ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at: www.ijdsir.com

Volume – 3, Issue – 2, April - 2020, Page No. : 23 - 35

PRF An Adjunct to Rejuvenation: A Review

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Citation of this Article: Dr. Asha Nara, Dr. Bodhyanga Kedare, Dr. Viddyasagar Mopagar, Dr. Sharanpriya, Dr. Mahadevi B Hosur, Dr. Sneha Ginimav, "PRF an Adjunct to rejuvenation. : A Review", IJDSIR- April - 2020, Vol. – 3, Issue -2, P. No. 23 – 35.

Type of Publication: Review Article

Conflicts of Interest: Nil

Introduction

In the field of tissue regeneration, vascularization plays a crucial role as it ensures a continuous supply of nutrients and the removal of waste products from the scaffold and the transplanted region.[1]A major objective of biomaterial research and tissue engineering is to promote a material-induced tissue reaction that leads to regeneration and an effective wound-healing process in the defective area. Thus, a biomaterial should serve as a temporary barrier to cover defects and promote tissue regeneration while being tissue compatible and, most importantly, clinically applicable.[1] Regenerative therapy in dentistry involves the replacement and/ or regeneration of oral tissues altered as a result of disease or injury.[2] These include both mineralized tissues such as the cementum, alveolar bone, and dentin, as well as soft tissues connected by ligaments (periodontal ligament), each comprising distinct populations from various tissue origins (ectodermal and mesodermal).[3] These cell populations reside in specialized extracellular matrices organized in complex fashions[4]

In the past a variety of regenerative procedures used biomaterials with barrier membrane to perform guided tissue/ bone regeneration and used bone grafting materials from human, animal and synthetic resources.[5] Researchers used bioactive growth factors such as bone morphogenetic proteins (BMPs) and enamel matrix derivative (EMD).[3] Investigators proposed that the use of three-dimensional scaffolds fabricated from the patient's own peripheral

blood could be utilized.[6] This new approach is based on the concepts that were introduced over a decade ago consisting of a platelet concentrate without the use of anticoagulants.[7] Platelet-rich fibrin (PRF) was therefore developed as an improved formulation of the previously utilized platelet-rich plasma (PRP).[2]

What is PRF

PRF is often named as Choukroun's PRF after its inventor.[8] It is a second-generation platelet concentrate.[9] The PRF constitutes components of blood sample that are beneficial to improve wound healing and immunity.[9, 10,] Ross et al. were amongst the pioneers who first described a growth factor from platelets.

Various research groups have shown that different biological properties of PRF collected from a given individual may result based on centrifugation speeds; i.e. relative centrifugal forces (RCFs).

Preparation of PRF

The procedure involves drawing of blood that is collected into test tubes without an anticoagulant and needs to be centrifuged instantaneously. A tabletop centrifuge can be used for this purpose for 2 minutes at 2,700 rpm.[11] The resultant product consists of the three layers.[10]

- Straw colored fraction of acellular platelet poor plasma (PPP) at peak level.
- PRF clot in intermediate level.
- Red fraction of red blood cells (RBCs) at the base level.

The blood coagulation starts instantaneously as it comes in contact with the glass surface due to the lack of anticoagulant. If the time necessary to collect blood and launch centrifugation is exceedingly prolonged, the fibrin will polymerize in a diffuse way in the tube and only a small blood clot without consistency will be obtained.[8]

Consequently, blood collection should be prompt and instant centrifugation is a prerequisite in the production protocol for PRF.

