



## Original article

# The effect of platelet-rich plasma on the repair of muscle injuries in rats<sup>☆</sup>



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### ABSTRACT

**Objective:** The need for therapeutic options for muscle injuries, which are increasingly frequent among sports practitioners, was the motivation for this experimental study, which had the aim of evaluating the histological effects of platelet-rich plasma (PRP) on repairs to muscle tissues of rats.

**Methods:** PRP was obtained by means of double centrifugation of blood from five animals. In 30 rats, an injury was produced in the middle third of the belly of the gastrocnemius muscle of each hind limb. These injuries did not receive any treatment in six rats (12 legs). In 24 rats, 0.9% physiological serum was injected into the injury in the left leg and PRP into the injury in the right leg. Samples from the treated and untreated tissue were evaluated histologically 7 and 21 days after the procedures.

**Results:** The quantity of collagen in the injuries treated with PRP was significantly lower than that in the other injuries, in the evaluation made 7 days after the procedure, but it became equal to the other groups in the evaluation done on the 21st day. There was a significant increase ( $p < 0.001$ ) in the quantity of collagen from the 7th to the 21st day in the injuries treated with PRP, but this was not seen in the injuries treated using other methods. The inflammatory process was shown to be more intense in the injuries treated with PRP than in the injuries of the other treatment groups, in the evaluation done 7 days after the procedure. However, the morphological aspects of these injuries were seen to be similar to those of the untreated injuries, 21 days after the procedure.

**Conclusion:** PRP promoted complete tissue restitution between the 7th and 21st days in experimental muscle injuries.

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## O efeito do plasma rico em plaquetas no reparo de lesões musculares em ratos

### R E S U M O

**Palavras-chave:**  
Músculos/lesões  
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Ratos

**Objetivo:** A necessidade de opções terapêuticas para lesões musculares, cada vez mais frequentes entre os esportistas, fundamentou este estudo experimental, cujo objetivo foi avaliar os efeitos histológicos do plasma rico em plaquetas (PRP) no reparo do tecido muscular de ratos.

**Métodos:** O PRP foi obtido por dupla centrifugação do sangue de cinco animais. Em 30 ratos, foi produzido um trauma no terço médio do ventre do músculo gastrocnêmio de cada membro traseiro. Essas lesões não receberam tratamento em seis ratos (12 patas). Em 24 ratos, injeções intralesionais de soro fisiológico a 0,9% e de PRP foram aplicadas nas patas esquerdas e direitas, respectivamente. Amostras do tecido tratado e não tratado foram avaliadas histologicamente sete e 21 dias após os procedimentos.

**Resultados:** A quantidade de colágeno nas lesões tratadas com PRP foi significativamente menor do que a das demais lesões na avaliação feita sete dias após o procedimento, mas se equiparou à dos demais grupos na avaliação feita no 21º dia. Houve aumento significativo ( $p < 0,001$ ) na quantidade de colágeno do sétimo para o 21º dia nas lesões tratadas com PRP, o que não ocorreu nas lesões tratadas de outra forma. O processo inflamatório se mostrou mais intenso nas lesões tratadas com PRP em comparação com as lesões dos outros grupos de tratamento na avaliação feita sete dias após o procedimento; todavia, os aspectos morfológicos dessas lesões se mostraram similares ao das lesões não tratadas 21 dias após o procedimento.

**Conclusão:** O PRP promoveu completa restituição tecidual entre o sétimo e o 21º dia em lesões musculares experimentais.

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## Introduction

Muscle injuries are defined as morphological or histochemical alterations that cause dysfunction of the locomotor system.<sup>1</sup> They can be caused by two mechanisms: direct trauma such as bruises and lacerations, and indirect trauma such as ischemia, denervation and strain.<sup>2</sup>

Approximately 30% of injuries diagnosed by doctors are related to the muscle system,<sup>3</sup> and muscle injury is one of the most common forms of trauma that occur during sport practice, causing 10–55% of all injuries.<sup>2</sup> Sport injuries appear to be a result of exercises performed in a strenuous, inadvertent or inappropriate manner. The prevalence and incidence of these episodes are underestimated because of the absence of notifications within the world of sports.<sup>4</sup> The reported incidence of injuries to hamstring muscles is of the order of 12% in soccer players,<sup>5</sup> 50.9% in sprint athletes<sup>6</sup> and 42% in breast-stroke swimmers.<sup>7</sup>

Depending on the severity and location of the injury, different forms of therapeutic management are used, from conservative and drug treatment to surgical treatment.<sup>8</sup> Except for cases of complete muscle tearing, avulsion and myositis ossificans, the standard treatment used for acute muscle injuries consists of resting, protection, ice, compression and elevation. Beyond these principles, there is no clear consensus about treatments for acute muscle injuries.<sup>9</sup> Thus, questions still remain, especially regarding the effects and

results of various commonly used treatments for stimulating the process of muscle repair.

