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ORIGINAL ARTICLE

# Effect of platelet-rich plasma on reconstruction with nerve autografts



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**Abstract** Despite advances in understanding of peripheral nerve injuries and regeneration and advances in surgical techniques, successful outcomes cannot be guaranteed after reconstructive surgery. Platelet-rich plasma (PRP) has been reported to have positive effects on nerve regeneration, as well as on tissue healing. The present study was designed to evaluate the effect of PRP on nerve-grafted defects. Sprague–Dawley rats were divided into four surgery groups ( $n = 7$  in each). A 1-cm long nerve defect was created in the upper thigh and then reconstructed using a nerve autograft in all groups. The wet muscle weights, electromyographic findings, and histomorphologic changes were evaluated 10 weeks later. As shown by both the electromyographic ( $p < 0.001$ ) and histomorphologic findings ( $p < 0.001$ ), PRP had more positive effects on nerve gap reconstruction in Group 3 than Group 4 as compared to the control groups. The present study is novel in that it evaluated the regeneration effect of PRP on a large nerve defect reconstructed with a nerve graft rather than primary repair. The results are encouraging for further experimental studies on the role of PRP in nerve healing.

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Conflicts of interest: All authors declare no conflicts of interest.

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## Introduction

Although there are several available methods to repair nerve defects, reconstruction using nerve grafts remains the gold standard and produces the most successful results [1–6]. However, even when nerve grafts are used for reconstruction of nerve defects, recovery rates may not be satisfactory [7]. Therefore, the potential of additional treatments, including growth factors, hormones, and mediators, has been tested in nerve regeneration studies to strengthen the current gold standard method [7].

Grafts survival depends on surface diffusion in the early stages, with thinner grafts more likely to survive than thicker grafts due to better diffusion ability. As reported previously, thick nerve grafts can result in central necrosis due to a lack of diffusion [8]. To address this issue, in the present study, we performed a modification in two groups, which increased the surface area of standard nerve grafts. This modification was partial resection of the grafts epineural layer.

Platelet-rich plasma (PRP) is easy to obtain and relatively cheap. It also has a low risk of immunological side effects as it can be obtained autogenously. The platelets in PRP are rich in growth factors. In clinical use, PRP is derived from the patient's own blood. The growth factors inside the granules of platelets are secreted locally after the activation of PRP, with a long effecting time [9].

In addition to its positive effects on the healing of many types of tissues, recent studies reported that PRP had positive effects on nerve regeneration [10–13]. These effects were attributed to the growth factors (platelet derived growth factor, fibroblast growth factor, vascular endothelial growth factor, and insulin like growth factor I) that PRP contains [14–17]. Studies in literature commonly have focused on the development of alternatives to nerve grafts.

The present study was designed to evaluate the effect of PRP on nerve graft reconstruction of a nerve defect. This is a novel study in that it evaluated the regeneration of a long nerve graft segment treated with PRP.

## Methods

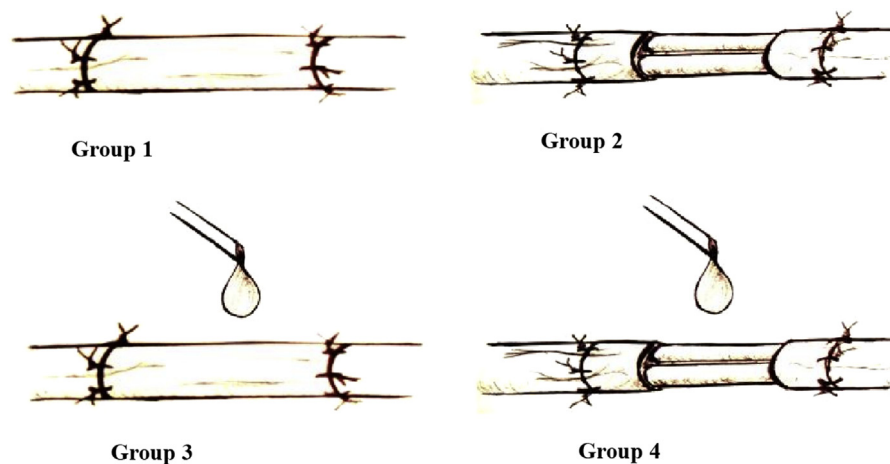
### Animals and laboratory

All experiments and protocols described in the present study were performed in accordance with the Guide for the Care and Use of Laboratory Animals, as adopted by National Institutes of Health (USA) and also approved by the Medical Faculty Experimental Ethics Committee of Dokuz Eylül University (Izmir, Turkey; Ethical Committee Number: 87/2012). Thirty-eight Sprague–Dawley rats were used in this study. The rats were kept under a 12-hour light/dark cycle (light from 07.00 to 19.00), in quiet rooms, with 22–24°C ambient temperature and provided free access to standard rat nutrients and purified drinking water *ad libitum*.

