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AN INSIGHT IN TO UPDATES ON PLATELET RICH FIBRIN

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ABSTRACT

Platelet Rich Fibrin (PRF) from its inception has undergone continuous improvement and precision as needed in various Regenerative fields. Compared with PRF, its derivatives have ushered with more supraphysiological levels of platelets, white blood cells and cell growth factors which are conducive by promoting cell proliferation and differentiation which are promising to healing and regeneration of tissues. Different types of PRF derivatives have differences in their preparation methods, retention time, transfer process, fibrin structure, platelets and growth factors release. All the modifications of PRF aim at maximizing the growth factor mediated biologic effects and the cellular activity. Thus PRF with its derivatives has gained tremendous acceptability as the best blood product able to better augment the soft and hard tissue, compared to other similar blood product. Regenerative field necessitates defined structure and consistency. The manipulation of PRF to suit the particular regerative area is tough during its application and the suspensions, gels are not retained effectively resulting in unpredictable outcomes. Till date several techniques for platelet concentrates are available, however their applications have been perplexing because each method leads to a different product with different characterization and potential uses. We here in this review an attempt is made to update the various forms of PRF with preparation technology, their usage and clinical guidance for the rational application.

KEYWORDS:- Platelet Rich Fibrin, Platelets, Centrifugation, Growth factors, Regeneration.

INTRODUCTION

Naturally derived blood and blood derived product have been used to seal wounds and to improve clinical situation started with the use of fibrin glue. To improve strength and also to promote neoangiogenesis and regeneration, platelets have been added, the benefits of which has been attributed to diversity of peptide growth factors released from alpha-granules on activation.[1] The preparation of these platelet biomaterials from the commercial available devices have a collective persistence that allow the isolation of plasma with high concentration of platelets which functionally differ.[2] This concentrated platelet derived from autologous source in plasma solution when utilized in the regenerative areas have capability to release 6 to 8 times the supraphysiological doses of growth factors responsible for wound healing locally.[3] There are various devices which uses different protocol, different type of machines and collection tube with different speed which has resulted in different variant of PRF of different function.[4] The clinical cases of PRF has demonstrated the different effects which cannot be attributed only to the different protocols of preparation but also to other dynamics.[5]

PRF and its derivatives

PRF the second generation platelet concentrate was developed during the year 2000 spanning of two decades with Platelet Rich Plasma being the First generation and titanium-PRF (t-PRF) being the third generation.[6] The concept of fibrin glue has given reason to the development of PRF through the Platelet rich plasma and Platelet rich in growth factors. All these platelet concentrates had their own drawn back which has ushered the development many modifications of PRF with different spinning cycle and time. Hence the usage of all these PRF in wound healing enhancement in surgical regenerative procedure is a boon to the regerative field.[7]

Standard PRF (S-PRF)

The simple protocol for the preparation of all PRF and its variant is the centrifugation of the whole blood, which is autologous without any external additives. Choukrans PRF is also known as Standard PRF (S-PRF). The protocol first introduced by Choukrans and co-workers is by Centrifuging 9ml of whole blood conveniently at 2700 revolutions per minute (rpm) at 325grams (gms) for 12minutes (min) duration in a sterile glass tube or glass coated plastic tube, which separates its components according to density using centrifugal and buoyant force.[8] The separation of blood elements and blood coagulation occur simultaneously during

the centrifugation process. Erythrocytes are of more density so they collect at the bottom of the tube forming the haematocrit layer. The thin, white-tinted buffy coat settles at the top of the erythrocytes and plasma forms the supernatant. This buffy coat is taken out with a sterile tweezer after platelet poor plasma is withdrawn from the glass tube, resulting in PRF gel form. The gel form can be easily packed in to bone defect sites or extraction sockets. This gel form can be conveniently formed in to membranous form by compressing between two glass slides or commercially available PRF box. Membranous form finds its multiple application in to recession sites, as barrier form and coverage of surgical fields.[9,10] Earlier the protocol used for L-PRF was 3000 rpm for a duration of 10min. More recently this S-PRF has also been termed as Leucocyte-PRF simply L-PRF.[11]

