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Functional evaluation of muscle-tendon repair in athletes treated with monomeric phase platelet-rich fibrin

Avaliação funcional do reparo músculo-tendão em atletas tratados com fibrina rica em plaquetas em fase monomérica

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ABSTRACT

The autologous platelet-rich fibrin in the monomeric phase (MP-PRF) is a matricellular product obtained from the selective separation of elements in the blood by centrifugation with very low relative centrifugal force, and it appears as a possible alternative in the treatment of muscle-tendon injuries caused by therapeutically functional molecules concentrate. The objective of this study was to verify the obtained concentration of leukocytes and platelets in the matrix according to the proposed centrifugation method (fibrin protocol) and to correlate it with the functional clinical condition in patients submitted to the application of this matrix. In the laboratory phase, according to the acquisition protocol, hemocytometry was performed with anticoagulated blood samples from 05 patients recruited from the sample universe of 14 participants, where the platelet concentration of 119.35% was higher than that observed in whole blood. For the clinical experimental phase, MP-PRF obtained from non-anticoagulated blood samples, were applied to pre-existing muscle-tendon injuries with more than 30 days of evolution in the 14 participants. For functional assessment, the validated DASH and LEFS were used. Of the 14 patients selected, 13 were athletes and showed significant functional improvement three weeks after the intervention. No complications resulted from the response. The infiltrations improved the functional clinical status of the patients. There were no cases of complications, revealing that the procedure is safe. New prospective clinical studies are needed to strengthen this new therapeutic modality.

Keywords: Platelet-rich Fibrin; Muscular Diseases; Wound Healing.

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RESUMO

A fibrina autóloga rica em plaquetas na fase monomérica (MP-PRF) representa um produto matricelular obtido a partir da separação seletiva de elementos no sangue por centrifugação com força centrífuga relativa muito baixa e surge como uma possível alternativa no tratamento de lesões músculo-tendíneas por concentrar moléculas terapeuticamente funcionais. O objetivo deste estudo foi verificar a concentração obtida de leucócitos e plaquetas na matriz segundo o método de centrifugação proposto (protocolo fibrin) e correlacioná-lo à condição clínica funcional em pacientes submetidos à aplicação desta matriz. De acordo com o protocolo, a hemocitometria foi realizada com amostras anticoaguladas de sangue dos 05 pacientes recrutados. A MP-PRF, obtida de amostras não-anticoaguladas, foram aplicadas em lesões músculo-tendíneas com mais de 30 dias de evolução em 14 participantes. Para avaliação funcional, foram utilizadas as escalas validadas DASH e LEFS. A concentração de plaquetas de 119,35% foi maior que a do sangue total. Dos 14 pacientes selecionados, 13 eram atletas e apresentaram melhora funcional significativa três semanas após a intervenção. Nenhuma complicação resultou da resposta. As infiltrações melhoraram o estado clínico funcional dos pacientes. Não houve casos de complicações, revelando que o procedimento é seguro. Novos estudos clínicos prospectivos são necessários para fortalecer essa nova modalidade terapêutica.

Palavras-chave: Fibrina Rica em Plaquetas; Doenças Musculares; Cicatrização de Feridas.

INTRODUCTION

The incidence of muscle-tendon injuries is one case per 10,000 people. It is believed that about 30 to 50% of the injuries related to some activities have muscular and tendinous origin, and in some cases, they demand a delicate approach and treatment (DIETRICH *et al*., 2015; MISHRA; WOODALL; VIEIRA, 2009).

Complete or partial ruptures of muscle and tendon structures are frequent indications for surgery, which leads to a long period of immobilization and recovery, in addition to causing a significant impact on health costs. In this scenario, studies of regenerative medicine seek to improve and accelerate the repair and regeneration of muscle-tendon injuries (MTI) and to explore the promising role of platelets in tissue regeneration (MISHRA; WOODALL; VIEIRA, 2009; DIETRICH *et al*., 2015).

Since the second half of the 1990s, there has been an expansion of therapeutic approaches using platelet rich plasma (PRP), which is a methodology for non-transfusion and autologous use of a selective fraction of platelet-enriched autologous anticoagulated human blood. Over the past few decades, new methods have emerged that have made it possible to obtain non-anticoagulated platelet concentrates for injectable therapeutic applications. The second generation of blood concentrates gave rise to platelet-rich fibrin (PRF), which can be obtained during the polymeric phase in gel and liquid, or during the

monomeric phase through coagulation delay (MOURÃO *et al*., 2015; CHOUKROUN and GHANAATI, 2018; de OLIVEIRA *et al*., 2020a; PETRONILHO *et al.*, 2022).

