



Platelet Concentrates and Different Preparation Methods of Versatile Forms of PRF

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Type of Publication: Original Research Paper

Conflicts of Interest: Nil

Abstract

The Healing is a complex process that involves cellular organization, chemical signals, and the extra-cellular matrix formation for tissue repair. A variety of materials are available for healing and regeneration of soft and hard tissues. Platelets play a very important role in both tissue hemostasis and wound healing. The regenerative potential of platelets was identified by release of platelet derived growth factor as a serum growth factor for fibroblasts, smooth muscle cells and glial cells. Upon activation, platelets release various growth factors that stimulate cellular growth, proliferation, healing, and cellular differentiation. Currently a variety of platelet concentrates has been developed and has shown promising results. This review describes about various techniques in preparation of versatile forms of platelet concentrates.

Keywords:

INTRODUCTION

PRP is the first generation platelet concentrates which could be used in periodontal surgery. Thus, the use of PRP at injury sites might be able to promote wound healing and the regeneration of periodontal soft tissues¹⁻³. However, the complexity of PRP preparation protocol and the risk of cross-infection due to the use of bovine thrombin led to development a newer generation of completely autologous platelet concentrates- platelet rich fibrin also called as Choukroun's platelet rich fibrin. PRF is an optimized blood clot, which could be considered as an immune organizing node. The PRF matrix can release various growth factors and cytokines locally at the wound site for a prolonged period of time which could play a very important role in various stages of wound healing and simultaneously promotes periodontal tissue regeneration.

PRF could be utilized for surgical procedures involving bone augmentation, sinus lifts, ridge preservation, and correction of intra-bony defects, gingival recession, guided bone regeneration, periapical lesions, regenerative pulpotomies and periapical surgeries and regeneration in open apex. Platelet concentrates could be considered as a source

of blood proteins enriched with growth factors to promote wound healing. The versatile forms of different platelet concentrates includes fibrin glue, PRP, PRF, A-PRF, T-PRF, I-PRF, PRF lysates, H-PRF or BIO-PRF, Alb- PRF and CGF8. The T-PRF membrane was able to remain in the tissues for at least 1 month, which was sufficient time for the initiation of formation of new bone⁸. A-PRF treatment induces proliferation in serum-starved fibroblasts so that it could influence bone and soft tissue as well as pulp tissue regeneration, especially with the presence of monocytes or macrophages and their growth factors⁹.

Classification Of Platelet Concentrates

Dohan Ehrenfest classification 2009¹⁰

Based on presence or absence of cell content and the fibrin architecture

1. Pure PRP or leucocyte-poor PRP
2. Leucocyte rich PRP
3. Pure PRF or leucocyte-poor PRF
4. Leucocyte-rich fibrin and PRF

Mishra classification 2012

There are 4 types of PRP based on presence or absence of leukocytes and whether or not the PRP is activated. There could be 2 sub-types: A: Platelets >

5 × baseline or B: Platelets < 5 × baseline. In all the following types “solution” means non-activated, PRP and gel means activated PRP.

Type 1	L PRP solution
Type 2	L PRP gel
Type 3	P PRP solution
Type 4	P PRP gel

De Long classification system - PAW 2012¹¹

Depending on the platelet quantity, the activation mode of the platelets and the presence of white cells. It is a limited classification which involves only the PRP group of families

Platelet-Rich Fibrin

Platelet-Rich fibrin (PRF) is known as three-dimensional (3-D) autogenous biomaterial derived by centrifugation process which was easy, simple, rapid where it could be obtained from the patient’s blood samples. This process does not involves the addition of agents like anti-coagulants, bovine thrombin, additives, or any gelifying agents. This autologous PRFs stimulates early bone formation and maturation along with accelerated healing of soft-tissue; it also

decreases the post-operative pain, edema, and discomfort during healing process¹².

PRF is considered as an advanced and original tool in regenerative dentistry. PRF acts as a strong alternative and cost-effective biomaterial for periodontal tissue repair and regeneration. PRF may accelerate, enhance, promote soft and hard tissue wound healing and regeneration due to its capacity to allow the gathering and concentration of platelets and many therapeutic blood constituents like growth factors, fibrinogen/fibrin, leukocytes, and other circulating cells¹³. PRF is stable, resilient, strong, adhesive, and malleable biomaterial which can be cut or adapted into different anatomical defects and applications.