L-PRF consists of three different components, all of which can influence angiogenesis and wound healing. The pro-angiogenic molecules secreted by white blood cells present in L-PRF neutrophils and Macrophages favors angiogenesis and inflammation thus favoring wound healing.[12] Castro et al. in his systematic review reported the favorable effects on hard and soft tissue healing and postoperative discomfort reduction when L-PRF was used.[13] L-PRF can also be considered as a living tissue graft due to its cellular content and its constant release of growth factors for more than 7 days. Khorshidi H. et al. in his study showed that the tensile strength, stiffness, and antibacterial activity were significantly higher in the Silver Nano Particles modified L-PRF membranes the reason being silver nano particles were densely mixed with the fibrin strands in the outer layer than the inner layer of the in the fibrin matrix. The precipitation of silver nano particles was patchy in the outer layer and homogenous in inner layers. This end results in improved mechanical properties.[14] Leukocytes in PRF has role in tissue healing, in regulating inflammatory process, antibacterial activity and switching from inflammatory process to regenerative Phase.[15] The potential effect suggested are leukocyte has a positive correlation between their count and Growth factors, hence they potentiate total amount of growth factors released.[16] By releasing anti-inflammatory cytokines (IL-4, IL-10 and IL-13) and opiod peptides (beta-endorphins, Met-enkephalin and dynorphin-A) it has anti-nociceptive action.[17] Switching from M1(pro-inflammatory) to M2(anti-inflammatory) phenotype have function such as tissue remodelling, cell proliferation, extracellular matrix deposition, promoting angiogenesis and resolution of inflammation.[18] Proteinase secreted by leukocytes modulate healing of tissue, matrix remodelling by converting inactive to active form.[19]

Advanced- PRF (A-PRF)

A-PRF formation comprises centrifugation of blood at a slower speed concept of 1500rpm at more time of 14min in a sterile glass tube. The concept for A-PRF was given by Ghanaati in the year 2014 used 1300 rpm for a duration of 14min to procure A-PRF. He showed the high concentration of growth factors such as vascular endothelial growth factors, platelet-derived growth factors, transforming growth factors- β and stimulation of tissue cicatrisation, vessel formation, bone cells proliferation and differentiation is by the anti-inflammatory cytokines. There are as well higher chemotactic molecules such as chemokine ligand-5 and eotaxin from these higher numbers of neutrophils and monocytes or macrophages. Thus all these mimic physiology and immunology of wound healing.[20] The uniqueness of this A-PRF is uniform distribution of platelets and leukocytes with more of B-lymphocytes and T-lymphocytes entrapment. As a result more of leukocytes, Monocyes and Macrophages are seen in this A-PRF. With this uniform platelet distribution, more of growth factors are released from the Platelet from A-PRF compared to L-PRF.[21] Ravi and Santhanakrishnan in their study clearly demonstrated the superiority of A-PRF when it comes to the sustained release of growth factors (PDGF-AA) which could prove beneficial in regenerative periodontal therapy.[22] Advanced -platelet-rich fibrin modified by Ghaznavi et al. by adding gold nanoparticles which enhanced the the osteoblastic differentiation of human mesenchymal stem cells by improving alkaline phosphatase acivity.[23]

A-PRF +

Another variation of A-PRF+ has been suggested by Fujioka- Kobayashi & co-workers in 2016 where they have reduced the centrifugation time to 1300 rpm (208gms) for 8 minutes. They have called this modification as A-PRF+. They claim that less time would result in a decrease in the amount of forces that the cells of the blood would be exposed to & hence, would increase the number of cells contained in the PRF matrix. The fact being the lower centrifugation force more uniform distribution of platelet can be achieved with the more entrapment of granules of neutrophil in PRF. Higher centrifugation force tends to push the platelets and leukocytes away from PRF clot. This overall augmented cellularity of A-PRF+ would translate into increased differentiation of macrophages which leads to osteoblastic differentiation and optimized growth factor release.[24] The fibrin matrix showed more porous structure, there was additional space for trapping of the immune cells and platelets thus releasing sustained growth factors. Improved and accelerating therapeutic development for

hard and soft tissue healing was seen with A-PRF+ in the management of alveolar osteitis as well as effective pain reduction.[25]

