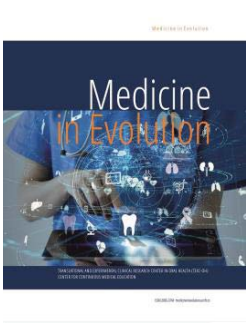
Comparison between the size of PRF clot obtained through horizontal centrifugation vs. fixed-angle centrifugation



Ardelean A.[1], Buzatu R.[1], Novac A. C.[1,2], Purv C.[1], Petrescu E. L.[1,2], Pop D. M.[1,2], Sinescu C.[1,2], Negruțiu M. L.[1,2], Leretter M.[1,3]

[1]Departement I, Faculty of Dental Medicine, University of Medicine and Pharmacy "Victor Babes", Timisoara
[2]Research Center in Dental Medicine Using Conventional and Alternative Technologies, Department of Prostheses Technology and Dental Materials, Faculty of Dental Medicine, "Victor Babes" University of Medicine and Pharmacy of Timisoara
[3]TADERP Research Center – Advanced and Digital Techniques for Endodontic, Restorative and Prosthetic Treatment, "Victor Babeş" University of Medicine and Pharmacy

Correspondence to:
Name: Buzatu Roxana
Address: Piata Eftimie Murgu 2, Timisoara, 300041
Phone: +40 721 236 147
E-mail address: drbuzaturoxana@gmail.com

Received: 19 July 2024; Accepted: 20 September 2024; Published: 30 September 2024

Abstract

Introduction: Over the years, centrifugation systems have undergone significant transformations, adapting in shape to facilitate their application in dental clinics and advancing in operational technology. While centrifuge designs may vary in numerous ways, one critical distinction between the centrifuge is the angle at which centrifugation takes place. **Aim of the Study:** The objective of this experimental study is to assess and measure the volume of the end product, specifically the PRF clot obtained using horizontal centrifugation and fixed-angle centrifugation to ascertain which method produces a larger quantity of PRF clot. **Material and Methods:** The study was conducted on a cohort of 6 male and female patients. Blood was drawn from each patient into two 10ml glass tubes without anticoagulants or additives, destined for centrifugation in the two centrifugation systems. The tubes were split into two sets of six tubes each, labeled with the letters H (for tubes to be centrifuged horizontally) and L (for tubes to be centrifuged at a fixed angle), along with numbers from 1 to 6 for each tube pair corresponding to a patient. **Results and Discussions:** It has been proven that horizontal centrifugation produces a bigger PRF clot. Considering the insights gained from this study alongside the ANOVA test results, we conclude that horizontal centrifugation using the Bio-PRF system results in a larger PRF clot compared to fixed-angle centrifugation. One possible explanation is the variance in g-force distribution between fixed-angle and horizontal centrifugation. This variance led to marginally smaller quantities of PRF clots in the second tube batch, albeit not significantly so. **Conclusions:** In conclusion, the appearance and methodology of the two types of PRF we obtained are highly similar but not identical, with disparities in PRF clot quantity and consequently, in cellular properties. Notably, H-PRF exhibited a greater clot quantity compared to L-PRF.

Keywords: PRF clot, fixed-angle centrifugation, horizontal centrifugation, L-PRF, H-PRF

INTRODUCTION

Platelet-Rich Fibrin (PRF) is a fibrin matrix in which platelet cytokines, growth factors, and cells are trapped and can be released over time, serving as a resorbable membrane. Choukroun and his associates were among the pioneers in using the PRF protocol in oral and maxillofacial surgery to enhance healing in dentistry. PRF is considered a healing biomaterial, and studies have shown its application in various dental disciplines [1][2].

The history of these products illustrates the evolving trends in research over the years. It began with an interest in fibrin matrix alone as a healing material, then shifted to the healing properties of platelets, and finally focused on growth factors (both circulating and from platelets) for tissue regeneration. Among all these elements, which can be considered the most important? Given our general understanding of coagulation and healing—and a certain degree of common sense—it is now considered that all these elements are important and should be properly combined to achieve the best clinical outcomes. Fibrin, platelets, growth factors with slow release, leukocytes, and other cells are the key players in the natural healing process, and together they form a kind of processed tissue derived from blood. This complex combination is key to optimal performance. For this reason, PRF has often been described as an "optimized blood clot" that can be surgically manipulated and used. This description is actually true (more or less) for all well-designed platelet concentrate products [3][4][5].