Centrifugation and Platelet concentrate

The advantages of various PRF includes clinical ease of use and handling, the biochemical composition of the PRF by-products provides favours the haemostatic, osteogenic, anti-inflammatory, anti-microbial, angiogenic, pain-inhibitory, and wound-healing properties during healing process.

Generation Of Platelet Concentrates

1st Generation

This includes the evaluation of Platelet concentrates and growth factors and development of various automated PRP preparation devices and techniques.

2nd Generation

The Modification of preparation protocols with developments of novel Platelet concentrates derivatives, such as platelet rich plasma and platelet-rich fibrin.

3rd Generation

When Comparing PRP derivatives ability to retain and release growth factors, biodegradability, mechanical strength, the PRF is considered as superior than PRP. Versatile types of different PRF could be considered as better than PRP formulations. T-PRF is considered as 3rd generation autologous platelet concentrate in which there is a polymerized matrix that includes platelet, cytokines, leukocytes, and various growth factors that are entrapped and act as a resorbable membrane. The growth factors within the T-PRF stimulates the cellular activity,

Different Preparation Techniques For Prf And Changes During Centrifugation

Protocol For Prf Preparation

The protocol for PRF preparation tries to increase the quantity of platelets along with the released cytokines in a fibrin clot. The PRF protocol involves centrifuged blood and it does not involves the addition of anticoagulant and bovine thrombin. Then, a blood sample is taken without anticoagulant in 10-mL tubes in a glass or glass-coated plastic tube or titanium tubes and then immediately centrifuged at 2700 to 3500 rpm for about of 10 minutes period. The Fibrinogen could be concentrated in the upper portion of the tube before the proper thrombin transforms it into fibrin. Due to the lack of an anticoagulant, blood begins to coagulate when it

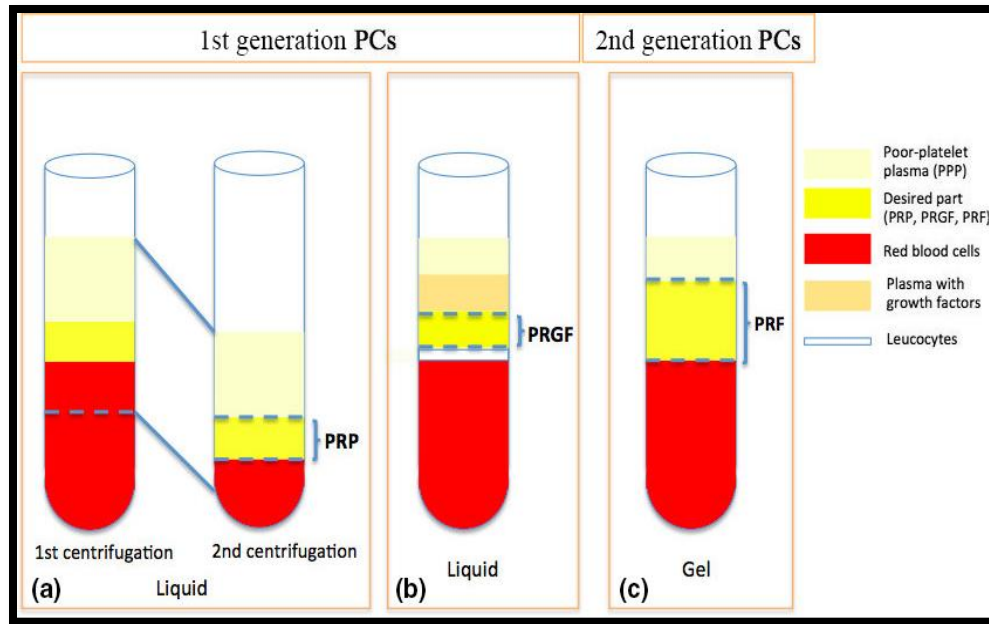
proliferation along with increased neoangiogenesis. They are effective in the enhancement of early wound healing acts as promoters of periodontal tissue regeneration.

