Platelet-Rich Fibrin: An Update

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Abstract

The development of bioactive surgical additives to regulate the inflammation and increase the speed of the healing process is one of the great challenges in clinical research. In this sense, platelet-rich fibrin (PRF) appears as a natural and satisfactory alternative with favorable results and low risks. The following review attempts to summarize the relevant literature regarding the technique of using PRF, focusing on its preparation, advantages, and disadvantages of using it in clinical applications and its updates. PRF alone or in combination with other biomaterials seems to have several advantages and indications both for medicine and dentistry, due it is a minimally invasive technique with low risks and satisfactory clinical results.

Keywords: *Blood Platelet; Fibrin; Platelet-Rich Fibrin; Hard Tissue Regeneration; Soft Tissue Regeneration.*

Introduction

Platelets are generally produced in the bone marrow by the megakaryocytes and their sizes vary from 2–3 μm in diameter. They are consists of several granules, mitochondria, and canalicularsystem. The re is also another structure known as theα granules which are oval or round in shapes with diameter 200 to 500 nm which is covered by a membrane (Figure 1 : Platelet). These create a mass storage of proteins that are vital for wound healing, including platelet-derived growth factor (PDGF), transforming growth factor (TGFb), and several others. The fusion of αgranulewith the platelet cell membrane occurs after activation. This again leads to the formation of bioactive proteins. Then these proteins allow them to bind with the target membrane or cells. After the attachment, intracellular signal proteins are activated. This ultimately creates a gene expression sequence which leads to cell multiplication, collagen synthesis, and new cell production.[1]

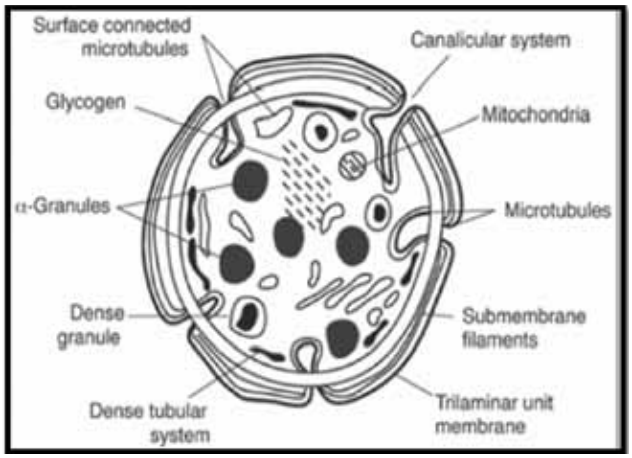

Figure 1: Platelet

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Figure 2 : Platelet Concentrates

Platelet Concentrates: PRF was first introduced by Ross et al in 1974 and it was also discovered that there are presence of several growth factors in PRF (Figure 2 : Platelet concentrates).Platelets play an important role in both the bleeding control and wound healing. From the early 90s, there are discoveries regarding numerous blood-related growth factors which can accelerate the wound healing procedure.[1]

Classification[1]:

1st Generation–PRP (Depending on the presence or absence of leukocytes and the activation or not of the PRP) Mishra et al, 2009

- Type 1, PRP is a L-PRP solution
- Type 2, PRP is a L-PRP gel
- Type 3, PRP is a P-PRP solution
- Type 4, PRP is a P-PRP gel

2nd Generation–PRF (Based on their fibrin architecture and cell content) Dohan Ehrenfest 2009

- Leukocyte poor or pure platelet-rich fibrin (P-PRF)
- Leukocyte and platelet-rich fibrin (L-PRF)-Choukroun's PRF
- Leukocyte poor or pure platelet-rich plasma (P-PRP)
- Leukocyte & platelet-rich plasma (L-PRP)

History of Platelet Concentrates:

Fibrin sealants: It was first available in the market since the late 1970s in Europe. Fibrin sealants or 'fibrin glues' are derivatives of plasma which helps in the blood coagulation process by the formation of a fibrin clot. The y are currently used as topical hemostaticagents. Widely used for flap closure in aestheticzones. As there is enhanced risk in cross-infection, nowadays tissue sealants are produced from the patient's plasma.[2]

Pure PRP or Leucocyte-Poor PRP: This is prepared without the addition of leukocytes, it results in low-density fibrin clot.[2]

Leucocyte-Rich Fibrin and Pr: Products are preparations with leucocytes and with a high-density fibrin network.[2]

Pure Prf or Leucocyte-Poor Prf: Preparations are without leucocytes and with a high-density fibrin network. Unlike pure PRP or PRP containing leukocytes, these products cannot be injected and exist in an activated gel form.[2]

Leucocyte-Rich Fibrin and Prf: Products are preparations with leucocytes and with a high-density fibrin network.[2]

Leukocyte and platelet-rich fibrin (l-prf)-choukroun'sprf

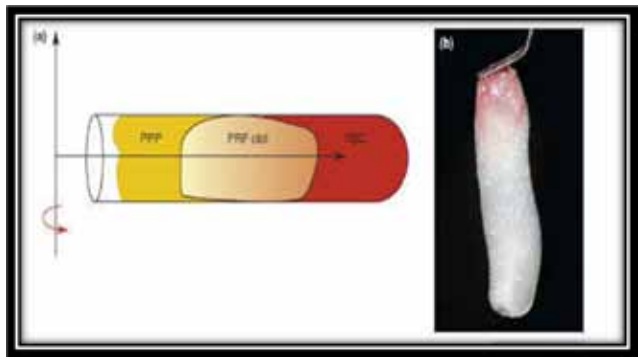

Figure 3 : Choukroun's Prf

PRF was first introduced by Choukroun et al, 2001 (Figure 3 :Choukroun'sPRF).

The procedure of making PRF uses neither anticoagulant nor bovine thrombin.[4]

Prf Protocol: Choukroun et al. in France produced the PRF by taking up venous blood (around 5 ml) in 2 test tubes without any addition of anticoagulant and the tubes are then centrifuged at 3,000 rpm, (approx. 400g) for 10 min, which creates 3 distinct layers.[4]Upper straw-colored acellular plasma, red-colored lower fraction containing red blood cells (RBCs), and the middle

fraction containing the fibrin clot. The upper straw-colored layer is discarded and the middle fraction is collected, which is PRF.[4] (Figure 4 : L-PRF)

Antimicrobial Property of Prf: An in vitro study, it was found that i-PRF has maximum antimicrobial efficacy rather than any other platelet preparations because of its high platelet count.[9]

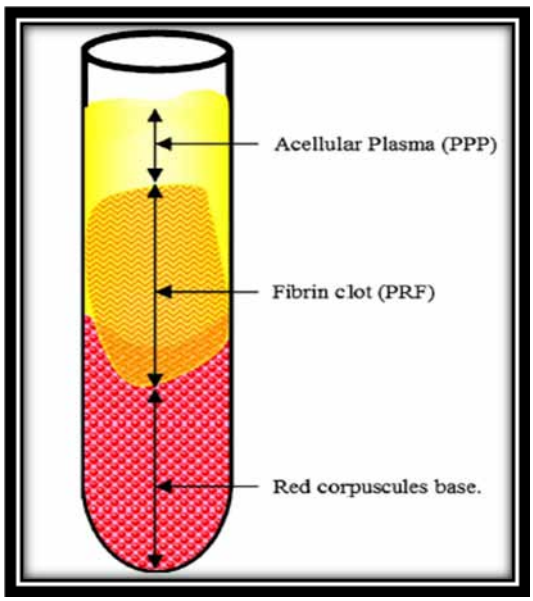

Figure 4 : L-PRF

Importance of Various Parameters for Centrifugation:

