoes degeneration.

PRF APPLICATIONS

PRF is a potent healing biomaterial and has intrinsic regenerative ability which can be utilized in various aspect, for instance:

- Treatment of periodontal intra-bony defects.^[8]
- Periodontal Furcation involvement therapy.^[48]
- Sinus lift procedures.^[49]
- Tissue engineering.^[50]

APPLICATION OF PRF IN PERIODONTIC FIELD

PRF used to be a scaffold matrix only or in mixture with other agents of biomaterials. Later, it has widely dental applications which accelerate the healing and regeneration of oral tissues. Many periodontists use PRF to regenerate the periodontal defects. Following regenerative periodontal treatment with PRF, these attempts showed significant enhancements in clinical attachment level gains as well as periodontal pocket depth. Moreover, improvement in the outcomes of furcation class II involvement treatment also achieved.^[39,40] Nowadays, the clinical trials establish that using PRF alone or combined with bone grafts

result in statistically higher findings in a comparison with conventional open flap treatment alone or the use of bone grafts alone.

USE OF PRF IN GUIDED BONE REGENERATION

PRF has been employed in combination with bone augmentation procedures. When PRF is used with a bone grafting material, additional graft stability as well as an increased vascular supply are improved. However, more research is needed to address effective potential of PRF for bone regeneration procedures.^[35]

DISADVANTAGES AND LIMITATIONS OF PRF

The disadvantages of PRF include but are not limited to:

- The preparation and storage of PRF are quite inadequate.
- Both time interval between handling and blood centrifugation is critical for clinical outcome.
- PRF membranes shrink and alter PRF structure if not used immediately after the preparation.
- Growth factor in PRF begins to drop right after the preparation changing PRF biologic properties.^[31]
- Bacterial contamination of membranes is likely to happen if PRF is stored in the refrigerator.
- PRF is very slippery and hard to handle and use.

However, these limitations can be avoided by followed the standardized preparation protocol.

PRF DEGRADATION PROPERTIES

The degradation rate of PRF is typically 10 to 14 days.^[35] PRF fast resorption time is a disadvantage for using PRF for dental procedures such as guided bone regeneration (GBR). It is recommended that resorption time with collagen membranes is 4 to 8 weeks. Consequently, the use of two PRF membranes or modifying the properties of PRF membranes through heating or other procedures is suggested.^[35] The effect of these double layering techniques or various modifications affect cell survival/behavior, growth factor release, or tissue regeneration. Further research is needed to explore the modification of the adjustments to standardized protocols.^[35]

Future recommendations

More studies should be conducted to relate the clinical outcomes of PRF use along with its biologic mechanisms which open up new uses of second generation of platelet concentrate. Limited studies are on PRF effect on cell proliferation, differentiation and other biological consequences. Hence, further studies should be carried out to open newer approaches for the platelet concentrate use.

CONCLUSION

The use of PRF either alone or with bone grafts, soft tissue grafts, and pharmacologic agents offered reliable

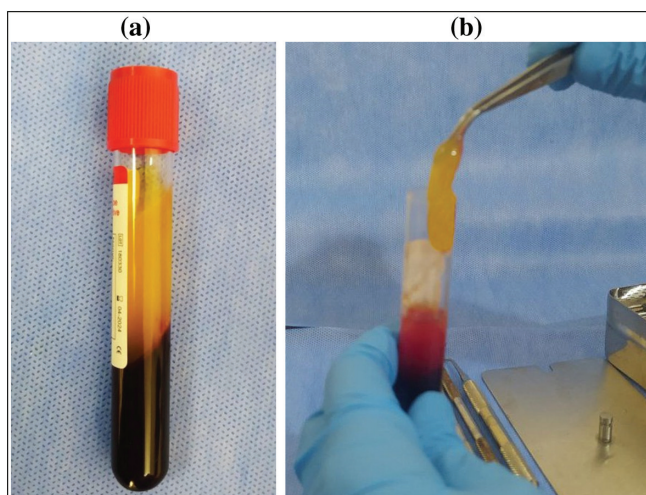


Figure 1: Formation of yellow gel like fibrin clot in the middle of tube after centrifugation

and promising outcomes via enhancements in clinical and radiographic parameters of periodontal therapy. However, long-term randomized control trials with large sample size are required to affirm the advantages and recognizing the unknown effects of PRF as a biomaterial agent.

Ethical approval

Not applicable.

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Conflict of interest

All the authors certify that they have no conflict of interest to disclose in relation to the subject matter or materials discussed in the present study.

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