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An insight in to Parameters influencing Platelet rich Fibrin characterization

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Abstract

Platelet concentrates originate from centrifugation of blood and are named according to their biological characteristics such as Platelet-rich plasma, Platelet-rich fibrin with its variants and concentrated growth factor. Use of these Platelet concentrates in Medical and Dental field dates back to three decades. These have gained considerable attention these days as soft and hard tissue regenerative material because of their improved techniques and have evolved to become one of the most widely used regenerative materials. Indeed, components of Platelets concentrates such as growth factors, fibrin matrix and cells principally Platelets have their unique roles and favors wound healing. Platelet concentrates for topical use are innovative tools for regenerative Medicine and Dentistry with excellent results. Having known these materials since many years, their effects in various therapeutic situations are fervently debated. Unfortunately these field of research have mainly focused on the Platelet growth factors. However the fibrin architecture as well as the leukocyte content of these products as well as other parameters are too often neglected, which also have very prominent role in regeneration. Getting insight in to these parameters will enhance knowledge and skills in their preparation techniques with promising results.

Keyword: Platelet concentrates, Platelet-rich fibrin, Platelet, Fibrin, regeneration.

Introduction

Platelet concentrates (PC) of which Platelet Rich Fibrin (PRF) and its variants are very vastly used as popular regenerative autologous preparation and is boon for Medical and Dental fields. The ambiguity of the terminology and the associated lack of characterization of the various PC led researchers for an improved terminology with the evolution of improvements in it. These PC of which PRF as a tools of regenerative material in surgical field is intended for the local release of Platelet

growth factors into a wounded or site of surgery, in order to encourage tissue healing and regeneration.(1)

Leukocyte content and fibrin architecture are two key characteristics of all Platelet concentrates which allow classifying these technologies in to four families. Conversely very less is known about the impact of these two parameters and other variables on the intrinsic biology of these products. The polymerization and final architecture of the fibrin matrix considerably influence the strength and the growth factor trapping or releasing potential of the membrane. It is also suggests that the leukocyte have a strong influence on the release of some growth factors, particularly Transforming Growth Fβ1. Further the various Platelet concentrates till date present have very different biological characteristics. An accurate definition and characterization of the different PRF is a key issue for the better understanding and comparing of the reported clinical effects of these surgical adjuvants.(2) it is seen from literature that till date the concepts or the information on the parameters which have influence on PRF are less. Hence in this literature review on PRF an attempt is made to understand PRF and various parameters which can influence PRF.(2)

Classification of Platelet concentrates.(3)

Depending on the presence or absence of leukocytes and the activation or not of the platelet Rich Plasma (PRP), classification was given by Mishra et al. in 2009

1st Generation–PRP

- 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

Based on their fibrin architecture and cell content classification given by Dohan Ehrenfest in 2009

2nd Generation–PRF

- 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)

Development of PRF

The concept of fibrin adhesives has ushered the development of Platelet concentrates. PC products are living biomaterials in fact blood concentrates. Their biology is as complex as blood itself. They are more difficult to handle and evaluate than synthetic materials shaped on order. The Platelet concentrates technologies started first in the field of oral and maxillofacial surgery by Marx et al. [4] He used the term PRP in mention to the phrase used for PC in hematology for the treatment of patients suffering from severe thrombopenia. PRP was first used in 1954 by Kingsley to designate thrombocyte concentrate during blood coagulation experiments.[5]

Various drawbacks of PRF in addition to overcome the French laws related to re-implantation of blood derived products, PRF came in to existence. Choukroun and co-workers introduced this simple protocol of PRF in 2001 which is completely autologous without any external additive. The basic protocol of PRF includes centrifugation of freshly drawn blood without any anticoagulant in glass or glass based collection tubes. This centrifugation process resulted in formation of three layers i.e. red blood corpuscles at the bottom, Platelet poor plasma at the top and PRF in between depending on differences in density.4 Further this basic protocol has been modified to obtain Advanced-PRF(A-PRF), Advanced PRF+(A-PRF+), injectable-PRF(i-PRF) and titanium-PRF(T-PRF) by modifying the centrifugation speed, time and the tube in terms of its design and protocols. (6,7)