Platelet-rich plasma (PRP) is a product from autologous blood that, since 1990, has been proposed for treatments because it promotes strong stimulation to tissue repair.<sup>10</sup> It is obtained through centrifugation of peripheral blood and the platelet concentration should ideally be higher than 338%, in comparison with that of the peripheral blood.<sup>11</sup> PRP has healing properties that have been attributed to the increased concentrations of autologous tissue growth factors and proteins at cellular level. These factors, when introduced to the area of the injury, are expected to increase recruitment, proliferation and differentiation of cells involved in tissue repair, and to promote accelerated repair with better tissue differentiation.<sup>12</sup>

Various clinical uses of PRP have been studied, including the repair of chondral<sup>13</sup> and tendon injuries,<sup>14</sup> repair of injuries and bone regeneration,<sup>15</sup> and treatment of plantar fasciitis<sup>16</sup> and severe diabetic foot ulcers.<sup>17</sup> The repair of chronic Achilles tendinopathy with intralesional injection of PRP has shown promising results from histological and morphological evaluation of the neoformed tissue.<sup>18</sup> Both experimental and clinical studies have revealed the effects of intralesional injection of PRP in muscle injuries and, generally, these studies have reported better muscle regeneration, increased neovascularization and reduced fibrosis.<sup>19–25</sup>

In view of the growing incidence of muscle injuries and the need for therapeutic options that promote faster and more

**Table 1 – Platelet quantification ( $10^3/\mu\text{l}$ ) observed in the four blood samples extracted from five rats for preparation of PRP.**

	Samples			
	1	2	3	4
Blood before preparation	236	214	250	249
Platelet-rich plasma	1.195	808	928	1.145
Platelet-poor plasma	148	141	138	182

effective muscle regeneration, the present experimental study was performed in order to ascertain the effect of homologous PRP on repairs to muscle lesions that were induced in rats through applying an impact. This study evaluated collagen deposition and made a qualitative morphological analysis on the tissue repair process, under a microscope, 7 and 21 days after treatment.

## Material and methods

This study was approved by the Ethics Committee under the protocol 334/2011. The experiment was conducted in a laboratory from April to July 2012. Thirty-five isogenic male Wistar rats (*Rattus norvegicus albinus*) of EPM-1 lineage from the central vivarium of the Federal University of Mato Grosso do Sul were used. The rats were 12 weeks old and their mean weight was  $320 \pm 20$  g.

Initially, over a 30-day period, the animals underwent a period of adaptation and weight gain, during which they were kept in standard boxes for five animals, made of polypropylene and with a galvanized metallic lid. The environment was climate-controlled, with a temperature of  $22 \pm 3^\circ\text{C}$ , artificial lighting with 12-h light/dark cycles and air humidity of  $56 \pm 13\%$ . The animals were fed with Nuvilab<sup>®</sup> CR1 feed (Nuvital Alimentos e Produtos Veterinários Ltda<sup>®</sup>, Curitiba, PR, Brazil) and filtered water, *ad libitum*.

Four study groups were formed randomly:

- Group 1: five rats underwent blood sampling in order to prepare PRP;
- Group 2: the left legs of 24 rats whose muscle injuries were treated with 0.9% physiological saline solution.
- Group 3: the right legs of 24 rats whose muscle injuries were treated with PRP.
- Group 4: the right and left legs (12 legs) of six rats whose muscle injuries were not treated.

### Protocol for producing the experimental injury

The device and the technique used for producing the experimental injuries were the same as described by Nogueira.<sup>26</sup> For creating muscle injuries, the device developed by Sene was used,<sup>27</sup> which consists of two adjustable telescopic metal rods, through which is possible to mark out a height of 30 cm, and a plastic base of area  $272.5\text{ cm}^2$ . A rectangular metallic surface of area of  $12.25\text{ cm}^2$  was attached to this base. This surface served as a support for dropping the weight and for attaching the animal's hind leg in a predetermined place, thus concentrating the weight in the central area of the leg. A metal structure was attached to the upper end of the metal rods, in order to provide stability and to hold a pulley wheel across

which a guidewire held the weight that was to be released. Transparent acrylic channeling was set up between the rods to guide the weight during the 30 cm freefall, in order to avoid deviation and oscillation of the weight.

The device for producing injuries through impact was fixed with clips to the surgical table in order to stabilize it in such a way as to avoid any oscillation as the weight dropped. In order to ensure that the injury would occur in the same area, the load released from 30 cm high was channeled by the acrylic guide and by a wire attached directly to the weight, which was released centrally by means of a pulley wheel that was placed on the rods of the device.

The animals were previously anesthetized with ketamine (60 mg/kg) and xylazine (15 mg/kg) and then underwent a single traumatic event in each limb, in the middle third of the belly of the gastrocnemius muscle and were separated according to the experimental group to which they would belong. The 24 animals whose legs comprised groups 2 and 3 underwent contusion injuries in their hind legs. In the central posterior area of the left legs, 0.1 ml of 0.9% saline solution was administered and, in the right legs, 0.1 ml of PRP.