4th Generation

It is obtained by exploration of coupling partner cells of PCs. The fourth generation comprises research on the tissue engineering triangle. Most conventional PC derivatives do not contain a sufficient number of circulating mesenchymal stem cells or CD34+ hemopoietic stem cells. The fourth generation PC involves CD34+ stem cells which favors the better regeneration of all kinds of periodontal tissues, like cementum, periodontal ligament and alveolar bone¹⁴⁻¹⁶.

comes in contact with the surface of glass. The contact with a silica surface is required for activation of the fibrin polymerization cycle.

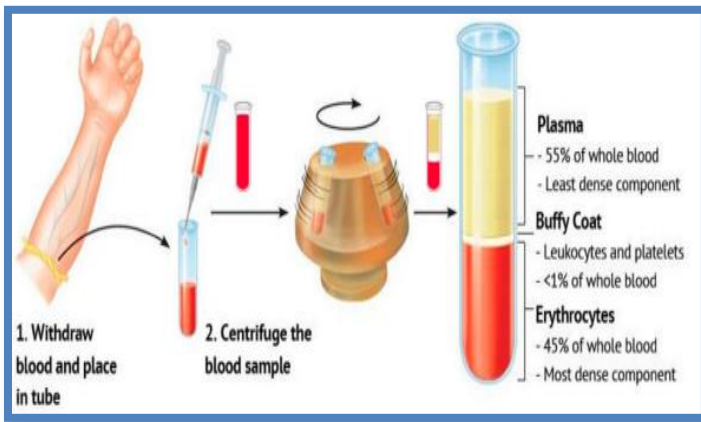
The disadvantages of this techniques includes, the silica in the glass tube is needed for the initiation of platelet aggregation and formation of fibrin matrix in the PRF. There could be a possible health hazards in glass-evacuated blood collection tubes with silica activators. Connell et al. have explained about the silica particles used in the glass tubes, although dense enough to sediment with the red blood cells, are sufficiently little in quantity for a fraction to stay in colloidal suspension of the buffy coat, fibrin, and platelet-poor plasma layers and also there will be chance of contamination during therapeutic application to the patient¹⁷⁻²¹.



Courtesy from' Castro AB, Meschi N, Temmerman A, Pinto N, Lambrechts P, Teughels W, Quirynen M. Regenerative potential of leucocyte- and platelet-rich fibrin. Part A: intra-bony defects, furcation defects and periodontal plastic surgery. A systematic review and meta-analysis. *Journal of clinical periodontology*. 2017 Jan;44(1):67-82.

The resultant product consists of the following three layers:

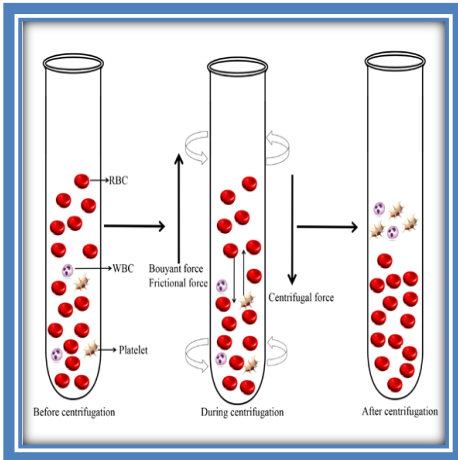
1. Top-most layer is consisting of cellular plasma.
2. PRF clot in the middle.
3. Red blood corpuscle could sediment at the bottom of the tube.



Blood derived by venipuncture and then centrifuged

Courtesy from' Mohan SP, Jaishangar N, Devy S, Narayanan A, Cherian D, Madhavan SS. Platelet-rich plasma and platelet-rich fibrin in periodontal regeneration: A review. *Journal of pharmacy & bioallied sciences*. 2019 May;11(Suppl 2):S126.

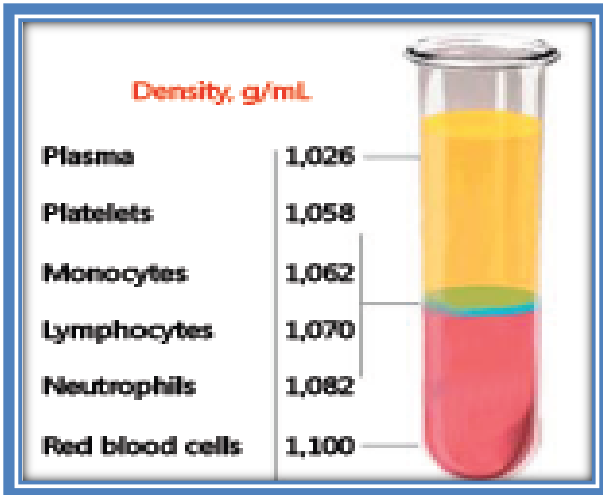
The result product contains three layers containing RBC, Buffy coat, Plasma.



