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Technical considerations in obtaining platelet rich fibrin for clinical and periodontal research

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ABSTRACT

Autologous platelet rich fibrin (PRF), is currently being widely used and investigated across the globe by clinicians and periodontal research. The technical aspect required for the procurement of PRF includes revolution per minute (RPM), relative centrifugal force (RCF) or G-force, rotor radius, rotor angle, stability or vibration in the centrifugal machine and material of test-tube, besides the systemic health of the individual may influence the final outcome. Present technical note intends to compile these aspects for better understanding and appropriate outcome while preparing PRF in varying clinical scenarios.

1. Introduction

Platelet rich fibrin (PRF), an autologous blood-derived biomaterial, is currently being widely investigated and used across the globe by clinicians not only in field of dental implantology,[1] periodontology,[2] oral and maxillofacial surgery,[3] pulp revascularisation[4] and regenerative pulp therapies,[5] but also by dermatologists,[6] orthopaedic surgeons and diabetologists[7] as a cost-effective biological healing agent. In last decade, studies have also correlated impact of demographic and systemic factors on quantitative and qualitative outcome of PRF with varying results.[8–12] Narayan & Malaiappan[9] found that quantity of PRF obtained is independent of age and gender. Hemlata et al.[8] reported significantly higher concentration of platelets in PRF clot in non-diabetic patients as compared to diabetic patients. In continuation, Das & Amaranath[11] also highlighted the possibility of alteration in fibrin clot formation due to variation in systemic and behavioural conditions.

Since its inception as second-generation platelet concentrate by Chaukroun et al.,[13–18] continuous evolution and modifications in centrifugation protocols have been suggested. These are Standard-PRF (S-PRF),[13,19,20] Leukocyte-PRF (L-PRF), Concentrated Growth Factor (CGF),[21] Titanium-PRF (T-PRF),[22] Advanced-PRF (A-PRF),[23,24] Advanced-PRF Plus (A-PRF+),[24] i-PRF (Injectable-PRF),[24,25] Albumin-PRF (Alb-PRF),[26,27] hyperacute serum (HAS)[28] and Horizontal-PRF (H-PRF) [Table 1].[29] To clarify further, POSEIDO have recommended a classification system that served as basis for evolutions of PRF. However, literature pertaining to the technical aspect of centrifugation speed (revolution per minute, RPM), timing of centrifugation, PRF tube (glass, glass-coated plastic or plastic tubes), and relative centrifugal force (RCF, G-force), is still contentious. Present technical note intends to highlights technical aspects to be taken care, while preparing PRF in different scenarios.

2. Technical concept

In original paper of Dr Joseph Choukran et al. in 2001,[13] it was advocated that the protocol for obtaining PRF is a common, simplified, free, acquiescently available for all clinicians and not linked to a medical device nor a specific machine, and the name PRF was copyright protected by the primary researchers.

Centrifugation: It is a procedure used for the separation of particles by sedimenting them under varying degree of gravitational forces. More dense particles or organelles or components of the solution or mixture migrate away from the axis of rotation of the centrifuge, whereas less-denser components of the mixture drift towards the centre axis. Various factors influencing the outcome of centrifugations are particle size, shape, density, viscosity, rotor radius, timing of centrifuge and

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Table 1
Timeline and technical specifications for evolution and modifications in platelet rich fibrin.