Injectable platelet-rich fibrin (i-PRF)

Liquid form of PRF was introduced in 2015 is injectable platelet-rich fibrin more commonly referred as i-PRF™. It is obtained by centrifuging blood at low-speed 700 rpm (60gms) for 3min. This results in PRF for use in liquid form which is conducive for injection than compared to the gel form of PRF.[26] This coagulates within few minutes after injection and is believed to contain not only white cells, platelets as well as circulating stem cells and endothelial cells thus called as blood concentrate than platelet concentrate.[27] The liquid form of i-PRF forms a dynamic gel upon application which acts as storage for growth factors and addition release of growth factors beyond 10days is seen compared to Platelet Rich Plasma. Miron et al. has shown i-PRF has capacity to stimulate tissue regeneration by secretion of various growth factors as well triggers fibroblast migration with promising outcomes.[28] The injectable form can be combined with various graft material or can be utilized solely. The shorter and slower centrifugation spin results in higher presence of regenerative cells and growth factors.[29] i -PRF is the preeminent for induction of osteoblast mineralization and is the choice for application in bone tissue engineering compared to other clinical grade hemoderivatives of standardized methods.[30] Karde et al. compared the content of platelets in i-PRF, PRF and PRP and their antibacterial activity and concluded that i-PRF contains the highest platelet content and the highest antibacterial activity. i-PRF is also used in the treatment of lichen planus and also has significant clinical effects suggesting that i-PRF may be an option when complications occur with glucocorticoids.[31]

PRF Lysate

Another application of PRF is PRF lysate. PRF was isolated from volunteer blood using the technique developed by Choukroun *et al.* 20 ml of peripheral blood was immediately centrifuged for 10 minutes in room temperature at 400 gms. Two layers would be formed in the tube at the end of centrifugation, the upper layer was PRF jelly and erythrocytes at the botttom. The jelly was pulled out of the tube with sterile tweezer and cut using sterile scissors on the border of the PRF jelly with erythrocytes. The PRF jelly was immediately transferred in to a sterile glass tube then incubated at 37°C in a humidified atmosphere of 5%CO2 or 95% air and the exudate thus collected is referred as PRF lysate.[32] This has a good amount of growth factors comprising Transforming Growth Factor-β (TGF), Vascular Endothelial

Growth Factors (VEGF), Platelet Derived Growth Factos (PDGF) and Endothelial Growth Factors(EGF). This has been shown to reverse the damage by increasing the proliferation rate, migration of fibroblast caused by UV radiation dermal Fibroblast.[33] Rosen et al. showed that PRF Lysate reduced osteoblast differentiation in calvaria derived osteoblast by reducing Alkaline Phosphatase activity.[34] TGF-β gene array revealed three TGF-β target genes namely Inteleukin 11, NADPH oxidase 4 and Proteoglycan 4 precursor among the 51 strongly regulated genes by PRF lysates which downstream the effects of TGF-β and play a role in bone homeostasis.[35] Nishimoto et al showed that PRF lysates encompassing growth factors and other bioactive molecules are released at the surgical site in the regenerative procedure.[36]

Titanium -PRF (T-PRF)

Tunali and co-workers used medical grade Titanium tubes for processing of blood to produce PRF and called it as T-PRF. They used Titanium vacutainers based on the hypothesis that Titanium is more effective in activating Platelets than silica of glass tubes. used for PRF preparation. Thus different biomaterial was used for processing of blood than Glass tubes or silica coated plastic tubes.[37] In a comparison study by Ravi and Santhanakrishanan , T-PRF was shown to have maximum Tensile strength, modulus of elasticity and least degradation compared to L-PRF and A-PRF whereas T-PRF showed rapid release of growth factor.[22] T-PRF membrane exhibited positive wound healing effect when applied to palatal mucosal wound.[38]