Platelet concentrate therapy was developed to naturally enhance the regenerative potential of platelets present in the blood. PRF is achieved by centrifuging blood into various components, including red blood cells, plasma, white blood cells, and platelets. The final PRF clot is a concentrate of white blood cells, platelets, and fibrin [6][7].

It is important to understand that inflammation and wound healing are regulated by a series of growth factors. These growth factors can stimulate or inhibit cellular migration, adhesion, proliferation, and differentiation. While growth factors are present in all tissues, it is important to note that blood serves as the primary reservoir for numerous growth factors and cytokines responsible for angiogenesis and tissue regeneration. Growth factors typically exist as inactive or partially active precursors that require proteolytic activation [8][9][10].

Over time, centrifugation systems have undergone numerous changes, both in terms of their shape to facilitate their use in dental clinics and in terms of their operating technology. The design differences between centrifuges can be numerous, but this aspect is not very important. One crucial aspect between the two types of centrifuges used in this experiment is the angle at which centrifugation occurs [11][12][13].

In horizontal centrifugation, as in the Bio-PRF centrifuge, the tube radius is larger, the RCF (Relative Centrifugal Force) is higher, and the forces are more efficient. Therefore, the time required for complete centrifugation is only 2/3 of the time required for centrifugation in the Duo Quattro Advanced PRF centrifuge by Choukroun, where the angle is fixed at 33 degrees and the tube radius is smaller [14][15].

Aim and objectives

The aim of the experimental study is to evaluate and quantify the amount of the final product, namely the PRF clot obtained through horizontal centrifugation (a newer technique) and fixed-angle centrifugation (a well-established technique in the market), in order to determine which method yields a greater quantity of PRF clot.

MATERIAL AND METHODS

The study was conducted on a cohort of 6 male and female patients who presented to a private clinic in Timişoara in the year 2021. Inclusion criteria for the study included ages between 25 and 30 years old, with patients not having chronic health issues. Blood was collected from each patient in two 10ml glass tubes without anticoagulant or additives, to be centrifuged in the two centrifugation systems. The tubes were divided into 2 sets of 6 tubes each, and each set was labeled with the letters H (for tubes to be centrifuged horizontally) and L (for tubes to be centrifuged at a fixed angle), along with numbers from 1 to 6 for each pair of tubes corresponding to a patient.

For the preparation of H-PRF, blood was collected from each donor in 10 ml glass tubes without additives and anticoagulant, and these were centrifuged at 700 RCF for 8 minutes in a centrifuge where centrifugation is performed horizontally (Bio-PRF system).

The time required for horizontal centrifugation is 2/3 of that for fixed-angle centrifugation. Therefore, the 12-minute protocol used in this study on a fixed-angle centrifuge perfectly equates to an 8-minute protocol used on a horizontal centrifuge, both set at 700g.

For the preparation of L-PRF, blood was collected from each donor in 10 ml glass tubes without additives and anticoagulant, and these were centrifuged at 2700 RPM for 12 minutes in a fixed-angle centrifuge (Duo Quattro Advanced PRF by Choukroun).

After centrifugation, the tubes were placed in a rack with a linear attachment to measure the size of the PRF clots. This allowed us to compare the quantity of PRF clots obtained through the two centrifugation techniques.

Figure 1. The tubes to be centrifuged horizontally are labeled with the letter H and numbers from 1 to 6, while their counterparts are found in the batch of tubes marked with L

RESULTS

To analyze the results obtained through the two techniques, after centrifuging the blood tubes, we photographed all the tubes. Using a ruler attached to the metal stand, we measured each PRF clot in centimeters.

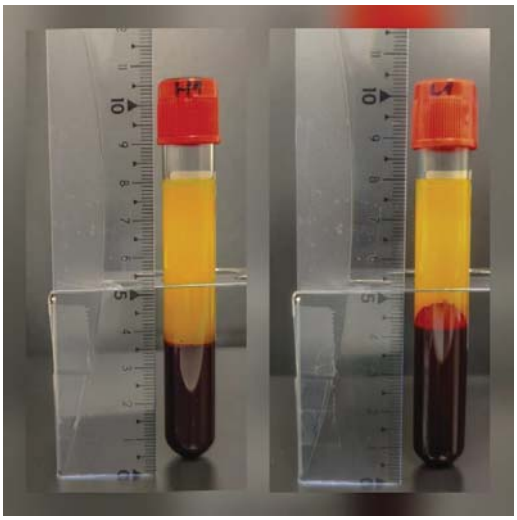


Figure 2. Comparative measurement of the two clots

To better observe and compare the results obtained, we created three charts. In Chart 1, we displayed the measurements in centimeters for each test tube from the two batches. At first glance, the graphical representation shows the difference between the H tubes (horizontally centrifuged) shown in blue and the L tubes (vertically centrifuged) shown in red.

