Original Article

Assessment of Platelet Rich Plasma Injection on Muscle Spindle Injury in a Rat Model (Histological and Immunohistochemical Study)

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ABSTRACT

Introduction: Nowadays, platelet-rich plasma injection (PRP) has become a common treatment in the scope of reconstructive plastic surgery and trauma. PRP contains varies growth factors that accelerate cell regeneration and differentiation.
Objective of the Study: Evaluate the effect of a single-shot injection of PRP on induced muscle spindle injury.
Materials and Methods: Forty rats were categorized into three groups; control, one and three weeks after muscle injury. The injured muscles were either treated with PRP or left without treatment. The muscle specimens were processed for histological and immunohistochemical staining for desmin and Ki-67 followed by computer image analysis.
Results: PRP administration resulted in appearance of muscle spindles among numerous regenerating muscle fibers and numerous newly formed blood vessels at 1st week of injury. Meanwhile, untreated group exhibited granulation tissue accompanied inflammatory cell infiltration with marked amount of deposited collagen. Three weeks PRP treated group showed well differentiated muscle spindles surrounded by outer multi-laminar connective tissue capsule which were similar to the control group. The desmin was expressed in regenerating spindle fibers as well as in intact muscle spindles and the expression pattern of these intermediate filaments was more intense in PRP treated groups. A significant reduction in the Ki-67 level was detected in the apparently normal newly formed spindle muscle in PRP treated group.
Conclusion: A single dose PRP plays a major role in promoting the proliferation, differentiation and has a neurotrophic function in muscle spindle regeneration. These findings provide useful and indispensable application of PRP in muscle spindle injury.

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Key Words: Muscle spindles, platelet rich plasma, rat model, regeneration.

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INTRODUCTION

Proprioception and movement control require proprioceptive mechanosensors (muscle spindles) that are sensitive to muscle length changes and speed-dependent amplitude modulation[1,2]. The muscle spindles comprise many specialized encapsulated intrafusal fibers distributed in parallel with extrafusal fibers and it consists of chain and bag intrafusal fibers[3]. The innervation pattern of muscle spindle was three different subtypes of nerve axons: type Ia, type II sensory nerve and motor nerve axons. The proprioceptive mechanosensors have a role in a variety of sensorimotor activities, including proprioception control, balance, gait, and the postural response, and a lack of muscle spindles may explain the disturbed proprioception, gait impairment, and ataxia of the sensory type[4]. Muscle spindle development and establishment of extensive synaptic connections require exchange of many factors among intrafusal muscle fibers and neurons. Loss or decrease of these factors ultimately leads to degeneration of muscle spindles and loss of control of movement[5,6].

Several methods of improving intrafusal muscle function in dystrophic patients can be exploited through the recently identified Piezo2 channel as the primary mechanotransduction channel[7]. Piezo2 channel target mechanosensitivity without any impact on the function of extrafusal muscle fiber function or with neuromuscular transmission[8]. Potential drugs are still under clinical trials and approval, on the other hand, their adverse effects caused by interfering with Piezo2 channels in non-muscle tissues may restrict their use[8]. Alternatively, proprioceptive sense training is a beneficial behavioral approach for improving the sensitivity of muscle spindles in patients with motor neuron diseases and in proprioceptive decline associated with aging[9,10].

Skeletal muscle injuries comprise the most prevalent orthopedic sports-related injuries, in athletes is approximately 12–16%[11]. The healing response may be augmented or stimulated by using endogenous or exogenous agents. Using platelet-rich plasma injections (PRP) has become a popular therapeutic procedure capable of enhancing the process of regeneration of various tissues, including striated skeletal muscles. Tsai *et al*.[12] reported that PRP administration promotes regeneration and reduces apoptosis and inflammation of injured muscle. Gigante *et al*.[13] reported that the platelet

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abundant with fibrin matrix promotes neovascularization in injured striated muscle. Basic science studies show many positive effects of PRP in *vitro* and in *vivo* on reducing the regeneration time and improving morphological outcome and functional recovery of the skeletal muscle[14,15,16]. However, there are interindividual differences of dosing, timing and number of PRP injections and muscle spindle recovery not investigated sufficiently.

PRP is a component of blood prepared by centrifuging whole blood to get a cellular constitute of platelet-enriched plasma. PRP contains several growth factors, for example insulin-like growth factors, transforming growth factor, hepatocyte growth factor and endothelial vascular growth factor. Further, PRP includes bioactive factors (non-growth factors); serotonin, dopamine, histamine, calcium, adenosine, *vitro*nectin, fibronectin, and fibrin. These growth factors accelerate epithelial regeneration, provoke angiogenesis and cell differentiation, increase the hemostatic response, assist cell migration and enhance collagen synthesis, thus enhance soft tissue healing[17,18].

Although impaired proprioception could result from abnormalities in neurons, axons, or mechanoreceptors, relatively little is known about how PRP mediates mechanoreceptor regeneration. Therefore, the current study aimed to evaluate the effect of a single-shot PRP injection on an induced muscle spindle injury.

MATERIAL AND METHODS

Forty adult male albino rats weighing 200–250 grams each were obtained from Ain Shams University, Faculty of Medicine, Research Institute (MASRI). They were left for 2 weeks to acclimatize before the experiment. During the acclimatization period, they were kept on a standard 12 hours light / dark cycle, well-ventilated cages with free access to a balanced laboratory diet and water. The experiment was approved by the Committee of Animal Research Ethics (CARE) of Faculty of Medicine, Ain Shams University.

The animals were divided into the following groups

Control groups (20 rats): the control groups were subdivided into 3 subgroups.

- Subgroup Ia (negative control): 10 rats were left uninjured.
- Subgroups Ib, Ic (positive control) were injected PRP in their right gastrocnemius. Subgroup Ib: 5 rats were sacrificed after one week. Subgroup Ic: 5 rats were sacrificed after three weeks.

Experimental groups (20 rats): Bilateral surgery to injury and repair the gastrocnemius muscles were performed (Right and left limbs). The right limbs of experimental groups were injected with PRP, while the left limbs were not injected. This group was further divided into 2 subgroups:

A. one-week group (10 rats): Specimens were taken after one week and were subdivided into: untreated group (untreated 1 wk.) and PRP treated group (PRP-treated 1wk).