The platelet-rich fibrin in a monomeric phase (MP-PRF), as well as its content, is added to mononuclear leukocytes by differential centrifugation through a low-speed blood concentration gradient. The increase in the number of platelets and mononuclear cells in this matrix provides supraphysiological concentrations of angiogenic growth factors and repair stimulators that enhance the healing physiology, as they also assist in the regeneration of various tissues (YUNG *et al*., 2017). Because it is obtained in an autologous manner, it reduces the chances of immune-mediated adverse reactions that may occur in allogeneic grafts, thus being a viable, effective, and low-cost option in regenerative procedures (MOURÃO *et al*., 2015; CHOUKROUN e GHANAATI, 2018; de OLIVEIRA *et al*., 2020b).

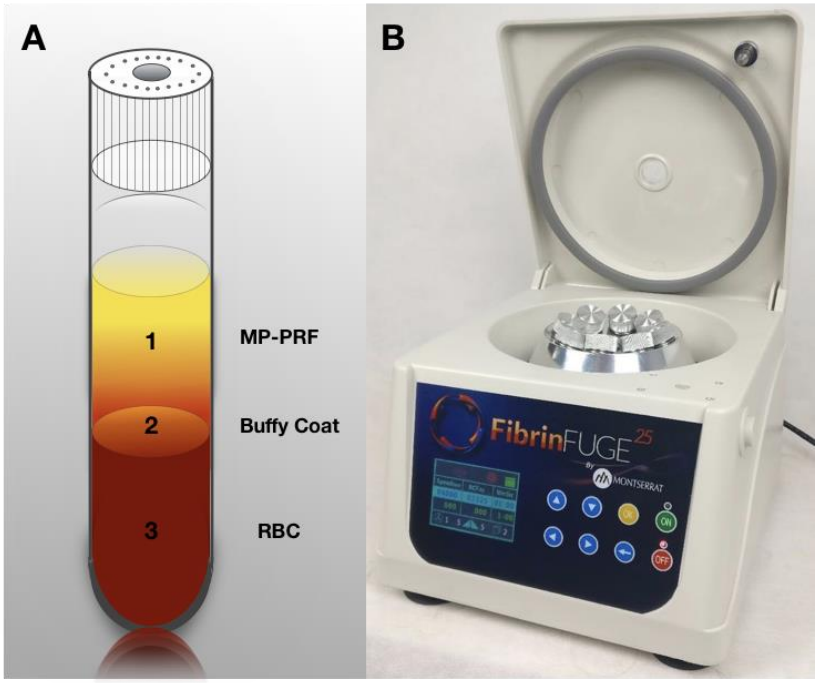
This study aims to verify the leukocytes and platelet count in the MP-PRF obtained by centrifuged autologous blood at 150 x g for 5 minutes and to evaluate its clinical effectiveness in patients with MTI undergoing the injectable application of this matrix.

MATERIAL AND METHODS

The present study consists of an interventional and prospective clinical piece of research of the case series type associated with the previous laboratory study of flow cytometry methodological validation on cell and platelet concentrations.

The laboratory test was performed by hemocytometry using the automatic hematology counter coupled to the peroxidase reaction staining method for leukocyte differentiation ADVIA 2120 Hematology System (Siemens, Germany) with anticoagulated peripheral blood samples from five patients drawn from the sample universe of this study. The piece of equipment was calibrated according to the hematological standards recommended by the manufacturer for erythrocyte, differential leukocyte series and platelet count. The samples were centrifuged in the FibrinFuge25 centrifuge (Montserrat, Brazil) according to the experimental proposal of Fibrin Protocol (OLIVEIRA *et al*., 2018), which uses the relative centrifugal force (RCF) of 150 x g for 5 minutes, figure 1b.

Figure 1. A. To laboratory phase the samples were collected in EDTA tubes to allow reading on the flow cytometer. After centrifugation, the samples showed fractions stratified by density differential. For clinical experiments, samples were collected in tubes without additives; B. FibrinFuge25 Centrifuge.



Source: Authors, 2022

Fourteen patients who were seen at the Shoulder, Knee and Sports Clinic (Instituto Balsini) located in Joinville, a city in Santa Catarina State – Brazil, between June and September 2019 were voluntarily recruited. They had muscle and/or tendon injuries in the upper or lower limbs with more than 30 days of evolution and had not undergone any surgical procedure to treat that injury or any previous orthopedic treatments. Participants who dropped out at some point in the research were excluded.

