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ORIGINAL ARTICLE

Evaluation of Platelet Rich Fibrin Obtained Using Different Centrifugation Parameters as a Tool for Regenerative Medicine

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SUMMARY

Background: **Platelet-rich fibrin (PRF) is a biomaterial widely used in the field of regenerative medicine. The purpose of this work was to analyze the structure and biomolecular characteristics of PRF through nine centrifugation parameters (CP) for its preparation, using a pool of blood samples of five volunteers.**
Methods: **The PRF obtained was analyzed by morphological and histological characteristics, as well as electronic and atomic force microscopy and growth factors determinations.**
Results: **A longer time of centrifugation showed taller clots and denser mesh fibrin in comparison with a short time ($p < 0.05$). The protocols with higher speed of centrifugation showed higher levels of PDGF-BB and VEGF. Higher levels of TGFβ1 were found in protocols with a shorter centrifuge time. The mean platelet count (916.05 ± 23.73 cells x 10^3 cells x cm^3) and its roughness (Ra) (616.5 ± 45.2 nm) did not show significant differences between different CP ($p > 0.05$). A significant correlation between fibrin density and levels of PDGF ($r = 0.57$) and VEGF ($r = 0.52$) was found. Additionally, the size of the clot had a significant correlation ($r = -0.47$) with TGFβ1 levels.**
Conclusions: **Different centrifugation parameters to obtain PRF have been reported. These results indicate that changes in the conditions to obtain PRF have a significant impact on their fibrin structure, cellular distribution, and biomolecular content, which can be decisive for its choice in the different clinical applications to be used. It is necessary to use a standardized centrifuge and protocol to guarantee high-quality PRF and clinical outcomes with less variability.**
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INTRODUCTION

Platelet concentrates are autologous biomaterials, blood-derived, used to improve wound healing and enhance tissue regeneration [1]. Platelet rich fibrin (PRF) is a second-generation platelet concentrate, free of anticoagulants and activators, which is obtained by a single centrifugation process and contains a high concentration of platelets and leukocytes. It can deliver platelet-derived growth factors, cytokines, and other biomolecules

useful in the tissue repair and/or regeneration process [2]. PRF has been widely used in different fields of the medicine and dentistry such as plastic and reconstructive surgery, repair of bone and cartilage defects, and oral and periodontal surgery, among others [3,4].
The preparation of the platelet concentrates is based on the principle of the centrifugation process to separate particles of different densities. The radial force generated by the rotor of the centrifuge is known as relative centrifugal force (RCF or g force), which is usually different in each machine and is determined by different centrifugation parameters (CP). The time (minutes) and speed (rpm) are adjustable CP of the centrifuge, which affects the quality of PRF [5,6]. Since the original PRF is an open access protocol, several modifications have been made to the CP for its preparation, resulting in variability of clinical outcomes [7].
The existence of both different protocols of CP and different commercial centrifuges tends to generate confusion in clinicians and investigators when using it in different applications, and it may lead to variability in the clinical results obtained. Therefore, differences of the equipment and CP used might generate alterations in the PRF quality [8,9]. Consequently, knowing about the modification of the structure and composition of the platelet concentrate after its preparation is important to determine its use in the best clinical application. The aim of this study was to analyze the macroscopic, microscopic, ultrastructural, and biomolecular characteristics of PRF, using nine different centrifugation parameters for preparation.

