Development of a Device to Obtain Platelet-Rich Plasma (PRP)

Desenvolvimento de um dispositivo para obter plasma rico em plaquetas (PRP)

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Abstract

Objective To present an innovative device that applies the double centrifugation method to obtain platelet-rich plasma (PRP), assessing whether there was an effective increase in the concentration of platelets.

Method Ten volunteers underwent blood collection. The samples were separated in 20 ml syringes, sealed and subjected to the double centrifugation protocol at 1,100 revolutions per minute (rpm) for 15 minutes, resulting in the separation of red blood cells, plasma with platelets, and leukocytes. Then, 10 ml syringes were added to remove 9 ml, respecting the "buffy coat" parameter, collecting 8 ml above and 1 ml below for the second centrifugation and transferring again to the 20 ml syringe. The plasma was again centrifuged at 1,550 rpm for 10 minutes; as a result, it was divided into two parts: at the top, consisting of low platelet plasma (LPP), and at the bottom, by the platelet button. Part of the LPP was discarded, leaving only 3 ml with the platelet button. The cells were then counted.

Results This innovative device was able to increase the concentration of platelets by almost three times compared with the baseline. In addition, the preparation time for the PRP was adequate, lasting only 35 to 40 minutes.

Conclusions Platelet-rich plasma was successfully obtained by the double centrifuge protocol, allowing its clinical use. In addition, obtaining through the presented device promotes greater applicability in the preparation of PRP in specific centers, furthermore, being a quick and economical way to obtain PRP.

Keywords
- device
- platelet
- platelet-rich plasma

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Resumo

Objetivo Apresentar um dispositivo inovador que aplique o método de centrifugação dupla para obter plasma rico em plaquetas (PRP), avaliando se houve um aumento efetivo na concentração de plaquetas.

Método Dez voluntários foram submetidos a coleta de sangue. As amostras foram separadas em seringas de 20 mL, seladas e submetidas ao protocolo de centrifugação dupla a 1.100 revoluções por minuto (rpm) por 15 minutos, resultando na separação de hemácias, plasma com plaquetas e leucócitos. Em seguida, foram adicionadas seringas de 10 mL para remover 9 mL, tendo como parâmetro a "buffy coat", coletando 8 mL acima e 1 mL abaixo para a segunda centrifugação e transferindo novamente para a seringa de 20 mL. O plasma foi novamente centrifugado a 1.550 rpm por 10 minutos; como resultado, foi dividido em duas partes: na parte superior, consistindo em plasma pobre em plaquetas (PPP), e na parte inferior, pelo botão plaquetário. Parte do PPP foi descartada, restando apenas 3 mL com o botão de plaquetas. As células foram então contadas.

Resultados Este dispositivo inovador foi capaz de aumentar a concentração de plaquetas em quase 3 vezes relação a linha de base. Além disso, o tempo de preparo do PRP foi adequado, com duração de apenas 35 a 40 minutos.

Conclusões O PRP foi obtido com sucesso pelo protocolo de centrifugação dupla, permitindo seu uso clínico. Além disso, a obtenção através do dispositivo apresentado promove maior aplicabilidade no preparo do PRP em centros específicos, além de ser, uma forma rápida e econômica de obter PRP.

Palavras-chave

- dispositivo
- plaquetas
- plasma rico em plaquetas

Introduction

Platelet-rich plasma (PRP), due to its regenerative capacity in different tissues, is considered a technology with great potential. The local action of growth factors, the results on cell differentiation and proliferation, as well as the modulation in the inflammatory response are some of the biological reasons for its clinical use.[1] Platelet-rich plasma is defined as a plasma volume with more platelets than that found in peripheral blood.[2] The most recent literature opts for a more quantitative definition, and it is believed that a platelet concentration between 200×10^3 and $1000 \times 10^3/\mu L$ is considered therapeutically effective, while higher concentrations would be biologically unfavorable.[3–5] Studies developed in the field of orthopedics point to the promising use of PRP. Sánchez et al.[6] demonstrated that, after 6 months of treatment of hip osteoarthritis with PRP, there was a decrease in pain intensity in 40% of patients. Mishra et al.[7] evaluated the effect of PRP when treating chronic elbow tendinosis, observing a reduction in pain, in addition to a therapeutic alternative before the surgical intervention. Sánchez et al.[8] treated patients with Achilles tendon rupture with PRP. In their study, athletes treated only with surgery were compared to those treated with PRP and surgery. It was observed that those treated with surgery and PRP showed improvement of healing and functional recovery. Platelet-rich plasma has also been used in orthopedic surgeries, such as total joint replacement and osteochondral defects correction by arthroscopy.[9,10]