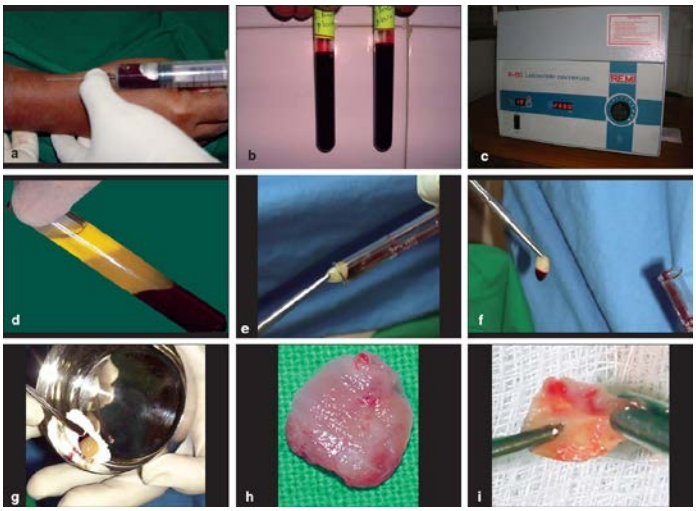

Figure1 : Blood collection procedure

Over the years, numerous reports, including those published initially by Choukroun *et al*. from 2001 to 2006 as well as others, have in fact misrepresented g-force values. These values have since been re-transcribed in a number of studies moving forward by many authors causing considerable confusion in the field[12–15]. One of the confusions that has been created in the field over the years is that 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).

Relative centrifugal force: Definition and calculation

One of the areas that have led to great confusion over the years is that RCF values have been calculated at various regions along a centrifugation tube. For these reasons, it is important to have a basic understanding of RCF values including calculations to obtain RCF-min, RCF-max, and RCF-av. The formula for RCF is as follows:

$RCF = 11.18 \times r \times (N/1000)^2$

Where *N* is revolutions per minute and r is the radius in mm.[16]

Recently, PRF box (Process, Nice, France) has been announced.[17] It is formulated to produce homogeneously thickened hydrated membrane and an exudate rich in platelets, leukocytes, vitronectin and fibronectin expressed from the fibrin clots.[18] It has improved the issues regarding the handling of the PRF clot.*

Various types of PRF are L (Leukocyte)-PRF, A (Advanced)-PRF, i (Injectable)-PRF, T (Titanium)-PRF. L-PRF has begun to be widely used for soft and hard tissue healing. It is a high density fibrin clot, serves as biological healing matrix by supporting cell migration and cytokine release, expanding the range of its potential. A-PRF is leukocyte rich and can fasten the healing of osseous surgeries in periodontics and endodontics procedures. Revascularization or maturogenesis can also be done by using i-PRF, it is injected in wide, open and blunderbuss canal, resulting in continued dentinogenesis and apex closure. T-PRF is prepared in titanium coated tubes and has more tightly woven matrix, thicker than classic L-PRF. It has a better hemocompatibility and lasts longer in the tissue. This article would be discussing preparation, application and effects of different types of PRF techniques in dentistry.

Table 1

Summary of growth factors released from platelets.

Growth Factors	Origin cells	Recipient	Action
PDGF	Platelets, Endothelial cells, macrophages, monocytes, smooth muscle cells.	Fibroblasts, glial cells, macrophages/ neutrophils, smooth muscle cells	Collagenase secretion, collagen synthesis, stimulates macrophage and neutrophils
TGF-b	Platelets, Macrophages/monocytes, T- Lymphocytes, Neutrophils	Fibroblasts, endothelial cells, epithelial cells, Preosteoblasts	Stimulates osteoblasts, fibroblasts, collagen synthesis, collagenase secretion
PDAF	Platelets, endothelial cells, osteoblasts.	Endothelial cells.	Increases permeability of vessels, Increases angiogenesis, and Cartilage growth.
IGF-1	Macrophages, monocytes, chondrocytes.	Fibroblasts, osteoblasts, chondrocytes	Replication of Preosteoblasts and osteoblasts, Bone matrix formation.
PF-4	Platelets	Fibroblasts, neutrophils.	Attracts neutrophils and fibroblasts.