MATERIALS AND METHODS

The subjects enrolled in this research responded voluntarily to an informed consent form previously approved by the Nariño Ethics Committee of the Universidad Antonio Nariño (code: 06252018), which is in accordance with the Helsinki Declaration.
Nine blood samples were taken from five healthy volunteers aged between 18 and 40 years (mean ± SD; 19 ± 9.54), no history of blood disorders or medication use that could affect platelet or bone marrow function for a minimum of two months. At the beginning of the study, a complete blood test was performed for each volunteer. Ninety milliliters of blood from each volunteer were taken by the Vacutainer® system (Becton Dickinson, NJ, USA). Then, the blood was pooled, mixed, and subsequently transferred to each centrifuge (SCILogex® DMO 412, SC, USA). The centrifugation was performed through nine experimental protocols, using three different centrifugation speeds: 2,700, 3,000, and 3,200 rpm or (~326, ~404, and ~455 g, respectively) and three distinct centrifugation times: 8, 10, and 12 minutes (Table 1). Finally, five tubes were obtained per protocol (n = 5). Then, one minute after each centrifugation protocol, the PRF clot was removed from the tube and the red blood cells were separated. Later, to obtain PRF membranes, each clot was treated on the PRF box allowing for constant compression force for five minutes [10]. The membranes, thus obtained, were collected and sectioned into four longitudinal sections and randomly distributed for histological analyses, scanning electron microscopy (SEM), atomic force microscopy (AFM), and growth factors determination.
Macroscopic images were analyzed using *Image J* software (National Institutes of Health, MD, USA). For microscopic analyses, the membranes were fixed and dehydrated. Next, for the histological analysis, the samples were imbedded in paraffin and cut into 4 µm sections. They were made longitudinally (along the clot axis) and then stained with Masson's trichrome stain [11]. After that, the membranes were observed under an optical microscope (Leica DM300, Wetzlar, Germany) at 40x and 100x magnification, and microscopic images were obtained.
The platelet count was performed with a manual technique by counting ten fields of vision. *Image J* software was used to quantify the percentage of the relative optical density (OD%) of the fibrin density. Five microscopic images (100x) of each protocol were analyzed.
A section of each sample was examined by scanning electron microscopy (FEI, Quanta 200 -r, USA). Photographs were taken with 15 - 20 Kv using magnifications of 200x, 500x, 2,000x, 6,000x, and 11,000x.
A section of the PRF (2 x 2 x 0.5 cm) was analyzed with atomic force microscopy (Asylum MFP-3D-BIO, Asylum Research, CA, USA) in the dynamic mode liquid medium (platelet poor plasma) and at environment at laboratory conditions (~21°C). Topographical images were captured (Olympus TR800PSA, NY, USA) using pyramidal silicon nitride AFM probes with a nominal resonant frequency ω~24 kHz, nominal spring constant k~0.15 N/m, and a nominal probe tip end radius R_{TIP}~15 nm. Then, images and data processing, qq and analysis were performed using *ARgyle Light* software (Oxford Instruments®, USA). The roughness average values (*Ra*) [12] were computed on five different 3D-images of the same fiber.
For the growth factors (GF) determination, the membranes were squeezed to collect plasma samples, which were frozen at -80°C until their use for the determination of GF (150 µL). Enzyme-linked immunosorbent assays (ELISA) were used for the quantification of the GF, platelet-derived growth factor, PDGF-BB, and the transforming growth factor betha-1, TGFβ1 (Quantikine® ELISA R&D Systems, Minneapolis, MN, USA - DBB00 and DB100B, respectively), the vascular endothelial growth factor, VEGF, (KHGO111, Invitrogen®, Thermo Fisher Scientific, MA, USA), according to the manufacturer’s instructions.

Statistics

The variables were described with mean and standard deviation. ANOVA and a post-hoc Tukey test were performed to determine the significant differences between

CP groups ($p < 0.05$). Pearson's correlation was performed between macroscopic, microscopic, and molecular variables ($p < 0.05$). For statistical analyses, SPSS statistics software (IBM v. 21) was used.

RESULTS

Macroscopic analysis

All PRF membranes studied macroscopically presented a morphological pattern characterized by a red blood cell zone (RBC), a leukocyte zone, and a supernatant, or poor-platelet plasma (PPP). However, they had different sizes and proportions (Figure 1a - i). The eight-minute protocols (Figure 1a, j; b, k; c, l) produced less dense and smaller clots with a greater amount of exudate compared with the twelve-minute protocols (Figure 1g, p; h, q; i, r). The ten-minute protocols (Figure 1d, m; e, n; f, o) produced the largest size clots on average (Table 1). Protocol number 6 produced PRF with a greater extension. Finally, the clot was denser and thicker was the seventh protocol (Figure 1).

Histological analysis

Histologically, differences were also found among the nine protocols analyzed. The low-speed protocols showed that the cellular content was variable. In the protocols of 2,700 rpm (Figure 1j, m, p), platelets and leukocytes were distributed throughout the membrane of PRF. Further, some other cell types such as erythrocytes and polymorphonuclear cells were found in the basal zone of the clot. In the 3,000 rpm protocols, a uniform distribution of platelets was also shown, but without evidence of other types of cells (Figure 1k, n, q). In protocols of 3,200 rpm (Figure 2c, f, i), most platelets were concentrated near the RBC zone of each membrane- a zone known as "Buffy Coat" (45% near the RBC zone, 30% leukocyte layer, 25% near the acellular plasma zone).