B. 3 weeks group (10 rats): Specimens were taken after 3 weeks and were subdivided into untreated group (untreated 3 wk.) and PRP treated (PRP-treated 3wk).

Induction of muscle injury

Under complete sterile condition, surgical exposure of the gastrocnemius muscle was achieved under general anesthesia (intramuscular injection of tiletamine and zolazepam (Zoletil) 3 mg/k). After hair shaving, a 1 cm skin longitudinal incision was made over posterior aspect of both left and right posterior limbs to expose the gastrocnemius muscles. The gastrocnemius muscle was visualized and a wedge-shaped lesion 4 mm long, 3 mm wide and 3 mm deep was excised transverse to fiber orientation. The injury sites of right limb muscle of the treated animals were immediately filled with PRP (50 μL)[16] whereas those of the left limb muscles were left without treatment. Contreras-Muñoz *et al*.,[16] found a significant improvement in muscle force together with a significant histological outcome in early muscular PRP injection 24 hours after injury. The suture was passed through the gastrocnemius muscle re-apposed to its anatomic position. To identify the position of muscle lesion for further examinations, the lesion was marked at both ends with a 4-0 Safil-suture[19]. The rats were not restrained after surgery, and were allowed free activity. The rats were sacrificed following the protocol of ethical committee at 1 and 3 weeks postoperatively.

Blood collection and preparation of platelet-rich plasma

Using a glass capillary tube or a Pasteur pipette, a sample of 2.0 ml blood was collected from the retroorbital plexus. Each rat's blood was gently taken into a syringe containing 1 mL of 3.8 percent sodium citrate. Each blood sample was centrifuged for 15 minutes at 250 g, yielding three layers. The lowest layer is composed of red blood cells, the middle layer is consisted of white blood cells, and the top layer contains plasma. The 1 ml plasma layer was centrifuged for 5 min at 1,000g to obtain two layers of plasma, the lower part consisting of PRP and the superior part consisting of poor-platelet plasma (PPP). The PPP was aspirated and avoided mixing with the PRP. The PRP was aspirated gently and put it in a sterile syringe. Platelet concentrations were confirmed using a Coulter counter. A double-step centrifugation protocol, as recommended by Dhurat and Sukesh[20], was used to quantify the platelets. PRP concentrate was injected immediately at site of muscle injury before skin wound closure.

Specimen collection

The animals were anaesthetized by intraperitoneal injection of sodium thiopental (40 mg/kg) then they were sacrificed and muscle specimens of marked muscle lesion of all groups were carefully excised taken after one and

three weeks of induction of injury. Specimens of all groups were fixed in 10% neutral-buffered formalin for 48 hours, washed in phosphate-buffered saline (PBS), dehydrated in ascending grades of alcohol, cleared in xylene to prepare paraffin blocks. Serial muscle sections were obtained with 5 µm thickness then processed to be stained with hematoxylin and eosin (H&E) and Masson's trichrome stain[21]

For immunohistochemistry techinque

Five µm thick sections were obtained, deparaffinized, and washed with phosphate buffered saline. The sections were incubated overnight in a humidified chamber with the primary antibody; mouse monoclonal anti-desmin (clone 33, BioGenex, USA) for detection of intermediate filaments in sarcomere architecture and rabbit polyclonal anti-Ki67 (ab15580; Abcam, Massachusetts, USA) for detection of cell proliferation, rabbit polyclonal anti-MMP1 (ab137332; Abcam, Massachusetts, USA). Then, the muscle sections were rinsed with buffered saline and treated with the biotinylated antibody for one hour. The sections were then incubated with streptavidin combined to horseradish peroxidase (Sigma, USA) and finally the reaction was established using DAB (3,3-diaminobenzidine tetrahydrochloride, Fluca)[22]. Stained muscle sections were examined using Olympus binocular microscope and photographed using a Canon camera connected to an IBM computer system.

Computer image analysis

Computer image analysis “TS View” version 6.2.4.5 was used to quantitate the number of encapsulated muscle spindles per microscopic fields (using 100 X magnification) and number of intrafusal fibers in the muscle spindle in samples of gastrocnemius muscles. In Masson’s trichrome stained sections at 400 X magnification, five fields per slide were examined to measure the collagen fiber surface area percentage (%).

Quantification of mean surface area percentage (%) of the immunoreactivity of desmin in muscle spindle per microscopic field (1000 X magnification), and the number of Ki-67positive cells between different groups (400X magnification) were performed (including Ki-67positive cells of fibroblast or dividing myoblasts or satellite cells). Measurements were taken from capsular regions of muscle spindle (including the equator & the juxta-equatorial portions, contained paraxial fluid space) of five microscopic fields per slide, five slides per rat[23]. Calibration for microscopic magnification was done in order to express pixels into micrometers[24].

Statistical Analysis

SPSS software (Version 13.0) was used. One-way analysis of variance (ANOVA) and the post-hoc Tukey test were used to compare the studied groups of the observed histomorphometric data. Values gained were reported as means ± standard deviation and the *p-value* was considered statistically significant with $P \leq 0.05$[25].

RESULTS

Histological results

Control group

Examination of muscle sections from positive and negative control groups revealed a similar histological picture. Muscle spindles are specialized muscle fibers that found in the belly of gastrocnemius muscle, between extrafusal muscle fibers (Figure 1a). These muscle spindles are surrounded by multi-laminar connective tissue capsule (Figures 1b,c,d).The capsule of muscle spindles is clearly seen as a rounded ring in the transverse sections. It consists of 2-3 lamellae of fibrous tissue with flat nucleated cells and is crossed by connective tissue trabeculae, nerve filaments and blood vessels. There was an inner capsule forming a delicate reticular network surrounding the intrafusal fibers. At the transverse section of the central (equatorial) region of the muscle spindles, the outer capsule bulges to form a periaxial space around the intrafusal muscle fiber. The main components of muscle spindles are nuclear bag fibers and nuclear chain fibers. Nuclear bag fibers could be distinguished by their larger size and large vesicular nuclei (Figure 1c). In contrast, the nuclear chain fibers exhibited a smaller diameter and contained closely aligned tightly packed, rounded nuclei (Figure 1b). The intrafusal myofibrils are oriented in the longitudinal direction and nearly similar to the extrafusal muscle fibers (Figure 1c). The morphometric parameters of muscle spindles showed statistically insignificant difference among the positive and negative control groups (Table 1).

