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HEALTH SCIENCES

Photobiomodulation and amniotic membrane for treat tendon injury in rats

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Abstract: Tendons, complex fibrous structures, are subjected to great tensions, which can give rise to the so-called tendinopathies. This study aimed to evaluate photobiomodulation and human Amniotic Membrane applied as single or combined therapies to treat induced Achilles tendon lesions. Seventy-five rats were divided into five groups (n=15): C- control Sham surgery; I- tendon injury; LA- tendon injury treated with photobiomodulation; AM- tendon injury treated with Amniotic Membrane; LAM- tendon injury + photobiomodulation and Amniotic Membrane, subdivided into three groups (n=5) with analysis at 3, 7, and 14 days. The tendon injuries were made with a 20 g weight released from a mini guillotine onto the ankle in dorsiflexion. AM and LAM groups received an Amniotic Membrane fragment while LA and LAM groups received transcutaneous photobiomodulation, using a 660 nm wavelength laser. The inflammatory cells showed statistical differences between groups C and I ($p<0.05$), I and AM ($p<0.01$), I and LA ($p<0.05$), and I and LAM ($p<0.01$). Both photobiomodulation and Amniotic Membrane were shown to enhance tendon repair, and the association of photobiomodulation plus Amniotic Membrane was the most effective treatment. We conclude that the association of photobiomodulation plus Amniotic Membrane was effective in accelerating and improving the tendon regeneration process.

Key words: Achilles Tendon, amniotic membrane, healing, LLLT, PBM, regeneration.

INTRODUCTION

Tendons are complex fibrous structures that attach muscle to bone, transmitting the force generated by the muscles to the bone where they are inserted. Tendons are subjected to great tensions, which can give rise to the so-called tendinopathies. The tendon tissue degeneration, called tendinopathy, could result from different processes generally related to high loads and/or repetitive strain, as occurs in sports activities. Tendinopathies are especially common in the calcaneus (or Achilles), supraspinatus, and patellar tendons. Histologically, Achilles tendinopathy is characterized by a degenerative process with damage to the tendon tissue integrity. There is a loss of parallel organization of the collagen fibers and an increase in tendon thickness, due to edema, causing pain or increased sensitivity to pain (Chimenti et al. 2017).

Running activity is the one that presents the highest risk of injuries due to overuse or repetitive effort (Francis et al. 2019) and studies reported that calcaneal tendinitis represents 10% of the real total of lesions in the foot/ankle segment (Kueckelhaus et al. 2014, Andres & Murrell 2008).

Severe cases of tendinopathy can lead to partial or total tendon tears, causing areas of hemorrhage, associated with fibrin deposition and local edema. Tissue damage can progress to ischemia and tenocyte necrosis, attracting

inflammatory cells like leukocytes/neutrophils to this region. The tendon tissue is characterized by its limited vascularization, presenting an atypical inflammatory process. Histological analysis of tendinopathies reveals increased cellularity, proteoglycan deposition, and extracellular matrix degradation probably due to the action of collagenases such as matrix metalloproteinases MMP1 and MMP13 (Andres & Murrell 2008).

Tendon injury treatment is usually time-consuming and complex. Tendons are formed by dense connective tissue, which requires the correct orientation of the fibers to acquire mechanical resistance to traction. Injured tendons rarely reach the mechanical and biological properties of healthy ones, even under the best conditions (Andres & Murrell 2008).

Nonsteroidal anti-inflammatory medications, physical therapy, and surgery were some of the various therapeutic modalities used in the treatment of tendon disorders (Andres & Murrell 2008). However, a consistent and effective treatment protocol for pain reduction and tissue regeneration has not yet been definitively established.