Platelet-rich plasma is obtained through the patient's blood through a process that uses cell separation by centrifugation, which can be classified as single or double centrifugation. Although there is no consensus as to which of them would be the most advantageous to obtain PRP, Macedo[11] demonstrated that double centrifugation has a higher platelet concentration capacity. In his study, when analyzing two protocols, he observed that the platelet count increased by 143% with the single centrifuge, while the double showed an increase of 327%. The obtainment of PRP by the various protocols existing in the literature is an object of interest, as it can result in compounds of different platelet concentrations.[12]

A study by Perez et al.,[13] which used 3.5 ml of blood and centrifugal force of 100 G during 10 minutes in a first centrifugation, and of 400 G during 10 minutes in the second centrifugation, resulted in a 5-fold higher concentration of platelets. Kececi et al.[14] used, as a protocol for the first centrifugation, parameters of between 250 and 270 G during 10 minutes; in the second centrifugation, the force varied to 300 G, 500 G, 750 G, 1000 G, 1500 G and 2000 G during 10 minutes. Platelet concentration followed the increase in the second centrifugal force, increasing by 1.92 times, 2.16 times, 2.80 times, 3.48 times, 3.67 times and 3.76 times, respectively. Thus, it was concluded that obtaining a certain concentration of platelets can be possibly adjusted, individually, according to the centrifugation force used. As seen, the different platelet concentrations obtained depend on the protocol used, making it difficult to evaluate which is best to obtain the PRP.

The present study aimed to present an innovative device for PRP preparation, composed by a set of parts adaptable to common-use syringes, which in turn are adaptable to the

centrifuge, accelerating the preparation time and reducing the chance of activation of platelets. This is a low-cost alternative. In addition, we tested its effectiveness through a double centrifuge protocol for PRP production, evaluating the effective increase in platelet concentration.

Material and Methods

Selection of patients

The PRP preparation process was carried out at the Unit Lab of Universidade de Tiradentes (Unit, in the Portuguese acronym), Farolândia Campus. The present study was approved by the Research Ethics Committee, under the number 3,801,134. Ten volunteers, aged between 18 and 70 years old, participated in the present study after undergoing a medical consultation in an orthopedic outpatient clinic. None of the participants used oral anticoagulants, nor had flu-like symptoms in the previous 30 days before the collection. In addition, only those who had total blood platelets greater than 50,000/µL and who did not manifest any type of blood dyscrasia were included in the present study.

Method of Obtaining PRP

First, with a 20 ml syringe, we aspirated 1 ml from the vial containing anticoagulant citrate acid dextrose (ACD). Then, after antisepsis in the antecubital region with alcohol-soaked cotton, a venous puncture of 14 ml of blood from each volunteer was performed with the 20 ml syringe, gently shaking to join the anticoagulant to the blood (►**Fig. 1A**). A 3 ml sample was separated into a sterile tube to determine the initial platelet count. The 20 ml syringe was used in the double centrifugation process to obtain the PRP in a laboratory centrifuge with a mold that allowed its easy adaptation (►**Fig. 1B**). The 20 ml syringes were taken to a centrifuge and, with the lid closed, subjected to 1,100 revolutions per minute (rpm) (224 G) for 15 minutes, giving rise to 3 columns due to differences in the density of blood components: the lower, red, with red blood cells; and the upper one, yellow, with plasma and platelets. Between these two columns is a narrow whitish band, known as the "buffy coat", which has white blood cells and concentrated platelets (►**Fig. 1C**). From the 15 ml contained in the 20 ml syringe, 8 ml were removed above the mist zone and 1 ml below it, totaling 9 ml. These 9 ml were subjected to a new centrifugation of 1,550 revolutions per minute (444 G) for 10 minutes, giving rise to 2 columns: the upper one, formed by platelet-poor plasma (PPP); and the lower one, by the erythrocytic-platelet button (►**Fig. 1D**). The volume of 6 ml that would correspond to the PPP was inspired with a 10 ml syringe, discarding it, leaving 3 ml next to the erythrocytic-platelet button. This material was slowly agitated to enable platelet resuspension, resulting in PRP. After this method to obtain PRP, the 3 ml were transferred to a sterile tube and the platelet count was performed in an automatic device.

