Review Article

# The Role of Centrifugation Process in the Preparation of Therapeutic Blood Concentrates: Standardization of the Protocols to Improve Reproducibility

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## Abstract

Investigations of endogenous blood-derived growth factors have increased in the past two decades. The initial protocols for blood concentrates, such as platelet-rich-plasma, utilized anticoagulants followed by bovine thrombin to catalyze fibrin polymerization. Subsequently, platelet-rich-fibrin (PRF) protocol was developed to eliminate anticoagulants and thrombin. The PRF production was described in a vertical rotor centrifuge with an angle of ~33°. Many commercial enterprises have attempted to replicate this protocol with a multitude of different centrifuges. These attempts have utilized revolutions per minute (RPM) to develop their protocol to generate PRF. However, RPM is a variable parameter, as it depends on the design and radius of the centrifuge. The separation of blood components is highly dependent on the relative centrifugal force (RCF) generated, which is based on the applied centrifugal force, expressed as multiples of earth's gravitation (*g*) field. RCF is a function of the acceleration due to the gravity of the earth, $g = 9.81$ m/s². RCF, not RPM, is the key factor for the sedimentation of the cells and proteins within blood concentrates. RCF is determined using the maximum centrifuge radius for calculation. The initial PRF was generated by applying an RCF of 700 ×*g*. Accordingly, so far, a large number of studies existing about PRF are not comparable, as they were technically prepared with different RCFs. In addition, due to the nonstandardized measurement methods, in some published studies, incorrect RCF values are published as the authors did not know how to calculate the RCF correctly. This is the case for a widespread blood concentrate called leukocyte-PRF, which is commercially available. Recently, we introduced the low-speed centrifugation concept (LSCC) for the production for solid and liquid PRF matrices. This concept is based on the above-mentioned initial PRF protocols, which represents a relatively high RCF. It shows that a systematic reduction of 700 ×*g* to 44 ×*g* can significantly increase the cells and growth factors within the same blood concentrates. The LSCC concept was established initially for a fixed rotor centrifuge with a radius of ~110 mm. The present narrative review highlights the necessity of standardization in the generation of blood concentrates, which utilizes RCF, rather than commercial protocols.

**Keywords:** Advanced platelet-rich fibrin, centrifugation, platelet-rich fibrin, relative centrifugal force, LSCC

## Introduction

The separation of blood components in a centrifuge is performed by the application of centrifugal force, measured by relative centrifugal force (RCF), which is expressed as multiples of earth's gravitational (*g*) field, which is equivalent to 9.81 m/s². Centrifugal force refers to the outward force applied to objects in circular motion [Figure 1], undergoing acceleration, responsible for changing the direction of movement, i.e., centrifugal acceleration. This force is directed outward in relation to the circular path. To determine the centrifugal force to which the samples will be subjected, a rotational speed in revolutions per minute (RPM), the speed of a centrifuge rotation, and the maximum radius (*R*) of rotation of the examples in millimeters (mm) are required, with the result of the RCF given in *g* (a measure of acceleration due to gravity of the Earth).

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In 1998, platelet-rich-plasma (PRP), considered the first generation of blood concentrates, was applied in oral and maxillofacial surgery.[1] Shortly thereafter, in 2000, a different technique from PRP was described that did not use anticoagulants, creating the blood-derived concentrate using a simple glass-based tube without additives, referred as platelet-rich-fibrin (PRF).[2] Subsequently, much research has been performed in the field of blood-derived concentrates, with the increasing focus on the second generation of blood concentrates. Various publications have highlighted the biological benefits of blood concentrates up to 2006.[3-6] In these studies, the concentration of platelets and leukocytes could be demonstrated as a result of the application of relatively lower (700 ×*g*) RCF.[3] In comparison, the PRP protocol required high RCF (3000 ×*g*) in the second centrifugation step.[7] Accordingly, the second-generation blood concentrates were referred to as leukocyte-PRF (L-PRF). Over time, L-PRF® became a trademark currently distributed under the IntraSpin® System (BioHorizons®, Boca Raton, Florida, USA).