### Preparation and application of the platelet-rich plasma

Cardiac puncture was performed using a BD needle (22 g  $\times$  1"; 0.70 mm  $\times$  25 mm) attached to a 20 ml disposable syringe (Viet Jet<sup>®</sup>; Labor Import Comércio, Importação e Exportação Ltda., Osasco, SP, Brazil) with 1 ml of 10% sodium citrate (Bioclin<sup>®</sup>; Quibasa Ltda.; batch 0067/2011). Four samples of 8 ml of blood with anticoagulant were obtained from the five rats that comprised group 1. The blood with anticoagulant immediately underwent cell counting in an automated device (Sysmex XE-2100D). After the first centrifugation, the plasma was separated from the red blood cell concentrate. During the second centrifugation, the supernatant portion was eliminated, such that only approximately 1 ml of the heavier centrifuged material remained. This fraction was called the PRP or platelet concentrate. The homogenized PRP and the platelet-poor plasma underwent automated cell counting again, as shown in Table 1.

### Sacrifice

After blood sample collection (group 1) and the 7 and 21-day post-injury period used for evaluation (groups 2, 3 and 4), the animals were sacrificed through lethal injection of a solution of a combination of S(+) ketamine hydrochloride (Cristália Produtos Químicos e Farmacêuticos Ltda., Campinas, SP), sodium thiopental (Cristália Produtos Químicos e Farmacêuticos Ltda., Campinas, SP) and sterile powder diluted in 0.9% physiological solution of sodium chloride, at a concentration of 100 mg/ml.

After dissection and muscle sample collection for analysis, the animals were discarded through incineration in an appropriate environment.

### Forms of evaluation

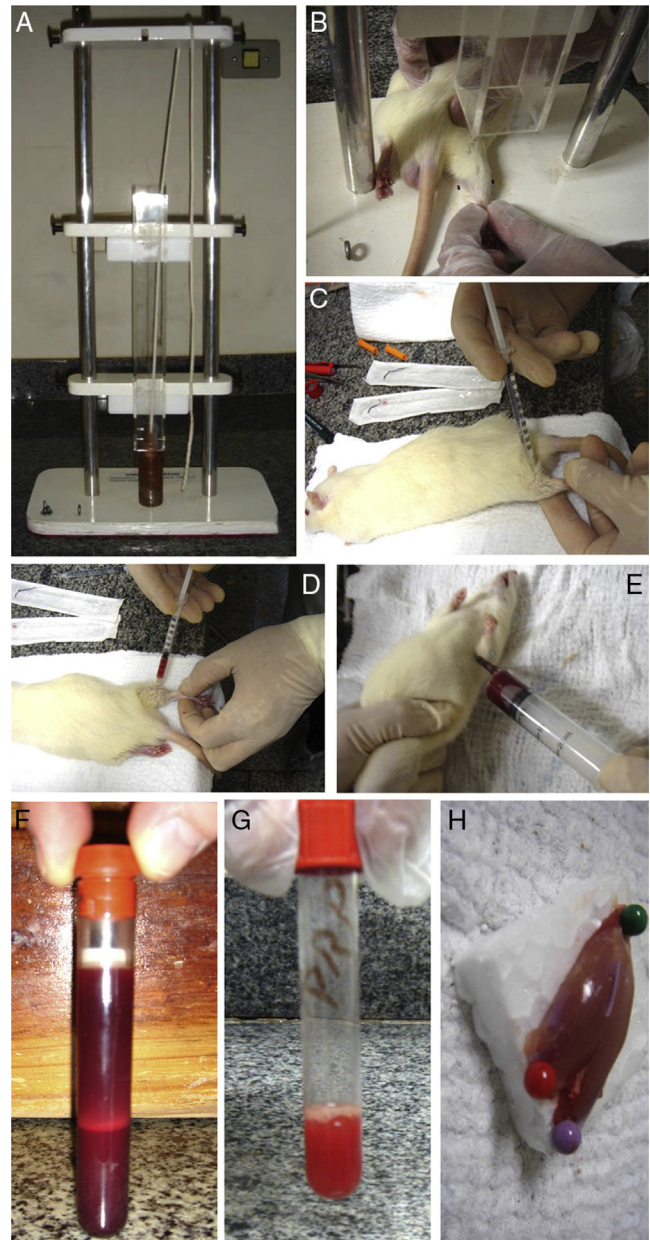
The gastrocnemius of each animal was removed through posterior incision in the hind legs of the animals in ventral decubitus position, with blunt dissection of skin and soft tissues. Muscle integrity was preserved, with maintenance of the origin and the insertion (femur–muscle–calcaneus). The pieces were attached to a solid surface using pins and were stored in 10% formaldehyde. They were sent for histological analysis, in which they received routine treatment consisting of progressive dehydration in alcohol, inclusion in paraffin blocks and cutting of sagittal and longitudinal sections of thickness 5  $\mu\text{m}$  using a microtome (in the central third of the muscle belly). The slides thus produced were stained with picosirius red and hematoxylin eosin (HE). Examination of the histological characteristics and quantity of collagen was performed in the Toxicology and Medicinal Plant Laboratory of Anhanguera University (UNIDERP).