The centrifugation protocol of PRF started with the introduction of L-PRF based on a high-speed protocol

(2700 rpm for 12 min).[8] Later on, A-PRF (1300 rpm for 14 min), A-PRF+ (1300rpm for 8 min) and i- PRF (700 rpm for 3 min) with lower g-forces and centrifugation times were introduced with the overall aim to increase the number of Platelets and leucocytes. To obtain T-PRF centrifugation at 2800 rpm for 12 minutes in medical grade titanium tubes was carried out.[9]

The various advantages of PRF include (Dohan et al.) [11-12,)]

1. Completely autogenous
2. Extended growth factor release for more than 7 days
3. Simple and faster technique
4. In-expensive
5. No requirement of any additive constituent such as bovine thrombin
6. No biochemical handling involved
7. No associated immune reactions
8. No associated infections
9. Acts as an 'immune regulation node'
10. Has anti- inflammatory effects

Various Parameters influencing characterization PRF

The characterization of PRF, a complex blood product seems to remain incomplete due to the number of parameters involved. Parameters that are taken in to consideration are with respect to Centrifuge Machine, Platelets, Fibrin, Leukocytes as well as other technical considerations which have shown to influence PRF. Parameters particular to the centrifuge used are very important as its size, vibration, the duration of centrifugation have a remarkable impact. Along with these the cost involved, ergonomics, the form and volume of final product etc, also need to be taken into consideration while evaluating newer techniques. A right understanding of its components and their connotation will make possible us to comprehend the clinical fallout obtained and next extend their fields of therapeutic application. The following are few of the parameters found to influence PRF.[13-15]

Relative centrifugal force (RCF), Radius of centrifuge and Rotation per minute

Relative centrifugal force (RCF) commonly known as g-force is the amount of accelerative force applied to a sample in a centrifuge, which is directly proportional to the revolutions per minute (RPM) a sample in a test-tube is subjected to. Because rotors are different from various manufactures, we use RCF to represent the centrifugation force.[16]

The formula for computation of relative centrifugal force is , $RCF = 11.18 \times r \times (N/1,000)^2$.

Where N is revolutions per minute and r is the radius in cm.

As the centrifugal force increases the particles move down the centrifuge tube. As a general rule, the greater the centrifugal force the shorter the separation time. However centrifugation also generates hydrostatic forces within the solution and so excessive centrifugal forces can disrupt some biological particles such as ribosomes. [17]

The multiplying role on final RCF values is played by radius, as at larger radiuses the values are grater. It is also to be understood that RCF values can easily be doubled between the RCF-min and RCF-max based on this increased radius distance. Standardizing and reporting of RCF parameters as a major factor should be emphasized in Centrifugation over which clinicians have control. [18]These are as follows.

1. Dimensions of the rotor (radius at the clot and end of the tube),
2. Rotor angulation for the tube holder,
3. Revolutions per minute (RPM) and time,
4. RCF value calculated at either the RCF-min, RCF-clot or RCF-max,
5. Composition and size of tubes used to produce PRF and

6. Centrifugation model used.

Confusion regarding acceptance of RCF values was seen as several articles have used RCF-min, RCF-clot, or RCF-max values without reporting exactly where these RCF values were derived. The RCF or g-force calculated at the fibrin clot (RCF-clot) is subjected to changes, owing to the centrifugation time even when centrifuged at the same speed. So this method of reporting g-force at RCF-clot is inferior in accuracy and not commonly reported internationally. Internationally reporting of g-forces is at the end of the centrifugation tubes or RCF-max as is not subject to these differences.[19]

Based on the fact that by high centrifugation speed tends to push the cells including Platelets and leukocytes away from the PRF clot is justified by many authors. By lowering the g-force applied, not only a more homogeneous Platelet distribution can be achieved but also the number of the neutrophilic granulocytes trapped in the PRF will be enhanced. This increased cellularity of the PRF would convert into increased macrophage differentiation which leads to increased osteoblastic differentiation. The enhance in the growth factor release was ascribed to possible raise in the number of leukocytes entrapped due to low centrifugation forces. So the use of low speed to produce PRF would optimize growth factor production and cellular response to PRF was demonstrated.[20]