Photobiomodulation (PBM), originally called low-level laser therapy, has been used to treat muscle-skeletal injuries for decades. Studies have reported positive results using PBM to reduce pain and improve the healing process in tendon injuries (Bjordal et al. 2001). PBM is an important tool to reduce inflammatory processes and, consequently accelerate tissue repair. Pain control, an increase in fibroblast proliferation, and a better collagen fiber organization at the injured Achilles tendons are reported after the use of PBM (Naterstad et al. 2018, Haslerud et al. 2017a, b, Marcos et al. 2014, 2012). Although the exact mechanisms of PBM in the inflammatory response may vary depending on the parameters used and specific experimental conditions, studies have shown that PBM can modulate several cellular signaling pathways, including those involved in inflammation, by improving mitochondrial function. PBM can influence signaling molecules such as cytokines, chemokines, and growth factors. Studies have reported that PBM inhibits the activation of Nuclear Factor-kappa B (NF-κB), reducing the expression of pro-inflammatory genes, such as COX-2 and PGE 2. As a consequence, there is a decrease in oxidative stress and an increase in the production of anti-inflammatory mediators, such as interleukin-10 (IL-10 (Bjordal et al. 2001, Haslerud et al. 2017b, Marcos et al. 2012)).

New therapies, and especially biomaterials have attracted the attention of researchers and clinicians. Studies have evaluated the benefits of using stem cells and the human amniotic membrane (AM), the innermost layer of the placenta. Studies have indicated that AM, besides presenting abundant stem cells, reduces the inflammatory process and helps tissue repair, acting as a substrate for cell proliferation and differentiation (Liu et al. 2018, Li et al. 2019, Cargnoni et al. 2009, Tseng 2001, Manuelpillai et al. 2011).

In addition to mechanical protection, AM contains in its microenvironment several growth factors that stimulate tendon repair, reduce fibrosis formation, and present no risk of rejection after transplantation, as it does not express most histocompatibility antigens (Manuelpillai et al. 2011, Niknejad et al. 2008). In this context, AM can be considered a promising biomaterial, easily obtained, processed, and used (Niknejad et al. 2008).

This study aimed to evaluate PBM and AM as single or combined therapies to treat experimental Achilles tendon lesions regarding the inflammatory process, proliferation of tenocytes and tissue organization.

MATERIALS AND METHODS

Animals

Seventy-five rats (*Rattus norvegicus albinus-Wistar*, male, 60 days, 200 ± 20g) were kept under standard conditions (20 ± 2°C, light cycle of 12:12 h light: dark, and food and water *ad libitum*). Experimental protocols were approved by Research Ethics Committee (CEP 350.427/2013) and Animal Ethics Committee (A06/CEAU/2013). The rats were randomly allocated into five groups (n=15): C (Control)- SHAM-operated rats; I- calcaneus tendon injury; LA- tendon injury + Laser irradiation; AM- tendon injury + AM; LAM- tendon injury + Laser + AM. Each group was subdivided into three groups (n=5), for a time-course analysis at 3, 7, or 14 days (Figure 1). Tendon injury was induced by direct trauma (groups I, AM, LA, and LAM) using a mini guillotine (Nicodemo et al. 2017). The sample size (5 animals per group) was validated by calculating the power (beta), obtaining values of 0.86 for the inflammatory cell counts and 0.99 for the tenocyte count. A power above 0.8 has statistical validity (Arifin & Zahiruddin 2017).

AM fragments

Placentae were collected from three pregnant women after signing the Consent Form. Inclusion criteria were a) placentae obtained from cesareans; b) gestational age equal or superior to 37 weeks; c) healthy medical history of the mother; and d) negative serological tests for syphilis, HIV-1 and Hepatitis B and C (Nicodemo et al. 2017, Sant'Anna et al. 2011). Immediately after surgery placentae were placed in a sterile plastic bag inside a thermal box (10 °C) and