One week experimental group

Examinations of H&E staining sections revealed extensive granulation tissue formation one week after injury in untreated group. Inflammatory cell infiltration accompanied the granulation tissue that comprised mainly of mononuclear leukocytes and macrophages that were identified by their size and single nuclei. No identifiable muscle spindles were noticed in the regenerating granulation tissue (Figure 2a). Masson's trichrome stained section of one week untreated group showed massive amount of collagen bundles in the area of regeneration (Figure 2b). On the other hand, one week PRP treated group showed numerous regenerating extrafusal myofibers with central myonuclei and the muscle fibers were still intervened with some inflammatory cells (Figure 2c). Few muscle spindles were observed among this group (Table 1). The outer capsule was thinner and the inner capsule was less developed. The regenerated muscle spindle fibers contained small centrally or peripherally placed nuclei. The regenerating myofibers were interspersed with many blood capillaries (Figures 2c,d). Additionally, a statistically significant increase in the number of muscle spindles per microscopic fields and number of intrafusal fibers in one week PRP treated group was observed when compared to the untreated ones (Table 1). One week PRP treated group contained fewer amount of collagen among the regenerating muscle fibers when compared with the

untreated ones (Figure 2d) that was statistically confirmed by collagen fiber surface area % (Table 1)

Three week experimental group

Three weeks untreated group showed numerous newly formed myotubes appeared pale acidophilic with multiple peripheral or central oval nuclei (Figure 3a). Muscle spindles of this group were identifiable, but their morphology differed from the intact muscle spindles of the control group. The regenerating spindle myofibrils were lacked typical nuclear bag or chain configuration (Figures 3a,b). A very striking feature of the regenerating muscle spindles is that they were surrounded by a very thick capsule of connective tissue in addition to the presence of some inflammatory cells. The collagen content of the capsule had increased and the collagen bundles had different directions than those of control group (Figure 3b, Table 1). Three weeks PRP treated group showed well differentiated muscle spindles which were the same as the control group. As regards the number of muscle spindles and intrafusal fibers number, the three weeks PRP treated group showed a statistically significant increase in comparison with the untreated ones (Table 1). The muscle spindles surrounded by outer multi-laminar connective tissue capsule (Figures 3c,d). The Masson's trichrome stained section of 3 weeks PRP treated group, the newly formed muscle spindle were seen separated by CT septa containing mild amount of collagen fibers (Figure 3d). Moreover, both 3 weeks group muscle spindles contained numerous capillaries among the outer capsule layers.

Immunohistochemical results

Desmin immunoreactivity

Desmin immunoreactivity was noticed in all muscle spindles, including the intact intrafusal myofibers as well as regenerating spindles. In intact muscle spindles, desmin was detected within the muscle spindle, as tiny spots with immunopositivity reaction (Figure 4a). One week after muscle injury of untreated group, desmin was not detected inside the granulation tissue (Figure 4c). On the other hand, numerous desmin positive muscle spindles were observed in the area of regenerated skeletal muscle fibers in one week PRP treated group (Figure 4d). In regenerating spindles in 3 weeks of untreated group, desmin was expressed among the few newly formed irregular spindle myofibers (Figure 4e) whereas the desmin immunoreactivity were increased in the apparently normal newly formed spindle muscle in PRP treated group (Figure 4f). Surprisingly, desmin was identified also in the wall of some blood vessels among different group.

Upon computer image analysis, the percentage of areas stained for desmin in the control group was 24.5 ± 1.38%. Desmin expression levels were significantly different between PRP treated and untreated rats at 1 week and 3 week after injury (Figure 4b). At 1 week, a significant increase was observed in PRP treated group (16.5±0.94) when compared to untreated (4.68±0.72) whereas a significant increase was still observed in PRP treated group (21.56±1.24) when compared to untreated (14.76±0.82) at 3 weeks of injury (Figure 4b).

Ki-67 immunoreactivity

Ki-67 expression levels of proliferating cells in general were significantly increased in untreated group compared to the levels detected in the control muscles & the PRP treated group (Figures 5a,b).One week untreated group showed positive Ki-67 immunoreaction detected in many regenerating cells in the granulation tissue (Figure 5c). In one week PRP treated group showed few Ki-67 positive regenerating cells among the newly formed spindle myofibers (Figure 5d). Three week after muscle injury of untreated group, apparent increase in the number of Ki-67 positive regenerating cells among the newly formed spindle irregular myofibers (Figure 5e) whereas the Ki-67 immunoreactivity were not detected in the apparently normal newly formed spindle muscle in PRP treated group (Figure 5f). Positive Ki-67 immunoreaction was identified also in some endothelial cells of blood vessels among different group.

Regarding the mean number of Ki-67 positive cells in the control group was (2.1±1.73). Ki-67 expression levels were significantly different between PRP treated and untreated rats at 1 week and 3 week after injury (Figure 5b). Ki-67 expression was statistically significant higher in untreated group (24.4±1.17) at first week after injury than that observed in PRP treated group (10.8±2.4). At 3 weeks; a significant increase was still observed in untreated group (13.83±1.5) as compared to PRP treated group (4.3±1.35) (Figure 5b).