Physical Changes and Forces during Centrifugation Process

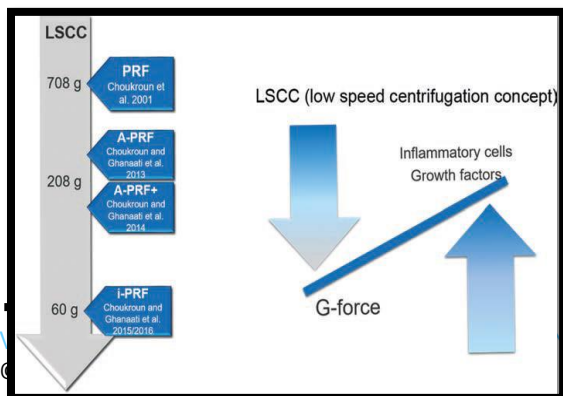
Courtesy from' Miron RJ, Pinto NR, Quiryren M, Ghanaati S. Standardization of relative centrifugal forces in studies related to platelet-rich fibrin. J Periodontol. 2019 Aug; 90(8):817-20.

Due to Centrifugation there could be an interaction of Frictional, buoyant, centrifugal forces which results in Sedimentation rate at different levels for each component of blood²².



Different Density for Various cells of Blood

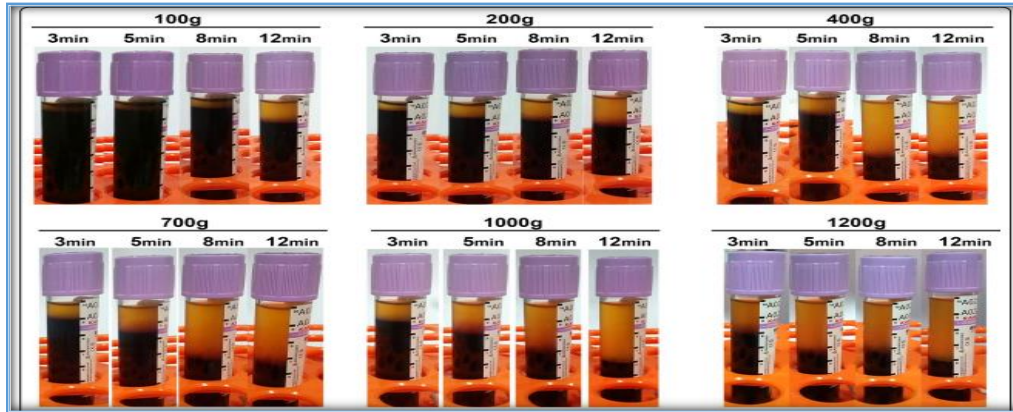
Due to different density, the cells sedimentation could occurs in layer by layer during centrifugation process. Following centrifugation, the blood components (RBC, WBC, and platelets) are separated from the plasma due to presence of their different densities. As the platelets have the lowest density it will sediments in upper portion of centrifuged blood²².



LSCC – LOW SPEED CENTRIFUGATION CONCEPT

Biologic Effect Of Platelet Rich Fibrin Matrix

PRF could have specific role in many aspects of healing process which includes angiogenesis, immune regulation, including the circulating stem cells, and finally the wound protection by complete epithelial coverage.



Clinical image explaining the separation of plasma layer for the 24 protocols.

Many of the protocols showed that they are roughly similar separation of plasma layer and the variation in lower cellular content in different protocols.

Courtesy from' Miron, R.J., Chai, J., Fujioka-Kobayashi, M. et al. Evaluation of 24 protocols for the production of platelet-rich fibrin. BMC Oral Health 20, 310 (2020).



Major Centrifuges used for PRF preparation.
From left to right: Eppendorf #5702 (for BIO-PRF), Medifuge (for CGF), Duo Quattro (for A-PRF), and Hettich EBA200 (original model of Intra-Spin) (for L-PRF).