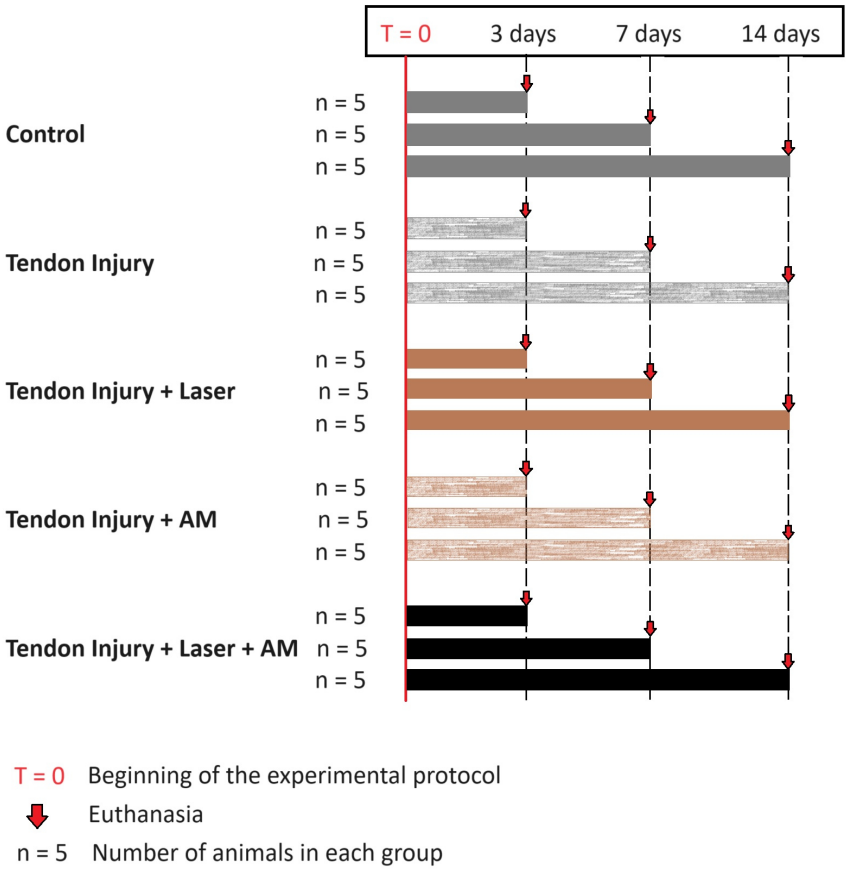

Figure 1. The experimental schedule. The time line indicates the tree observational experimental times (3, 7 and 14 days), the treatment protocol of each group (respectively), the number of animal in each group (n=5) and the euthanasia day.

were transported according to standardized rules cited in previous studies (Cargnoni et al. 2009, Sant'Anna et al. 2011).

The amniotic membrane, after manually separated from the chorionic membrane, was washed with phosphate-buffered saline-PBS (Sigma, St. Louis, MO, USA) containing 100 U/mL penicillin, 100 µg/mL streptomycin and amphotericin (Lonza, Basel, Switzerland). The amniotic membrane was sectioned into fragments of 1 cm^2, slightly larger than the injured area in the Achilles tendon[19]. Fragments were stored separately in 50 mL vials filled with serum-free and phenol red-free DMEM in sterile conditions until application. The AM fragments were used within 24 h (Hennerbichler et al. 2007).

Injury protocol

Animals were anesthetized with a combination of xylazine hydrochloride (0.01 ml/kg, i.m.) and ketamine hydrochloride (0.005 ml/kg, i.m.). I, AM, LA, and LAM groups were submitted to tendon injury induction by a non-surgical method according to previous studies (Oliveira et al. 2009). The protocol used for tendon injuries was described in a study published by our group (Nicodemo et al. 2017). Briefly, the right paw of the rat was positioned with the ankle in dorsiflexion at the base of a mini guillotine. A 20 g weight was released on the flexed leg from a fixed height of 20 cm, and removed immediately after injury. The impact of the weight caused a transverse crushing of the tissue fibers concerning the long axis of the Achilles tendon. The control group (SHAM) underwent Achilles tendon exposure and the simulated application of the AM fragment, followed by tissue suture. After surgery, animals from all groups received antibiotic- Amoxicillin (0.001 ml/kg, i.m.), and analgesic- Metamizole (0.001 mg/kg orally for 3 days). The experimental procedures were performed at the Biostimulation and Tissue Repair laboratory (Univap, São José dos Campos - São Paulo, Brazil)