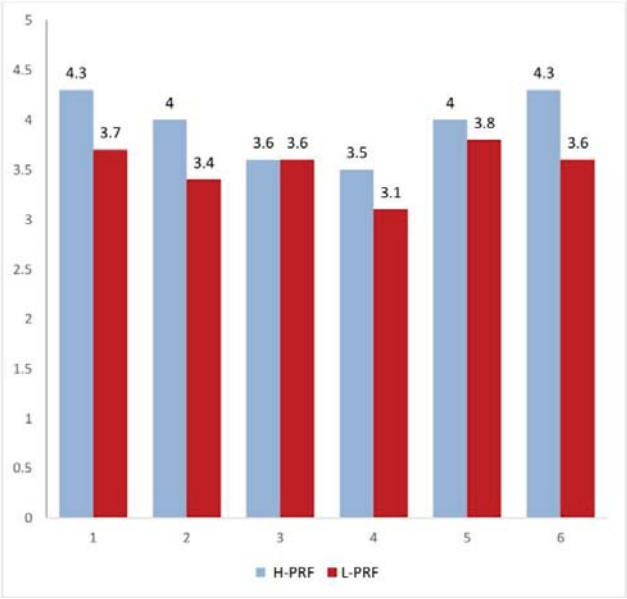


Chart 1. Quantity of PRF clots measured in centimeters

In Chart 2, we displayed the average measurements in centimeters: the H tubes (horizontally centrifuged) are shown in blue, and the L tubes (vertically centrifuged) are shown in red.

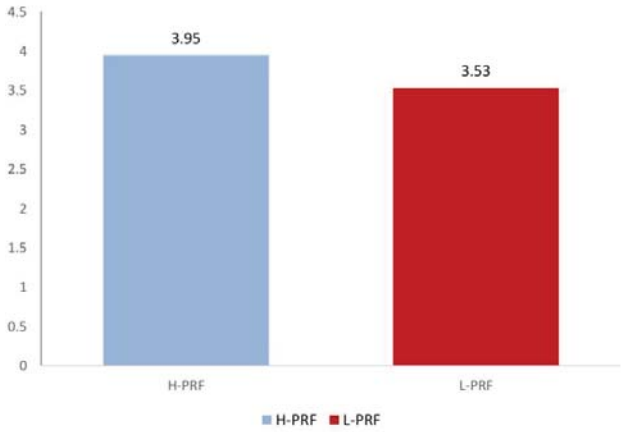


Chart 2. Average clots of H-PRF vs. L-PRF in centimeters

In Chart 3, we displayed the difference in centimeters between the clots of H-PRF and L-PRF for each pair of test tubes.

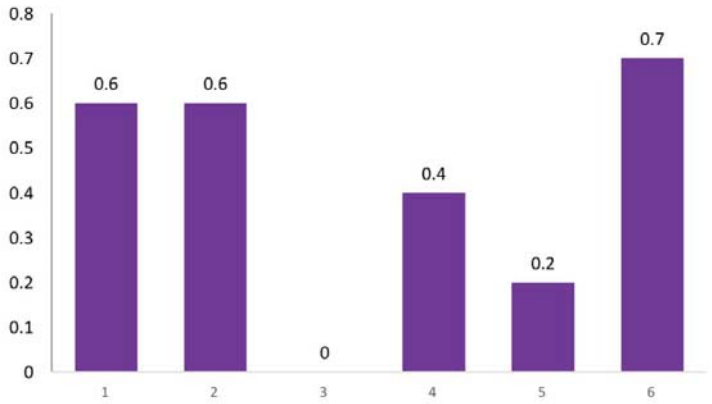


Chart 3. Difference between H-PRF and L-PRF clots for each pair of tubes, measured in centimeters

From Table 1, the following observations can be made: the smallest values belong to the L-PRF tubes, while the largest values belong to the H-PRF tubes.