Year	Author	Protocol	Equipment used	Nomenclature
2001	Choukron et al.[13]	3000 rpm (750 g) for 10 min		Chaukroun's PRF
2006	Choukron et al.[14]	3000 rpm (750 g) for 12 min	Fibrinet	Pure Platelet Rich Fibrin (P-PRF) or Standard Platelet Rich Fibrin (S-PRF)
2006	Dohan Ehrenfest et al.[16,17]	2700 rpm (400 g) for 12 min	• Open-access method, IntraSpin, Intra-Lock International, BocaRaton, Floroda; • Salvin 1310, Charlotte, NC, USA; • LW-UPD8 (LW Scientific) • A-PRF 12 (Advanced PRF, Process)	Leukocyte-and platelet-rich fibrin (L-PRF)
2006	Sacco[27]	Acceleration for 30 s, followed by 2 min centrifugation at 2700 rpm (692 g), 4 min at 2400 rpm (547 g), 4 min at 2700 rpm (592 g), 3 min at 3000 rpm (855 g) and finally 36 s deceleration and stopped	• Programmed spin cycle, Medifuge, Silfradent, Sofia, Italy	Concentrated growth factor (CGF)
2012	Tunali et al.[22]	2800 rpm for 12 min (prepared in 10 ml titanium tube)	EBA 20, Andreas Hettich GmbH & Co. KG, Tuttlingen Germany	Titanium Platelet Rich Fibrin (T-PRF)
2014	Ghanaati et al.[23]	1500 rpm (200 g) for 14 min	PROCESS for PRF, Nice, France; Advanced PRF Process, France	Advanced-Platelet Rich Fibrin (A-PRF)
2015	Mourao et al.[25]	3300 rpm for 2 min	Duo Process, France	Injectable PRF (i-PRF)
2015	Kawase et al.[26]	700 g for 8 min, Heat compression of PRF	Medifuge centrifugation system (Silfradent S.r.l., Santa Sofia, Italy)	Alb-PRF
2017	Fujioka-Kobayashi et al.[24]	1300 rpm (200 g) for 14 min	Duo Centrifuge, Process for PRF, Nice, France	Advanced-Platelet Rich Fibrin (A-PRF)
2017	Fujioka-Kobayashi et al.[24]	1300 rpm (200 g) for 8 min	Duo Centrifuge, Process for PRF, Nice, France	Advanced-Platelet Rich Fibrin (A-PRF +)
2018	Simon et al.[28]	1710 g for 5 min	With the use of a flat forceps, the serum portion squeezed out of the fibrin clot	SPRF (serum from platelet-rich fibrin) or HAS (hyperacute serum)
2021	Fujioka-Kobayashi et al.[28]	700 g for 8 min Followed by 10 min cooling of PRF with albumin gel	Eppendorf centrifuge 5702 machine (Hamburg, Germany)	Albumin-PRF (Alb-PRF)
2022	Zheng et al.[29] Dashore et al.[6]	700 g for 8 min	Bio-PRF, Venice, Florida	Horizontal PRF (H-PRF)
2023	Bains et al.[18]	3000 rpm for 18 min	REMI-R-303	PRF in patients with uncontrolled diabetic

RPM. Compliance to the standard in manufacturing of Centrifuge is maintained with International Electrochemical Commission (IEC) standard 61010-2020.[30]

Centrifugal Machine: Centrifugal machine (class II medical device, requiring general and special control) is used to separate substances (like blood cells from serum and plasma) of different densities in a liquid by rotating at a certain speed measured as revolutions per minute (RPM) by the generation of centrifugal force, termed as Relative Centrifugal Force (RCF or G-force). Although the common way of expressing centrifugation is rotor speed that is stated in terms of RPM, however it does not take into account the radius (i.e. distance of the tubes to the axis of rotation) of the centrifuge.[31] Rotor radius plays an important role in the generation of G-Force. Centrifugal machine used for the preparation of PRF originally have a rotor distance of 5 cm.[32] Under the influence of G-force, denser particles move outwards radially and settle down at the bottom of the tube, whereas low-density substances move to the top.[33]

Another important feature of the centrifuge is the type of rotor, and most commonly available rotors are fixed angle rotor (33°), swinging bucket rotor and vertical rotor. Amongst these, fixed angle and swinging bucket rotors are most commonly used for table-top, low speed and high-speed floor model centrifuge, whereas vertical rotors are most commonly used in ultracentrifugation. Dashore et al.[6] suggested swingout bucket model of centrifuge or the horizontal centrifuge is considered as the ideal machine for the preparation of both PRF and liquid or injectable PRF. In India different types centrifuge commonly available are Labtech Centrifuge with fixed rotor radius of 8 cm [Fig. 1 (a) and (b)], Remi R-303 with fixed rotor radius of 7.5 cm [Fig. 1(c) and (d)], and Remi-8C, Remi-C854 and Neva-2 (Remi, India) with swinging bucket rotor radius of 12 cm [Fig. 1(e) and (f)]. Fig. 2(a)–(c) represents the fixed angle, vertical and swinging bucket types of rotors in table-top centrifugal machine, whereas, Fig. 2(d)–(f) represents the positioning of tubes in fixed angle, vertical and swinging bucket rotors, respectively.