The platelet count average was $916.05 \pm 23.73 \times 10^3$ cells x cm^3. Statistically significant differences between protocols were not found ($p > 0.05$) (Figure 1t). The fibrin mesh was denser in the protocols where more centrifugation time was employed. The twelve-minute protocols (Figure 1q, r) showed a percentage of fibrin density significantly higher ($p < 0.05$) in comparison with eight and ten-minute protocols. The time and speed affected the density of the fibrin mesh, but time was more relevant (Table 1).

Scanning Electron Microscopy (SEM) evaluation

The SEM analysis confirmed that the time and speed of the experimental protocols affects different characteristics in the fibrin surface, as well as the leucocyte and platelet distribution. In longer time protocols (protocols 4, 5, 6, 7, 8 and 9; Figure 2d, e, f, g, h, i), a well-distributed and polymerized coarse fibrin matrix with normal cellular content was observed. However, protocols 5 and 6 (Figure 2e, f) showed a higher cellular concentration towards the center of the membrane than the other protocols. In shorter time protocols (Figure 2a, b, c), the fibrin layout seems less dense, irregular, and thinner and some fibrin fibrils were incompletely formed. The platelet content showed facets of cell activation and inactivation and was evenly distributed (Figure 2k). Even so, cellular damage would be difficult to ascertain through this technique.

Sample characterization by Atomic Force Microscopy (AFM)

The dynamical mode image of the fibrin surface is shown in Figure 2j. A maximum 256 x 256 pixel sample size and 2 Hz scan rate were used. The microstructure of the PRF sample, as revealed through AFM (Figure 2j), showed a rough surface morphology with no evident periodic or directional trends. However, AFM images reveal a distinct layer of fibrin on the surface. The topographical analysis showed that tubular fibrin has an average diameter of 3.8 ± 0.43 µm. Furthermore, the fibrin average roughness (*Ra*) was computed to be 616.5 ± 45.2 nm for a section of 20 x 20 µm. The differences between the points analyzed do not present significant statistical differences ($p = 0.732$).

Determination of growth factors

There were also differences in the concentrations of the growth factors (GF) analyzed. Protocols 1, 6, and 9 showed the highest GF concentrations, but most differences were not significant between the other protocols ($p > 0.05$) (Table 1, Figure 2l). Finally, we found a positive significant correlation between fibrin density and levels of PDGF ($r = 0.57$) and VEGF ($r = 0.52$). The size of the clot has a significant correlation ($r = -0.47$) with TGFβ1 levels.

DISCUSSION

Platelet rich fibrin (PRF) is a blood-derived biomaterial with multiple clinical uses and has applications in medicine and dentistry, particularly in surgery. It is obtained from whole blood by centrifugation, but since it is an open protocol, there are reports of many variations in centrifugation parameters (speed and time). These variations may affect the clinical results because of the structural variations and composition of PRF. Consequently, this could affect the biomechanical and biofunctional properties of PRF. The objective of this study was analyzing the macroscopic, microscopic, and ultrastructural characteristics, as well as the growth factor contents after preparation of PRF using nine different centrifugation parameters.

We evaluated nine different protocols (with time and speed variations) for obtaining PRF, like the study of Magalon et al. They evaluated various preparation systems of platelet rich plasma (PRP) and found biological differences [13]. Thus, the changes in the centrifugation parameters may affect the concentration of molecules

Table 1. Size, platelet count, fibrin density, expression of PDGF, TGFβ1, and VEGF growth factors of nine protocols with different centrifugation parameters.