Courtesy from Kawase T, Mubarak S, Mourão CF. The platelet concentrates therapy: from the biased past to the anticipated future. Bioengineering. 2020 Sep;7(3):82.

T-PRF

In 2014 Tunali et al. have developed the Titanium – Platelet rich fibrin (T-PRF). Tunali recommended force was 2700-3500 rpm for about of 12min. Titanium tubes was used for collection and centrifugation purposes. The T- PRF could causes greater osteoconduction, bone induction for greater period of time by action of various growth factors.

Titanium-induced platelet aggregation similar to glass tube induced platelet aggregation and the clot produced in the titanium tubes was clinically identical when compared to glass tubes stimulated clot. The fibrin carpet formed with titanium had a firmer network structure, and the resorption in the tissue was longer. This material is also used to avoid any short and/or long-term negative effects of dry glass or glass coated plastic tubes and to eliminate the concerns regarding silica. T-PRF membrane have capacity to remain in the tissues for minimum of 1 month period, which could be considered as enough time for the initiation new bone formation. The T-PRF could produce excellent amount of new bone formation. The new bone maturation was greater and faster in the T-PRF than other bone graft materials. It is due to slow and steady resorption with continuous osteoconductive features of the T-PRF⁸.

I-PRF

In 2015 Mourao developed the I-PRF. Injectable PRF (I-PRF) is one of the recently introduced platelet concentrates. As the name suggests, it is available in injectable form and coagulates few minutes after the injection. It is also called “blood concentrate “because in addition to platelets and leukocytes, it also contains stem cells and endothelial cells. There will be 700 rpm for about of 3min period of time for preparing Injectable form of PRF. The main advantage of this technique is allowing maximum cell migration and increased amount of production of various growth factors. This results in formation of more number of type-I collagen by induced signaling of m-RNA. Therefore, several modifications of I-PRF preparation, such as 600 rpm/8 min/44 g and 2,700 rpm/3 min/408 g have been proposed. In addition, horizontal centrifugation has also been evaluated to improve I-PRF with higher number and concentration of platelets/leucocytes compared to fixed-angle centrifugation²³.

A – PRF

In 2014 Choukroun have introduced the Advanced Platelet rich fibrin (APRF) by slow centrifugation process which has the advantage of release of growth factors for longer period of time. This cause’s earlier vascularization, faster soft tissue growth, more cytokines and release of BMPs. Blood was drawn into a 20- ml injector and separated immediately into two grade IV sterile tubes, each of which received 10 ml of blood. The tubes were placed opposite to one another and centrifuged at 1500 rpm for 14 min at room temperature. Increasing the centrifugation time causes production of the A-PRF. This allowed for the amplification of neutrophilic granulocytes in the distal part of the clot.

Capturing the total number of monocytes in PRF may make PRF become more active in stimulating bone grafts and increase the transformation of monocytes into macrophages, hence amplifying the bone stimulation effect. Neutrophilic granulocytes contribute to monocyte differentiation into macrophages. Accordingly, a higher presence of these cells might be able to influence the differentiation of host macrophages and macrophages within the clot after implantation. The gingival fibroblasts, periodontal ligaments, and osteoblast proliferation were stimulated by A-PRF²⁴⁻²⁵.

L-PRF AND L-PRF BLOCK

Totally 9–10 mL of blood samples were obtained in sterile glass or plastic coated tubes. The tubes are kept in pairs and at 400g RCF centrifuged for 12 min centrifugation (400g RCF is equivalent to 2700 rpm). Ideally, the tubes should be centrifuging within 60 seconds after the start of the venipuncture. Immediately after centrifugation, due to the activation of autologous thrombin, it is converted to fibrin and a fibrin clot is created. The clot by itself contains a great amount of exudate, which is rich in growth factors. This exudate can be expressed by gentle compression of the clot (about 5 min) in order to obtain stronger L-PRF membranes. For this compression, one can utilize a specially engineered box. It forms standard 1-mm-thick L-PRF membranes. The membranes remain stable at room temperature for several hours. The clots could be placed in tiny cylinder of the metal Xpression box. With the help of piston compression of the clot could yields L-PRF membrane. The L-PRF act as a scaffold that promotes cellular migration, which acts as initial

step in the process of tissue regeneration. The L-PRF membranes preserved as solid membrane, this could release higher amount of various growth factors in continuous manner for about seven to fourteen days period²⁶⁻²⁸.