Heat-compression of the PRF membrane

The biodegradability of PRF membrane can be reduced by heat compression of the PRF membrane. PRF membrane compressed with gauze was further compressed with an electric straightening iron for hair (ITH520, TESCOM, Tokyo, Japan). Surface temperature was fixed at 60 to 120 degree centigrade and was checked before the procedure using infrared thermometer (AD-5614; A&D Company, Tokyo, Japan). The PRF membrane preparations were wrapped with a ultraviolet (UV)-sterilized plastic wrap, which is a sealing film of polyvinylidene chloride (Saran Wrap; Asahi Kasei Home Products Corp., Tokyo, Japan), and compressed with the electric heat-compression device for 2–15sec. The resulting membrane had the ideal quality for barrier membrane such as plasmin resistant, stable for more than 10days invitro and had reduced biodegradability.[39] Animal implantation studies of heat-compressed PRF showed the membrane to be in place for at least 3weeks postimplantataion

compared to the control PRF which got resorbed within 2weeks. The additional advantage is that the barrier membrane can be prepared chair-side and applied during the surgical ptocedure. [40]

Table: 1 Advancement in platelet Concentrates and Procedure.

Advancement of PRF technologies	Protocols and Procedures
Leukocyte Platelet Rich Fibrin (L-PRF)	2700 RPM for 12 minutes in a plain glass tube.[11]
Advanced Platelet Rich Fibrin (A-PRF)	1500 RPM for 14 minutes in sterile plain glass based tubes.[20]
Advanced Platelet Rich Fibrin+ (A-PRF+)	1300 RPM for 8 minutes in sterile plain glass.[21]
Injectable Platelet Rich Fibrin (i-PRF)	700 RPM for 3 minutes in plastic tubes.[27]
Titanium – platelet rich fibrin (T-PRF)	2800 RPM for 12 minutes in medical grade Titanium Tubes.[37]
PRF Lysate	20 ml of blood centrifuged for 10 minutes in room temperature at 400 gms and the resultant jelly incubated at 37°C in a humidified atmosphere of 5%CO2 or 95% air.[37]
Heat-compressed PRF membrane	PRF membrane compressed with gauze was further compressed with an electric compressed with the electric heat-compression device for 2–15 seconds.[40]

Table: 2 Various growth factors of PRF and their functions.

Growth factors	Functions	References
PDGF	Fibroblast proliferation. Collagen synthesis. Chemotaxis for Neutrophills, Macrophages, fibroblasts and Endothelial cells. Wound contraction.	Etulain J. et al. 2018.[41] De Pascale MR et al. 2015.[42]
TGF-β	Involved in fibroblast proliferation, fibroblast/monocyte migration, collagen and collagenase synthesis, modulates angiogenesis.	Broughton G. et al. 2006.[43]
FGF	Calls macrophages, fibroblasts, endothelial cells, regulates fibroblast/monocyte/ epithelial/endothelial migration, fibroblast/epithelial/endothelial proliferation, collagenase synthesis, Induces angiogenesis, contributes in wound contraction.	Broughton G. et al. 2006.[43]
EGF	Regulates epithelial migration,	Ratajczak J.et al. 2018.[44]

	fibroblast/epithelial/endothelial proliferation, promotes M2 differentiation.	
VEGF	Induces angiogenesis stimulating migration and proliferation of endothelial cells, regulates collagenase synthesis and collagen secretion, calls macrophages & granulocytes.	Gonzalez AC. et al 2016.[45]
CTGF	Stimulates leucocyte migration, promotes angiogenesis, activates myofibroblasts stimulating ECM deposition and remodeling.	De Pascale MR.et al. 2015.[46] Lipson KE. Et al. 2012.[47] Sonnylal S et al. 2010.[48]
IGF	Facilitate proliferation of osteoblasts, can enhance the osteogenic differentiation of periodontal stem cells and enhances osteogenic mineralisation through the regulation of MAPK pathway.	Li X. et al. 2018.[49]