Using an optical microscope coupled to a computer, the images of the slides were digitized and captured by an image processing and analysis system (ImageLab™). This system was developed for morphometric analysis and image subtraction and can be used for specimens at both macroscopic and microscopic scale. There are many unit conversion systems, image correction filters, exportation formats and means of communication with other software. On the computer screen, the system presents the original image digitized from the histological slide and, alongside this, three frequency histograms showing the image intensities R (red), G (green) and B (blue). From the representation of these histograms, the system calculates the desired quantifications. All the data relating to these calculations are presented in a spreadsheet within this software, which can be converted into a spreadsheet of the Microsoft Office Excel 4.0 software.

The main instruments and procedures of the study are shown in Fig. 1.

### Statistical analysis

The data were tabulated in spreadsheets in the Microsoft Office Excel (2010) software and the normality of the samples was evaluated using the Bioestat 5.0 software. Calculations for comparing the data and producing graphs were made through the GraphPad Prism 4.0 software. The measurements of the numerical variables were expressed as means  $\pm$  standard deviations. Intragroup comparisons were performed using the Student t test on the samples with normal distribution and the Wilcoxon test on those of non-normal distributions. Inter-group analyses were performed using analysis of variance (ANOVA) and the *post hoc* Tukey test on samples with normal distribution and the *post hoc* Dunn test on samples with non-normal distribution. The normality of the groups was evaluated through the Shapiro–Wilk test. The value of  $p \leq 0.05$  was adopted for determining the significance level of the differences found.



**Fig. 1 – (A) Device used for producing muscle injuries in animals. (B) Position of the load released on each limb of the animals. (C) Administration of saline solution in the posterior area of the left leg of the animals. (D) Administration of PRP in the posterior area of the right leg of the animals. (E) Cardiac puncture to obtain blood samples for preparing PRP. (F) Preparation presenting a liquid column with two predominant fractions: supernatant plasma and red blood cell concentrate at the bottom, separated by a buffy coat. (G) Preparation containing only approximately 1 ml of the heavier centrifuged fraction (PRP), after elimination of the supernatant portion. (H) Sample of whole gastrocnemius attached to a solid surface and stored in 10% formaldehyde for histological analysis.**

**Table 2 – Means (and standard deviations) of the quantification of collagen fibers observed in the legs of the control rats and the rats that received PRP and saline solution, 7 and 21 days after muscle injury.**

	Control group n = 12	PRP n = 24	Saline solution n = 24
7 days	34.44 ± 6.65	30.69 ± 4.99	35.35 ± 5.19
21 days	38.29 ± 6.58	38.52 ± 6.47	35.02 ± 6.73