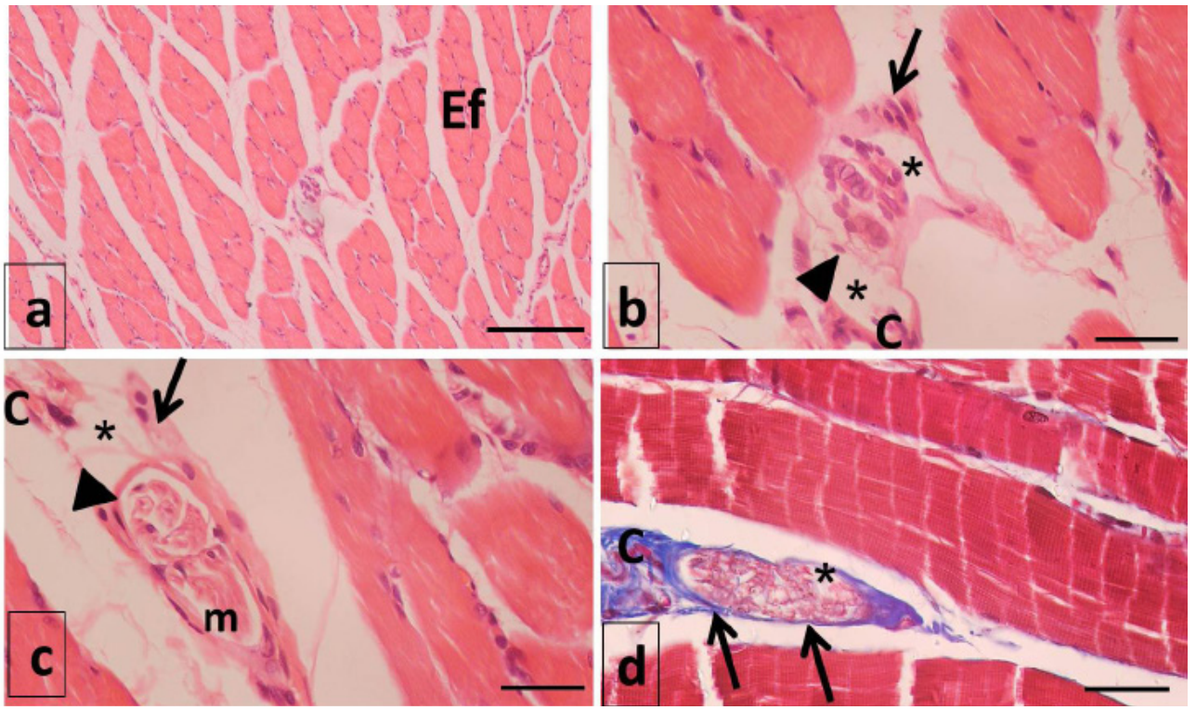

Fig. 1: photomicrographs of muscle spindle of control groups showing the components of muscle spindles. (a) cross-section of a spindle in between extrafusal muscle fibers (Ef). (b-d) the outer multi-laminar connective tissue capsule (black arrows). Capillaries (C) often course between the capsule's layers. There is an inner one forming a fine reticular network (arrowhead) surrounding the intrafusal fibers. The periaxial space (Black asterisks) lies between the capsule elements. The intrafusal myofibrils (m) oriented in the longitudinal direction. Scale bar: (a) uninjured control group H & E 200 µm, (b) uninjured control group H & E 50 µm, (c) uninjured PRP group H & E 50 µm (d) Masson's trichrome stain 50 µm.

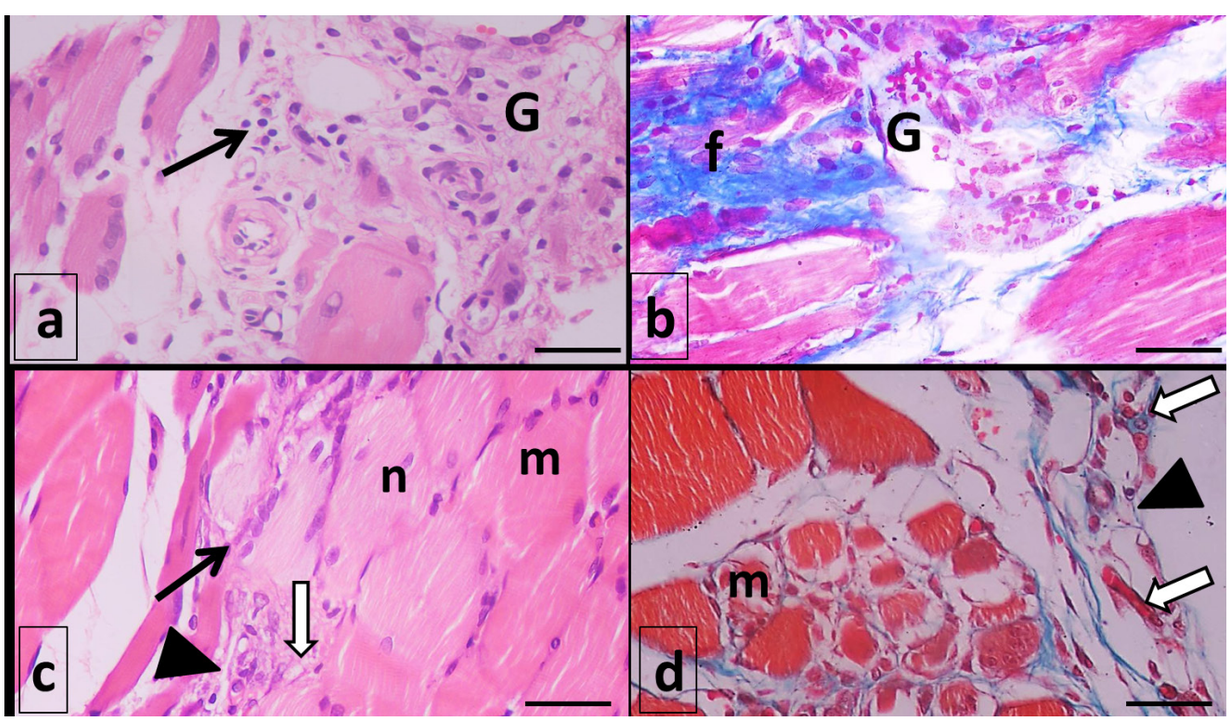

Fig. 2: photomicrographs of histological features observed for muscle spindle after 1 week of muscle injury. (a & b) untreated group showing extensive granulation tissue (G) that comprised mainly mononuclear leukocytes (black arrow) with deposition of marked amount of collagen fibers (f). (c& d) PRP treated group showing numerous regenerating extrafusal myofibers (m) with central myonuclei (n) with some inflammatory cells (black arrow). Few regenerated spindle fibers (arrow head) with small centrally placed nuclei among many blood capillaries (white arrow). Scale bar: (a & c) H & E 50 µm, (b& d) Masson's trichrome stain 50 µm.