Non growth factors

Various non-growth factors present in the PRF have their role adding their effect on regeneration and wound healing. Histamine and Seratonin found to increase capillary permeability, which in turn allows inflammatory cells a greater access to the wound site and resultant macrophage activation. serine protease inhibitors, such as alpha-1-antitrypsin, alpha--antichymotrypsin, alpha-1-acid glycoprotein, inter-alpha-trypsin-inhibitor, protease C1 inhibitor, and complement proteins are Involved in inflammatory response, clotting, complement activation. In addition, abundant presence of immunoglobulin G was observed. The abundance of albumin, haptoglobin, ceruloplasmin vitronectin, fetuin-A, ficolin-3 and transthyretin help in colloidal osmotic pressure maintenance, substrate transport, buffering capacity, free radical scavenging, coagulation, and wound healing.[19,45]

Clinical usage of PRF, Advantages and disadvantages

PRF finds its usage in most of fields of dentistry as well as medicine. As the research is growing PRF is extensively used in all fields of dentistry, orthopaedic surgeries, plastic surgery, acute injuries of muscle, ligament injury, tendon injury, osteoarthritis, skin rejuvenation, hair loss, surgical repairs, esthetic procedures and others. For oral application as a sole material in socket preservation sinus lift. As adjunctive use it has innumerable application like as a regenerative material in intra bony defect, for guided bone regeneration, in socket preservation, in hard and soft tissue augmentation around implants, in endodontic periodontal lesions, in oral surgical procedures.[13,28] It can also be mixed with bone graft to enhance volume and root coverage procedures. In membrane form can be placed after

depigmentation of gingiva, recession coverage, as scaffold in tissue engineering and as GTR membrane.[7] Advantages of PRF being autologous, safe, easy to prepare, cost-effective, minimal biochemical handling of blood.[40] It has few drawbacks like, shorter shelf life, limited availability, inadequate storage facility for later use. Miron in his systemic review has shown the use of PRF which has been most investigated in periodontology for the treatment of periodontal intrabony defects and gingival recessions. Majority of studies have demonstrated favourable results in soft tissue management and repair.[50] Strauss et al. in his review commented on the role of PRF to improve other implant therapy outcomes.[51] Castro et al has shown the regenerative potential of L-PRF concluding that it has a positive effect on bone regeneration and osseointegration.[52] PRF offered benefits when applied in regenerative therapy as a natural scaffolds and was promising in regenerating bone and periodontal tissues with histological confirmation.[53] Franscisco et al. suggests that the application of platelet derivatives in the extraction socket can accelerate orthodontic tooth movement and PRF can improve alveolar cleft reconstruction and orthodontic tooth movement.[54] Thus the literature appraisal has concluded that PRF and its variants are beneficial bioactive adjuncts in regenerative dentistry.

Clinical variables which affect the outcome of PRF

Major variables which affects the outcomes are rotor size, centrifuge speed, duration of centrifugation. So the centrifugation model (dimension of rotor and rotor angulation for tube holder), composition and size of tube, temperature, time laps between of collection and start of centrifugation affects outcomes of PRF.[55] The time between blood draw and start of centrifugation has to be kept between 60-90 second so as to optimize the size outcomes of PRF. Female and older patients tend to yield a larger membrane.[56] Its known that relative centrifugation force (g force or RCF) which is derived from calculating the Radius of centrifuge rotor and the revolution of the rotor considered in calculating also affects the standardisation of PRF.[57] Also with concern for standardisation, preparation, nomenclature of type of biomaterials and the ability to reproduce consistent clinical results is pressing priority.

CONCLUSION

PRF and its technological advanced derivatives are biological surgical additives which have been used in various dental and medical applications. Advancement of PRF from its gel formulation to its membrane form or liquid formulation has made the versatile application of

PRF in regenerative and tissue engineering field. Thus PRF matrix, growth factors and the cells synergistically toil to affects soft and hard tissue healing in dentistry. PRF remains the most investigated material for wound healing and regeneration because of its varied clinical outcome on its application and the parameters which influence it. Different protocols have given PRF as required for clinical applications have been updated in this review. Further research is needed to standardise the preparations, composition and nomenclature of each type of PRF are needed.

DECLARATIONS

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CONFLICT OF INTEREST

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