Relative centrifugal force, Radius of centrifuge and Rotation per minute: Various authors have reported centrifugal g-force at the PRF clot (referred to as relative centrifugal force (RCF)-clot – location at which the PRF clot is formed), whereas others have utilized the international standard method to report g-force calculated at the bottom of centrifugation tubes (RCF-max).[6]

Biological Differences of Platelet-Rich Fibrin Clots Based on Centrifugation Parameters[6]: PRF clots fabricated at lower centrifugation speeds and times improve growth factorrelease and cellular behavior owing to higher cellular content and growth factor accumulation. PRF clots fabricated at lower centrifugation speeds and times are smaller in size, however, contain more platelets and leukocytes, as well as with more growth factor release. The g-force calculated at the fibrin clot (RCF-clot) is subject to change owing to the centrifugation time – even when centrifuged at the same speed. For these reasons, this method of reporting g-force is inferior in accuracy and not commonly reported internationally. The g-force calculated at the end of the centrifugation tubes (RCF-max) is not subject to these differences – hence, why it is internationally utilized to report g-forces.

PRF Box:

Figure 5 : PRF Box

Figure 6 : PRF Box with PRF

The PRF Box (Process Ltd., Nice, France) is commercially available to prepare the PRF membrane. The PRF clot is placed on the grid in the PRF box and covered with compressor lid which squeezes out the fluid from the clot (Figure 5 : Prf Box). The membranes formed using this method had constant thickness which remain hydrated for several hours and have recovered the serum exudate expressed from the fibrin clots.[4] (Figure 6 : Prf Box with PRF).

Application of PRF:

Socket preservation in case of tooth extraction: A study was performed to check the efficacy of PRF in extraction sockets andthat study showed faster-wound healing, bone regeneration along with the high-density bone formation.[12]

L-PRF block for bone augmentation procedure: The L-PRF block seems a successful new protocol for horizontal alveolar bone augmentation. This procedure is safe, predictable, with a high feasibility and a low morbidity.[7]

L-PRF in intrabony defects: The use of PRF as the sole grafting material seems to be an effective modality of regenerative treatmentfor periodontal intrabony defects at 6 months after the surgery.Greater reduction in pocket depth, more gain in clinical attachment level and greater intrabony defect fill at sites treated with PRF than those treated with open flap debridement alone.[14]

L-PRF in furcation defects: PRF has shown good results in reducing probing depth, improved clinical attachment level, and defect fill. Also, it was proven as an effective regenerative material for treating class II furcation defects.[15]

PRF as a Barrier Membrane in Guided Bone Regeneration: The use of PRF has popularity because it's biological scaffold capable of improving tissue healing. It is always advised to place PRF membranes over-top of the collagen barrier membranes. In this way, PRF membranes can be utilized to speed defect closure, improve soft-tissue wound healing/regeneration, and further bear the added defense component from pathogen-fighting leukocytes capable of significantly reducing the rate of infection and complication.[5]

L-PRF in fenestration and dehiscence: Guided tissue regeneration in combination with platelet-rich fibrin can successfully be used to treat fenestrated root apices. Satisfactory healing of the gingival fenestration defect with excellent color, texture match with the surrounding area along with the keratinized tissue formation emphasizing the importance of using PRF as a membrane in esthetically demanding areas.[16]

L-PRF application in delayed implant placement: A study showed that PRF may improve the post-insertion stability of dental implants during the time of healing.[17]

L-PRF in implant placed in sinus lift: It was seen that sinus floor elevation can be done by PRF placement alone as a regenerative material. The potential clinical implications for this inexpensive and autologous material are promising. Treatment approach with PRF was effectuation active in treatingthe peri-implant defects.[18]

L-PRF in wound healing: The use of PRF in a case of delayed and complicated healing following subepithelial connective tissue graft harvest action has proved to be effective in reducing donor site morbidity of free gingival grafts.[13]

Figure 7: Titanium-PRF

Titanium-PRF[3]: A new method for the formation of L-PRF was introduced in which normal test tubes are replaced by titanium test tubes which can omit the silica particles from dry glass. This new formulation is known as T-PRF which is a modified PRF. The material itself forms an adhesive oxide layer and quite popular because of its enhanced property of osseointegration in dental implants (Figure 7 : Titanium-PRF)

Usage of different materials for blood processing during PRF preparation[3]: Tunali & co-workers used medical-grade titanium tubes to produce PRF and called it T-PRF. On examination, it was observed that T-PRF samples have more organized structure and integrity compared to the L-PRF samples. Reddy et al, 2018 concluded that T-PRF helped in the treatment of intrabony defects by showing radiographic defect fill.