Protocol (min x rpm)	Clot size mm (SD)	Platelet 10^3 x cm^3 (SD)	Fibrin %RD (SD)	PDGF pg/mL (SD)	TGFβ1 pg/mL (SD)	VEGF pg/mL (SD)
1 (8 x 2,700)	43.7 (1.2)	884.9 (22.8)	61.9 (4.9)	6.54 (0.03)	14.23 (1.63)	0.54 (0.01)
2 (8 x 3,000)	52.7 (1.6)	890.2 (16.5)	63.7 (2.7)	7.83 (0.27)	14.02 (0.31)	0.56 (0.02)
3 (8 x 3,200)	45.2 (3.4)	915.8 (70.1)	68.2 (2.3)	8.37 (0.74)	15.06 (1.99)	0.57 (0.04)
4 (10 x 2,700)	63.4 (1.2)	905.3 (75.4)	68.5 (2.7)	6.83 (0.09)	13.01 (0.31)	0.54 (0.00)
5 (10 x 3,000)	71.1 (2.2)	930.5 (64.5)	81.5 (1.4)	8.39 (0.59)	13.51 (0.20)	0.56 (0.02)
6 (10 x 3,200)	71.2 (2.3)	908.5 (52.3)	79.3 (3.6)	8.98 (0.35)	13.44 (0.51)	0.57 (0.00)
7 (12 x 2,700)	69.3 (0.9)	963.1 (24.5)	65.5 (2.0)	6.67 (0.15)	13.37 (0.20)	0.54 (0.01)
8 (12 x 3,000)	55.3 (2.1)	932.3 (61.1)	86.5 (4.3)	8.29 (0.09)	13.08 (0.82)	0.56 (0.04)
9 (12 x 3,200)	51.3 (6.3)	914.1 (73.3)	89.7 (2.6)	9.21 (0.38)	16.13 (0.18)	0.56 (0.01)

Data are presented as Mean Value and Standard Deviation (SD), Relative Density (RD).

