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CHARACTERIZATION OF PHYSICAL AND CELLULAR PROPERTIES OF MEMBRANES OF L-PRF PRODUCED BY DIFFERENT CENTRIFUGES/ CENTRIFUGATION PROTOCOLS.

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ORIGINAL ARTICLE

ABSTRACT

Leukocyte and platelet-rich fibrin (L-PRF), an essential source of cytokines and growth factors, has been widely used to regulate the healing process. The quality of the L-PRF obtained is dependent on technical factors and the centrifugation protocol used. Thus, the present study aimed to macro and microscopically evaluate the formation of the L-PRF membrane/clot in two centrifuge models and protocols recommended by manufacturers. Four blood samples from 8 healthy volunteers undergoing rehabilitation surgery with dental implants were used. In group 1 of the Montserrat centrifuge (model 80-2B 15mL) 400g of centrifugal force were used for 10 minutes, 27ºC, and 2700 rpm, while in group 2 of the Intra Spin-Intra-Lock centrifuge (IntraSpin™, Intra-Lock Iberia) 400g of centrifugal force were used for 12 minutes, 27ºC and 2700 rpm. After obtaining 32 L-PRF clots/membranes, the pieces were recorded, measured, and weighed, separating the L-PRF from the supernatant/clot of red blood cells, remaining in the tubes. The specimens were then histologically processed for Hematoxylin/Eosin (H/E) staining and each slide was divided into three called areas: proximal, center, and distal. The presence of leukocytes was analyzed semi-quantitatively in each area. The results revealed no significant macroscopic differences in L-PRF between the two groups. However, the central areas of the L-PRF membranes produced in group 2 showed higher intensity of leukocytes (p=0.012), as well as the presence of concomitant leukocytes in at least 2 of the evaluated areas (p=0.003). In the proximal area, no significant differences were observed between the two groups. The results allow us to postulate that technical protocol factors influence the presence and distribution of leukocytes between the L-PRF membranes, obtained in different centrifuges.

Keywords: Platelet Rich Fibrin, Platelet Rich Plasma, Regenerative Medicine, Healing.

CARACTERIZAÇÃO DE PROPRIEDADES FÍSICAS E CELULARES DE MEMBRANAS DE L-PRF PRODUZIDAS POR DIFERENTES CENTRÍFUGAS/ PROTOCOLOS DE CENTRIFUGAÇÃO.

RESUMO

A Fibrina Rica em Plaquetas e Leucócitos (L-PRF) importante fonte de citocinas e fatores de crescimento tem sido amplamente utilizada como reguladora do processo cicatricial. A qualidade da L-PRF obtida é dependente de fatores técnicos e do protocolo de centrifugação utilizado. Desta forma, o presente estudo teve como objetivo avaliar macro e microscopicamente a formação da membrana/coágulo de L-PRF em dois modelos de centrífugas e protocolos recomendados pelos fabricantes. Foram utilizadas 4 amostras de sangue de 8 voluntários saudáveis submetidos à cirurgia de reabilitação com implantes dentários. No grupo 1 da centrífuga Montserrat (modelo 80-2B 15mL) foram utilizadas 400g de força centrífuga por 10 minutos, 27ºC e 2700 de rpm, já no grupo 2 da centrífuga Intra Spin-Intra-Lock (IntraSpin™, Intra-Lock Iberia) foram utilizadas 400g de força centrífuga por 12 minutos, 27ºC e 2700 de rpm. Após a obtenção de 32 coágulos/membranas de L-PRF, as peças foram registradas, mensuradas e pesadas, separando-se as membranas L-PRF do sobrenadante/coágulo de glóbulos vermelhos remanescente nos tubos. Os espécimes foram então processados histologicamente para a coloração por Hematoxilina/Eosina (H/E) e cada lâmina foi dividida em três áreas denominadas de proximal, centro e distal. A presença de leucócitos foi analisada semi-quantitativamente em cada área. Os resultados não revelaram diferenças macroscópicas significantes nas L-PRFs pelos dois grupos. Entretanto, as áreas centrais das membranas de L-PRF produzidas no grupo 2 demonstraram maior intensidade de leucócitos (p=0,012), assim como presença de leucócitos concomitantes em pelo menos duas das áreas avaliadas (p= 0,003). Não foram observadas diferenças significativas entre os dois grupos na área proximal. Os resultados permitem postular que fatores técnicos protocolares influenciam na presença e distribuição dos leucócitos entre as membranas de L-PRF, obtidas em diferentes centrífugas.