The scientific inquiry into the field of blood concentrates has demonstrated the significant role of centrifugation protocol on the composition and biologic properties of the isolates. A study by Ghanaati *et al*. showed for the first time an increase in the number of neutrophils and macrophages in the PRF fibrin mesh by reducing the RCF from 710 ×*g* to 208 ×*g*.[8] Accordingly, after reduction of RCF, higher cellular accumulation within the fibrin clots of equal volume. This new concept protocol has been adopted by Advanced-PRF (A-PRF) protocol, distributed under the A-PRF® trademark distributed by Choukroun (Process, Nice, France).

There are increasing efforts on the commercialization of PRF with a growing number of brands and protocols available on the market, such as L-PRF®,[9] A-PRF®[8], or Concentrated Growth Factors (CGFs®)[10] among others. These protocols may be distinguished on the bases of different centrifuge equipment (rotors, etc.), different protocols (RCF and centrifugation time), and tubes with varying material. Each of these aspects can affect the properties of the blood isolates. This narrative review seeks to present some relevant aspects for the preparation of blood concentrates, correlating them with their variations, with focus on RCF.

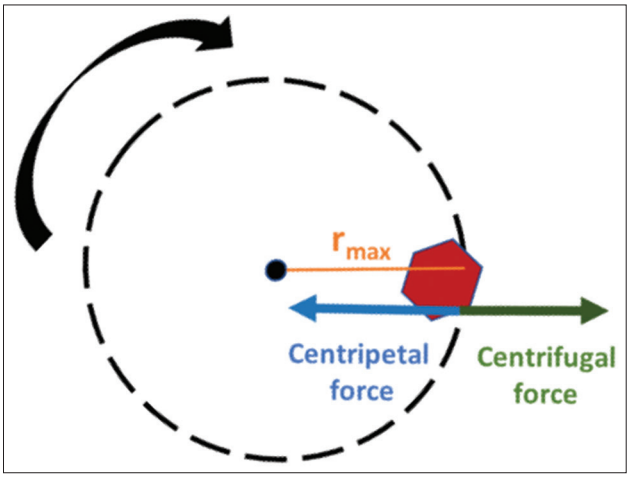


**Figure 1:** Schematic illustration of the centrifugation process during the rotation of a body (red symbol) and the resulting centrifugal force (green arrow) and centripetal force (blue arrow) in relation to the maximum radius (orange line)

## Platelet-rich Fibrin

Following blood centrifugation in glass-based tubes, a yellowish clot containing a fibrin mesh is obtained, the red blood cell portion is disposed, and the yellowish clot is used. This clot occurs by the polymerization of fibrinogen present in the blood during the centrifugation process. The polymerization of fibrin is influenced by the composition and surface characteristics of the collection tubes. This fibrin mesh has a three-dimensional shape, with good strength and elasticity, which clinically facilitates its implantation during surgery[3] [Figure 2a and b].

Entrapped within polymerized fibrin, PRF contains leukocytes and platelets. These cells release growth factors and cytokines once implanted in the host surgical site. The growth factors and cytokines typically include transforming growth factor (TGF-β), insulin-like growth factor 1, platelet-derived growth factor, vascular endothelial growth factor (VEGF), fibroblastic growth factor, epidermal growth factor, and platelet-derived epidermal growth factor. These mediators are essential in modulating the inflammatory response and promoting wound healing. Given the repair processes, the leukocytes present in this fibrin mesh will also contribute to angiogenesis and lymphogenesis through the expression of cytokines.[11] Accordingly, the PRF matrix will serve as a drug delivery system for cells and promotes tissue recovery.[12]

Another protocol for the isolation of blood concentrates includes generation of liquid PRF.[13,14] This requires the application of a relatively low RCF for a shorter duration, prior to polymerization of fibrinogen within plastic-based tubes[15] [Figure 2c].