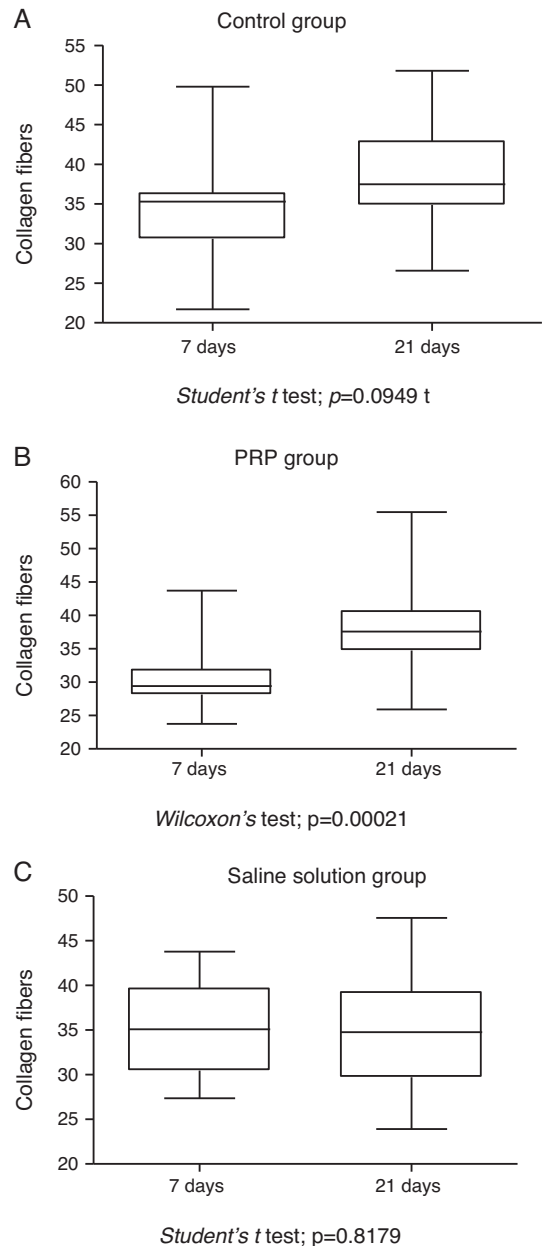
## Results

A total of 60 legs were evaluated and the mean values (and standard deviations) of the quantity of collagen fibers in each group at each evaluation time (7 and 21 days) are shown in Table 2. In the intragroup analysis, comparing the counts that were made on the 7th and 21st day after injury, there were no differences in the mean quantities of collagen fibers in the control group ( $p=0.094$ ) or in the group of rats that received saline solution ( $p=0.817$ ), as shown in Fig. 2A and C. In turn, the quantity of collagen observed 21 days after the injury was significantly greater ( $p=0.00021$ ) than the quantity observed 7 days after the injury in the group of rats that received PRP (Fig. 2B). In the intergroup analysis, in the count performed 7 days after the injury, the mean quantity of collagen fibers was significantly smaller ( $p=0.014$ ) in the rats treated with PRP than in those treated with saline solution, but not in comparison with the control group. On the other hand, in the count performed 21 days after the injury, there was no difference between the groups regarding the mean quantity of collagen fibers (Fig. 3).

Regarding the morphological findings, circumstantial macrophages were observed in the interstitial tissue of the skeletal muscle in the control group on the 7th day. The muscle fibers had normal appearance at this time. Also, myoblasts appeared among the muscle fibers (Fig. 4). At the same time (7 days), in the group of rats treated with PRP, an evident inflammatory process due to mononuclear cells, red blood cells of normal appearance and myotubes were observed among the muscle fibers (Fig. 5). In the rats treated with saline solution, myotubes, macrophages and red blood cells of normal appearance were observed (Fig. 6). At 21 days, the muscle fibers and blood vessels already presented the normal appearance of the tissue in the control rats and in the rats treated with PRP (Figs. 5 and 6). In the rats treated with saline solution, foci of macrophages and some myotube formation were observed (Fig. 7).

## Discussion

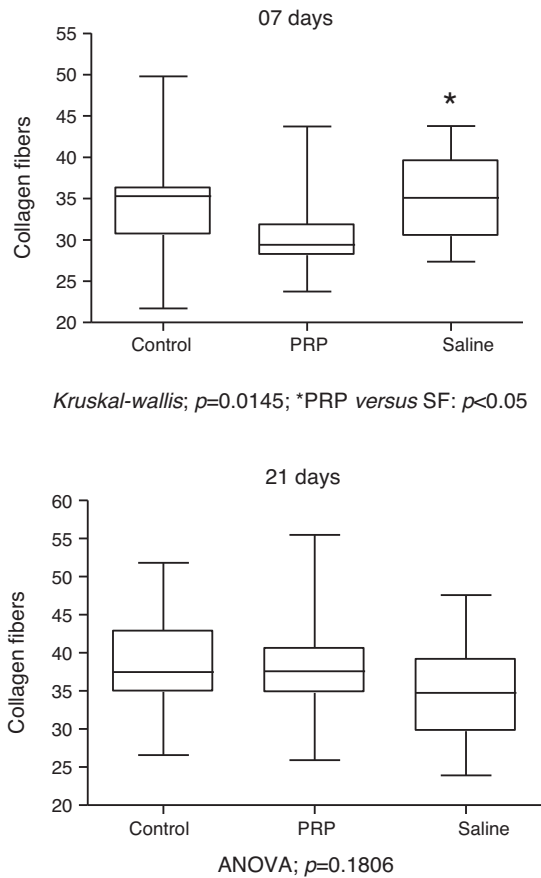
There is evidence that growth factors play an essential role in the healing process of tissues.<sup>28</sup> However, in addition to the fact that use of many growth factors separately is still impossible in clinical practice, the mechanisms of action of all the different factors involved in this process are not completely clear. From knowledge that the alpha-granules of platelets concentrate large quantities of specific growth factors such as PDGF and TGF-beta, a technique for obtaining high concentrations of growth factors through preparation of autologous PRP



**Fig. 2 – Graphic representation of the means and standard deviations of the quantities of collagen fibers 7 and 21 days after the injuries caused in the rats of the control group (A), those treated with PRP (B) and those treated with saline solution (C).**

was proposed.<sup>11</sup> This technique basically consists of sequestering and concentrating platelets from the blood plasma, which results in a product that can be applied to the injury healing area.<sup>29</sup>

Despite widespread use of PRP for treating muscle injuries in athletes and the fact that some studies have shown that PRP can shorten the time taken to return to sport activities after injury,<sup>14,19,24</sup> few experimental studies have been conducted on the standardization of what PRP is or on understanding the mechanisms involved in use of PRP, specifically in relation to muscle injuries. For this reason, little evidence