Experimental protocols

Amniotic Membrane- AM and LAM groups received a AM fragment with the mesenchymal face contacting the injured tissue, based on previous studies (Niknejad et al. 2014). The AM fragment was fixed at the lesion site with the application of a single drop of methacrylate (Loctite®) (Nicodemo et al. 2017, Sant'Anna et al. 2011).

Photobiomodulation: LA and LAM groups received PBM transcutaneously, using a gallium–aluminum–arsenide laser device (TWIN LASER, MM Optics, São Carlos, SP, Brazil) calibrated according to Brazilian medical equipment standards (NBR 60601–1, NBR IEC 60601-2-22, and IEC 825–1) according with the parameters: Continuous Wave (CW), wavelength of 660 nm, output power of 40 mW and spot area of 4 mm^2. The time of irradiation was 10s applied in two points at the rat tendon enough to cover the entire area of the lesion. Total Energy delivered was 0.4 Joules and Energy density was 1 Joule/Cm^2. Treatment was performed three times a week until 3, 7 or 14 days as mentioned above. The laser pointer was protected with PVC film changed for each application.

Histomorphological analysis

Rats were euthanatized with an overdose of 10% Ketamine Hydrochloride (300 mg/kg) and 2% Xylazine Hydrochloride (30 mg/kg) intramuscularly, followed by the administration of 10% intracardiac Potassium Chloride (2 mmol/kg) at the end of each experimental period (3, 7 and 14 days). The tendons were excised and fixed in 10% neutral buffered formalin (48 hours, room temperature, Synth, Diadema-SP, Brazil), and underwent routine histological processing (Paraplast, Oxford, St. Louis, MO, USA). The

histological and quantitative analysis used five-micron-thick sections with the fibers in the longitudinal direction, stained with hematoxylin-eosin (HE), and under optical microscopy (Leica DM 2500 microscope coupled to the Leica DFC 425 camera and Leica Application Suite LAS v3.7 program). Images were captured and digitized at 1024x768 pixels with 24 bits/pixel and 20x magnification. We quantify the number of inflammatory cells and tenocytes of digital images using Image-J™ (Software program-Version 1.32 for Windows- National Institute of Health, NIH, Bethesda, USA). The cells at the grid intersection were scored at ten fields per slide, with a 200x magnification, using manual counting of cell nuclei. The average score obtained determined the single score for each sample from each experimental group.

Data from the histomorphometric analysis were statistically analyzed by comparison tests using Minitab 17 software. The normal Kolmogorov-Smirnov two-way ANOVA test was used presenting two distinct factors: more than one experimental time (3, 7, and 14 days) and different experimental protocols (Control/Injury/AM/LA/LAM) with a significance level of 0.05. Variable dependence was analyzed by the one-way ANOVA test with the post-Tukey test ($p \leq 0.05$).

The organization of collagen fibers was used to generate a score, in order to assess the alignment of these fibers in all groups and experimental times[25]. The tendon has an extracellular matrix (ECM) composed of collagen fibers (type I) with parallel orientation along the load-bearing axis. A 20x magnification was used to analyze the area of the injured tendon, performed in 5 samples per group, in the three experimental times studied (3, 7, and 14 days) generating the scores:

1. Disorganized collagen fibers;

2. Collagen fibers beginning to organize along the longitudinal axis of the tendon;

3. Collagen fibers aligned and parallel to the longitudinal axis of the tendon.

RESULTS

Histological and quantitative analysis

3 days

All injured groups presented inflammatory cells and disorganized collagen fibers (I, AM, LA, and LAM – Table I). We observed granulation tissue (Figure 2) with inflammatory cells, specifically neutrophils (with a multilobulate nucleus and condensed chromatin), a few macrophages (characterized by abundant cytoplasm and large nuclei), and tenocytes (characterized by ovoid shape and large nucleus centrally located). The injured groups (I- 22,6 ± 2,70; AM- 29,0 ± 2,35; LA-26,6 ± 2,70; LAM- 29,6 ± 4,04) presented a greater number of inflammatory cells compared to the Control group (12.4 ± 3,65), with a significant difference ($p<0.01$). Table II shows a significant difference ($p<0.05$) regarding the number of inflammatory cells observed between group I and groups AM and LAM. The proliferation of tenocytes is also analyzed showing significant values (< 0.01) comparing groups LA (15,4 ± 2,70) and LAM (15,60 ± 1,82) to the C group (6,6 ± 1,14).

7 days

At 7 days, group C showed a slight inflammatory infiltrate (19,0 ± 3,54) compared to the other groups (Figure 2). Groups I (33,0 ± 1,41) and AM (33,0 ± 4,30) showed a higher number of inflammatory cells ($p<0.01$) compared with the C group and groups LA and LAM ($p<0.05$), while group AM also presented significant values ($p<0.01$) compared with LA and LAM groups (Table II). In addition, group I showed a smaller number of tenocytes (11,2 ± 2,05) and thin and

Table I. Score of the collagen fibers organization detected in the hematoxylin-eosin staining in each group, where 3 is very organized, 2 is intermediate grad; and 1 is significantly disorganized: Control (C), Injured (I), AM fragment (AM), PBM (LA) and AM associated with PBM (LAM).

		Score Number		
Groups	**Experimental times**	**1**	**2**	**3**
C	3			+++++
	7			+++++
	14			+++++
I	3	+++++		
	7	+++++		
	14	++	+++	
AM	3	+++++		
	7	++++	+	
	14	+	++++	
LA	3	+++++		
	7	+++	++	
	14	+	++++	
LAM	3	+++++		
	7	+++	++	
	14		+++++	

Score 1 - damaged and disorganized collagen fibers. Score 2 - neoformed collagen fibers with beginning of parallel orientation. Score 3 - collagen fibers organized and oriented along the axis. The intensity of the presence of these characteristics is represented by the symbol +.

unaligned collagen fibers, while in groups LA (15, 8 ± 2,39) and LAM the tenocytes appeared in greater numbers (19,0 ± 1,87) and the collagen fibers were thicker and showed a beginning of organization and alignment (Table I, Figure 2).

The two groups treated with AM (AM and LAM) presented the inflammatory cells located at the central region of the lesion, close to the site where the membrane was applied, while at the group LA and I, these cells were spread diffusely throughout the injured area (Figure 2).

14 days

The inflammatory cells decreased in all groups at 14 days (C= 18,8 ± 3,11; I= 25,4 ± 4,72; AM= 13,4 ± 1,14; LA= 18,8 ± 3,77; LAM= 14,8 ± 1,64) compared to 7 days (Figure 2). Groups I, AM, LA, and LAM showed an increased number of tenocytes (I= 17,0 ± 1,22; AM= 17,2 ± 3,35; LA= 20,6 ± 2,30; LAM= 24,8 ± 3,56) associated with visible immature collagen fibers (Figure 2). The groups that received some type of treatment (AM, LA and LAM) showed better organization of collagen fibers (Table I), highlighting the results observed in the LAM group. Table II shows the statistically significant difference in relation to the number of inflammatory cells between groups C and I ($p<0.05$), groups I and AM ($p<0.01$), groups I and LA ($p<0.05$), and between group I and LAM ($p<0.01$).

DISCUSSION

This is the first study that investigated the effects of photobiomodulation associated with the amniotic membrane, a semipermeable biomaterial, to evaluate the inflammatory process and tissue repair in injured tendons.