Leukocyte Rich PRF (L-PRF)

Leukocytes are the cells of the immune system that are involved in protecting the body against infections or foreign bodies. Different types of leukocytes are concentrated in the fibrin matrix, namely lymphocytes (T-lymphocytes, B-lymphocytes), monocytes and neutrophilic granulocytes.[19]

Leukocyte enriched PRF (A-PRF and L-PRF) is reported to be an ideal provider of leukocytes.[20] Leukocytes are enriched in A-PRF and L-PRF, primarily to exploit their antibacterial and osteoconductive actions. Most leukocytes are found in the first 25– 30% proximal part of the clot. The leukocytes enmeshed into the dense fibrin network are alive and functional as an immune node that is able to stimulate defence mechanisms.[21] Leukocytes living in the fibrin matrix are also involved in the production of significant amounts of growth factors, particularly TGFβ. Studying the L-PRF matrix, stated that it dissolves slowly,

allowing the progressive release of cytokines and platelet-derived growth factors, acting as an anti-infective agent with a key role in immune regulation.[22] Therefore, it accelerates the healing of epithelial wounds, promotes tissue vascularization and improves soft tissue regeneration.

Sharma and Pradeep in 2011 conducted a study and observed that L- PRF + OFD (Open Flap Debridement) demonstrated greater probing depth reduction, clinical attachment gain and bone fill in comparison to OFD alone.[23]

A-PRF

Modification of the preparation protocol by reducing the applied RCF resulted in an improved preparation protocol for advanced solid PRF (A- PRF) using 208g. PRF clots formed with the A-PRF centrifugation protocol (1500 rpm, 14 minutes) shows a porous structure with more interfibrous space, and more cells could be counted in the fibrin-rich clot.[24, 25]

Furthermore, the cells were more evenly distributed throughout the clot as compared to S-PRF, and some cells could be found even in the clot's more distal parts.

A-PRF might influence bone and soft tissue regeneration, especially through the presence of monocytes/ macrophages and their growth factors. It was established that preparation protocol other than A-PRF led to either comparable dense structure as PRF or no clot formation.[22] Therefore, attention was directed towards centrifugation to improve the growth factor release along with maintaining the porous and stable A-PRF structure. A slight decrease in centrifugation period by maintaining the RCF range within 208 g resulted in an improved clot termed Advanced PRF plus (A-PRF+), indicating its supplemented characteristics. The low speed centrifugation concept indicates that, by reducing the relevant centrifugation force (RCF), the regenerative capacity of PRF matrices can be improved.[26, 27]

Applications:

1) A-PRF+ is widely used either as prophylactic measure in terms of socket preservation [28] after tooth extraction to prevent jaw atrophy and support the wound healing or in combination with bone substitute materials. [29]

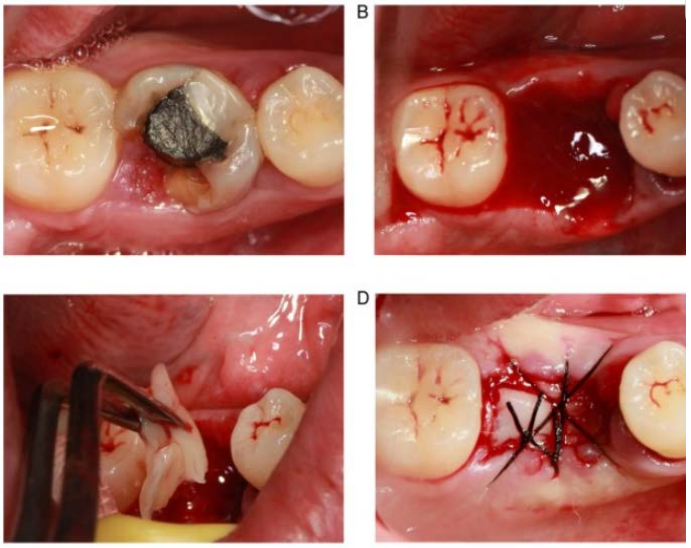

Fig. 2: Process of minimally invasive tooth extraction and application of PRF. A] Clinical manifestation of the tooth prior to tooth extraction. B] Extraction socket. C] Application of PRF in the extraction socket. D] Suturing of the extraction socket.