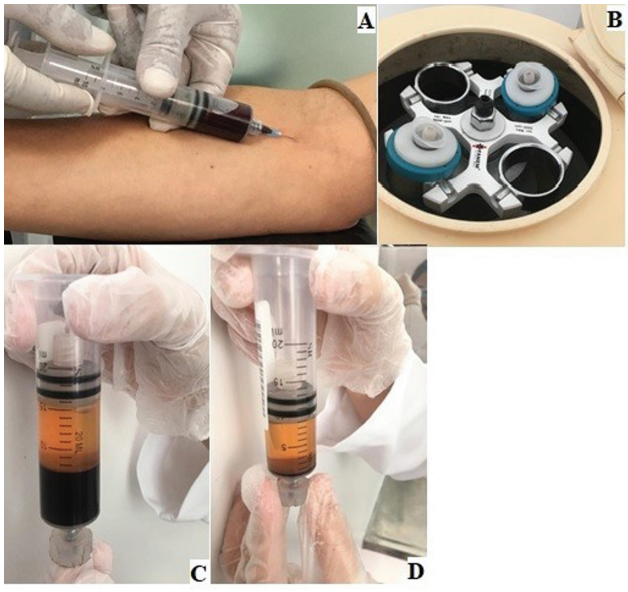

Fig. 1 Images of the platelet-rich plasma (PRP) preparation. (**A**) Positioning of venipuncture. (**B**) Adapted centrifuge used to obtain the PRP. (**C**) Blood sample after the first centrifugation. (**D**) Blood sample after second centrifugation.

Statistical Analysis

Data analysis was performed using the Student t-test for dichotomous variables and the Pearson correlation coefficient for categories, with a significance level of 5% ($p < 0.05$). The software handled was the IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA).

Results

The sociodemographic profile of the volunteers is shown below (►**Table 1**).

Platelet Count after Application of the Protocol

In the blood samples of the 10 volunteers, the initial amount of platelets was within normal parameters (200,000–-385,000 platelets/µL). After double centrifuging the 14 ml of blood samples, we obtained an average value of 694,200 platelets/uL (standard deviation [SD] = 57,019 platelets/µL). The platelet count obtained initially and subsequently the application of the platelet concentration protocol using this new device is explained in ►**Table 2**.

This double centrifugation protocol led to an increase in platelet count of at least 2 times over the initial platelet value in the blood sample. The mean platelet values before and after the application of our protocol for obtaining PRP are shown in ►**Figure 2**.

Table 1 Volunteer data

	Female	Male
Gender	8	2
Average age	29.30	
Average body mass index	25.7783	

Table 2 Results of the initial platelet count and after application of the PRP obtainment protocol

Voluntary	Initial platelet count ($x10^3$ platelets/μl)	Platelet count in PRP ($x10^3$ platelets/μl)	Increase in platelet count ($x10^3$ platelets/μl)
1	291	595	304
2	224	623	399
3	291	849	558
4	222	549	327
5	266	760	494
6	200	507	307
7	318	673	355
8	385	829	444
9	339	717	378
10	283	840	557
Average	281.9	694.2	412.3

Abbreviation: PRP: platelet-rich plasma.

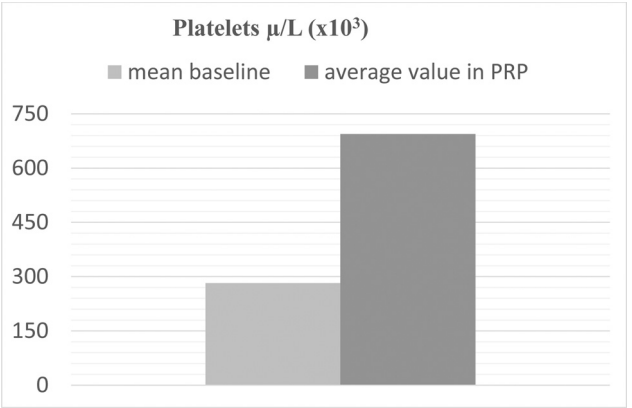

Fig. 2 Result after the application of the protocol for obtaining platelet-rich plasma, promoting an increase in the mean platelet count of at least twice the initial value.