All patients with lesions in the lower limbs were examined before and after treatment, and the data were used to complete the lower extremity functional scale (LEFS). This scale is self-applied and composed of twenty items, each with a minimum score of four points and a maximum score of eighty points, which means a normal functional state (PEREIRA *et al*., 2013).

In cases of changes in the upper limbs, the disabilities of arm, shoulder and hand (DASH) scale was used. This self-administered questionnaire (SAQ) contains thirty items designed to measure functional capacity and restrictions on leisure, work and sports activities. The items are graded according to the difficulty of accomplishment; the score

ranges from 0 to 100, and it is calculated using previously established formulas. The higher value indicates greater functional disability (ORFALE *et al*., 2005).

The MP-PRF samples in the liquid phase for clinical applications were prepared according to the Fibrin centrifugation protocol proposed by OLIVEIRA *et al*, 2018. Under antisepsis precautions, autologous peripheral venous blood was collected from the antecubital fossa of each patient in sterile polyethylene vacuum tubes without additives and with 9 mL capacity (Greiner Bio-One, Brazil). The tubes were centrifuged with RCF of 150 x g for 5 minutes. After centrifugation, the supernatant fraction of the approximately 4mL tube was aspirated. Concomitantly, antisepsis and anesthesia were performed with 2% xylocaine without a vasoconstrictor at the injury site. Under the effect of anesthetic, MP-PRF was injected over the entire injured area.

The patients were instructed to keep the injured area at rest for 48 hours after the procedure and to use simple analgesic medication and cryotherapy twice a day, if necessary. Progressive return to doing physical activities with reduction in load and training volume was oriented in the three weeks following the intervention.

After three complete weeks, the patients were reevaluated and the validated assessment scale previously used was reapplied, according to the injury site. The use of such an interval was based on the process of regeneration and remodeling of muscle fibers, which occurs between two and three weeks after treatment (PADULO *et al*., 2016; SANTOS *et al*., 2016).

This study followed the criteria of the Brazilian CNS Resolution nº 196/1996 and its complementary ones from the National Health Council of Brazil, submitted to the analysis of the Research Ethics Committee at Hospital Municipal São José in Joinville – Santa Catarina, Brazil, approved under nº 2818186/2018. It was performed in accordance with the ethical standards of the Journal (EHRENFEST *et al*, 2012).

RESULTS

The cell count included the total erythrocyte counts, total and differential leukocyte counts and platelet counts in whole blood, both in the sediment and in the supernatant fraction obtained after centrifugation.

The RCF of 150 x g for 5min generated a total volume of 4.2 mL of MP-PRF. In the sediment, the erythrocyte concentrations were equivalent to those of the whole blood,

as well as its expressive reduction in the supernatant fraction. In MP-PRF, supraphysiological concentrations of platelets were found, corresponding to an increase of 119.35% in relation to the concentration found in the whole blood. The total leukocyte count showed a drop of 67.4%. The differential count showed that the smallest drop was that in lymphocytes (25.1%) and the highest one was that in granulocytes (55.7%), as shown in table I.

Table 1. MP-PRF hematological Indices

Hematological Indices (x 10^3 cells/µL)	Total Blood	PRF-MP
Platelets	263.50	578.33
Leukocytes	8.45	5.70
Neutrophils	5.22	2.91*
Lymphocytes	2.67	2.00
Monocytes	0.50	0.46

Data are mean and standard deviation (n = 5) for each hematological index counted by flow cytometry and peroxidase cytochemistry in the sediment and in the PRF-MP after centrifugation (supernatant) of 9mL samples of whole blood to RCF 150 x g for 5 minutes. (*) significant reduction in granulocyte concentrates, p> 0.05 Anova.

Source: Authors, 2022

In the present study, 14 patients received experimental therapeutic intervention, ten of whom had lesions on the lower limbs and four had lesions on the upper limbs. Out of the total number, thirteen were male and one was female. The average age in the total sample was 37.5 years. The average duration of injuries was 3.6 months, ranging from one to six months. Among the patients, thirteen did regular physical activities with a frequency of up to four times a week. It is noteworthy that there was no loss of follow-up of patients in this study.

Regarding the patients with lower limb injuries (table II), nine were male and one was female. The average age was 37.4 years, ranging from 18 to 47 years. Among sports, four did running, two did jiu-jitsu, two played football and two did triathlons. The lesions were located as follows: six in the thigh region, three in the calcaneal tendon and one in the patellar tendon. The average duration of the injury was 3.5 months, ranging from one to six months.