T-PRF

Successful clinical results have been reported with L-PRF[22, 14, 15], but some physicians worry about a possible health hazard with glass-evacuated blood collection tubes with silica activators. O'Connell[30] described the unavoidable silica contact. The silica particles in the tube, although dense enough to sediment with the red blood cells, are small enough for a fraction to remain colloidally suspended in the buffy coat, fibrin, and platelet-poor plasma layers; therefore, these particles might reach the patient when the product is used for treatment.

Although this issue is still debated, the cell composition and three-dimensional organization of L-PRF were

evaluated by the influence of different collection tubes (dry glass or glass-coated plastic tubes) and compression procedures (forcible or soft) on the final L-PRF-membrane architecture. It was shown that the type of tested tube (dry glass or glass-coated plastic tubes) and the compression process of the clot (forcible or soft) did not influence the architecture of this second generation platelet concentrate.

Titanium-prepared platelet-rich fibrin (T-PRF) is the third-generation platelet concentrate. It was developed by Tunali *et al*. in 2011.[31] Titanium was tried to eliminate the speculations about the potential negative effects of silica from dry glass or glass-coated plastic tubes. Titanium has one of the highest strength-to-weight ratios and corrosion resistance among metals[32]. Due to its noncorrosive properties, titanium has excellent biocompatibility [33]. The material passivates itself in vivo by forming an adhesive oxide layer. Titanium also displays a unique property of osseointegration, connecting both structurally and functionally with the underlying bone, and is commonly used in total joint replacements[34], dental implants, internal and external fixators, artificial heart valves, spinal fusion, and medical devices[34]. Titanium induced platelet aggregation similar to glass tubes, and the clot produced in titanium tubes was clinically identical to that in glass tubes. Preparation of T-PRF is done by centrifugation at 2,800 rpm for 12 minutes.[35]

Light microscopy reveals that the T-PRF samples have a highly organized network with continuous integrity compared to the PRF samples. The fibrin border between the cellular structures and the fibrin network is thicker and more prominent in the T-PRF samples than in the L-PRF samples. Fluorescence microscopy shows that the fibrin network is mature and dense in both the T-PRF and L-PRF groups. However, the fibrin is thicker and better organized in the T-PRF samples. T-PRF has well-organized matrix and fibrin maturation and also fibrin network of T-PRF occupies larger area than the L-PRF.This can be attributed to better hemocompatibility of titanium compared to glass, leading to the formation of more polymerized fibrin. This may also result in longer life of T-PRF in the tissue [36]

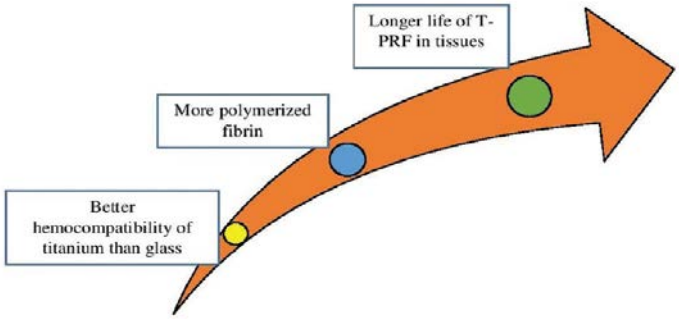

Fig. 3: Properties of Titanium prepared platelet rich fibrin. Reddy S. et al in 2018 concluded that T-PRF is efficacious clinically and radiographically in the treatment of a periodontal intrabony defect[37]. (Fig 3)