Advanced PRF[10]: For creating A-PRF, slow speed(1500 rpm) for a longer duration (14 mins) is used in vacuum sterile glass tubes. Enhanced B- & T-lymphocyte entrapment, more even distribution of platelets, neutrophils. The number of platelets is significantly more in A-PRF and is better deployment of resident monocyte, macrophages, and lymphocytes. Clinically, this would be beneficial as it would translate into an increased amount of growth factor and cytokine release. Pinto & co-workers demonstrated that A-PRF protocol produced lighter, shorter, narrower clots with

light polymerization and more squashed bodies. A-PRF application has proven more effective than PRF for reduction of probing depth,CAL as well as increasing bone fill.

Advanced PRF + (A-PRF+)[19]: Fujioka- Kobayashi & co-workers in 2016 formulated this combination by reducing both RPM and centrifugation time (1300 rpm for 8 minutes). The authors argued that decreased centrifugation time would lead to the production of more viable platelets in the PRF matrix. When they assessed the PRF produced by this protocol to L-PRF and A-PRF in terms of growth factor release, biocompatibility & cellular activity, they observed that A-PRF+ demonstrated the highest release of PDGF, TGF- β1, EGF and IGF. Also, in culture, gingival fibroblasts exposed to A-PRF+ revealed significantly higher levels of PDGF, TGF-b, and collagen-1 at three and seven days measured in terms of mRNA expression.

Injectable PRF (I-PRF)[19]: Injectable PRF (i-PRF) formulation is one of the recent development. For producing i-PRF, blood-filled test tubes without anticoagulants are centrifuged 700 rpm (60 g) for 3 minutes. Another set of authors has proposed a similar protocol where they centrifuge plain blood in non-coated test tubes at 2400-2700 rpm for around 2 minutes. The supernatant is collected and they have named it concentrated growth factors (GCF).Used along with bone grafts for regenerative purposes as it forms a gel-like consistency. Mixing the bone graft with i-PRF also gives the benefit of growth factor release at the recipient site. It was also seen that by mixing PRF clot, i-PRF and bone graft combination has shown improved efficacy in regeneration.

PRF Lysate: A novel preparation of PRF based products is the PRF lysate. In this, after PRF preparation, it is incubated at 37°C in a humidified atmosphere of 5% CO2/95% air and the exudate thus collected has been referred to as PRF lysate.[19]

Current Updates (Recent Studies Carried Out by Different Authors): Miron et al in 2019 revealed a new method for the preparation of PRF, rather than using normal centrifugation technique, a horizontal method is used which can result in higher platelets/leukocytes. The y have also shown a 10 fold increase in the platelets and leucocyte count by utilizing only 0.3–0.5 mL of sample. Tsujino et al, 2019 suggested that tube types and centrifugal speeds affect the types of growth factors that are released over the periodand the retention of those factors in the periodontal defect. Along with that, they also added that even though silica-coated testtubes are easily available, they should not be used to avoid health hazards. Wang et al, 2019 showed that combining L-PRF with bone marrow stem cells has the potential to replace the normal human bone structure.

Conclusion

PRF as a biologic surgical additive has been successfully used for varied applications in dentistry. Technological advancements in the field of PRF have paved the way for the versatility in the applications of the platelet concentrates. Therefore, in the future, more advances can be expected in the field of PRF which will be beneficial in different aspects.

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Conflict of Interests: None

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