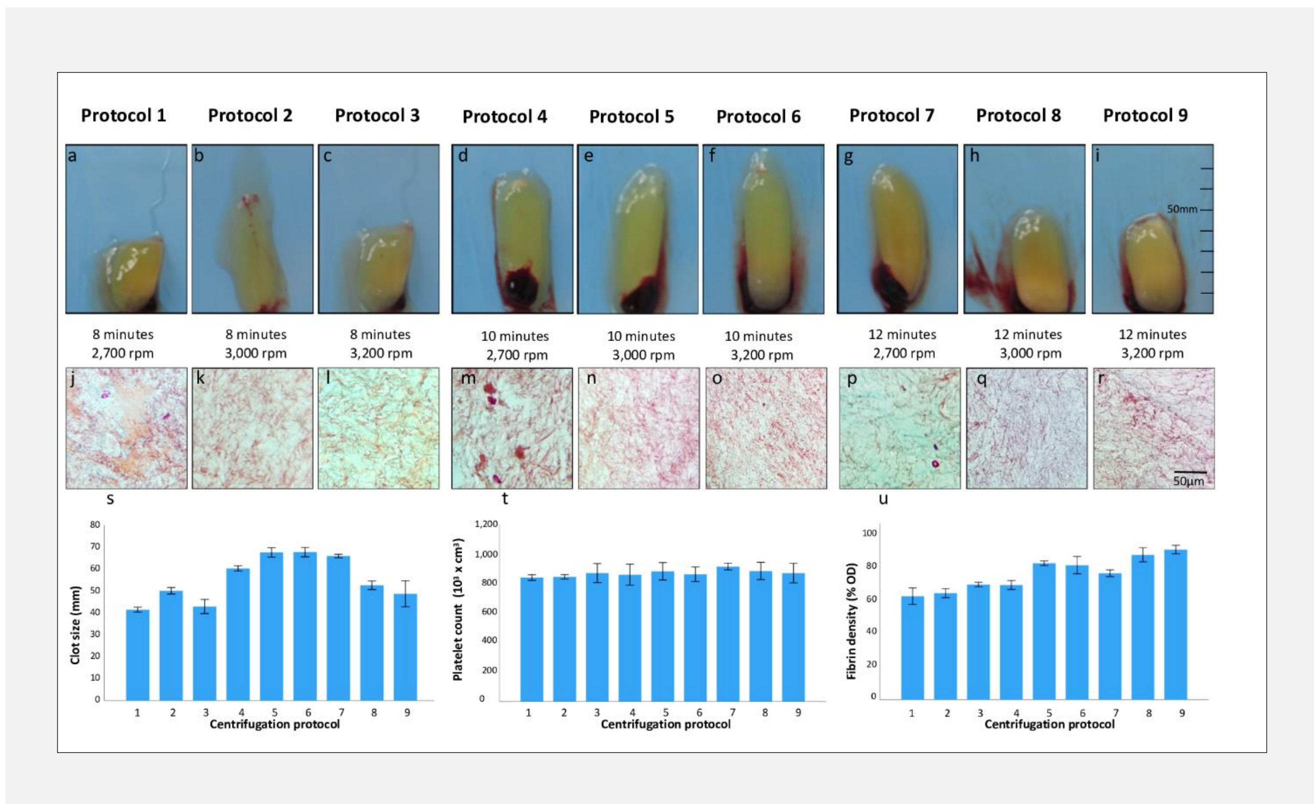

Figure 1. Macroscopic (a - i) and histological (j - r) analysis of nine PRF centrifugation protocols (Masson staining. 100 x). Quantification of: (s) size of clot fibrin, (t) platelet number, and (u) percentage of fibrin density.

such as growth factors, cytokines, and others. However, the cell and molecular content also may have variability associated with the donor [14]. To avoid this bias and increase the reproducibility, we used a similar methodology that Oh et al. [15] and Leitner et al., who evaluated different systems in order to obtain PRP from blood samples from three donors [16]. We also mixed the blood samples from five volunteers to obtain a pool before the centrifugation process of each protocol.

The present study shows that speed and time are associated with platelet distribution, which is in turn related to the fibrin network structure and concentrations of the

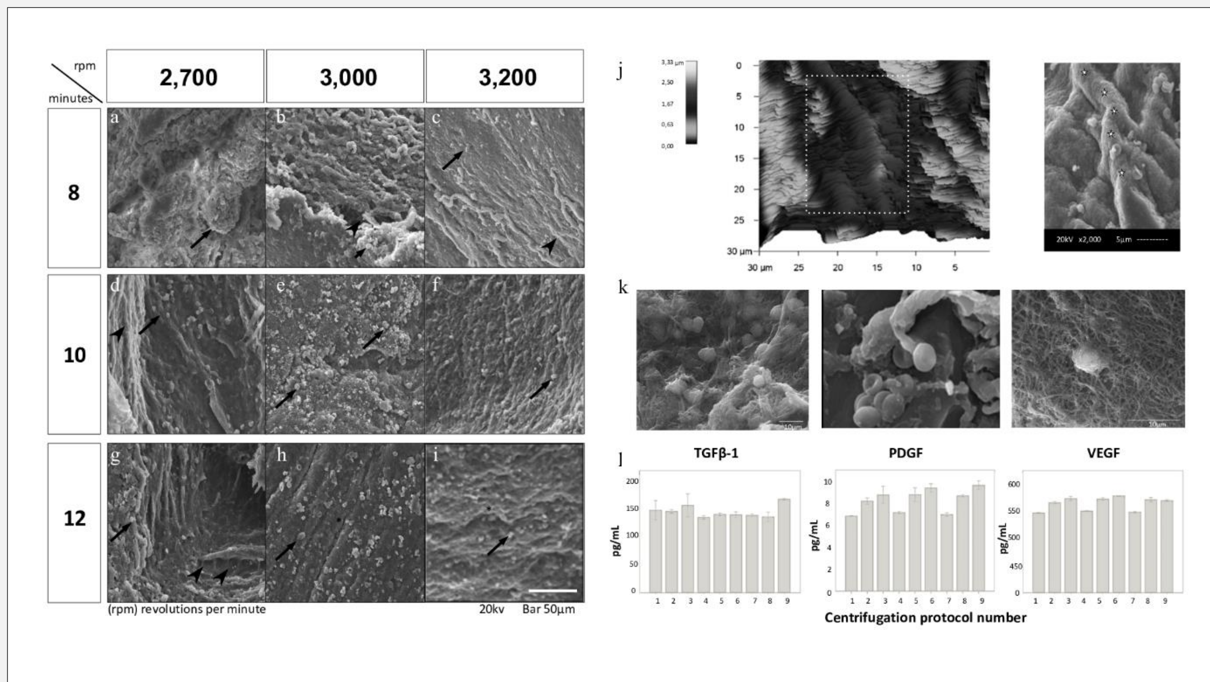

Figure 2. SEM analysis of nine PRF centrifugation protocols (a - i). Black arrows show platelets, and the asterisks indicate polymerized fibrin. Arrowheads show fibrils of fibrin, (500 x 20 kV). (j) left, 3D-AFM rendering image with Argyle software; right, microphotography in SEM (2,000 x 20 kV), the stars show the measured points. k) PRF image of SEM 1,000 x (left), inactive platelets (middle), and an active platelet (right). (l) concentrations of TGFβ1, PDGF, and VEGF in nine different PRF preparation protocols.