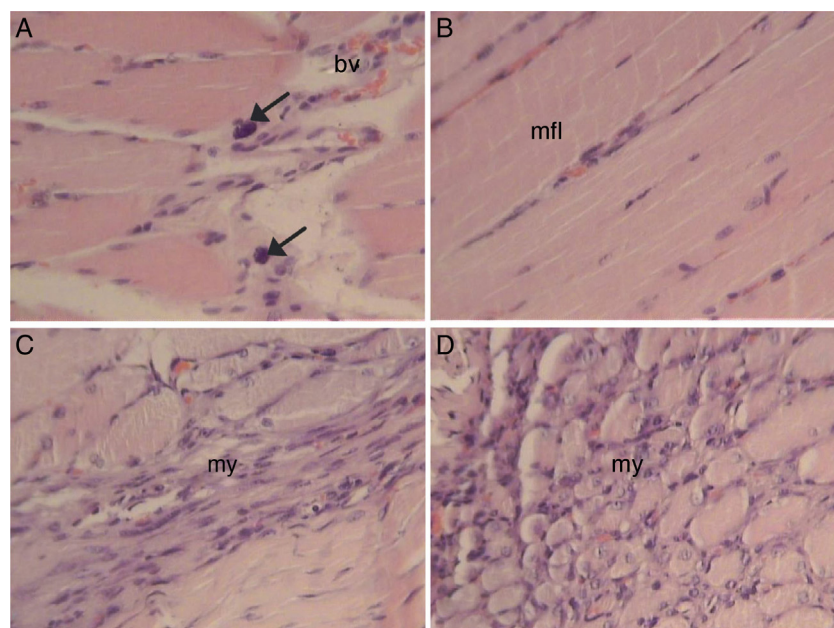
**Fig. 3 – Graphic representation of the means and standard deviations of the quantities of collagen fibers 7 and 21 days after the injuries caused in the rats of the three different study groups.**

can be discussed from the results of this study. Nonetheless, in an experimental model of bruising that was also conducted on the gastrocnemius muscle of rats, whose injuries were injected with autologous platelet-rich serum, Wright-Carpenter et al.<sup>19</sup> observed accelerated activation of satellite cells, 30–48 h after injury, and an increase in the diameter of the muscle fibers undergoing regeneration, during the first week after injury. These authors also observed increased concentrations of FGF-2 (460%) and TGF-beta1 (82%) through ELISA and suggested that these could, at least partially, have been responsible for the accelerated regeneration, due to their proliferative and chemotactic characteristics.

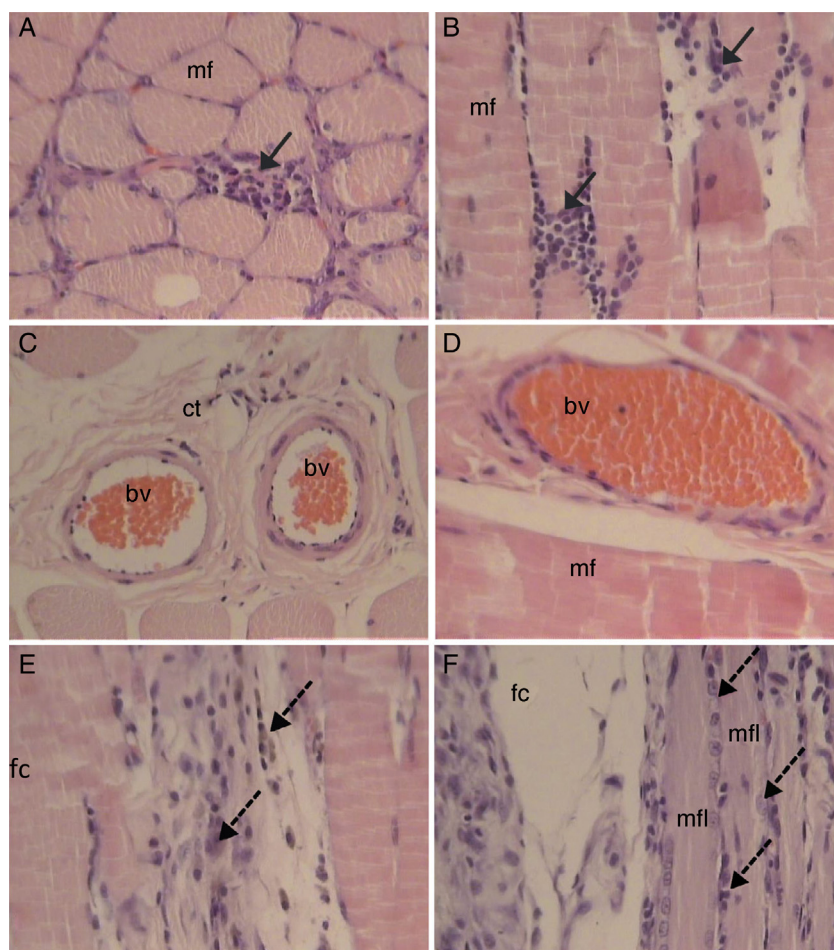
In major sprains in the anterior tibial muscle of rats treated with PRP or placebo, Hammond et al.<sup>20</sup> observed that the time taken for muscle regeneration was significantly shorter in the group treated with PRP and suggested that acceleration of myogenesis was probably the mechanism responsible for this effect from PRP, because of the higher concentrations of different growth factors in the product.