In most of clinical scenarios PRF is prepared with small and light table centrifuge in which there are risk of vibrations and resonance during centrifugation, which can be perceived easily by hearing and via tactile sensation with hand during centrifugation. Study suggested that most of centrifuge produce radial vibrations of above the threshold of 1 when used at the speed of 2700 or 3400 rpm, resulting in resonance in centrifuge tubes, that can significantly damage the blood cell content of the tube.[20]

Relative Centrifugal Forces (RCF or G-force): Relative centrifugal force (RCF or G-force) refers to the amount of radial force generated by the spinning rotor (e.g. in Centrifuge) that is expressed relative to the earth's gravitational force. It is dependent on the speed of rotation (RPM: Rotation/Revolution Per Minute) and the distance of the particles from the centre of rotation [i.e. R: Radius of the rotor in centimetres (cm)] that can be calculated by formula RCF or G-Force= $(RPM)^2 \times 1.118 \times 10^{-5}$ x R. Simply, doubling the speed of rotation (RPM) increases the G-Force by a factor four, and increase in the rotor-radius results in exponential increased values of G-force.[34]

However, for the preparation of PRF, G-force also depends on rotor angulation besides speed (rotation/revolutions per minute, RPM) and the rotor radius. Due to angulation of rotor, the radius of the rotor is calculated at the clot and end of the tube. For fixed angle rotor, average of G-force (RCF^{Avg}) can be deducted from the minimal G-force at the top inside ie the shortest distance to rotor (RCF^{min}), Clot G-force calculated at the distance from centre of rotation axis to the middle of the fibrin clot ie the middle of the tube (RCF^{Clot}), and maximal G-force value at largest

Fig. 1(a). Labtech Centrifuge with; (b) Fixed rotor of radius of 8 cm; (c): Remi R-303 with; (d) Fixed rotor of radius of 7.5 cm; and (e): Neva-2 (Remi, India) with; (f): Swinging bucket rotor of radius 12 cm.

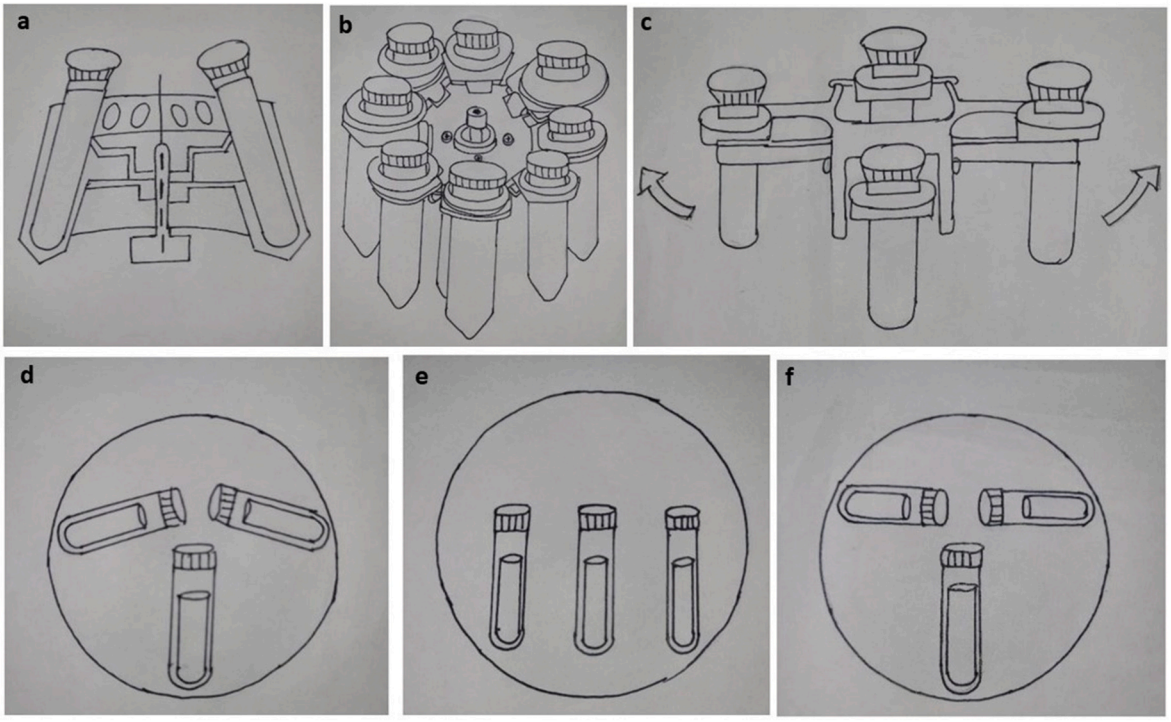

Fig. 2(a). (b) and 2(c) showing types rotor in various table-top centrifugal machine available; and 2(d), 2(e) and 2 (f) represents the positioning of tubes in fixed angle, vertical and swinging bucket rotors.