Table 1. ANOVA test results for PRF clot measurements

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
H PRF	6	23.7	3.95	0.115		
L PRF	6	21.2	3.533333	0.062667		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.520833	1	0.520833	5.863039	0.035977	4.964603
Within Groups	0.888333	10	0.088833			
Total	1.409167	11				

Thus, it has been demonstrated that horizontal centrifugation produces a larger PRF clot. Combining the experience gained from this experiment with the data obtained from the ANOVA test in Table 1, we can state that horizontal centrifugation using the Bio-PRF system results in a larger PRF clot compared to a PRF clot obtained through fixed-angle centrifugation.

DISCUSSIONS

In the experimental study we conducted, we analyzed the amount of PRF clot obtained based on the centrifugation system used. The Choukroun Duo Quattro Advanced PRF system has stood the test of time, and the benefits of PRF obtained with this system are indisputable. However, as technology evolves over the years, new approaches emerge offering additional benefits and advantages. The Bio PRF system promises these new benefits and advantages through horizontal centrifugation.

Choosing the best centrifugation system to obtain the optimal PRF clot can be challenging and requires extensive knowledge in this field. Since the Bio-PRF system is relatively new, there are not many studies on this system that uses horizontal centrifugation, making it difficult for clinicians to choose the best system currently available on the market. To assist clinicians in making an informed decision, we decided to conduct this study. Based on our findings, we can state the following [16].

By measuring the PRF clots, we demonstrated that the clots obtained with the Bio PRF system through horizontal centrifugation have a larger volume and better cellular properties compared to those obtained with the Choukroun Duo Quattro Advanced PRF system using fixed-angle centrifugation. Since the Bio PRF system is relatively new to the market, there are not many studies that clarify this precisely. One explanation is that the distribution of g-forces differs between fixed-angle and horizontal centrifugation, as explained in more detail by Richard J. Miron in his book. This difference resulted in slightly smaller PRF clot quantities from the second batch of tubes, but not significantly.

We emphasize that the statements about the superior cellular properties of H-PRF clots from batch 1 are based on recent studies by Masako Fujioka-Kobayashi, Michihide Kono, and Richard J. Miron, without us conducting these histological and microscopic studies in this paper. [17]

This study highlights the quantitative difference between PRF obtained using a fixed-angle centrifugation system and PRF obtained using a horizontal centrifugation system. By correlating the amounts of PRF clots, we can assert that PRF clots obtained through horizontal centrifugation provide clinicians with a larger and higher-quality PRF membrane in terms of cellular properties, resulting in a significantly improved treatment. The advantages of this technique are not only related to the clot but also to shorter working times, which enhance treatment efficiency. Reducing working times is always advantageous for clinicians as it saves time, allowing for the treatment of more patients and offering them shorter waiting times.

The results of this study align with our expectations based on an in-depth review of the specialized literature.

The Choukroun Duo Quattro Advanced PRF system, which has been long established in the market, may lose ground to these new horizontal centrifugation systems due to their technique yielding a superior final product in all respects. [18][19]

In conclusion, while the Choukroun Duo Quattro Advanced PRF system has successfully stood the test of time and its benefits cannot be contested, the advantages offered by the Bio-PRF system through horizontal centrifugation cannot be overlooked. This system can be considered essential for current clinical situations. [20][21]

CONCLUSIONS

In conclusion, the appearance and technique of the two types of PRF we obtained are very similar but not identical, with a difference in the quantity of the PRF clot and, consequently, in the cellular properties. H-PRF was found to have a larger clot quantity than L-PRF. The specialized literature lacks sufficient data on this aspect; however, a study by Fujioka-Kobayashi and collaborators in 2020 demonstrated that H-PRF obtained through horizontal centrifugation has better separation and a larger PRF clot quantity, as well as better cellular properties compared to PRF obtained through fixed-angle centrifugation. These properties need to be studied more deeply, and a comparison between horizontal and fixed-angle centrifugation in terms of both clot quantity and cellular properties would bring many advantages to clinical dental practice.