Harris et al.<sup>22</sup> injected PRP or saline solution into various muscle tissues of healthy rabbits (without injuries), and samples were histologically evaluated 2, 6 and 12 weeks after the procedure. After 6 and 12 weeks, they observed persistent but decreasing amounts of inflammatory infiltrate at sites that received PRP, but not at those that received saline solution. Likewise, they only observed fibroblasts, collagen formation and neovascularization in the focal areas of the scar tissue of the legs that received PRP. This was not observed at any site in which saline solution was injected. These authors concluded that PRP promoted an inflammatory response in normal soft tissues of rabbits. Their assertion supports the hypothesis defended by the present study, i.e. that PRP initially promotes intensification of the inflammatory process in muscle injuries.

In contrast, Gigante et al.<sup>23</sup> did not observe any difference regarding the inflammatory process when comparing injuries



**Fig. 4 – Photomicrograph of samples of the control group after 7 days. Macrophages (arrows), muscle fiber in longitudinal section (mfl), myoblasts (my) and blood vessels (bv) can be seen. HE/200x.**



**Fig. 5 – Photomicrograph of rats treated with PRP after 7 days. An inflammatory process due to mononuclear cells (continuous arrows), muscle fiber in transverse section (mf), muscle fiber in longitudinal section (mfl), myotubes (dashed arrows), blood vessels (bv), fat cells (fc) and connective tissue (ct) can be seen. HE/200 $\times$ .**

produced in the longus muscle of rats, between those treated with PRP and those that were not treated. However, better muscle regeneration, increased neovascularization and slight fibrosis reduction were observed among the treated injuries.

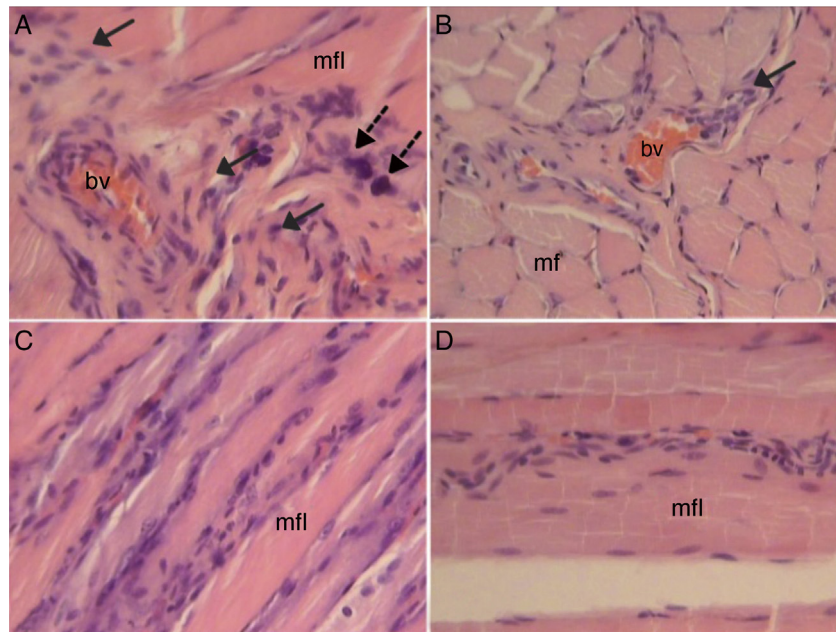
It needs to be emphasized that the concentrations of different growth factors in PRP obtained from different species (rats, rabbits, sheep and humans) present significant variation. This has a direct influence both on experimental studies and on clinical trials.<sup>30</sup> Hence, there is a need for standardized protocols in order to achieve real expansion of knowledge regarding the effects of PRP in treating muscle injuries.

The use of PRP for regenerating bones and soft tissues has been a focus of attention among clinical doctors and researchers. Its use in different surgical specialties has also been reported. Since the time of the initially proposed technique for producing autologous PRP, in which the discontinuous method of cell separation demanded a great amount of blood, several other protocols have been proposed and have contributed toward the evolution of the original technique. The advances have included lower blood volume needs, use of benchtop centrifuges, lower costs, shorter production time, easier application in outpatient settings and lower stress on the patient's cardiovascular system. The safest and most

effective protocols seem to relate to the double centrifugation techniques that were used for performing the present study.<sup>29</sup>

Some studies have shown that platelet concentrations, 338% higher than normally found in the blood, boost bone and soft tissue healing in human beings.<sup>11</sup> Other authors have maintained that the expected effects of PRP would only be reached when the platelet concentrations were eight times higher than the concentrations in the circulating blood. In our experimental study, in the four blood samples collected from five rats for producing PRP, we obtained a mean platelet concentration that was approximately four to five times higher than what was observed in the blood.