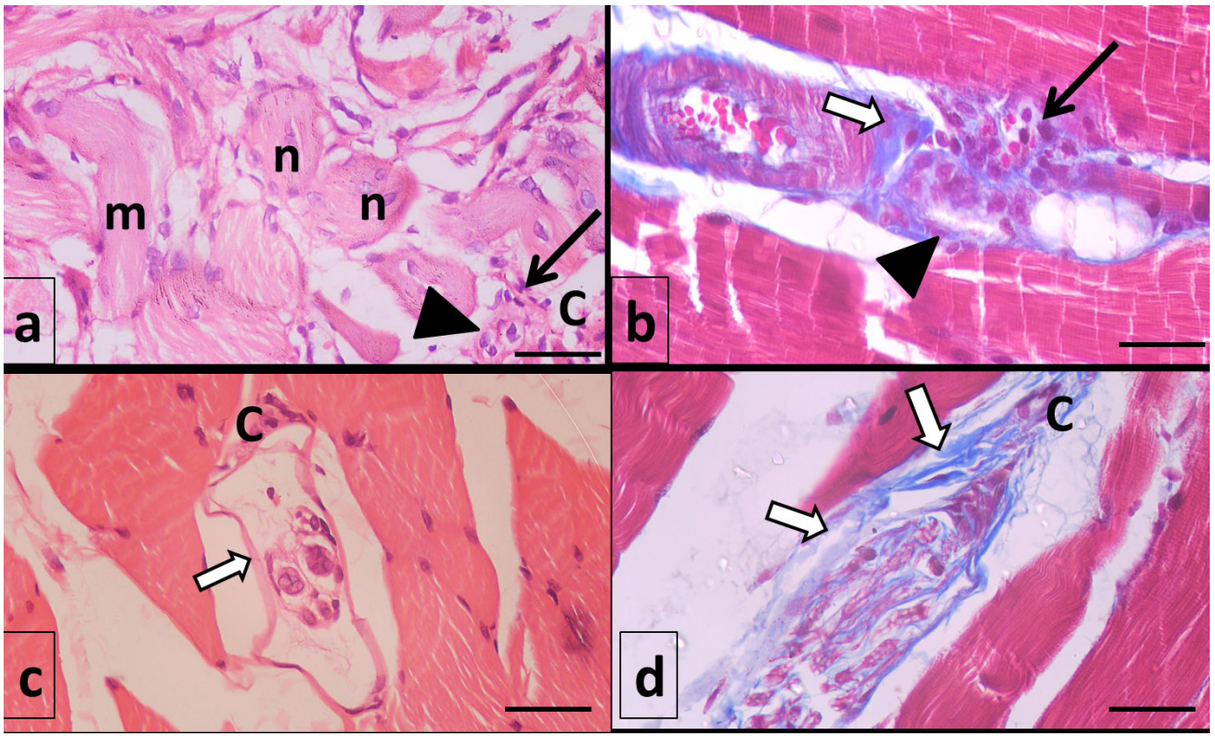

Fig. 3: photomicrographs of histological features observed for muscle spindle after 3 weeks of muscle injury. (a & b) untreated group showing numerous myofibers (m) with central nuclei (n). The regenerating spindle myofibrils (arrow head) surrounded by a very thick capsule (white arrow) with some inflammatory cells (black arrow). (c& d) PRP treated group showing well differentiated muscle spindles surrounded by outer multi-laminar connective tissue capsule (white arrows). Numerous capillaries (C) appear among the outer capsule layers in both groups. Scale bar: (a & c) H & E 50 µm, (b& d) Masson's trichrome stain 50 µm.