Palavras-chave: Fibrina Rica em Plaquetas, Plasma Rico em Plaquetas, Medicina Regenerativa, Cicatrização.

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INTRODUCTION

The development of protocols for modulating biomaterials of the inflammatory process that act by accelerating tissue repair has been the subject of continuous research in recent decades. In this context, Leukocyte and platelet-rich fibrin (L-PRF) has shown to be promising[1,2]. L-PRF has three main components capable of promoting tissue regeneration, including autologous cells, such as leukocytes and platelets, a provisional three-dimensional extracellular matrix scaffold made from autologous fibrin, and bioactive molecules, such as cytokines and growth factors[3,4].

Platelets play an important role both in hemostasis and in the tissue repair process, since they have growth factors responsible for cell mitosis, induction of cell differentiation and increase in collagen production. Thus, the therapeutic strategies carried out with the use of platelet concentrates (PCs) aim to achieve these effects[5,6,7].

The PCs correspond to autogenous products prepared by centrifuging the patient's blood sample, allowing components rich in growth factors, fibrin and leukocytes, to be collected and become amenable to clinical use. Its surgical use has been improved, aiming to optimize its clinical applicability[8].

Initially described by Choukroun et al. (2001) in France, L-PRF, characterized as an autologous scar matrix, is considered a second-generation platelet concentrate technology because it is a simpler and less expensive technique compared to other protocols[9]. In the technique developed by Choukroun et al. (2001), blood was collected without the addition of anticoagulant in 10 ml tubes and immediately centrifuged at 2700 RPM for 12 minutes using a PC-02 desktop centrifuge and a collection kit from Process (Nice, France), leading to the formation of a clot of PRF in the middle of the tube. The clotting process occurred naturally after the blood came into contact with the wall of the collecting tube[10].

The success of the L-PRF preparation technique depends on the fast and efficient processing of the collected blood. After centrifugation, blood is separated into three phases. The first phase is the acellular plasma, the second phase corresponds to the L-PRF clot, and the third phase is the series of red blood cells[10]. The simplicity of the technique for obtaining L-PRF and, consequently, the greater accessibility of the use of

this concentrate in routine surgical procedures, allow the acquisition of an autologous platelet concentrate for clinical use, which is seen as an advantage of L-PRF compared to other platelet concentrates[8].

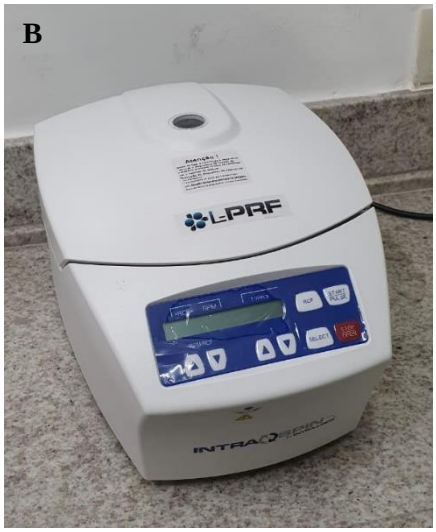
With the evolution of studies on L-PRF, variations on centrifuge models and different protocols that can be used for this technique have emerged in the literature. Given this context, questions arose about the differences found in the L-PRF clots produced by different centrifuges/protocols. It is postulated that variations in the quality, size and weight of clots can influence tissue repair[11].