MPM-MINERALIZED PLASMATIC MATRIX

PRF can be mixed with autologous bone and is known as MPM, which can be used for various periodontal regenerative purposes with the advantage of Cohesion, increased Retention with Homogeneity. Elasticity of MPM allows more amount of Cell Migration with increased Osteoblastic activity and better bone regeneration.

The PRF can be mixed with dentin particles and could be used as sticky bone. This gives better advantages than regular bone graft techniques. The sticky bone could be used in place of periodontal defects for better soft tissue and hard tissue regeneration.

STICKY BONE

Sticky bone is a combination of human tooth allograft and autologous fibrin glue. Dentin has shown to contain BMPs, IGF-II, VEGF, (TGF)- β and type I and type III collagen, this improved osteoinductive potential. The use of "sticky bone" in alveolar ridge augmentation favours the bone regeneration²⁹⁻³¹.

LYOPHILIZED PLATELET CONCENTRATE

Lyophilization, a process comprising sublimation and desorption of water from the frozen sample, may improve protein stability, extend shelf-life and preserve the biological activity of samples. lyophilization may not influence cell sheets in vitro due to the constant levels of GF in lyophilized and fresh PRF preparations. However, structural changes occur due to lyophilization, such as enlarged pore size, which serve pivotal roles in wound healing and influence proliferation, adhesion and migration of stem cells in vivo.

STEM CELLS AND PLATELET CONCENTRATES

Adipose-derived stem cells (ADSC). ADSC have a high potential to differentiate into several types of cells. In addition to their multipotent differentiation

ability, the paracrine functions of ADSC also influence tissue regeneration

PRF LYSATE

A newer application 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 were known as PRF lysate. It is said to be a good source of several growth factors including PDGF, TGF, VEGF& EGF. It could be used to reverse the damage caused by chronic UV radiation exposure to human dermal fibroblasts by significantly increasing the proliferation rates, migration rates, and collagen deposition equal to those of normal fibroblast.

PRF AND NANOMETAL PARTICLES

Nanometal particles, such as nano-silver, have significant anti-bacterial properties. Combination of nano-silver and platelet concentrates provides integrated methods to inhibit bacteria and promote tissue regeneration for patients with burn damage.

H-PRF or BIO-PRF

The idea of horizontal centrifugation to produce the PRF was introduced by Lourenço et al. Horizontal spinning allows for blood samples to be resolved in density gradients and promotes the elective separation of individual blood cells, while fixed angle rotors are useful for a variety of applications, from pelleting blood cells to the isopycnic separation of Macro molecules, horizontal centrifuges reduce the probability of cell-cell and cell-inner wall collision, thereby preventing accelerated cell adhesion and potential injury. In fact, horizontal centrifuges at higher speeds are able to recover platelets in PRF matrices at higher levels.

Additionally, the PRF prepared using conventional horizontal centrifuges and glass tubes, without modification on the surface could not be considered as an innovative procedure and distinguished. It is another branded preparation protocol that requires their specific devices and has been competing with other companies to occupy the market. The horizontal position produced from a swing-out bucket allows for the greatest differential between the minimum and maximum radius found within a centrifugation tube. This effect allows for a greater ability to separate cell layers based on disparities

between the RCF-min and RCF-max produced within a tube. Second, a fixed-angle centrifuge results in more trauma to cells along the back walls of centrifugation tubes.

The speed and time for centrifugation could have major impacts on the resulted cell counts in the upper layers of PRF. So that comparing leukocytes cells, the platelets could be more easily separated because of its lower cellular density. The Protocol time could have impact on the final cell layer separation when compared to speed. The optimal centrifugation speed and time ranged between 400 and 700g for 8 min for solid-PRF protocols (greater yield with evenly distributed cells) and 200–400g for 5 min for liquid-PRF (highest concentration of platelets/leukocytes). Notably, variability in patient baseline platelet/leukocyte counts significantly affected cell layer separation^{32, 24, and 27}.