According to data presented in table II, the initial score of the validated questionnaire that was applied ranged from 32 to 76. Three weeks after the intervention, the score ranged from 39 to 80 (with 80 being the maximum possible). Only one of the patients worsened in his injury, thus decreasing eight points. All the others showed clinical and functional improvement. Three patients showed complete improvement, obtaining the maximum score on the scale.

Table II. Characteristics of patients analyzed with TMI in lower limbs seen in Joinville – SC, Brazil.

Patient	Sex	Age	Sport	Injury site	Time of lesion (months)	Initial LEFS* score	Final LEFS* score (after 3 weeks)
1	F	50	Running	Right calcanea tendon	6	69	74
2	M	44	Jiu-jitsu	Back right thigh	2	35	80
3	M	47	Triathlon	Back right thigh	1	42	78
4	M	33	Jiu-jitsu	Previous left thigh	2	63	72
5	M	32	Soccer	Medial left thigh	6	45	77
6	M	37	Running	Left calcanea tendon	1	76	80
7	M	34	Running	Right patellar tendon	6	47	39
8	M	39	Running	Right calcanea tendon	3	32	80
9	M	18	Soccer	Previous left thigh	3	60	75
10	M	40	Triathlon	Back left thigh	5	63	78

*LEFS - lower extremity functional scale
Source: Authors, 2022

Regarding the four patients with upper limb injuries, all were male. Their average age was 38 years, ranging from 29 to 49 years. Of those analyzed, three did physical activities, two of whom did jiu-jitsu and one did bodybuilding. The injury site was varied, two on the lateral side of the triceps brachii muscle and two on the right lateral epicondylitis. The average injury period was four months, ranging from one to six months.

The validated DASH scale used is subdivided into three areas: general functionality, functionality in sport and functionality at work. The data of the patients approached in this group are shown in table III. All of them presented a decrease in the final score of each evaluated area.

Table III. Characteristics of patients analyzed with TMI in upper limbs seen in Joinville - SC, Brazil.

Patient	Sex	Age	Sport	Injury site	Time of lesion(months)	Initial DASH* score	Final DASH* score (after 3 weeks)
1	M	41	Jiu-jitsu	Lateral face of triceps brachii	3	General – 9.16 Sports– 75 Work – 18.75	General – 2.5 Sports - 43.,75 Work - 0
2	M	33	Jiu-jitsu	Right lateral epicondylitis	1	General – 27.5 Sports – 81.25 Work - 0	General – 4.16 Sports – 37.5 Work - 0
3	M	29	Bodybuilding	Lateral face of triceps brachii	6	General – 30.83 Sports - 100 Work - 0	General – 10.83 Sports – 12.5 Work - 0
4	M	49	*	Right lateral epicondylitis	6	General – 42.5 Sports - * Work - 0	General – 30.2 Sports - * Work - 0

*DASH - disabilities of arm, shoulder, and hand scale.
Source: Authors, 2022

DISCUSSION

The monomeric phase presents a temporary liquid form. The MP-PRF is a blood concentrate obtained by very low relative centrifugal force centrifugation in a single step without chemical additives. The method does not require the use of additional anticoagulation because the centrifugation takes place in a polyethylene terephthalate (PET) tube free of silicon oxide (SiO2), which slows down the activation of the coagulation system (OLIVEIRA *et al*., 2020b).

The laboratory experiment showed that in the centrifugation of blood using tubes that are free of additives, the short period of time and the low RCF led to obtaining a resulting blood supernatant that is free of erythrocytes; with reduced concentrations of granulocytes; increased concentrations of platelets and mononuclear leukocytes; and which presented a temporary liquid form, thus allowing for workable time for its injectable therapeutic application (KHRISTE and TARI, 2013). This blood concentrate can be understood as an autologous biomaterial that intensifies the processes of intercellular communication due to the growth factors released by platelets and leukocytes in the recipient tissue (CALOPRISCO *et al*., 2010). These properties provide acceleration in healing and tissue regeneration (KARDE *et al*., 2017).

The present study, in its preclinical approach by experimentation with flow cytometry, demonstrated that the centrifugation methodology was efficient to concentrate platelets and mononuclear cells in a temporarily liquid matrix, allowing for its injectable therapeutic application. Such experimental conditions associated with the fact that this is a low cost autologous biomaterial obtained by simplified minimal processing in a sterile environment with low risk of contamination served to increase the expectation of improvement of the translational clinical response in tendon injuries.