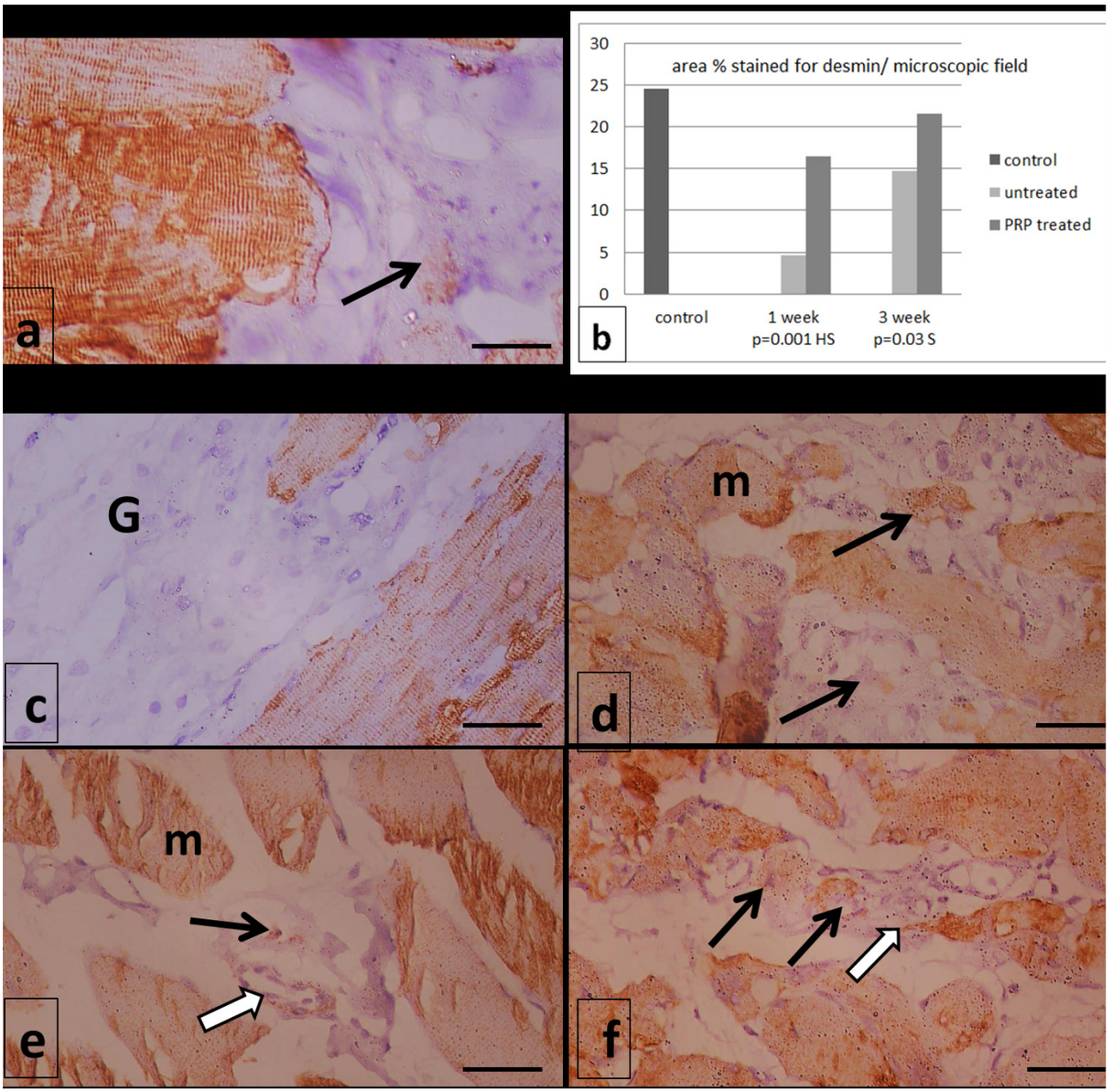

Fig. 4: photomicrographs of desmin immunohistochemical staining features of intrafusal fibers. (a) control group with desmin immunopositivity of muscle spindle (black arrow). (b) Assessment of desmin immunohistochemical results, in control and experimental group by computerized image analysis; S – significant; HS – highly significant. (c) 1 week untreated group, desmin is not detected in the granulation tissue (G). (d) 1 week PRP treated group showing, numerous desmin positive muscle spindles (black arrow) in between regenerating skeletal muscle myotubes (m). (e) 3 week untreated group, desmin is detected among the few newly formed irregular spindle myofibers. (f) 3 week PRP treated group, desmin is detected in apparently normal spindle muscle (black arrow). Desmin appear in the wall of some blood vessels (white arrow) among different groups. Scale bar: (a-f) desmin immunohistochemical 50 µm.

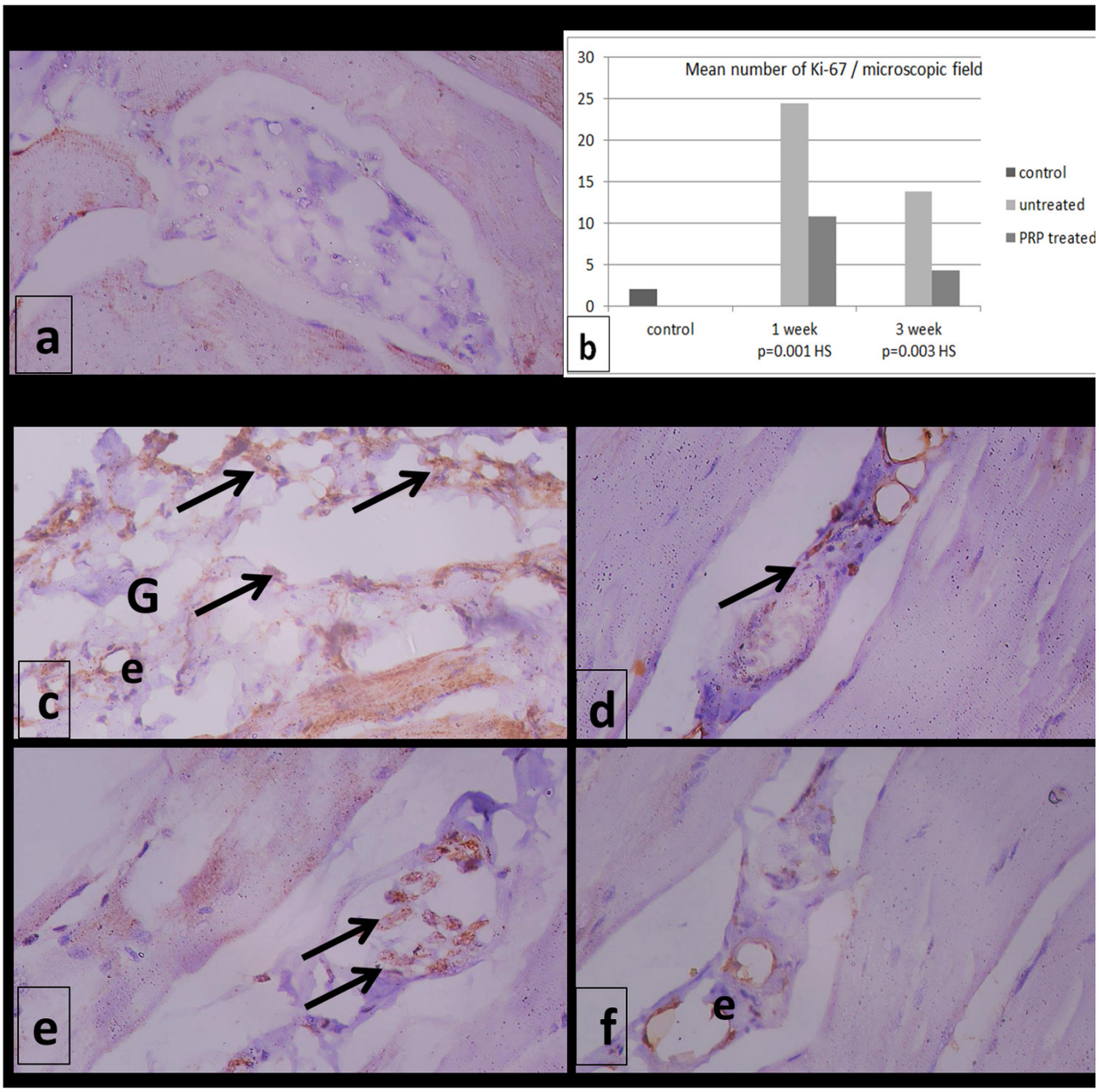

Fig. 5: photomicrographs of Ki-67 immunohistochemical staining features of intrafusal fibers. (a) Ki-67 expression in control group. (b) Assessment of Ki-67 immunohistochemical results, in control and experimental group by computerized image analysis; HS – highly significant. (c) 1 week untreated group, positive Ki-67 (black arrow) is detected in the granulation tissue (G). (d) 1 week PRP treated group showing, few Ki-67 positive among the newly formed spindle myofibers (black arrow). (e) 3 week untreated group, apparent increase in Ki-67 positive regenerating cells among the newly formed spindle irregular myofibers (black arrow). (f) 3 week PRP treated group, Ki-67 is not detected in apparently normal spindle muscle. The Ki-67 appears in some endothelial cells blood vessels (e) among different groups. Scale bar: (a-f) Ki-67 immunohistochemical 50 µm.