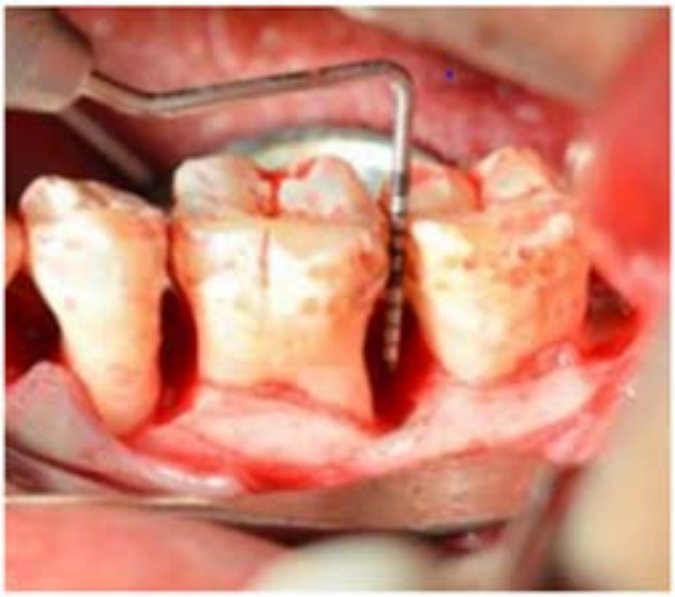

Fig 3a: Intra oral Pre- Operative probing depth.

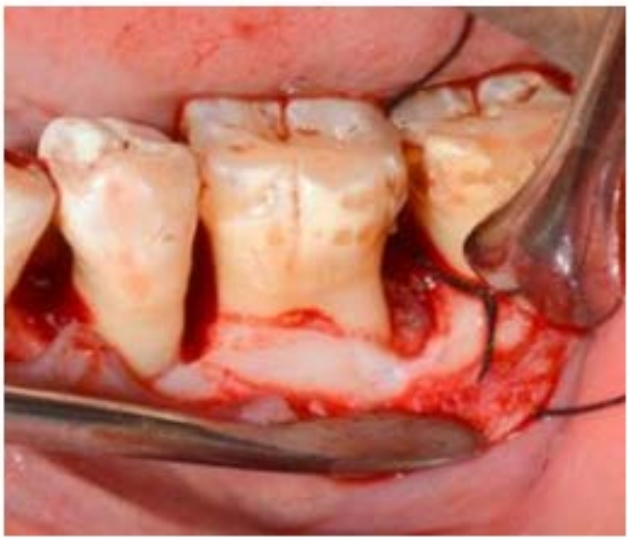

Fig 3b: Minced T- PRF covering bony defect.

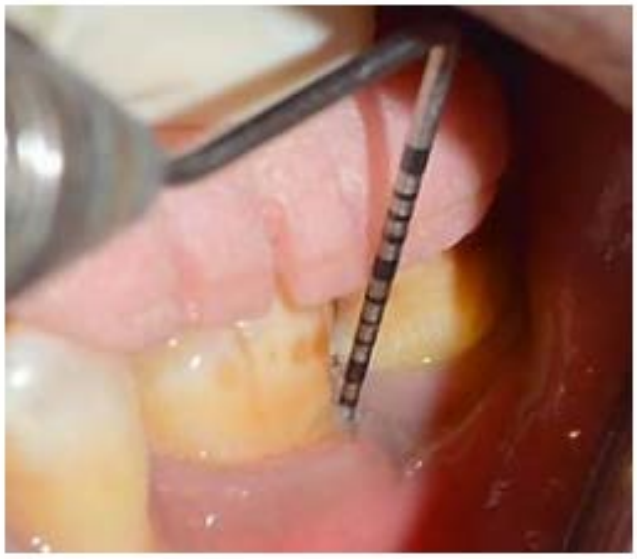

Fig 3c: Postoperative probing depth after 6 months.

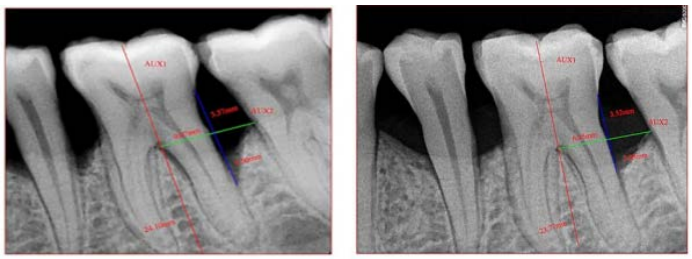

Fig 3d: Radiographic defect depth at baseline and after 6months post treatment.