Counting of other Cells after Application of the Protocol

When analyzing hemoglobin (HB), hematocrit (HCT), leukocytes (LEUCO) and neutrophil (NEUTRO) values, we observed a reduction of 25.12% in the HB value and of 24.1% in the HCT value. Regarding LEUCO and NEUTRO, there was an increase of 104.98% and 72.58%, respectively.

Some Correlations

There was a correlation between the amount of platelets and the amount of basal hemoglobin ($p = 0.023$). In addition, the percentage increase in platelets has a higher mean among men (169.5%), and there was no significant correlation in relation to age, weight or body mass index (BMI).

Platelet Preservation and Time to Obtain PRP

The volume of ACD used in platelet conservation was 1 ml. Obtaining the PRP, from the initial stage with anticoagulant aspiration to obtaining PRP, lasted from 35 to 40 minutes.

Discussion

In the literature, the clinical applications of PRP are diverse; they involve skin, bone, dental, ophthalmic injuries, nerve injuries, burns, vascular and aesthetic surgeries, as well as tendon, muscle and cartilage injuries. The results found were optimistic and promising in most studies. However, it is difficult to choose the method to be used due to numerous alternative protocols for obtaining PRP and the standardization absent among them, which may justify the lack of effectiveness observed by some authors.[15] The methods described for obtaining PRP can be divided into two groups: centrifugation and apheresis. A study by Malavolta et al.[16] analyzed the use of PRP obtained through apheresis in arthroscopic repair of complete rotator cuff tears, showing that it is associated with significant functional improvement and incomplete tear. By this method, ~ 400 ml of blood were drained into a separation device under continuous centrifugation at 5,800 rpm for 15 minutes, through a peripheral access. After separation of blood elements by density, an optical analyzer determined the platelet layer by the characteristic of its refraction and separated it in a sterile collection bag, obtaining ~ 30 ml of PRP; the other blood components were returned via the same venous access. Apheresis, although it can generate higher concentrations of platelets compared to centrifugation, is not a practical and less expensive method like centrifugation. Thus, by using centrifugation, the present study presents a less expensive and highly practical method for the preparation of platelet-rich plasma (PRP), together with a protocol that aims to guarantee a minimum effectiveness of the final product. The blood punctured for PRP formulation was added to the syringe containing ACD, which has a greater capacity for platelet concentration compared to other anticoagulants, such as sodium citrate (CS).[17] The use of anticoagulants is important because, by binding with calcium, they prevent the beginning of the coagulation cascade, making it impossible to transform prothrombin into thrombin. It is important to emphasize that

the use of an anticoagulant is not in fact mandatory in the creation of PRP; however, in its absence, the coagulation cascade will begin within 30 seconds to several minutes.[3] The choice of ACD in the present study aims to preserve platelets, avoiding changes in their structure and functionality.

The initial platelet count was at least 2 times lower than that present after PRP preparation. The conclusions of the present work are in line with those presented by Nagata et al.,[18] in which the final amount of PRP platelets was substantially greater than the platelet count of the initial blood sample. In addition, Franco[19] also obtained similar values when assessing the effectiveness of PRP after total knee arthroplasty. A volume of 20 ml of peripheral blood was collected and centrifuged at 1,200 rpm for 10 minutes. The plasma (the most superficial layer) was transferred to another sterile 10 ml tube and centrifuged at the same rotation for 5 minutes. In the end, half of the upper plasma layer obtained was discarded, leaving a platelet concentrate of two to four times the value of the plasma. The study pointed out that, although PRP was not effective in reducing bleeding or improving knee function after arthroplasty, there was a better response in assessing pain after 24 and 48 hours, 1 and 3 weeks and 2 months after the operation ($p < 0.05$). Regarding the centrifugation method, it can be single or double centrifugation. The present study opted for the double centrifugation protocol, recommended by Landsberg et al.[20] and by Garcez et al.,[21] having been shown to be effective, since an increase of at least 100% in the number of platelets was observed. There were also increases in the amount of platelets close to 200%, making it possible to obtain even more favorable results. Furthermore, the double centrifugation proved to be superior in a study comparing both methods. A volume of 8.5 ml of blood was submitted to 1,300 rpm for 10 minutes and, for a second centrifugation, 2,000 rpm for 10 minutes. In the single centrifugation, the volume was 3.5 ml, with parameters of 1,500 rpm for 7 minutes. At the end, there was an increase of 336% in the number of platelets with 2 centrifugations and of 227% with only one.[22] Platelet-rich plasma is more difficult and expensive when obtained by automated methods that require exclusive kits, which require greater amounts of blood to obtain an adequate platelet concentration and high PRP volumes.[23,24]