growth factors. Similarly, Dohan et al. evaluated four different centrifuges and different PRF centrifugation parameters. They concluded that both the equipment and the centrifugation parameters have an impact on cell integrity, the release of growth factors, and the level of fibrin polymerization of the clot and membrane [17]. Our study confirms that the centrifugation parameters are an important aspect associated with the quality of PRF. Thus, it is necessary to establish a better protocol for each centrifuge and for each clinical application, or to use specialized equipment to obtain PRF with the pre-established protocols.

Our results do not show a significant difference between the platelets counted after the different PRF preparation protocols. The mean obtained ($916.05 \pm 23.73 \times 10^3$ cells x cm^3) was similar to the reports for other platelet concentrates [16,18,19]. However, changes in morphology and platelet activation were found in some of the protocols analyzed here. It is difficult to consider structural damage without testing different variables that allow the evaluation of the viability of the cells studied such as rate of cell division, metabolic activity or DNA integrity [20]. Most of studies of PRF determine the number of platelets in the concentrates, but analyses of cell viability and DNA integrity usually have not been performed.

In general, we found that the speed affects the cell distribution. In protocols with higher speed (3,200 rpm), the distribution of platelets and leukocytes was concentrated adjacent to the red corpuscular zone of the membrane. Furthermore, in protocols with lower speed (2,700 rpm), the cell concentration was uniformly distributed in circular patterns along the clot. The platelet content and their distribution through the membrane guarantees the availability of cytokines and chemokines, in such a way as to favor the patient immunological processes after the corresponding surgical technique has been performed [21], for this a homogenous platelet distribution may be involved in a higher biological activity of the membranes and therefore have better clinical results [22,23].

The decrease in RCF may favor leukocyte flow to the bottom of the PRF, allowing a high percentage of cells to be collected inside the plasma acellular zone; this is then used clinically [24]. This is important because the cells involved in guided tissue regeneration (platelets, leukocytes, among others) have the ability to secrete angiogenic and lymphogenic factors responsible for cross-

talk in regenerative processes. Our study emphasizes the influence of the reduction of the RCF demonstrating a more equitable cellular distribution in the experimental membranes used (Figure 2b, c, h). Therefore, the RCF is a useful tool for the control of the cellular distribution according to the specific needs of individual patients with respect to the required clinical therapeutics [25]. The topographic analysis of platelet rich fibrin showed that the roughness is stable and does not have variability in the assessed points. This condition is important because the material may be applied to coat other materials or scaffolds, and in some cases, can be used to generate a surface with more roughness. The polymerized fibrin is a useful and modifiable mesh that may improve the properties of the other materials [26] or can be used as a molecular carrier [27]. To understand the PRF´s physical and mechanical properties more studies are necessary.

We observed differences in the GF levels. The results show that time does not significantly affect it, but speed does. The protocols of 3,200 rpm showed higher concentrations of growth factors; this could be because the g force affects the platelet concentration and could also affect the secretion of these molecules. Our results are similar to another study of PRF growth factor determination in which higher values were obtained with the protocol 3,200 rpm x 12 minutes [28]. Nishimoto et al. performed quantification of growth factors, but their methodology was different, and their results are not comparable with ours. Although, similar to the present report, the tendency was that the TGFβ1 levels are highest in comparison with PDGF [29]. Similar patterns of GF concentration (TGFβ1 > PDGF > VEGF) were reported in a study that evaluated PRF obtained with different centrifuge equipment and protocols [24], and two other studies that assessed platelet rich plasma with different protocols of incubation [30] and with different patients [11].

One of the limitations of this study was that we used a different centrifuge than the one scientifically tested by Dohan DM et al. [31]. It has been proven that centrifugation can modify cell characteristics and the fibrin architecture of PRF [17]. For future research, it is important to rely on the biological bases since there are a variety of centrifuges and commercial brands that promote different kits and automated equipment [8,10].