Alb –PRF

Heat-compression technique was used as a way to reduce the biodegradation of PRF and increasing the period of growth factor release. Because of the heating, it could modify the native PRF membrane to be used as a barrier for guided bone regeneration procedures. Heating the blood serum and a portion of plasma could modify properties of platelets. Heating and subsequently incorporating PRP or liquid PRF for the inclusion of cells; at the time, the product was called Alb-CGF, corresponding to the albumin produced by the heating process, with the incorporation of the concentrate of growth factors. Alb-PRF membrane degrades, after 21 days in subcutaneous tissue, indicating its slow degradation and the potential use of this blood by-product as a barrier.

C-PRF

The Concentrated PRF defined as the 0.5-mL buffy coat directly above the red blood corpuscle in PRF. Standard high g-force L-PRF and low g-force i-PRF protocols were utilized to separate blood layers. Above the red blood corpuscle layer, sequential 100- μ L layers of plasma were harvested. The liquid PRF that was directly collected from the buffy coat layer following L-PRF protocols was referred to as C-PRF. This layer is referred to as concentrated PRF (C-PRF) because it was harvested from the concentrated buffy-coat layer. The maximum concentration of

leukocytes and platelets in PRF that could be obtained by harvesting this specific concentrated region of cells (C-PRF). There could be a > 500% increase in leukocytes and a > 1500% increase in platelets when utilizing this novel concentrated PRF (C-PRF) harvesting technique³³⁻³⁴.

DRUGS AND PRF COMBINATION

The PRF could be used with antibiotics, which can serve as a novel antibiotics slow-release biological device. PRF was mixed with the antibiotics (5 mg/ml metronidazole; 150 mg/ml clindamycin; 1 mU/ml penicillin). Platelet-rich fibrin incorporated with antibiotics showed long-term antibacterial effect against *Fusobacterium nucleatum* and *Staphylococcus aureus*. This modified PRF preparation may be used to reduce the risk of post-operative infection in addition to the beneficial healing properties of PRF. The addition of antibiotics to the PRF demonstrated significant inhibition of both aerobic (*S. aureus*) and anaerobic (*F. nucleatum*) growth. PRF with clindamycin or penicillin also significantly inhibited the growth of *S. aureus*.

These results confirm that the antibiotics incorporated into the PRF preserved their activity for at least 4 days, suggesting its use as post-surgical slow-release anti-bacterial agent. Since the added antibiotics may be trapped in the liquid phase of the PRF or within the PRF protein structure.

ANTAGONISM EFFECTS OF PRF

The bisphosphonates are used to manage osteoclast-mediated osseous resorption and to preserve the activity of osteoblasts. Different types of platelet concentrates may be able to inhibit bisphosphonates on different levels. The stilbenoid which is a plant-derived resveratrol and it has anti-inflammatory, antioxidant and bone-protective properties. In addition, it may enhance the therapeutic outcomes of CGF treatment in bisphosphonates-related osteonecrosis of the jaw.

SYNERGISM EFFECTS OF PRF

Ginsenoside Rg1 (G-Rg1) and PRF can be utilized to human breast adipose-derived stem cells (HBASC) prior to being seeded in a 3-dimensional porous sponge scaffold of collagen. Reasonable combinations of platelet concentrates with drugs may reduce side effects and enhance the effects of drugs

due to the mutual effects between drugs and cytokines in platelet concentrates. The use of Concentrated Growth Factors (CGF) and Stem Cells CD34 combined with the simultaneous use of bone graft can have on the sinus membrane following intentional perforation³²⁻³⁴.

CONCLUSION

Platelet concentrates have been utilized in dentistry and medicine for over five decades due to their ability to release supra-physiological doses of autologous growth factors. In dentistry alone, Platelet Concentrates has been utilized for the treatment of extraction sockets, gingival recessions, palatal wound closure, the regeneration of periodontal defects, hyper plastic gingival tissues and for implant supportive therapy. In other medical fields, PRF has been utilized for the successful management of hard-to-heal leg ulcers, diabetic foot ulcers, venous leg ulcers, and chronic leg ulcers. The reported advantages include faster healing, increases in angiogenesis, lower costs (when compared to PRP), and complete immune biocompatibility. By utilizing various centrifugation cycles, different centrifugation tubes and by modifying tube angulation different types of Platelet Concentrates could be produced and utilized for various regenerative purposes. Further research and studies are needed for future PC therapy to standardize the quality, centrifugation protocols.

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