Table 1: The effects of PRP on number of muscle spindles per microscopic fields, number of intrafusal fibers and collagen fiber surface area (%)

			Nº of muscle spindle	Nº of intrafusal fib	Collagen fib surface area (%)
Control groups		Untreated group	4.4±0.8	4.1±0.14	10.33 ± 1.8
		PRP 1 week	4.0±0.91	3.8±0.4	9.93 ± 1.9
		PRP 3 weeks	4.3±0.31	3.9±0.97	10.12 ± 1.2
Experimental groups	1 wk	Injured group	0±0.0*	0±0.0*	38.63 ± 12.2*
		PRP treated	2.5±1.07#	1.3±0.41#	19. 8 ± 1.97#
	3 wk	Injured group	2.6±0.22#	1.8±0.5#	25.4 ± 1.7#
		PRP treated	3.9±0.62ab	3.7±0.94ab	14.27 ± 0.59ab

Values are mean±SD; One-way ANOVA followed by Tukey's multiple comparison test.
* $P < 0.001$ compared to untreated control group.
# $P < 0.001$ compared to injured 1 week group.
a $P < 0.001$ compared to PRP 1 week group.
b $P < 0.001$ compared to injured 3 week group.

DISCUSSION

PRP has been used for tissue healing for a long time and offers a number of benefits over other products and methods being entirely safe procedure. Clinical use of PRP does not cause any adverse events or postoperative complications[26]. PRP also contains a high concentration of a natural range of growth factors that stimulate various processes[27]. Meanwhile, mammalian intrafusal and extrafusal myofibers have a considerable capacity of regeneration after injury from surviving satellite myoblasts[22].

Platelet density in PRP preparations was different. As reported by Han *et al*.,[28], percentage increase in platelet density was associated with significant variations in growth factor concentrations between individuals. Mosca and Rodeo,[29] assumed that variation in cell types and growth factors in PRP preparations may have different effects on muscle healing phases of (inflammatory, proliferative phase, and remodeling) when compared to other tissues. Platelet-derived growth factor, for example, promotes myogenesis and may be found in different concentrations in PRP generated by various commercial systems. Accordingly, we used a double-step centrifuge technique in platelet preparation system. This double-step centrifugation method provides high concentrations of growth factors for tissue regeneration and an easy and cost-effective method[30].

PRP injection in uninjured muscle (positive control) showed a similar histological picture as the negative control. This proves that PRP preparations are safe to use in patients, as PRP is an autologous preparation. Similarly, Taylor *et al*[31] proved that neither allergic reactions nor disease transmission has been reported during PRP injection under clean aseptic conditions.

In the current work, PRP administration resulted in appearance of muscle spindles among numerous regenerating muscle fibers and numerous newly formed blood vessels in the first week of injury. Meanwhile, untreated group exhibited granulation tissue accompanied inflammatory cell infiltration. PRP appeared to accelerate the clearance of necrotic tissue, by modulating the inflammatory process. This finding is consistent with Mosca and Rodeo,[29] who stated that PRP are known as a chief source of biologically active metabolites that modulate inflammation, antimicrobial action, cellular proliferation, migration, angiogenesis, vascular remodeling and ECM synthesis. Also, PRP had a potent effect on the proliferation and differentiation of human muscle-derived precursor cells (hMDPCs) and keep their stemness[32]. This could be clarified by Tidball,[33] who mentioned that the invasion of neutrophils and macrophages share in phagocytosis of the debris and liberation of inflammatory cytokines such as IL-1b, IL-6, IL-10, TNFa & TGF β1. These cytokines play a main role in chemotaxis, cell proliferation and cell differentiation.

Previous studies have shown that the intrafusal muscle fibers respond to stretch even though the sensory endings are morphologically immature[34]. In the current work, regenerating muscle spindles within intact capsules were detected in all PRP groups, possibly well-differentiated intrafusal fibers. This could be supported by Hippenmeyer *et al*[5] who mentioned that an intracellular signaling pathway leads to the final differentiation of intrafusal fibers and also their survival. Intrafusal fibers provide neurotrophin-3 (NT3) to proprioceptive sensory neurons ensuring their survival during cell death via the NT3 TrkC receptor (TrkC; known as neurotrophic tyrosine kinase receptor type 3)[35]. Also, Inoue *et al*.[36] added that many transcription factors were highly expressed by dorsal root ganglia neurons (DRG) and is essential for their survival, axonal projection, and connectivity to the spinal cord. Similarly, there was an increase in gene expression of NGF and GDNF in the PRP-treated groups. NGF and GDNF are neurotrophic factors that secreted by SCs, which have biological functions in maintaining the survival of peripheral neurons[26].

In the current study, muscle spindle quantification has involved in cross sections of the spindle capsule using light microscopy or by immunohitochemical techinque.

This method of visualizing the spindle does not distinguish proprioceptive axons from the surrounding fibers and does not allow intricate analysis of axon morphology. Moreover, the proprioceptive nerve afferents have axons of smaller caliber (group III and IV) more widely distributed in the muscles[37]. A limitation of this study is the lack of neurological and functional evaluation of intrafusal muscle fibers. Future studies should visualize muscle spindle nerve afferents longitudinally that could provide more data on axonal width and inter-rotational distance of annulospiral endings.