Studies by CALOPRISCO *et al*. (2010), demonstrated that the enrichment of therapeutic blood matrices, such as PRP and PRF, with mononuclear leukocytes, increased their effectiveness in the clonogenic assay of undifferentiated CD34+ cells, as their data pointed to a strong therapeutic potential in injured tissues. It is also believed that circulating stem cells and endothelial cells are present in these matrices, so it can be considered a blood concentrate and not only platelets (ZUMSTEIN *et al.*, 2014; KARDE *et al.*, 2017).

Platelet-rich Plasma (PRP) is obtained through a sensitive methodology that can present sanitary weaknesses by opening the system, double centrifuging and adding chemical agents to control clotting time. It also presents an early and intense release of growth factors, while MP-PRF trap them in its bed for a longer period (7-21 days), making the environment favorable to accommodation, chemotaxis, migration, and cell signaling, thus inducing early fibroblast migration and intensity by the synergistic action of different growth factors of leukocytes and platelet origin materials (DIETRICH *et al*., 2015; OLIVEIRA *et al*., 2020b; KARDE *et al*., 2017).

Tendon injuries promote changes in repair and anomalous tissue regeneration. Types I and III collagens are the main components of the extracellular matrix in tendons, corresponding to 65-95% and 10% of the composition, respectively. Type III collagen synthesis increases during the early repair stages, and it is believed that, when it decreases, type I collagen is synthesized and organized (DIETRICH *et al*., 2015).

In a clinical study, Dietrich and his collaborators compared the clinical effect of MP-PRF and PRP. At 14 and 28 days, the group that received MP-PRF showed a significant difference between the areas of types I and III collagen, obtaining tissue organization similar to normal tendon conditions. The group undergoing the application of PRP did not show significant improvement in the same observation period, thus suggesting that MP-PRF has a tendency to improve and accelerate the cure when compared to PRP (DIETRICH *et al*., 2015).

The study by ZUMSTEIN *et al.* (2014), observed an improvement in vascularization, a higher cellular response, and a higher rate of healing of patients with chronic involvement in rotator cuff tendons when compared to patients who did not receive treatment with PRF. An improvement in the pain test and a simple shoulder test score also showed an improvement in pain and early function in the group with PRF.

Tendons and ligaments have a longer healing time than other tissues due to their sparse vascularization. Furthermore, in a healing process, new tissues may not have the same structural and functional pattern since poor vascularization leads to an inadequate release of growth factors and cell healing at the injury site (ABD *et al*., 2019).

In this study (CALOPRISCO *et al*., 2010), patients showed improvement in their functionality after three weeks of follow-up. Regarding the lower limbs, three patients showed maximum functional improvement. Such data corroborate with the findings in the support literature.

Most of the patients undergoing the application played sports regularly and this significant improvement in functionality allowed them to return to their activities early. In this perspective, the use of fibrin in the liquid phase was presented as a source of growth factors and healing cells, thus improving the speed and quality of the healing process of these tissues (ABD *et al*., 2019).

The development of different physical activities by the participants, as well as their rest time and the way to return to their activities were limiting factors in the evaluation of the results. Only one of the patients that was followed up showed clinical worsening during the follow-up period, but he did not stick to the guidelines for rest and progressive return to his activities. It is noteworthy that none of the patients had local reactions or had the need for other medical interventions at the site.

It is important to mention that lack of specific protocols for application time, follow-up and unguided application may have been limiting for the evaluation of results. Therefore, we suggest that comparative studies are performed to seek for more knowledge about the effectiveness of the proposed methodology in different clinical settings, as well as for performing ultrasound-guided infiltrations with the aim to improve the quality of the results found.

The use of autologous platelet aggregates is an innovative reality in medical procedures, and it has brought hope to the treatment of muscle and tendon injuries (MOURÃO *et al*., 2015; MISHRA; WOODALL; VIEIRA, 2009). This material can be an interesting therapeutic option, as it is easy to obtain and to apply at low cost. Therefore, it is one more option that fits the reality of the Brazilian population.

CONCLUSION

This study presented the cell concentration and demonstrated the therapeutic potential of MP-PRF in TMI. MP-PRF infiltrations provided an improvement in the patients' functional clinical status. Thus, its clinical use within this context presents itself as a viable, effective, and low-cost option. Therapies using autologous MP-PRF represent a safe strategy of simplified and convenient use with significant regenerative potential. However, new prospective clinical studies are needed to strengthen this therapeutic modality in TMI.

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