Finally, we propose that with the centrifuge evaluated, the protocol with stronger macroscopic characteristics was the sixth one (3,200 rpm x 10 minutes) because the fibrin was polymerized, the platelet distribution was homogeneous, the inter-fiber space is ideal for cell migration, and the quantity of growth factors is sufficient. However, the protocol with stronger molecular conditions was the ninth one (3,200 rpm x 12 minutes) because it showed the highest levels of growth factors. Besides, we observed that the fibrin mesh is denser in longer centrifugation protocols. This information is relevant for clinicians because in some surgical cases, it is necessary to use PRF as a dense membrane, for example in sinus perforation; in these cases, PRF is used as a barrier. In other cases, it is required to be less dense, for example when PRF is mixed with bone fillings or another biomaterial, as in bone reconstruction surgeries. Our recommendation is to use the protocol based on the clinical requirement. Also, it is important to use centrifuges with previous standardizing of the centrifugation parameters to guarantee a PRF with high level of quality, as well as reproducible clinical results.

CONCLUSION

Changes in the centrifugation parameters (time and speed) to obtain platelet-rich fibrin, different to those proposed in the original protocol, have a significant impact on fibrin structure, distribution, and cell integrity and the content of biomolecules. As a result, it is probable that mechanical properties could change between each protocol and therefore their possible clinical applications as well.

The platelets, leukocytes, fibrin, and growth factors evaluated in this study revealed important information about the characteristics of the different protocols to obtain PRF. Only using the centrifuge tested in this study, clinical decisions can be taken based on the obtained results; with other centrifuges, the results could be different.

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Declaration of Interest:
The authors declare no conflict of interest.

References:

1. Del Corso M, Vervelle A, Simonpieri A, et al. Current knowledge and perspectives for the use of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in oral and maxillofacial surgery part 1: Periodontal and dentoalveolar surgery. Curr Pharm Biotechnol 2012 Jun;13(7):1207-30 (PMID: 21740371).

2. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006 Mar;101(3):e45-50 (PMID: 16504850).

3. Miron RJ, Fujioka-Kobayashi M, Bishara M, Zhang Y, Hernandez M, Choukroun J. Platelet-Rich Fibrin and Soft Tissue Wound Healing: A Systematic Review. Tissue Eng Part B Rev 2017 Feb; 23(1):83-99 (PMID: 27672729).

4. Karimi K, Rockwell H. The Benefits of Platelet-Rich Fibrin. Facial Plast Surg Clin North Am 2019 Aug;27(3):331-40 (PMID: 31 280847).

5. Miron RJ, Xu H, Chai J, 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 2020 Mar;24(3):1171-82 (PMID: 3132 1574).

6. Melo BAG, Luzo ÂCM, Lana JFSD, Santana MHA. Centrifugation conditions in the L-PRP preparation affect soluble factors release and mesenchymal stem cell proliferation in fibrin nanofibers. Molecules 2019 Jul 27;24(15):2729 (PMID: 31357568).

7. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Trends Biotechnol 2009 Mar;27(3):158-67 (PMID: 19187989).

8. Dohan Ehrenfest DM, Pinto NR, Pereda A, 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 Mar;29(2):171-84 (PMID: 28437133).

9. Gutierrez Ramire DA, Hinojosa Sarria JP, Restrepo Dorado AI, Munoz Ramírez AL, Velarde Trochez NF FE. Structural Analysis of Platelet-Rich Fibrin and its Applications in Regenerative Dentistry. Univ Odontol 2018;37(79). http://hdl.handle.net/10554/42031

10. Dohan Ehrenfest DM. How to optimize the preparation of leukocyte- and platelet-rich fibrin (L-PRF, Choukroun's technique) clots and membranes: introducing the PRF Box. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010, Sep;110(3):275-8; author reply 278-80 (PMID: 20727495).

11. Marck RE, Gardien KLM, Vlig M, Breederveld RS, Middelkoop E. Growth factor quantification of platelet-rich plasma in burn patients compared to matched healthy volunteers. Int J Mol Sci 2019, Jan 12;20(2):288 (PMID: 30642068).

12. Gadelmawla ES, Koura MM, Maksoud TMA, Elewa IM, Soliman HH. Roughness Parameters. Journal of Materials Processing Technology 2002 Apr 123;(1):133-45.

13. Magalon J, Bausset O, Serratrice N, et al. Characterization and comparison of 5 platelet-rich plasma preparations in a single-donor model. Arthroscopy 2014 May;30(5):629-38 (PMID: 247253 17).