To date, few studies have investigated the impact of different centrifuge parameters and models on the characteristics and quality of L-PRF clots with regard to cellular and fibrin architecture[4,12]. Considering that the characteristics of centrifuges can somehow interfere in the biological result of L-PRF, this study aimed to evaluate the macroscopic and microscopic characteristics of the L-PRF clot/membrane produced by two different commercial centrifuge models.

MATERIALS AND METHODS

L-PRF membranes were produced according to protocols established by the manufacturer of each centrifuge. The study included 8 volunteers classified as ASA I and ASA II according to the American Society of Anesthesiologists (ASA) Physical Status Classification System, with no history of use of any medication correlated with the clotting process and who agreed to sign the Free and Informed Consent Form (FICF). Four tubes of blood were collected from each volunteer for immediate production of clots and L-PRF membranes (1 for use as a sample and 1 for storage in each centrifuge). Blood was collected by a qualified professional.

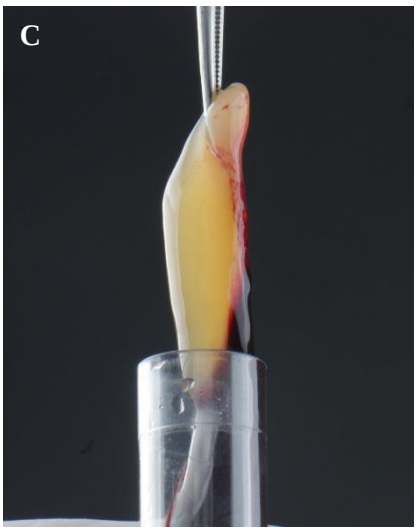
The material was stored in plastic tubes, without anticoagulants (BD Vacutainer Serum of 10,0 ml, Becton Dickinson, Franklin Lakes, NJ, USA), and the collection procedure was performed according to Andriolo et. al (2010) according to the standards recommended by the Brazilian Society of Clinical Pathology Laboratory Medicine for venous blood collection.

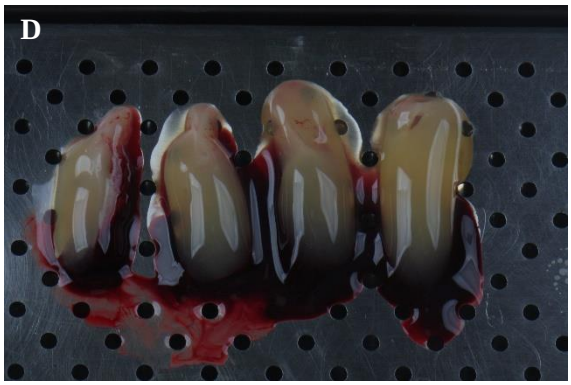


For the preparation of L-PRF, two types of centrifuges were used, characterizing the two experimental groups of this work: Group 1 (Montserrat Centrifuge, model 80-2B 15ml) (Figure A) e Group 2 (IntraSpin™ Centrifuge, Intra-Lock Iberia) (Figure B). Prior to blood collection, the centrifuges were programmed with the protocols indicated by the manufacturer (Intra Spin-Intra-Lock: 400g of centrifugal force, 12 minutes, 27ºC, 2700 RPM; Montserrat: 400g of centrifugal force, 10 minutes, 27ºC, 2700 RPM). After the end of the centrifugation, the tubes remained inside the respective centrifuges for 10 minutes. Four clots were obtained per volunteer, two from the Intra Spin-Intra-Lock centrifuge and two from the Montserrat centrifuge, totaling 32 L-PRF clots.

Macroscopic analysis

After centrifugation, the L-PRF fibrin clots (Figure C) were properly measured and weighed. Then, they were placed in a surgical box in which, through gentle compression, these clots were characterized as L-PRF membranes (Figure D). Then, measurements of the weight/size of the L-PRF membranes and the weight of the supernatant and red blood clot remaining in the tubes were performed.





Evaluations were then carried out between the groups with continuous numerical variables on the volume of the tube containing the L-PRF clot produced, the size of the clot and the L-PRF membrane, the weight of the L-PRF membrane and also, supernatant and red blood cell clots remaining in the tube.