The formula for calculation of RCF requires information on the radius of the rotor and RPM according to the following formula:

$$RCF = 1.12 \times r \times (RPM/1000)^2$$

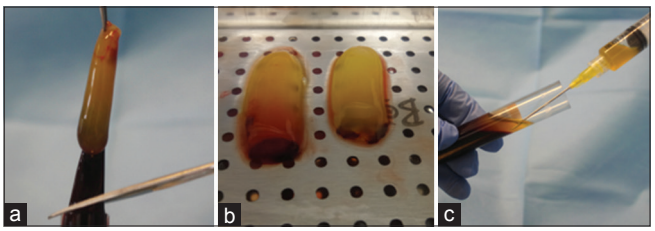


**Figure 2:** Preparation of platelet-rich-fibrin in clinical setting. (a) Platelet-rich-fibrin clot directly after centrifugation showing the interface between the fibrin clot and the red phase. (b) Separated platelet-rich-fibrin clots. (c) Liquid platelet-rich-fibrin collection after blood centrifugation

It is of paramount importance that scientific and clinical protocols communicated in a standard format that may be replicated by others. For this reason, the reporting of the L-PRF protocol may be considered inaccurate because it lists the centrifugation RCF at 400 × *g*.[3] However, the location of the rotor at which the RCF was measured was not reported.

Recently, some studies from our group presented an approach to standardize the terminology for PRF production by focusing on RCF obtained from minimum radius, clot radius, and maximum radius[16,17] [Figure 3]. It became important to identify the correct protocol for each centrifuge.[18,19] The maximum RCF is commonly used for the calibration of centrifuges to achieve a more reliable standardization. Using the average radius to calculate the RCF, i.e., the mean between the maximum and minimum, will make the measurement variable. The same is applicable when using the PRF clot as a reference for the radius,[18] as the position of the formed clot is not constant. This is due to the different angles and distances from the center of the rotors in different centrifuge brands. The location of the fibrin clot may also vary based on how much blood is contained in each tube. Therefore, if one uses the standard rotor location, i.e., of maximum RCF, the L-PRF® protocol may be considered to be produced by RCF of ~ 700 ×*g* and not ~ 400 ×*g*. A letter to the editor questioning these new findings on L-PRF[20] could be answered based on an editorial by our group, highlighting the importance of standardization of the PRF protocol.[18]

Accordingly, the maximum radius should be considered for the determination of the RCF value in blood concentrate production, using various centrifuges available on the market.

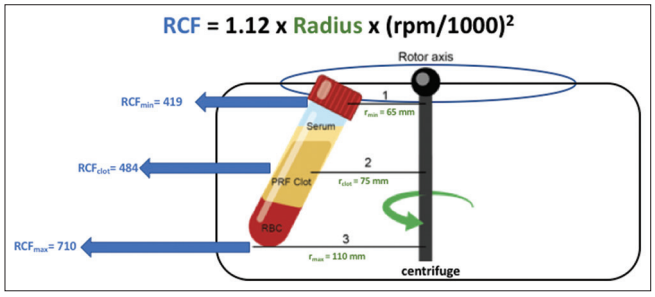


**Figure 3:** Schematic illustration of the blood centrifugation process shows the differences between the minimum radius (1), clot radius (2), and maximum radius (3). 1 <2 <3 and the different relative centrifugal force values. The maximum radius (3) has to be considered to calculate the relative centrifugal force for platelet-rich-fibrin production

Otherwise, the PRF production process would be different and the quality of the fibrin mesh, as well as the number of cells and growth factors, will not be comparable. Consequently, the gained scientific data obtained might be useless as they remain incomparable.

## Low-Speed Centrifugation Concept

In reviewing the literature over the past 15 years, there have been relatively few controlled clinical trials investigating the effect of PRF in dental surgery.[21] Moreover, the existing data have been obtained by different PRF protocols, using different tubes and centrifuges operated at different RCFs.[21] As a result, both health organizations and insurance companies in many countries have denied to accept the positive contribution of PRF as a blood concentrate beneficial for wound healing processes.

In an effort to improve the therapeutic outcomes, the low-speed centrifugation concepts (LSCC) have been developed.[13] The main principle of LSCC is a universal approach to obtain blood concentrates from 710 × *g* to 44 × *g* in different physical conditions, i.e., solid or liquid, and for different applications in medicine and dentistry. LSCC was established on a specific fixed angled centrifuge with a maximum radius of 110 mm (PRF for Process, Nice, France). A number of *in vitro* and *in vivo* studies have been conducted to establish this concept.[22-24] The effects of three different RCFs carried out for 8-min centrifugation on cells and growth factors within the same blood concentrate have been investigated. The reduction of RCF from 710 × *g* to 44 × *g* led within the same blood volume to an eightfold higher platelets and a sixfold higher leukocyte concentration, while TGF-β and VEGF experienced a sixfold and threefold increase, respectively [Table 1]. The same concept has been studied for 3-min centrifugation time.[25]