REFERENCES

1. Naik B, Karunakar P, Jayadev M, et al. Role of Platelet rich fibrin in wound healing: A critical review. Journal of conservative dentistry. 2013 August; 16(4): p. 284-293.
2. Dohan Ehrenfest David M, Isabel Andia, Matthias A. Zumstein et al. Classification of platelet concentrates (Platelet-Rich Plasma-PRP, Platelet-Rich Fibrin-PRF) for topical and infiltrative use in orthopedic and sports medicine: current consensus, clinical implications and perspectives. Muscles, ligaments and tendons journal. 2014 May; 4(1): p. 3-9.
3. Kawase T, Tanaka T. An updated proposal for terminology and classification of platelet-rich fibrin. Regenerative Therapy. 2017 June; 7: p. 80-81.
4. Kawase T. Platelet-rich plasma and its derivatives as promising bioactive materials for regenerative medicine: basic principles and concepts underlying recent advances. Odontology. 2015 May 16; 103(2): p. 126-135.
5. Peck M, Hiss D, Stephen L. Factors affecting the preparation. South African Dental Journal. 2016 August; 71(7): p. 298-302.
6. Isobe K, Watanebe T, Kawabata H, et al. Mechanical and degradation properties of advanced platelet-rich fibrin (A-PRF), concentrated growth factors (CGF), and platelet-poor plasma-derived fibrin (PPTF). International Journal of Implant Dentistry. 2017 December; 3(1): p. 2-6.
7. Richard J. Miron. Understanding Platelet Rich Fibrin. 1st ed. Huffman L, editor. USA: Quintessence Publishing USA; 2021 Cap. 1: 1-4.
8. Miron RJ, Bosshardt DD. OsteoMasc: Key players around bone biomaterials. Biomaterials. Biomaterials. 2016; 82(1-19).
9. Tsirogianni AK, Moutsopoulos NM, Moutsopoulos HM. Wound healing: Immunological aspects. Injury; 2016 Cap. 37: S5-S12.
10. Davis VL, Abukabda AB, Radio NM et al. Platelet-rich preparations to improve healing. Part I: Workable options for every size practice. J. Oral Implantology. 2014; 40(4): p. 500-510.
11. Davis VL, Abukabda AB, Radio NM et. al. Platelet-rich preparations to improve healing. Part II: Platelet activatios and enrichment, leukocyte inclusion, and other selection criteria. J. Oral Implantology. 2014; 40(4): p. 511-521.
12. Kulkarni MR, Thomas BS, Varghese JM, et al. Platelet-rich fibrin as an adjunct to palatal wound healing after harvesting a free gingival graft: A case series. Journal of Indian Socienty of Periodontology. 2014 May; 18(3): p. 399-404.
13. Borie E, Oliví DG, Orsi IA, et al. Platelet-rich fibrin application in dentistry: a literature review. International journal of clinical and experimental medicine. 2015 August; 8(5).

14. Richard J. Miron, Joseph Choukroun. Platelet Rich Fibrin In Regenerative Dentistry: biological background and clinical indications. 1st ed. Richard J. Miron JC, editor.: Wiley Blackwell; 2017, Cap.2: 16-20.
15. Ghanaati S, Booms P, Orlowska A, et. al. Advanced platelet-rich fibrin: a new concept for cell-based tissue engineering by means of inflammatory cells. The Journal of oral implantology. 2014; 40(6): p. 679-689.
16. Kobayashi E, Fluckiger L, Fujioka-Kobayashi M, et al. Comparative release of growth factors from PRP, PRF, and advanced-PRF. Clinical Oral Investigation. 2016 January 11; 20(9): p. 2353-2360.
17. Masako Fujioka-Kobayashi, Richard J. Miron, Maria Hernandez, et al. Optimized Platelet-Rich Fibrin With the Low-Speed Concept: Growth Factor Release, Biocompatibility, and Cellular Response. Journal of periodontology. 2017; 88(1): p. 112-121.
18. Effect of centrifugation time on growth factor and MMP release of an experimental platelet- rich fibrin-type product. Platelets. 2016 February 1; 27(5): p. 427-432.
19. Masako Fujioka-Kobayashi, Michihide Kono, Hiroki Katagiri, et al. Histological comparison of Platelet rich fibrin clots prepared by fixed-angle versus horizontal centrifugation. Platelets. 2020 April 18; p. 1-7.
20. Richard J. Miron. Understanding Platelet-Rich Fibrin. 1st ed. Huffman L, editor.: Quintessence Publishing USA; 2021 Cap. 3: 51-70.
21. Richard J. Miron. Understanding Platelet-Rich Fibrin. 1st ed. Huffman L, editor.: Quintessence Publishing USA; 2021 Cap. 4: 71-81.
22. Olariu I., Buzatu R., Azar R.R., Luca M., Buzatu B.L.R., Azar I., Azar E.R., Ardelean V.A., Leretter M. PRF in modern dentistry: An innovative approach to oro-dental tissue regeneration, Medicine in Evolution Volume XXIX, No. 3, 2023