Histological processing and microscopic analysis

After histological processing, the slides were stained with Hematoxylin-eosin and evaluated under an optical microscope (Axiostarplus® / ZEISS, Germany 2008) at 40X magnification. Each slide was divided into three areas of equal size: Proximal (Head and Face), corresponding to the area in contact with the red blood cells where the largest cell population is found; Center (Body); and Distal (Tail), which corresponds to the area opposite the packed red blood cells. The evaluation of the slides was performed through a blinded study, by an experienced pathologist, in a semiquantitative way, to analyze the presence of leukocytes.

Regarding the microscopic characteristics, a discrete numerical variable was used regarding the number of areas with the presence of leukocytes. Descriptive evaluations and the following categorical variables were also performed between the groups: area with the highest concentration of cells; cell intensity in the proximal, central and distal; score in percentage of the presence of cells in the proximal, central and distal; distribution of cells in the proximal, central and distal; and location of cells in the proximal, central and distal region.

Statistical analysis

For all analyzes performed between groups, statistical tests were applied, for an error probability of 5% ($p < 0,05$). The Chi-square test was used to compare categorical and discrete numerical variables, assessing how likely it is that any observed difference happens by chance. For continuous numerical variables, Student's t test was applied. All data obtained were tabulated using the Microsoft Excel program and statistically analyzed using the Minitab® program.

RESULTS

Macroscopic analysis

When comparing Group 1 (Montserrat Centrifuge) with Group 2 (Intra Spin-Intra-Lock Centrifuge) it was observed that there was no difference in the volume of the tubes containing the L-PRF clots (p=0.734), in the size of the clots (p=0.893) and the L-PRF membranes produced by the centrifuges (p=0.341), the weight of the L-PRF membranes (p=0.843), and the supernatants and red blood cell clots remaining in the tubes (p=0.466) (Table 1).

TABLE 1- Comparison of macroscopic characteristics between groups 1 and 2.

Variable	Group	Mean	SD	p-value
Tube volume (g)	1	18,670	0,788	0,734
	2	18,814	0,814	
L-PRF membrane weight (g)	1	0,541	0,152	0,843
	2	0,521	0,233	
L-PRF clot size	1	33,88	4,22	0,893
	2	34,25	6,45	
Membrane size L-PRF	1	31,13	5,22	0,341
	2	33,63	4,90	
Tube supernatant (g)	1	16,35	1,23	0,466
	2	16,82	1,31	

SD= standard deviation

Microscopic analysis

Regarding the number of areas with cells, it was observed that, in Group 1, 62.5% of the L-PRF membranes produced had cells in only 1 area and, in none of the membranes produced in this group, there were cells in the 3 areas concurrently. In group 2, most of the L-PRF membranes produced (62.5%) showed cells in at least 2 areas at the same time and, in this group, in none of the membranes the presence of cells was observed in only 1 area (Table 2) (p=0.003).

TABLE 2- Number of areas with cells

Areas number	G1	G2
1	62,5%	0%
2	37,5%	62,5%
3	0%	37,5%
p-value	0,003	

G1= Group 1
G2= Group 2

Regarding cell concentration, a certain homogeneity was observed. In group 1, all L-PRF membranes had the proximal area as the one with the highest concentration and, in group 2, about 87.5% of the samples had the same area as the one with the highest concentration of cells. However, this result was not statistically significant (p=0.89) (Table 3).

TABLE 3- Microscopic Analysis

Variable	Classification	Proximal		Central		Distal	
		G1	G2	G1	G2	G1	G2
Area with > concentration of cells	--------	100%	87,5%	0%	12,5%	0%	0%
p-value		0,89					
Cell intensity	0 = absent	0%	0%	62,5%	0%	100%	62,5%
	1 = few	12,5%	25%	25%	37,5%	0%	37,5%