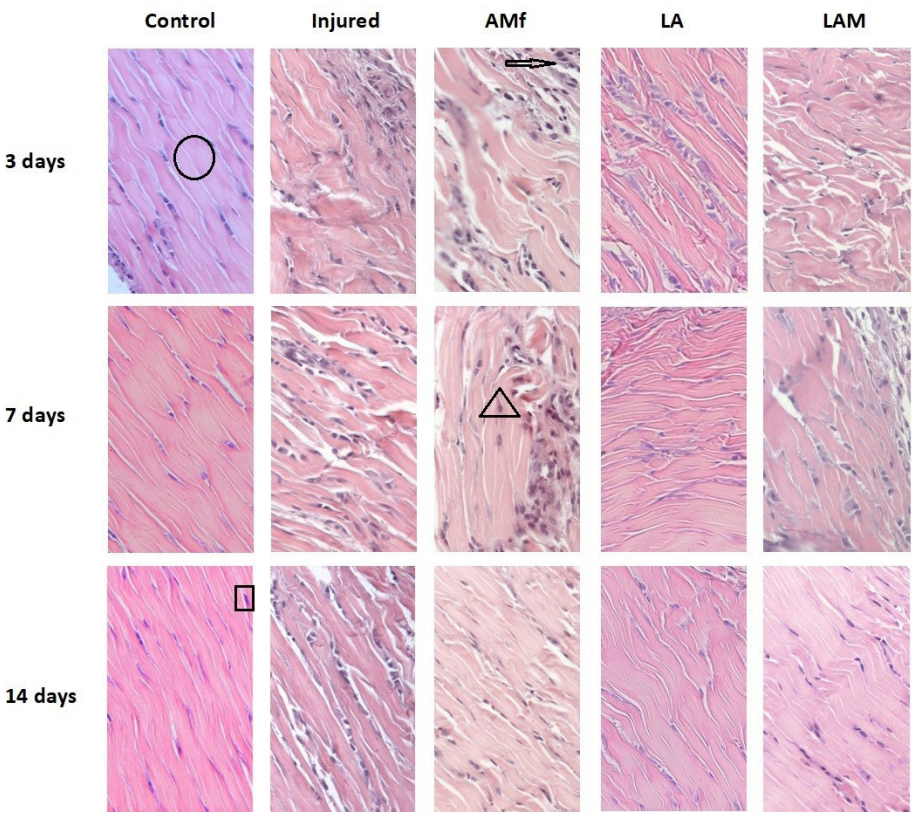

Figure 2. Histological aspects of the HE stained sections in the experimental times of 3, 7 and 14 days of all groups: C (SHAM), I (Injuried) AM (AM fragment), LA (PBM) and LAM (AM associated with PBM) (20X). Collagen fibers (O); Inflammatory cell (→); Tenocytes (□); Activated fibroblasts (△).

Some studies demonstrated a positive effect of AM (Manuelpillai et al. 2011, Niknejad et al. 2008, Nicodemo et al. 2017) or photobiomodulation (Naterstad et al. 2017) on the regeneration process, however, no survey associated the application of the amniotic membrane to photobiomodulation.

Consistent with other studies, tendon trauma produced an intense inflammatory infiltrate associated with disorganization of the collagen fibers as observed in groups I, AM, LA, and LAM at 3 days, confirming that the lesion was effectively induced (Oliveira et al. 2009, Joensen et al. 2012).

In comparison to other soft tissues, the tendon is poorly vascularized, and it heals slowly. Treatment often tends to be a long-lasting process that can continue for several months, with variable results, and high rates of re-injury (Tohidnezhad et al. 2011, Bosch et al. 2011, Lyras et al. 2009, Mos et al. 2008). To achieve the best results from the tendon repair process, effective therapies must induce cell proliferation and production of cell differentiation factors. It is already well known that photobiomodulation has the ability to improve both, tissue healing and mechanical strength of tendons. In addition, it is important to recover tendon slippage, allowing the return to normal function, while avoiding tendon opening or rupture and extensive adhesions (James et al. 2008).