Acetylcholine receptors (AChRs) are localized in the muscle fiber plasma membrane in the central region of intrafusal fibers, at site of contact with the sensory nerve endings[38]. Also, collagens, nidogens, laminins and proteoglycans are specifically concentrated at neuromuscular junctions[39]. In the present work, PRP treated group contained fewer amount of collagen among the regenerating muscle fibers at the site of muscle injury when compared with the untreated ones denoted decrease fibrotic response to the muscle injury. Many authors observed neovascularization and decreased fibrosis among PRP treated injuries[40,41]. Harris *et al*.,[40] reported that PRP triggers the cascade of wound healing leading to cellular proliferation and migration, collagen and glycosaminoglycan accumulation, collagen maturation and remodeling of the tissue. Harmon[42] added that fibrous tissue restricts the regeneration of the muscle by preventing the stumps of the myofibers from rejoining and may prevent axons from creating new neuromuscular junctions. Muscle fibers that are not innervated will ultimately undergo atrophy. Further, the protein levels of a7 nicotine acetylcholine receptor were maximized at 9 days post muscle injury, which is involved in muscle fiber regeneration through regulation of satellite cell status[43], neurotransmitter release and attenuation of fibrosis[44].

Desmin is the major constituent of intermediate filaments in sarcomere architecture and is expressed in the myofibers forming an interlinking scaffold with connections to the sarcolemma and the nuclear membrane. Desmin contributes to maintaining cell integrity, mechanochemical signals within the myocyte and efficient transference of force[22]. The present study showed that the desmin was detected in regenerating spindle fibers as well as in intact spindles and the expression pattern of these intermediate filaments was more intense in PRP treated groups that was confirmed by computer image analysis. These are likely to reflect a wider range of contractile properties of the intrafusal fibers and the more complex architecture and functions of PRP treated groups. Similarly, Cízková *et al*.,[22] found that spindle muscle cells expressing desmin strongly should be more mature than elements revealing weak desmin immunoreactivity. Hammond *et al*.,[14] founded that the PRP injections significantly shortened the time to complete recovery and improved contractile function.

From the cell cycle point of view, factors controlling the cell cycle and cellular proliferation can be easily detected by immunostaining using Ki-67 expression which is a measure of cell growth fraction. Nuclear antigen Ki-67 is lacking in resting cells (G0 phase) and hence exclusively positive in the nuclei of proliferating cells[24]. In addition, Johnson Chacko *et al*.[45] stated that Ki-67 expression is restricted to the undifferentiated cells, whereas the differentiated cells are negative for this marker. In the current work, significant reduction in the ki-67 expression level was detected in the apparently normal newly formed spindle muscle in PRP treated group. These results indicated that the PRP increased cell proliferation and differentiation. These findings are in line with those previously reported in Guitart *et al*,[46] who stated that muscle recovery following splint removal result in a significant rise in regeneration events along with a reduction in the expression of satellite cell activation factors (active cells as identified by Ki-67 expression levels) and a concomitant rise in terminal muscle differentiation expression. Many researchers showed that PRP injected into injured muscles accelerates regeneration compared to controls[13,14]. Mammoto *et al*.[47] reported that PRP promotes lung regeneration as it maintains vascular integrity in *vivo* and in *vitro*, stimulates new blood vessel formation and enhances phosphorylation of low-density lipoprotein receptor and thus activates angiogenic factor receptors in the endothelial cells and accelerates endothelial cell sprouting. PRP can not only promote the muscle recovery process but also decrease the apoptotic cells[12].

CONCLUSION

PRP plays a major role in promoting the proliferation, differentiation and has a neurotrophic function in muscle spindle regeneration. These findings provide useful and indispensable application of PRP in muscle spindle injury.

AUTHORS CONTRIBUTION

MMES designed research; YR performed research; MMES, YR and AMD Analyzed data; MMES, YR and AMD Wrote the article and revising it critically. The authors declare no conflict of interest.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تقييم حقن البلازما الغنية بالصفائح الدموية على إصابة المغزل العضلي في نموذج الفئران (دراسة هستولوجية وهستوكيميائية مناعية)

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خلفية الدراسة: يعد حقن البلازما الغني بالصفائح هذه الأيام من العلاجات الشائعة في الجراحات التكميلية والحوادث. ويحتوي البلازما على عوامل نمو متنوعة والتي تعجل التئام الخلية وتشكلها.

الغرض من الدراسة: تقييم تاثير جرعة واحدة من حقن البلازما في تنشيط التئام العضلة المغزلية المصابة.

المواد وطرق البحث: اربعون من الفئران يتم توزيعهم الى ثلاث مجموعات : مجموعة ضابطة ومجموعة بعد أسبوع من الإصابة ومجموعة بعد ثلاثة أسابيع. المجموعة العضلات المصابة سواء المعالجة بالحقن او غير محقونة سيتم معاالجة العينات بصبغات للانسجة وأخرى صبغات للانسجة المناعية عن طريق الديسمن وال ki-٦٧ وتحليلها الصورة عن طريق الكمبيوتر.

النتائج: ساهم العلاج بتلك الطريقة في ظهور الياف عضلية والعديد من تكون الاوعية الدموية في الأسبوع الأول من الإصابة. بينما في المجموعة الغير معالجة بالحقن ظهرت تحببات صاحبتها تجمع لخلايا ملتهبة وزيادة الياف الكولاجين. بينما تلك المعالجة بعد ٣ أسابيع أظهرت الياف عضلية مغزلية واضحة محاطة بغلاف نسيجي متعدد الطبقات كتلك الموجودة في المجموعة الضابطة . كما ظهرت صبغة الدسمن في الالياف المعالجة بالحقن كتلك الظاهر في الياف المجموعة الضابطة الغير مصابة كما لوحظ أيضا انخفاض ملحوظ في مستويات ki ٦٧ في تلك المجموعة المعالجة واظهرت التئام كبير.

الخلاصة: حقن البلازما الغني بالصفائح يلعب دور كبير في تحفيز نمو النسيج العضلي وتشكله بالإضافة الى التغذية العصبية في التئام الالياف العضلية المغزلية.

وهذه النتائج يعد تطبيقا مفيدا لحقن البلازما الغني بالصفائح في إصابة الالياف العضلية المغزلية.