14. Taniguchi Y, Yoshioka T, Sugaya H, et al. Growth factor levels in leukocyte-poor platelet-rich plasma and correlations with donor age, gender, and platelets in the Japanese population. J Exp Orthop 2019 Feb 2;6(1):4 (PMID: 30712144).

15. Oh JH, Kim W, Park KU, Roh YH. Comparison of the Cellular Composition and Cytokine-Release Kinetics of Various Platelet-Rich Plasma Preparations. Am J Sports Med 2015 Dec;43(12): 3062-70 (PMID: 26473014).

16. Leitner GC, Gruber R, Neumuller J, et al. Platelet content and growth factor release in platelet-rich plasma: a comparison of four different systems. Vox Sang 2006 Aug;91(2):135-9 (PMID: 16907874).

17. Miron, R.J., Chai, J., Fujioka-Kobayashi, M. et al. Evaluation of 24 protocols for the production of platelet-rich fibrin. BMC Oral Health 2020 Nov 7;20(1):310 (PMID: 33160335).

18. Suchetha A, Lakshmi P, Bhat D, Mundinamane DB, Soorya KV, Bharwani GA. Platelet concentration in platelet concentrates and periodontal regeneration-unscrambling the ambiguity. Contemp Clin Dent 2015 Oct-Dec;6(4):510-6 (PMID: 26681857).

19. Karde PA, Sethi KS, Mahale SA, Khedkar SU, Patil AG, Joshi CP. Comparative evaluation of platelet count and antimicrobial efficacy of injectable platelet-rich fibrin with other platelet concentrates: An *in vitro* study. J Indian Soc Periodontol 2017 Mar-Apr;21(2):97-101 (PMID: 29398852).

20. Piccinini F, Tesei A, Arienti C, Bevilacqua A. Cell Counting and Viability Assessment of 2D and 3D Cell Cultures: Expected Reliability of the Trypan Blue Assay. Biol Proced Online 2017 Jul 20;19:8 (PMID: 28814944).

21. Passaretti F, Tia M, D'Esposito V, et al. Growth-promoting action and growth factor release by different platelet derivatives. Platelets 2014;25(4):252-6 (PMID: 23855408).

22. El Bagdadi K, Kubesch A, Yu X, 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 Jun;45(3):467-79 (PMID: 28324162).

23. 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. J Oral Implantol 2014 Dec;40(6):679-89 (PMID: 24945603).

24. Fujioka-Kobayashi M, Miron RJ, Hernandez M, Kandalam U, Zhang Y, Choukroun J. Optimized Platelet-Rich Fibrin With the Low-Speed Concept: Growth Factor Release, Biocompatibility, and Cellular Response. J Periodontol 2017 Jan;88(1):112-21 (PMID: 27587367).

25. 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 Feb;44(1):87-95 (PMID: 28 283682).

26. Brown AC, Barker TH. Fibrin-based biomaterials: modulation of macroscopic properties through rational design at the molecular level. Acta Biomater 2014 Apr;10(4):1502-14 (PMID: 240560 97).

27. Miron RJ, Zhang Y. Autologous liquid platelet rich fibrin: A novel drug delivery system. Acta Biomater 2018;75:35-51 (PMID: 29 772345).

28. Su CY, Kuo YP, Tseng YH, Su CH, Burnouf T. *In vitro* release of growth factors from platelet-rich fibrin (PRF): a proposal to optimize the clinical applications of PRF. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108(1):56-61 (PMID: 19451 002).

29. Nishimoto S, Fujita K, Sotsuka Y, et al. Growth Factor Measurement and Histological Analysis in Platelet Rich Fibrin: A Pilot Study. J Maxillofac Oral Surg 2015 Dec;14(4):907-13 (PMID: 26 604462).

30. Durante C, Agostini F, Abbruzzese L, et al. Growth factor release from platelet concentrates: analytic quantification and characterization for clinical applications. Vox Sang 2013 Aug;105(2):129-36 (PMID: 23639058).

31. Dohan Ehrenfest DM, Del Corso M, Diss A, Mouhyi J, Charrier JB. Three-dimensional architecture and cell composition of a Choukroun's platelet-rich fibrin clot and membrane. J Periodontol 2010 Apr;81(4):546-55 (PMID: 20373539).