Various research groups have further shown that different biological properties of PRF collected from a given individual may result based on centrifugation speeds i.e. RCF.[21,22]

Centrifuge angle rotor

In fixed angle rotor the RCF at the top and bottom of the centrifugation tube might differs. Thus RCF also varies at different level in the test tube, from the supernatant level to pellet level. [23] 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 resulted in higher Platelets or leukocytes. A 10 fold increase in the Platelets and leucocyte count by utilizing only 0.3–0.5 ml of sample was also seen.[9]

Centrifugation speed and times

PRF clots fabricated at lower centrifugation speeds and times there was an improved growth factor release 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 it contain more Platelets and leukocytes with more growth factor release.[24]

The time at which a centrifugation procedure begins following blood draw is critical to optimize the size outcomes of PRF membranes. In general about 15 sec is required per tube to harvest 9-10cc of blood. Therefore interval between blood draw and the start of centrifugation should be 60 to 90sec, which is appreciated by clinicians to avoid significant changes in the macroscopic morphology or size of fabricated PRF membranes. Shortly thereafter a significant reduction in size is observed. More than a minute or two waiting may cause the fibrin to polymerize in a diffuse way leaving behind only a small poorly formed clot in the test tube. [25]

Centrifugation tubes

Centrifugation tubes are available in plastic, silica coated plastic, glass and titanium. Commercially available silica-coated blood-collection tubes contain cytotoxic silica microparticles. These silica microparticles are incorporated into the resulting PRF matrix and implanted for regeneration and repair of injured tissues and causes acute cytotoxic effects. [26] Plastic tubes are not generally used as they have a hydrophobic surface and do not efficiently activate the coagulation process. [27]

However Tunali et al in 2014, introduced a new product called T-PRF. The use of titanium tubes for collection and centrifugation instead of glass tubes was established on the hypothesis that titanium may be a more efficient Platelet activator than silica for preparing L-PRF. Based on light, scanning electron and fluorescence microscopy analysis, he concluded that T-PRF has immensely organized network along with a continuous integrity and even the fibrin network was thicker and also it covered larger area. [28,29]

Fibrin

Fibrin is a bridging molecule that allows a series of cell interactions and supplies a provisional matrix for migration of fibroblasts and endothelial cells, in which cells may proliferate, organize, and carry out their functions like angiogenesis, healing new tissue formation mainly in sites that suffered injury or inflammation. [30,31]

A homogeneous 3-dimensional organization of fibrin network incorporates circulating cytokines in the fibrin meshes which are conducive to regeration.[32] Fibrin is derived from Fibrinogen which is a soluble fibrillary molecule that plays a decisive role in Platelet aggregation during hemostasis and it is transformed into a insoluble fibrin by thrombin which is a cicatricial matrix at the wounded site. This gelling action occurs with the silicon dioxide present in the glass coating of the PRF tube without any gelling agent.[33] Physiologic thrombin induces slow and natural polimerisation which gives it the crucial three dimensional organization of fibrin network. This provides the matrix with great elasticity, thus forming a very strong PRF membrane. Waiting for more than a minute or two for centrifugation may cause a small poorly formed clot in the test tube as the fibrin polymerizes in diffuse way. Multiple variations in fibrin structure may be due to different physiological situations, such as the concentration of calcium ions and fibrinogen. Moreover fibrin may change its structure in patients with certain comorbidities, such as diabetes and nephrotic syndrome, among others. Thus variation in these and their effect on PRF has to be elucidated clearly.[34]

Few more factors are also presumed to have an effect on fibrin clot formation and structure and presumed to influence PRF. These include genetic factors, acquired factors such as abnormal concentration of thrombin and factor XIII in plasma, blood flow, Platelet activation, oxidative stress, hyperglycemia, hyperhomocysteinemia, medications, and cigarette smoking and other parameters such as microgravity, pH, temperature, reducing agents, and concentration of chloride and calcium ions, which do have their role.[35] Scanning electron microscopic results have show that long-term cigarette smoking does affect the thickness and arrangement of fiber architecture in both leukocyte and Platelet rich fibrin and advanced Platelet-rich fibrin membranes and also could have an impact on activation of Platelets.[36]