distance to rotor (RCF^{Max}).[35]

Revolution Per Minute (RPM): It was observed that using low-speed centrifugation, platelets were distributed homogenously within the PRF matrix regardless of tubes types, whereas at high-speed centrifugation, platelets were distributed on mainly on one surface region of the PRF clot in glass tube as compared to more diffuse distribution of platelets in silica-coated plastic tubes. However, same is true for growth factor also is still not clear. Kobayashi et al.[36] reported that

A-PRF prepared at low RPM and G-force has more retention and releasing capability of growth factors than L-PRF prepared at higher RPM and G-force. In contrast, studies have reported that A-PRF has a low capacity as a growth factor carrier as compared to L-PRF due to too low centrifugation force that does not allow a good separation of the blood components. They further advocated that adequate G-force triggers the platelets to produce growth factors via stimulation of leukocytes.[20,37]

It was observed that cells like leucocytes, shift to the bottom of collection tube by increasing the speed of centrifugation (and hence G-force). Therefore, by reducing the G-force and centrifugation speed as in A-PRF, an increase number of leukocytes can be entrapped within the PRF clot and hence more release of growth factors may be observed at low G-force.[24]

Timing of Centrifugation: It was hypothesised that less centrifugation time may increase the total number of cells (platelets, leucocytes and macrophages) entrapped in the PRF clot due to reduced cell pull-down effect by centrifugation force. Ghanaati et al.[23] reported an increased existence of neutrophilic granulocytes in the distal part of the clot by decreasing the RPM and increasing the centrifugation timing in A-PRF- prepared in glass-tube. More chances of penetration into deeper part of clot during the centrifugation process may be due to smaller average diameter of neutrophilic granulocytes (8.5–10 μm) as compared to monocytes (15–20 μm). Sammartino et al.[38] based on their experience recommended extended centrifugation timing of 18 min for preparation of L-PRF clot instead of 12 min in patients on anticoagulants. Similarly, in a recent study Bains et al.[12] reported the extended duration of centrifugation of 18 min for preparation of PRF in well-established or uncontrolled diabetic patients. This increased centrifugation time mostly gives a bit more time for fibrin clot stabilisation.

Material of PRF Tube (Plastic Tube, Glass-Coated Plastic Tubes or Glass Tubes): As per initial advocated protocols, PRF is best obtained in plain chemical free dry glass tubes.[39–41] Since, most of oral health clinicians were not trained to accomplish blood harvesting, henceforth use of disposable plastic tubes was suggested to circumvent tube breaking and contamination in dental clinic.[39] Mirion et al.[35] revealed that the centrifugation tubes are central to the quality production of PRF. They advocated that glass-tubes produced an approximately 200–250% larger PRF clot than the glass-coated plastic tubes.[40–42] As with other biomaterials used in dentistry and medical field (eg bone grafts, glass-ionomer cements etc and flasks or perfusion phials for storing drugs), dry glass or glass-coated plastic tubes are advocated for PRF preparation without any health hazard by minute amount of release of silica.[39] However, Tsujio et al.[43,44] investigated that glass-coated plastic tubes used for obtaining PRF actually shed their silica-coatings into PRF membranes, thus embedding silica particles at quite significant levels within PRF clots. Masuki et al.[45] investigated the biosafety effect of the silica microparticles and reported that these particles are adsorbed onto the cell surface with ostensibly high affinity and brought apoptosis of primary human periosteal cells derived from alveolar bone cells, causing substantial decrease in cellular viability and proliferation.[45] Furthermore, silica nanoparticles were recently reported to induce cytotoxicity and inflammatory responses in lung epithelial and endothelial cell lines and in hippocampal cells by the generation of reactive oxygen species. It was also observed that specific types of amorphous silica nanoparticles can act as tumor-promoting substance. Even chemical addition of silicone on A-PRF glass tube has been shown to be detrimental and resulted in 2-fold reduction in clot size formation as compared with standard plain glass tubes alongwith the production of a form-like residue over the clot.[40,41] Therefore, clinicians must not overlook the possibility of negative influence of silica microparticles on tissue regeneration, until safety of glass-coated plastic tube is guaranteed. But there is another consideration, due to release of silica microparticles, ubiquitous initiation of coagulation and platelet activation was observed resulting in relatively wide distribution of platelets in PRF matrix regardless of centrifugation speed. This is in contrast to platelet distribution in PRF clot obtained in glass tubes, where platelet distribution is speed dependent.[43,44]