	2 = moderate	50%	50%	12,5%	37,5%	0%	0%
	3 = high	37,5%	25%	0%	25%	0%	0%
p-value		0,763		0,012		0,028	
Cells percentage	Score 0 = <5%	0%	0%	62,5%	12,5%	100%	87,5%
	Score 1 = 6-25%	0%	12,5%	25%	25%	0%	12,5%
	Score 2 = 26-50%	12,5%	0%	0%	50%	0%	0%
	Score 3 = >50%	87,5%	87,5%	12,5%	12,5%	0%	0%
p-value		0,91		0,037		0,89	
Cell distribution	0 = absent	0%	0%	62,5%	0%	100%	62,5%
	1 = difuse	100%	87,5%	37,5%	75%	0%	37,5%
	2 = focal	0%	12,5%	0%	25%	0%	0%
p-value		0,89		0,005		0,028	
Cell location	0 = absent	0%	0%	62,5%	0%	100%	62,5%
	1 = edges	37,5%	12,5%	0%	12,5%	0%	0%
	2 = center	0%	0%	37,5%	37,5%	0%	25%
	3 = edge and center	62,5%	87,5%	0%	50%	0%	12,5%
p-value		0,240		0,037		0,32	

With regard to cell intensity, in the proximal area there was no statistically significant difference in L-PRF membranes (p=0.763). However, statistical significance was found in the central area (p=0.012), where most samples from group 1 (62.5%) did not have any cells in the area (intensity 0), while in group 2 no sample had intensity 0 in this portion. It was also observed that, in group 1, 100% of the membranes produced had intensity 0 in the distal portion, while in group 2, 37.5% of the sample had low cellular intensity (intensity 1) in this portion (p=0.028) (Table 3). Furthermore, it was observed that while in group 1 no membrane produced was classified with high cell intensity (intensity 3), in group 2, 25% of the samples presented intensity 3 in the central portion.

Regarding the percentage of cells, it was observed that 87.5% of the membranes produced in both groups presented a score >50% in their proximal portions (p=0.91), indicating a homogeneity of results. The same occurred in the distal (p=0.89), where 93.75% of the total samples of the groups obtained a score of <5% of cells. Thus, there was no statistically significant difference between groups in either the proximal or the distal. Regarding the central portion (p=0.037), a statistical difference was found between the groups, observing that in group 1, 62.5% of the L-PRF membranes had <5% of cells in their central portion, and only 12.5% had >50% cells in this portion. Meanwhile, in group 2, 50% of the membranes produced had between 26-50% of cells in their central portion, and only 12.5% had >50% of cells in this portion (Table 3).

When analyzing the cellular distribution between the groups, it was noted that in the proximal region (p=0.89) there was no significant difference between the variables studied. In the central (p=0.005) and distal (p=0.028) regions, a statistically significant relationship was found in the cellular distribution between the groups. In the central region (p=0.005) in group 1, 62.5% of the membranes produced had no cell distribution, and the other 37.5% had a diffuse cell distribution. In group 2, it was observed that 75% of the sample had a diffuse distribution in the central region and, in the remaining 25% of the sample, a focal distribution of cells in the region was observed. Concerning the distal portion (p=0.028), 100% of the membranes produced in group 1 did not show cellular distribution in this region and, in relation to group 2, it was noticed that 37.5% of the membranes showed a diffuse distribution of cells (Table 3).

In the location of cells between the groups analysis, it was not possible to find statistical relevance in the proximal (p=0.240) and distal (p=0.32) regions. In the central region (p=0.037), it was possible to observe that there was a significant difference between the variables. In this region, 62.5% of the L-PRF membranes produced in group 1 did not present cells in any location and, in the remaining membranes (37.7%), it was possible to observe the presence of cells in the center of this region. In group 2, 50% of the membranes had cells located on the edges and in the center, 37.5% had cells only in the center and, in 12.5% of these membranes, it was possible to observe the presence of cells only on the edges of this region (Table 3).

DISCUSSION

PRF has gained evidence in recent years due to its ability to promote tissue regeneration[13]. In the early stages of technology development involving L-PRF, it was observed that the choice of centrifuge type and protocol used interfered in the final product of the L-PRF clot/membrane[5,14]. Despite this, scientific data comparing the impact of different parameters and centrifuges on the final result of PRF-based matrices are scarce[13], only recently some studies have investigated the use of different parameters in centrifuges and how their effects can interfere with the quality of L-PRF membranes[12,13,15].