Platelet

Biologically active proteins and growth factors are enclosed in Platelets which bind on to a developing fibrin mesh or to the extracellular matrix. Platelets have mitogenic properties create a chemotactic gradient for recruitment of stem cells. [6] PRF regeneration is because of these stem cells which differentiate and promote healing. Hence proper entrapment of Platelet in the fibrin mesh is successfully carried out with quick handling , speedy blood collection and immediate centrifugation before the initiation clotting cascade is absolute essential for clinical success of PRF.[37] Platelet size, Platelet activation are also considerably influenced by cigarette smoking which inturn affects characterization of the L-PRF and A-PRF membranes. [36]

Role of Leukocytes

Neutrophils are influenced by the fibrinogen degradation products, which stimulate migration of neutrophills thus modulating phagocytosis and enzymatic degradation of the neutrophils. Fibrin controls wound colonization by macrophages because of chemotactic agents trapped in fibrin. [39] PRF which has leukocytes in it acts as an immune regulation node and provides antiinfectious effect. All key immune cytokines like IL 1β, IL-6, IL-4 and TNF have the ability to control the inflammatory response at the wound site. Variation in Neutrophils which could be congenital or acquired could affects the characterization of PRF.(38)

Other variation which affects PRF

Other sensitive issues that may influence the nature of PRF include variation in quantity and quality of PRF with aging, influence of systemic diseases (thrombocytopenia, bleeding disorders, diabetes, leukocyte adhesion syndromes etc.), nutrition, environmental or racial differences, blood profile, autoimmunity and genetic predisposition.(39) Furthermore, females and older patients produced larger membranes, likely due to lower red blood cell counts derived from their peripheral blood. Additionally, females and older patients were found to produce larger PRF membranes. Centrifugation protocols may therefore be adapted accordingly.(40) Trauma and surgical intervention led to significant alterations of PRF, partially in direct relationship to alterations of peripheral blood composition. It was seen that the percentage of inflammatory monocytes was raised significantly and higher release of proinflammatory (IL-6) and anti-inflammatory (IL-10) cytokines was found in the PRF matrices obtained from Trauma and Surgical Patients. Even the quality of PRF matrices is different in these traumatized patients and patients undergoing a surgical intervention compared to those from healthy people.(41)

Applications of PRF

The vast benefits of PRF have led to its applications in different fields of medicine and dentistry. Ear, nose, throat, plastic surgery, Oral and maxillofacial surgery, Pre-implant and implant surgery. In other medical fields, PRF has also been utilized for the management of hard-to-heal leg ulcers including diabetic foot ulcers, venous and chronic leg ulcers, facial soft tissue defects, laparoscopic cholecystectomy, deep nasolabial folds, superficial rhytids, acne scars, chronic rotator cuff tears, and acute traumatic ear drum perforations . Application of PRF finds dental application in Regerative endodontics, Oral surgery, Orthodontics, Periodontics, in sinus lift procedures and implant procedures.(42)

Conclusion

PRF as a biologic surgical additive has been successfully used more than two decades for varied applications in dentistry and Medicine. The key issue in Platelet concentrate technologies is not only the cells and fibers but how Platelet, leukocytes, fibrin and growth factors are interlinked in the final product. With the present scientific literature, lot of understanding about parameters which can influence PRF has to be kept in mind before planning for the regenerative procedures. Great concern has to be taken regarding their preparation failing which predictability of PRF will be difficult. Technological advancements in the field of PRF have paved way for the versatility in their applications. With the increase in our understanding about the biology of PRF, in future we can expect improved additives which will further enhance the wound healing experience. The overall question of whether the in vitro PRF research reflects the clinical reality serving as a surrogate parameter to adapt the current PRF protocols remains to be clarified. In future rationale with respect to PRF study has to be addressed with various parameters which can influence PRF.

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