Therefore, the present evidence suggests that a reduction in the RCF from 700 × *g* to 44 × *g* can lead to properties that may be clinically beneficial. Knowing that systematic reduction of RCF will significantly increase the biological capacity of blood concentrates, increasing the number of leukocytes an platelets, as a consequence increasing the number of growth factors released, such as VEGF and TGF-β,[13] further ongoing clinical trials will show to what extent the two other lower RCF protocols of the LSCC, i.e., 177 × *g* and 44 × *g*, will also have a clinical benefit. Multiple clinical studies on the three LSCC protocols are ongoing and will answer this question.

**Table 1: Standardized protocols according to the low-speed centrifugation concept**

| Protocol | RPM | Time (min) | G-force (xg) | Cell composition (/μl) | Growth factors (pg/mL) |
|---|---|---|---|---|---|
| High RCF | 2400 | 8 | 710 | Leukocytes $<2.0\times10^3$<br>Platelets $<2.0\times10^5$ | TGF-β1 <2000<br>VEGF~0 |
| Medium RCF | 1200 | 8 | 177 | Leukocytes $<2.0\times10^3$<br>Platelets $>4.0\times10^5$ | TGF-β1 >2000<br>VEGF >10 |
| Low RCF | 600 | 8 | 44 | Leukocytes $>4.0\times10^3$<br>Platelets $>6.0\times10^5$ | TGF-β1 >4000<br>VEGF >20 |

RCF: Relative centrifugal force, TGF: Transforming growth factor, VEGF: Vascular endothelial growth factor, RPM: Revolutions per minute

## Conclusion

Currently, there are different PRF protocols in the literature for the production of PRF using centrifuges with different rotors. These protocols are based on the RPM value and not on the RCF, which is calculated out of the maximum rotor radius with a standardized mathematical formula. When the RCF is calculated at the maximum radius of the rotor, which is generally considered standard for centrifuge calibration, the RCF for the L-PRF® protocol is approximately 700 × *g* and not 400 × *g*, as previously reported. When looking at the PRF literature, there are many different protocols, so the existing data are not comparable. Recently, the LSCC was established to show a systematic reduction of RCF from 710 × *g* to 44 × *g*, which leads to a threefold to eightfold increase of cells and their growth factors within the same blood concentrates. LSCC provides three different protocols to the scientific community to produce liquid and solid PRF regardless of the centrifuge used. Ongoing controlled clinical studies are necessary to examine the clinical effects of modulating cells and growth factors by the PRF preparation protocol on wound healing.

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### Conflicts of interest

There are no conflicts of interest.