Dohan Ehrenfest et al. (2017) tested four different types of L-PRF centrifuges available on the market, the Intra Spin-Intra-Lock centrifuge and three other tabletop centrifuges[4]. Using the same centrifugal force (400g) and the same type of L-PRF collection, the authors aimed to evaluate the mechanical vibrations that appear during centrifugation in the four models and the impact of the characteristics of each centrifuge on macroscopy and microscopy of a clot /L-PRF membrane, regarding cellular and fibrin architecture [4,12,15].

Regarding the macroscopic characteristics of the L-PRF clots/membranes produced in the Montserrat and Intra Spin-Intra-Lock centrifuges, the results of this study differ from what was found by Dohan Ehrenfest et al. (2017) in its most recent publication on this topic[4]. While in the present study, when comparing the two centrifuges, it was not possible to find any macroscopic differences in the characteristics of the L-PRF clots/membranes that were produced, with regard to tube volume, membrane weight, clot/membrane size and supernatant in the tubes, Dohan Ehrenfest et al. (2017) found a significant macroscopic difference in the L-PRF produced by the 4 different centrifuges tested, in which they report that the L-PRF clots/membranes produced in the Intra Spin-Intra-Lock centrifuge were considered significantly superior in relation to the weight, size and also length of those that were produced in the other centrifuges[4]. This can be explained by the fact that the same centrifuges were not tested in both studies. About that, Miron et al. (2019) tested the Intra Spin-Intra-Lock and two other centrifuges using different speeds to assess the difference between clots due to centrifugation force[13]. That is, in addition to comparing different centrifuges, the

authors compared different protocols in the same centrifuge. These authors evaluated the three different brands at two centrifugation speeds each (700g and 200g) and observed that the clots formed were larger and heavier at higher speeds. Despite differing in terms of centrifugation speed, since two speeds were compared, this study corroborates with what Dohan Ehrenfest et al. (2017) defends, regarding the fact that in both the Intra Spin-Intra-Lock showed superior results in the macroscopic analysis of the clot/L-PRF membrane[4]. This finding is important and suggests further research comparing different centrifugation protocols, aiming to study the macroscopic differences of L-PRF clots/membranes produced in the Intra Spin-Intra-Lock centrifuge compared to other centrifuge models, so that this difference can be better evaluated.

As Dohan Ehrenfest et al. (2017) observed, through scanning electron microscopy (SEM), considerable differences between the L-PRF membranes produced in the four different centrifuges, we can say that, at the cellular level, this study corroborates ours[4]. According to Dohan Ehrenfest et al. (2017), while the Intra Spin-Intra-Lock L-PRF membrane showed a thick fibrin matrix strongly polymerized and with all the living cells and their normal shape, including the appearance of activated lymphocytes, the L-PRF membranes of the three other centrifuges showed a thin, slightly polymerized fibrin gel with destruction of most of the visible cell bodies, demonstrating a significant difference at the cellular level in the quality of the L-PRF produced in the different centrifuges[4]. The SEM evaluation by Miron et al. (2019) revealed that the fibrin network produced at high speed had a denser morphology when compared to those from low-speed protocols[13]. In addition, in protocols of greater centrifugal force, a greater amount of visible cells was observed in the proximal portion. Once again, suggesting the importance not only of comparing different models of centrifuges, but also of different protocols in the same centrifuge for better evaluation and possible establishment of an ideal protocol to obtain increasingly effective clots/membranes in tissue regeneration.

In comparison with the microscopic analysis obtained in this study, it was possible to observe that the presence of leukocytes was also statistically different between the groups. Most of the L-PRF membranes produced in the Intra Spin-Intra-Lock centrifuge showed leukocytes in at least two areas at the same time. In addition,

the variables used for microscopic evaluation of leukocytes in the L-PRF membranes produced in the two different centrifuges showed that the intensity, percentage, distribution and location of leukocytes in these membranes from the Intra Spin-Intra-Lock centrifuge occurred more intensely and more homogeneous, involving in most cases the proximal, central and distal areas. In most membranes from the Montserrat centrifuge, using these same variables, it was not possible to identify leukocytes in their distal area.