After countless studies in search of the most appropriate collection method linked to the lowest cost, such as those bt Weibrich et al.[25] and by Vendramin et al.,[23] which originated protocols for preparing PRP using laboratory centrifuges, the present study was carried out to develop a new device for obtaining PRP, whose method is simple, fast and low-cost. Although a centrifugation protocol has not yet been produced, some studies suggest single centrifugation, while others indicate double centrifugation.[20,26–28] Since there is no consensus regarding the number of centrifugations (single or double) and the speed of rotation and established time, this protocol standardized double centrifugation to obtain PRP, guaranteeing double the initial platelet count in peripheral blood. A speed of 1,100 rpm was used for 15 minutes in the first centrifugation and of 1,550 rpm for 10 minutes in the second. The choice of double centrifugation was due to its applicability in studies with promising results, such as the use of PRP adjuvant to the treatment of tibial pseudoarthrosis. Through peripheral access puncture, 55 ml of blood was collected and passed through a first centrifugation of 3,650 rpm for 12 minutes, discarding the erythrocyte layer, the remainder being subjected to a second centrifugation of 3,000 rpm, where the two upper thirds of PPP were discarded and the rest was mixed with thrombocyte concentrate, producing PRP. In this study, the use of PRP led to a shorter consolidation time (7 months) when compared to 13 months in patients who did not use it ($p > 0.01$).[29] A study by Denieli[30] also strengthens the choice for double centrifugation, in which the effect of the surgical application of PRP on chondral lesions of the knee was evaluated. A total of 20 ml of blood was punctured in a peripheral vein and centrifuged at 1,200 rpm for 10 minutes. The most superficial layer (plasma) was used, discarding the "buffy coat" and red blood cells in the second centrifugation for 5 minutes at the same speed. After the second centrifugation, the lower half (PRP) was used for application, noting better and faster results of the functionality compared with those who did not use the PRP. These results already appear 3 months after surgery and last up to 12 months.

The present work achieved an increase in the initial platelet count between 104.47% and 196.82% by using the double centrifugation protocol and applying the mentioned speeds. Although some authors state that the number of platelets contained in the PRP must be > 1,000,000 platelets/μL, others claim that it is possible to obtain a therapeutic effect with a minimum value of 300,000 platelets/μL.[31] In the present work, a final platelet count in the PRP between 507,000 and 849,000 platelets/μL was achieved, which is similar to the literature.[32] The preparation time for the PRP in the present study, from the collection of the anticoagulant before the venipuncture until obtaining it, was of 35 to 40 minutes. Time is a crucial factor when considering the use of PRP in surgical procedures. Regarding the duration to obtain the PRP, the present study showed similarities to those of Garcez et al.[33] and Morato et al.,[34] who observed a variation of 30 to 50 minutes in this time.

Several devices for preparing PRP are commercially available, which operate from a small collected blood volume (20–60 ml) and use the single or double centrifugation protocol. By varying the method and time of centrifugation for preparing the PRP, these systems differ in relation to their potential for concentrating platelets. As an effect, there is variation in the amount of platelets present in the PRP. Thus, it becomes complex to assess which kit would be the best for obtaining the PRP.[35] PRGF (BTI Biotechnology Institute, Porto, Portugal), Harvest SmartPReP (Harvest Technologies Corporation, Viena, Áustria), Vivostat (Vivostat A/S, Copenhague, Dinamarca) and Regen (Regenlab, Lausanne, Suíça) are some of the automated systems commercialized for PRP preparation; however, they are expensive and difficult to handle, which can lead to sterility compromised by an inexperienced operator or to shifting quality.

Conclusions

The conclusions presented from the present study indicate that obtaining PRP by the device presented, following this double centrifuge protocol, is of excellent applicability in

specific centers that operate in the production of PRP, in addition to being an easy and economical way to prepare it. By using the double centrifugation method, we achieved a significant increase in platelet count in the PRP compared to the initial amount of platelets in the peripheral blood. In addition, obtaining PRP takes approximately 35 to 40 minutes, which can be considered an acceptable and fast time.

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Conflict of Interests
The authors have no conflict of interests to declare.

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