## References

1. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85:638-46.
2. Choukroun J, Adda F, Schoeffler C, Vervelle A. Une opportunité en paro-implantologie: Le PRF. Implantodontie 2001;42:e62.
3. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, *et al*. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part I: Technological concepts and evolution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:e37-44.
4. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, *et al*. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part III: Leucocyte activation: A new feature for platelet concentrates? Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:e51-5.
5. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, *et al*. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part V: Histologic evaluations of PRF effects on bone allograft maturation in sinus lift. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:299-303.
6. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, *et al*. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part IV: Clinical effects on tissue healing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:e56-60.
7. Lucarelli E, Beccheroni A, Donati D, Sangiorgi L, Cenacchi A, Del Vento AM, *et al*. Platelet-derived growth factors enhance proliferation of human stromal stem cells. Biomaterials 2003;24:3095-100.
8. Ghanaati S, Booms P, Orlowska A, Kubesch A, Lorenz J, Rutkowski J, *et al*. Advanced platelet-rich fibrin: A new concept for cell-based tissue engineering by means of inflammatory cells. J Oral Implantol 2014;40:679-89.
9. Dohan Ehrenfest DM, Pinto NR, Pereda A, Jiménez P, Corso MD, Kang BS, *et al*. The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors, and fibrin architecture of a leukocyte- and platelet-rich fibrin (L-PRF) clot and membrane. Platelets 2018;29:171-84.
10. Zhao QM, Gao J, Huang XX, Chen XP, Wang X. Concentrated growth factors extracted from blood plasma used to repair nasal septal mucosal defect after rhinoplasty. Aesthetic Plast Surg 2019: [Epub ahead of print].
11. Qiao J, An N, Ouyang X. Quantification of growth factors in different platelet concentrates. Platelets 2017;28:774-8.
12. Verboket R, Herrera-Vizcaíno C, Thorwart K, Booms P, Bellen M, Al-Maawi S, *et al*. Influence of concentration and preparation of platelet rich fibrin on human bone marrow mononuclear cells (*in vitro)*. Platelets 2019;30:861-70.
13. Choukroun J, Ghanaati S. Reduction of relative centrifugation force within injectable platelet-rich-fibrin (PRF) concentrates advances patients' own inflammatory cells, platelets and growth factors: The first introduction to the low speed centrifugation concept. Eur J Trauma Emerg Surg 2018;44:87-95.
14. Mourão CF, Valiense H, Melo ER, Mourão NB, Maia MD. Obtention of injectable platelets rich-fibrin (i-PRF) and its polymerization with bone graft: Technical note. Rev Col Bras Cir 2015;42:421-3.
15. Albilia JB, Herrera-Vizcaino C, Weisleder H, Choukroun J, Ghanaati S. Liquid platelet-rich fibrin injections as a treatment adjunct for painful temporomandibular joints: Preliminary results. Cranio 2018;20:1-13.
16. Miron R, Choukroun J, Ghanaati S. Controversies related to scientific report describing g-forces from studies on platelet-rich fibrin: Necessity for standardization of relative centrifugal force values. Int J Growth Factors Stem Cells Dent 2018;1:80-9.
17. Miron RJ, Choukroun J, Ghanaati S. Reply from authors: RE: Optimized platelet-rich fibrin with the low-speed concept: Growth factor release, biocompatibility, and cellular response: Necessity for standardization of relative centrifugal force values in studies on platelet-rich fibrin. J Periodontol 2019;90:122-5.
18. Miron RJ, Pinto NR, Quirynen M, Ghanaati S. Standardization of relative centrifugal forces in studies related to platelet-rich fibrin. J Periodontol 2019;90:817-20.
19. Miron RJ, Xu H, Chai J, Wang J, Zheng S, Feng M, *et al*. Comparison of platelet-rich fibrin (PRF) produced using 3 commercially available centrifuges at both high (~ 700 g) and low (~ 200 g) relative centrifugation forces. Clin Oral Investig 2019; [Epub ahead of print].
20. Pinto N, Quirynen M. Letter to the editor: RE: Optimized platelet-rich fibrin with the low-speed concept: Growth factor release, biocompatibility, and cellular response. J Periodontol 2019;90:119-21.
21. Ghanaati S, Herrera-Vizcaino C, Al-Maawi S, Lorenz J, Miron RJ, Nelson K, *et al*. Fifteen years of platelet rich fibrin in dentistry and oromaxillofacial surgery: How High is the level of scientific evidence? J Oral Implantol 2018;44:471-92.
22. El Bagdadi K, Kubesch A, Yu X, Al-Maawi S, Orlowska A, Dias A, *et al*. Reduction of relative centrifugal forces increases growth factor release within solid platelet-rich-fibrin (PRF)-based matrices: A proof of concept of LSCC (low speed centrifugation concept). Eur J Trauma Emerg Surg 2019;45:467-79.
23. Wend S, Kubesch A, Orlowska A, Al-Maawi S, Zender N, Dias A, *et al*. Reduction of the relative centrifugal force influences cell number and growth factor release within injectable PRF-based matrices. J Mater Sci Mater Med 2017;28:188.
24. Ghanaati S, Al-Maawi S, Herrera-Vizcaino C, Alves GG, Calasans-Maia MD, Sader R, *et al*. A proof of the low speed centrifugation concept in rodents: New perspectives for *in vivo* research. Tissue Eng Part C Methods 2018;24:659-70.
25. Kubesch A, Barbeck M, Al-Maawi S, Orlowska A, Booms PF, Sader RA, *et al*. A low-speed centrifugation concept leads to cell accumulation and vascularization of solid platelet-rich fibrin: An experimental study *in vivo*. Platelets 2019;30:329-40.