Furcation defect regeneration with PRF

T-PRF has also been utilized in three studies investigating periodontal regeneration of class II furcation defects [38, 39, 40]. In all studies, PRF was compared to open flap debridement (OFD) alone, thereby fully characterizing its regenerative potential utilizing appropriate well-designed controls in all human clinical studies. In all three studies conducted by Sharma et al. 2011, Bajaj et al. 2013, and Pradeep et al. 2016, the use of PRF led to a significant improvement in CAL gains when compared to controls. These findings report a gain in vertical CAL of 2.33, 2.87, and 4.17 mm in test PRF groups when compared to 1.28, 1.37, and 1.82 mm, respectively, in OFD controls. These results demonstrate the potential for tissue repair utilizing PRF for furcation defects. One remaining issue to address is that the results have not confirmed the regenerative potential of PRF via histological evaluation and therefore the process can solely be defined as tissue repair. Furthermore, to date, no study has compared the use of PRF to other effective regenerative materials such as bone grafting materials or other regenerative bioactive growth factors. In the future, its clinical performance could be better assessed if compared to other leading regenerative agents.

Guided bone regeneration and extraction socket management with PRF

Several advantages have been reported when filling extraction sockets with PRF. Hauser et al. found in a study of 23 patients that PRF reduced dimensional changes prior to implant placement when compared to natural socket healing.[41] Furthermore, it was reported that raising a peri-mucosteal flap reduced the effectiveness of PRF. Girish Rao et al. found that following third molar extractions, the filling of sockets with PRF led to a non-significant increase in bone volume.[42] Hoaglin et al. reported that filling third molar extraction sockets with PRF led to a nearly tenfold decrease in osteomyelitis infections when compared to natural healing. This study was conducted bilaterally in 200 patients, thus providing some of the highest scientific evidence for the reduced rate of infection

following use of PRF.[43] Lastly, Suttapreyasri et al. found that PRF reduced dimensional changes in premolar extraction sites when compared to blank controls.[44]

i-PRF

The use of platelet aggregates in injectable form is widespread, especially in orthopedics and in plastic surgery, where it was possible to obtain favorable results, but these concentrates use venous blood collection tubes with anticoagulants or separating gel. However, the tubes used in the technique to be presented in this work have no additives that interfere in the process. With this test, fibrin clot is obtained in a short centrifugation time[10], only one minute, using the same spin speed used in the standard method.

The preparation of i-PRF is done by centrifugation for two minutes at 3300 rpm.

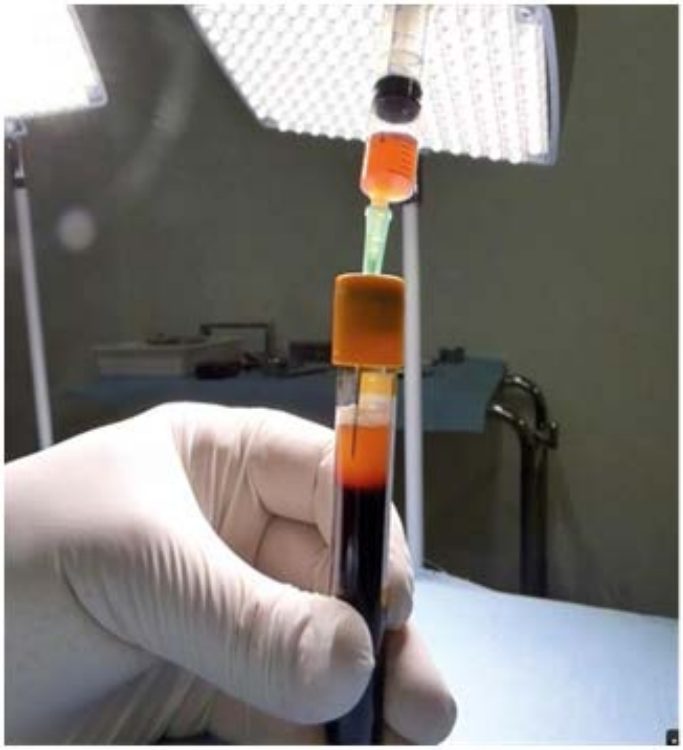

Fig. 5 : Showing collection of i-PRF[11]

The i-PRF is a new alternative to the platelet aggregate in different areas of Medicine and Dentistry, enabling experts to further research this product. Because it is autogenous, it decreases the chances of adverse reactions to the implanted material[45, 46, 47], especially immune-mediated ones, as with other types of grafting, which qualifies it as a viable option in regenerative procedures.