Several studies have already shown the benefits of amniotic epithelial cells and AM fragments in the regeneration process of several tissues (Manuelpillai et al. 2011). The effects seem to be related to the anti-inflammatory and pro-angiogenic properties besides the capacity for preventing peritendinous adhesions. In this context, AM has been demonstrated as a promising alternative to be used in order to accelerate tendon repair (Niknejad et al. 2008, Nicodemo et al. 2017). PBM, on the other hand,

has been widely used to treat tissue damage and inflammatory processes, and clinical studies have consistently shown positive outcomes from PBM on musculoskeletal and neuropathic conditions, including tendon injuries (Bjordal et al. 2001).

The tendons typically present dense and regular connective tissue composed of bundles of collagen fibers longitudinally aligned as observed in group C (James et al. 2008, Kannus 2000). In our study, the few inflammatory cells detected in Group C probably resulted from the manipulation of the tendon performed to simulate the application of the MA fragment.

Table II. Statistic differences in the comparison of the number of inflammatory cells and tenocytes between groups at experimental times of 3, 7, and 14 days.

TENOCYTES			
GROUPS	**EXPERIMENTAL TIMES (days)**		
	3	7	14
C - I	< 0.01	ns	ns
C - AM	ns	ns	ns
C - LA	< 0.01	ns	< 0.05
C - LAM	< 0.01	< 0.01	< 0.01
I - AM	< 0.01	ns	ns
I - LA	< 0.05	< 0.05	ns
I - LAM	< 0.05	< 0.01	< 0.01
AM - LA	< 0.01	< 0.01	ns
AM- LAM	< 0.01	< 0.01	< 0.01
LA - LAM	ns	ns	ns
INFLAMMATORY CELLS			
GROUPS	**EXPERIMENTAL TIMES (days)**		
	3	7	14
C - I	< 0.01	< 0.01	< 0.05
C - AM	< 0.01	< 0.01	ns
C - LA	< 0.01	ns	ns
C - LAM	< 0.01	ns	ns
I - AM	< 0.05	ns	< 0.01
I - LA	ns	< 0.05	< 0.05
I - LAM	< 0.05	< 0.05	< 0.01
AM - LA	ns	< 0.01	ns
AM - LAM	ns	< 0.01	ns
LA - LAM	ns	ns	ns

ns: not significant values.
ns: not significant values.

Tendons of group I, without treatment, presented a significant increase of inflammatory cells after 7 days, decreasing at 14 days. However, the groups that received PBM showed a reduction at 7 days, demonstrating the anti-inflammatory potential of laser therapy in agreement with other studies (Jesus et al. 2015). PBM has excellent results on the resolution of the inflammatory process (Haslerud et al. 2017a, Marcos et al. 2014) modulating inflammatory markers at biochemical and molecular levels with no undesirable side effects (Haslerud et al. 2017a, Marcos et al. 2012). Studies reported that the beneficial PBM actions including reduction of the inflammatory process are attributed to the inhibition of cycloxygenase-2 enzyme and prostaglandin E2 (PGE2) mediator, a similar action observed by the administration of anti-inflammatory drugs (Albertini et al. 2007, Marcos et al. 2011).

The groups treated with FBM also showed a greater number of fibroblasts, cells that produce extracellular matrix and collagen fibers, at all experimental times studied. Furthermore, the PBM group showed greater organization in the arrangement and distribution of these fibers after only 7 days of treatment. The increase in the number of tenocytes observed in the PBM groups could be explained by the fact that photobiomodulation stimulates fibroblast proliferation and promotes the release of fibroblast growth factor, increasing collagen deposition and collagen reorganization in the lesion area (Allahverdi et al. 2015). Published studies have stated that laser therapy also can stimulate angiogenesis, restore blood flow to the lesion site, and thus limit ischemic necrosis and accelerate tissue repair (Laraia et al. 2012). PBM increases GAGs and collagen types I and III, improves the healing process, and increases the migration of tenocytes to the injured area (Guerra et al. 2012, Tsai et al. 2012).