PRP produced in this manner was injected into traumatic injuries (bruises) that had been produced in the middle third of the belly of the gastrocnemius muscle of the right hind legs of 24 rats, while the same injuries in the left legs were treated with 0.9% saline solution. The findings showed that, in the group of injuries treated with PRP, the mean quantity of collagen was significantly lower than in the other groups (controls and rats treated with saline solution) in the evaluation performed on the 7th day after injury, but that there was a significant increase in this quantity of collagen from the 7th to the 21st day after injury only in the group with injuries



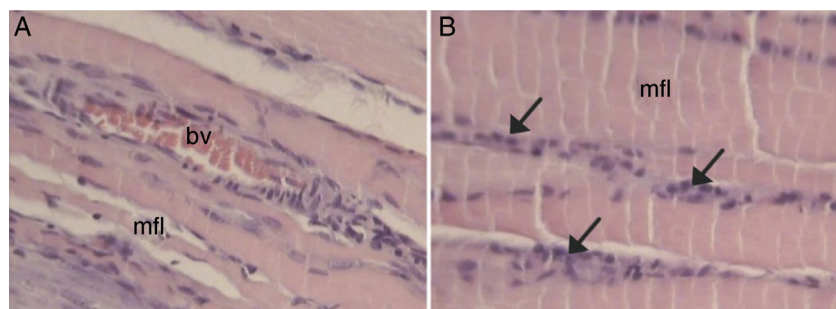
**Fig. 6 – Photomicrograph of rats treated with saline solution after 7 days. Muscle fiber in longitudinal (mfl) and transverse (mf) sections, myotubes (continuous arrows), macrophages (dashed arrows) and blood vessels (bv) can be seen. HE/200 $\times$ .**

treated with PRP, which, in this last evaluation, presented a mean quantity of collagen that was similar to that of the other groups.

Degradation of collagen is known to begin early and there is intense activity in the inflammatory process. This event occurs during the first stage of healing. In fact, apart from the injuries treated with saline solution, a lower mean quantity of collagen was observed in the evaluation performed on the 7th day after injury than in the one performed on the 21st day. However, in the first evaluation (7 days), the mean quantity of collagen was significantly lower in injuries treated with PRP than in the control injuries and in those treated with saline solution. This finding seems to ratify the theory according to which the inflammatory process is probably altered in the presence of PRP, thereby sometimes reducing the period of inflammation of the injury and sometimes altering the release of cytokines.<sup>20</sup> Thus, the results make it possible to put together a new hypothesis that can be tested, thereby suggesting that the inflammatory phase is extended or has higher intensity. This would lead to more intense

degradation of collagen in the first phase of muscle healing. However, no studies have yet tested these hypotheses more rigorously.

On the other hand, during the repair and remodeling phases, deposition of collagen in an organized and gradual manner is the most important characteristic for assuring balance between lysis of the old cell matrix and synthesis of the new matrix. This is an essential condition for successful regeneration of the injured muscle tissue. In addition, the initially produced collagen is thinner than the collagen from the healthy tissue; this initial collagen is then reabsorbed and thicker collagen is produced along the tension lines, and this is positively correlated with increases in tensile strength. In this study, the mean quantity of collagen significantly increased from the 7th to the 21st day after the injection of PRP, but this did not occur in the untreated injuries or in those treated with saline solution. The increase of collagen from the 7th to the 21st day in the animals that received PRP seems to have helped in the collagen degradation–deposition balance, through mechanisms that still need to be elucidated.



**Fig. 7 – Photomicrograph of control rats after 21 days. Muscle fibers in longitudinal section (mfl), nuclei of muscle fibers (arrows) and blood vessels (bv) can be seen. HE/200 $\times$ .**



In injuries treated with PRP, the inflammatory process was more evident than in the other groups of the study, in the evaluation performed 7 days after the procedure. This observation seems to confirm that PRP can intensify the inflammatory process.

In the final evaluation at 21 days, the morphological findings from the control injuries and the injuries treated with PRP were similar, which indicates that good regeneration of the injured muscle occurred in both groups. The administration of PRP does not seem to have shown differences in morphological features after 21 days, at the end of the muscle repair process, in comparison with the control group, since the same characteristics were observed in the untreated injuries. On the other hand, in the injuries treated with saline solution, the tissue still did not show full regeneration, with muscle fibers still undergoing formation and without vascularization.

This morphological descriptive analysis corroborates the quantitative findings regarding collagen fiber deposition in muscle scars because it shows that in the group that received saline solution alone, there was still an inflammatory reaction after 21 days, while in the other two groups, the tissue had already been completely regenerated. This perhaps suggests that there was better organization of the muscle repair process in the group treated with PRP.

The contribution of the present study is limited to the observation that PRP significantly activated the inflammatory process 7 days after being injected into blunt injuries that had been produced in the gastrocnemius muscle of rats. The reason for this seems to have been greater degradation of collagen over this period. On the other hand, this resulted in a significant increase in collagen between the 7th and the 21st day after the procedure, which suggests that full tissue recovery was achieved.

## Conflicts of interest

The authors declare no conflicts of interest.

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