A previous SEM analysis of leukocyte-PRF showed multi-different plasma layers that constituted of a fibrin-rich layer at the upper most layer, followed by an enriched platelet layer, and the buffy coat layer with numerous leukocytes before the base layer erythrocytes.[48] The SEM analysis conducted by Prakan Thanasrisuebwong in 2019 demonstrated a dense, organized, acellular fibrin network as in the fibrin-rich layer at the plasma top layer of leukocyte-PRF.[49] This characteristic might bear as on for the superior physical properties in the yellow i-PRF compared to the red i-PRF. Meanwhile, the red i-PRF showed a greater number of cells and platelets attached to the fibrin network similar to a combination of all the middle layers and the erythrocyte base layer of leukocyte-PRF together. This additional cell and platelet content in the red i-PRF might lead to better biological properties as we could observe a greater release of the growth factors.

Bains et al. reported the applicability of i-PRF for the management of an iatrogenic perforation of pulpal floor in the furcation region of mandibular first molar.[50] According to the authors, the autologous and biocompatible nature of PRF and mineral trioxide aggregate (MTA) appeared to be favorable for the long-term clinical results.

Shivashankar et al. reported a case of revitalization of tooth with necrotic pulp and open apex using i-PRF.[51] They described evidence of continued thickening of the dentinal walls, root lengthening, and regression of the periapical lesion and apical closure with use of i-PRF. The authors considered PRF to be an excellent biomaterial for pulp-dentin complex regeneration.

Huang et al. conducted an investigation into the biological effects of i- PRF on human dental pulp cells.[52]

i-PRF was found to increase dental pulp cell proliferation as well as osteoprotegerin (OPG) expression in a time-dependent manner. Alkaline phosphatase (ALP) activity was also significantly up-regulated by PRF. These findings might serve as a basis for preclinical studies that address the role of PRF in reparative dentin formation

Jayalakshmi et al. used PRF in combination with beta tricalcium phosphate (β-TCP) bone graft in the treatment of periapical cyst.[53] The authors reported progressive, significant, and predictable clinical and radiographic bone regeneration/healing with the use of PRF. The authors suggested that the combined use of PRF and β-TCP for bone augmentation in treatment of periapical defects is a potential treatment alternative for faster healing than using biomaterials alone. Similar results were reported by Kim et al. using combination therapy of PRF with β-TCP.[54]

Applications of PRF in Dentistry

In recent times a lot of research has been done on PRF and numerous cases have been reported regarding the use of PRF clot and PRF membranes. The release of growth factors from the PRF clot commences 5 to 10 minutes after clotting and continues for at least 60 to 300 minutes. Majority of the research has been concentrated on the use of PRF in oral surgery for bone augmentation, sinus lifts, avulsion sockets etc. and in periodontics to correct intrabony defects, gingival recession, guided bone regeneration, periapical lesions etc.[55] It has also been used for regeneration in open apex, regenerative pulpotomies, periapical surgeries etc.

In Oral and Maxillofacial Surgery

Studies show that PRF can be used as filling material in extraction sockets. As a filling material in extraction sockets, PRF will act as a stable blood clot for neovascularization and accelerated tissue regeneration. This can be used to improve wound healing in immunocompromised and diabetic patients. Also, as PRF stimulates coagulation (with thrombospondin) and wound closure, it can be used as an adjuvant in patients on anticoagulant therapy[56]

PRF has been extensively used in sinus lift procedures. Some studies show the use of PRF as the sole filling material during sinus lift and implantation. Some studies show the use of PRF in combination with other bone graft materials in various direct and indirect sinus lift techniques like bone-added sinus floor elevation, osteotome-mediated sinus floor elevation, minimally invasive antral membrane balloon elevation etc.[57] Some studies also show the use of PRF in combination with beta Tricalcium phosphate (beta TCP) without bone graft in sinus lift procedures and chronic periodontal lesions.