The number of inflammatory cells continues to decrease at 14 days in the groups treated with PBM (LA and LAM). In 14 days, the AM group showed a sharp decrease in the number of inflammatory cells reaching values close to the LAM group. Therefore, there was no statistically significant difference between the LA, LAM, and AM groups, while there was a significant difference between these groups compared to group I.

The decrease in inflammatory cells, in group AM at 14 days, shows that AM also had an anti-inflammatory effect, as cited by other studies (Manuelpillai et al. 2011, Muttini et al. 2010). This effect is due to the presence of interleukin-10 (IL-10), hyaluronic acid, and prostaglandin E2 (PGE2) in the AM stroma (Manuelpillai et al. 2011).

The reduction of the inflammatory process allows the continuous evolution of the tendon repair process, favoring both the increase in the diameter of the collagen fibers and their correct alignment in the area of the lesion (Alaseirlis et al. 2005). These characteristics are essential for the performance of tendon function and our results indicate the effectiveness of both treatments.

However, groups LA and LAM presented a better organization of their collagen fibers in the experimental time of 7 days, when compared to the other groups, which may be related to the reduction of edema and inflammatory cells. A similar organization of collagen fibers was only detected in the AM group at 14 days, considering the anti-inflammatory effect of the microenvironment present in the amniotic membrane. It should be noted that the best result in the evolution of tissue repair was observed with combined therapies, the LAM group. The reduction of edema, induced by PBM, associated with the release of growth factors of AM, favored the acceleration of tissue regeneration.

The combination of both therapies (PBM plus AM) has the advantage of associating the benefits and properties of each of them. In addition to the effects of laser in reducing the inflammatory process, stimulating the multiplication of tenocytes, angiogenesis, and reorganization of collagen fibers, as previously discussed, AM also has very important effects. The amniotic membrane has been shown to promote anti-inflammatory effects, and scarless wound-healing processes *in vivo* (Nicodemo et al. 2017). The AM is a biomaterial biologically active and contains several growth factors and cytokines that play essential roles in tissue development in utero and as a wound dressing (Hortensius et al. 2018, Huang et al. 2023). The release of several factors, including the basic fibroblast growth factor, favors cell migration, an essential step in the healing process (Koizumi et al. 2000). The application of the AM patch with the mesenchymal side facing the lesion aimed to release angiogenic factors, which are essential for the nutrition and survival of the newly formed tissue (Niknejad et al. 2014, James et al. 2008). This combination of factors released in the injured area would justify the improvement in the tissue repair process observed with the isolated application of AM.

Moreover, our study showed that AM induced a centripetal repair process in the first 7 days after the experimental lesion. Tendons treated with AM presented higher cellularity at the central area of the lesion. On the other hand, the distribution of inflammatory cells in the I and LA groups was more generalized, as also observed in a similar study (Barboni et al. 2012). The presence of the AM fragment around the injured region stimulated the migration and cell proliferation to this region.

There are some limitations to this study regarding it should be cautious when extrapolating animals *in vivo* studies to clinical

study conditions. Further animal studies are mandatory to verify the biochemical, molecular, and functional analyses regarding the effectiveness of the application of PBM and AM, isolated or in association. However, our histological results showed that treatment with PBM or AM alone has significant advantages compared to the untreated animals. However, the association of AM and PBM was even more effective, leading to an improved reconstruction of the Achilles tendon.

CONCLUSIONS

The association of photobiostimulation and amniotic membrane as an innovative protocol for the treatment of tendon injuries has proven to be a promising option based on the evaluation of histological aspects. The group treated with the combination of these therapies showed a reduction in the inflammatory response and an increase in the proliferation of tenocytes and collagen fibers, favoring the regeneration of tendon tissue with reduced healing time.

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