3. Discussion and recommendations

Numerous studies have been published to understand the technical aspect of centrifugation required for the generation of PRF that include RPM, G-force, rotor radius, rotor angle, stability or vibration in the centrifugal machine and type of test-tube used, besides the systemic health of the individual. For obtaining sufficient quantity and quality of PRF, recommended RPM for procurement of PRF is 430–3500. Most of time recommended G-force calculated for obtaining optimal PRF is 430 g–750 g. However, in most of Indian studies centrifugal machine used consist of rotor radius of 7.5 cm–12.0 cm producing G-force within range of 700 g–1000 g. Most of the studies advocated the use of fixed angled centrifugal machine (33°), however, horizontal centrifugation using centrifugal machine with hanging bucket rotor reported to release higher number of growth factors as compared to fixed angle centrifugal machine.[46] On comparing the various machine with different centrifuge type including the DUO Quattro (Nice, France), Remi-8C (Remi, India) and Remi-C854 (Remi, India) and rotation dynamic (reduction in RPM to adjust g-force to 400) on PRF generation, it has been suggested that the principles of centrifugation is critical as the quality, regenerative capacity and quantity of PRF may be affected by the G-force. By reducing the G-force to 400 in Remi-8c and Remi-C854 and by decreasing RPM, the quantity and quality in terms of regenerative potential and physical characteristics of the various platelet concentrates are significantly improved.[47]

PRF membranes produced with fixed angle centrifugation accumulated cells along the back distal walls of centrifugation glass tubes. Although having handling issue in dental clinic, yet glass tubes are considered material of choice for PRF generation as compared to silica coated plastic tubes or plastic tubes.[40] Recent studies also advocated use of titanium tubes to achieve higher strength of PRF membrane. Controversies still existed regarding calculation of G-force (RCF^{avg}, RCF^{min}, RCF^{clot} and RCF^{max}). Miron et al.[34] advocated that internationally G-force values should be calculated as RCF^{max} at the bottom end of the centrifugation tubes.

Dohn Ehrenfest et al.[48] suggested that all the systems for the production of platelet concentrates in the market require a specific centrifuge and collection kit system e.g. Intra-Spin centrifuge and kit (Intra-lock, Boca Raton, FL, USA) is used preparation of L-PRF. Following are the recommendations for future research publications on PRF. All platelet derivatives are regrouped under the general term of “platelet concentrates”. It is important to highlight the key influence of the leukocyte content and fibrin architecture. Miron et al.[37] suggested that in order to upsurge the transparency, at least following 6 parameters must be described in all research papers published pertaining to PRF. These are rotor radius or dimensions of the rotor (radius at the clot and end of the tube); angulation of the rotor for holding the tube; revolutions per minute (RPM) and centrifugation time; relative centrifugal force (G-force or RCF) value; composition and size of tubes used to produce PRF; and centrifugation model used. They further reported that PRF prepared in one centrifugation device is considered as “biological signature” for that device. Therefore, PRF properties may differ when prepared with two different centrifugal machines with dissimilar rotor radius, angulation of rotor, tubes size and material-composition. They proposed to avoid use of trade names (eg L-PRF or A-PRF) until specific proprietary protocols and devices are not exclusively used.[37]

4. Conclusion

Quantum and quality of PRF obtained definitely depends on the technical components of the centrifugation including centrifugal machine, centrifugation protocol as well as the material tube in which it is prepared. However, recent studies[11,12,38] have compelled the researchers to also think about the influence of systemic conditions on

PRF, and further studies are required to comprehend the same. This paper, is a compilation of standard guidelines for the novice clinicians and researches as understanding and utilisation of appropriate technical specifications is of paramount importance to maintain sustainable transparency in forthcoming research work and publication.

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Declaration of competing interest

None.

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