The filling of avulsion sockets with PRF leads to very favorable results when bony walls are intact. A combination of PRF with bone substitutes and other adjuncts may be necessary in residual defects where one or several walls are missing or damaged in order to provide an adequate reconstruction of bone volume. PRF increases the cohesion between the graft materials as fibrin act as physiological glue between the wound tissues.[58] Natural blood coagulation leads to the formation of a fibrin matrix that biologically links wounded tissue together along with cell proliferation, cell migration, neomatrix apposition and remodeling. Therefore, the combination of PRF with other graft materials should improve the integration of graft material, since PRF is an optimized blood clot.

In Periodontics

In periodontics, PRF has been used to treat gingival recession, intra-bony defects and periapical lesions. Some case reports show the use of a combination of PRF gel, hydroxyapatite graft and guided tissue regeneration (GTR) membrane to treat IBD. Some studies show the use of PRF gel and PRF membrane in combination with a bone

graft for treating a tooth with a combined periodontics-endodontic lesion. Some studies show use of two layers of PRF membrane to cover the defect. The membranes are very thin and inhomogeneous and leucocytes and platelet aggregates are believed to be concentrated in end of the membrane. Therefore, two layers of membrane in opposite sense can be used to prevent the resorption of the thin membrane and to allow the entire surgical area to be exposed to same components (leucocytes and platelet aggregates).[59] Platelet rich fibrin as a potential novel root coverage approach has been reported by Anil Kumar et al. for covering localized gingival recession in mandibular anterior teeth using combined laterally positioned flap technique and PRF membrane.[60]

In Endodontics

Studies have shown that PRF can be used as a scaffolding material in an infected necrotic immature tooth for pulpal regeneration and tooth revitalization.[51] Also, some case reports show that the combination of PRF membrane as a matrix and MTA in apexification procedures prove to be an effective alternative for creating artificial root-end barriers and to induce faster periapical healing in cases with large periapical lesions. . Use of PRF in regenerative pulpotomy procedures have also been documented where coronal pulp is removed and the pulp wound is covered by PRF followed by sealing it with MTA and GIC.[51] PRF has also been used to fill in the bony defects after periapical surgeries like root end resection etc.

PRF might serve as a potentially ideal scaffold in revascularization of immature permanent teeth with necrotic pulps as it is rich in growth factors, enhances cellular proliferation and differentiation, and acts as a matrix for tissue ingrowth. The potential theory behind the success of the use of PRF for regeneration of open apex could be attributed to a study conducted by Huang et al, who concluded that the PRF causes proliferation of human Dental Pulp Cells and increases the protein expression of these Dental Pulp Cells differentiate into odontoblasts like cells. OPG and ALP expressions are generally regarded as markers of odontoblastic differentiation.

Conclusion

Thus, with this article we can conclude that the new and recent generation of platelet concentrate-PRF, would be a good friend to Oral and Maxillofacial Surgeons in the near future. The clinical experience also confirms that PRF can be considered a healing biomaterial, as it features all the necessary parameters permitting optimal wound healing. It already has a list of intraoral applications, and numerous extraoral applications can also be imagined. PRF can be used for all types of superficial cutaneous and mucous healing. This material is already being used widely in France, and considering its advantages, its popularity should increase here too. More clinical, histological and statistical studies are now required from different parts of the world to understand the benefits of this new platelet concentrate better.

However, it cannot be ignored that since it is obtained from an autologous blood sample, the quantity of PRF produced is low and only a limited volume can be used. This fact limits the systematic utilization of PRF, as in general surgery.

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