According to Dohan Ehrenfest et al. (2010), the highest density of leukocytes and platelets is seen in the first millimeter of the yellow clot of the PRF (proximal region-head and face), right after the red clot and the distribution of platelets and leukocytes becomes increasingly scarce at the same time that approaches the end of the clot (distal-tail region), not being able to be found, in most cases, beyond the first half of the yellow clot[11]. However, Miron et al. (2019) observed, in the Intra Spin-Intra-Lock centrifuge protocol, a higher proportion of platelets and leukocytes only in the fifth layer 5 (5ml) of their L-PRF clots, finding almost no cells in the first 4ml of the PRF[13]. Since even small lymphocytes are considered to be efficient in regulating inflammatory reactions, the number of leukocytes within each membrane can have a significant impact on the process[11]. Thus, the present study may have an important clinical impact, since the L-PRF membranes produced in the Intra Spin-Intra-Lock centrifuge showed a relevant and superior presence of leukocytes also in the central and distal regions, an uncommon finding[16] .

It is well known that leukocytes are cells that play an essential role in wound healing because of their anti-infective action as well as the immune regulation that occurs through the secretion of cytokines such as interleukins (IL-1β, IL-6, IL-4) and the tumor necrosis factor-alpha (TNF-α). In this context, it is important to emphasize that L-PRF is a natural blood concentrate composed of approximately 97% of platelets and more than 50% of leukocytes within a high-density fibrin network compared to the whole blood[5]. Although the role of leukocytes in immune defense is already very well defined, they also play a role as regulators that control the ability of biomaterials to adapt to new host environments[17-19]. Thus, it can be said that it would be pretty

interesting in terms of accelerating the healing process if these cells were present in more areas of the L-PRF membranes/clots.

Given the scarcity of published studies that investigate how the different parameters in different centrifuge models may influence the quality of the clots/membranes of L-PRF made, it is important that factors that have already been studied, such as the use of the same centrifugal force, which must be calculated and adjusted for each centrifuge model, the use of the same type of blood collection and centrifuge vibration must be taken into account as possible influences on the quality of clots and L-PRF membranes produced[4,12,15].

Therefore, this study suggests that there are microscopic differences in leukocytes between the L-PRF membranes from Intra Spin-Intra-Lock centrifuges and from the Montserrat centrifuge. And, although no statistically significant difference was found in the proximal areas of these membranes, in the central and distal areas there was statistical significance in the presence of leukocytes in the membranes produced in the Intra Spin-Intra-Lock centrifuge. However, it is necessary that more studies are carried out, involving more parameters and investigating more and more centrifuges so that these results can be replicated.

CONCLUSION

In the present study, it was observed that there was no difference between the weight, size and supernatant of the clots/membranes of L-PRF produced in the different centrifuges used. The percentage, distribution and location of leukocytes in the L-PRF membranes made in the Intra Spin-Intra-Lock centrifuge were statistically higher in the central areas when compared to those produced in the Montserrat centrifuge. This difference in cellular characterization may contribute to the biological properties of the L-PRF membranes produced.

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PICTURE'S DESCRIPTION

FIGURE A: Montserrat Centrifuge

FIGURE B: Intra Spin Intra-Lock Iberia Centrifuge

FIGURE C: L-PRF L-PRF clot being removed from collection tube.

FIGURE D: L-PRF membranes.

APPENDICES

LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

REC	Research Ethics CommitteE
FICF	Free and Informed Consent Form
ASA	American Society of Anesthesiologists
L-PRF	Leukocyte and platelet-rich fibrin
PCs	Platelet Concentrates
ml	Milliliters
RPM	Rotation per minute
kg	Kilograms
g	Grams
PRF	Platelet Rich Fibrin