### Surgical groups

The present study consisted of four experimental groups (2 surgery-only groups and 2 surgery plus PRP groups), with seven rats in each group. Another 10 rats were sacrificed, and whole blood was taken to obtain PRP. In Group 1 (Control group), a standard nerve graft was used for reconstruction. In Group 2, a partially peeled nerve graft was used for nerve reconstruction. In this group, only the central part of the graft epineurium (~6 mm) was resected to increase graft surface area. In Group 3, a standard nerve graft was used for reconstruction, and PRP was then applied around the reconstruction area. In Group 4, a partially peeled nerve graft was used for nerve reconstruction, and PRP was then applied around the reconstruction area. The make-up of the groups is described in Figure 1.

In all the groups, only the left sciatic nerves were operated upon, with 1-cm long nerve segments resected as nerve autografts and then used in the nerve reconstructions of the same nerve defect. Similarly, in all the rat groups, the grafts were not rotated or turned upside down but



**Figure 1.** Schematic views of experimental groups. Group 1: 1 cm long nerve graft is used in original position for defect reconstruction with six epineural sutures on each anastomosis. Group 2: 1 cm long nerve graft is used in original position for defect reconstruction with 6 epineural sutures on each anastomosis. Additionally, from the mid-area of graft, about 6 mm long epineural sheet is resected. Group 3: Same surgery procedure as Group 1 and 0.5 mL PRP applied around graft. Group 4: Same surgery procedure as Group 2 and 0.5 mL PRP applied around graft.

replaced in their original positions. After the surgery, the rats were placed in separate cages under the care of a specialist veterinarian.

## PRP

As rats have a limited amount of blood for autologous PRP preparation, the whole blood of 10 rats was used for PRP production. To prevent graft versus host reaction, the blood was irradiated using 25 Gy ionized radiation on the same day (immediately before) as the PRP preparation. PRP and autologous thrombin solution was prepared using the protocol described by Franco et al. [14].

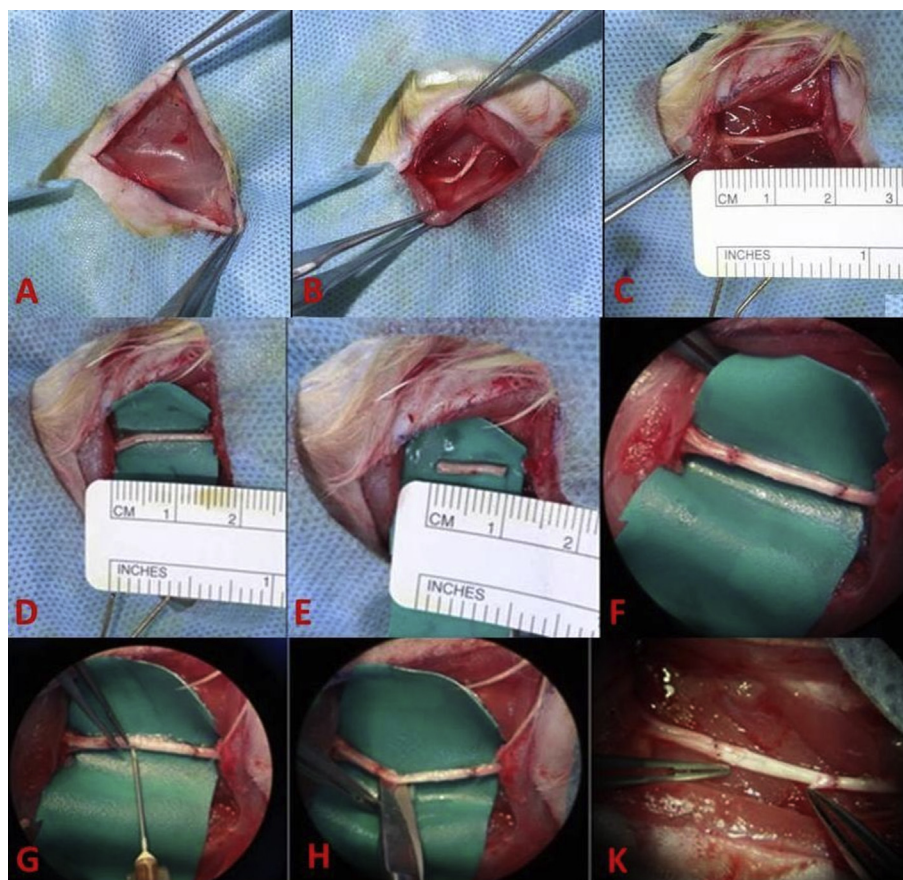
## Surgical technique

Under intramuscular ketamine-HCl and xylazine HCl anesthesia, the same surgeon operated on the left legs of the rats, and the right sides were used as controls.

The skin and superficial gluteus and biceps femoris muscles were incised to reveal the sciatic nerve from the sciatic notch to the popliteal branching level. The sciatic

nerve was then dissected and freed from the surrounding tissue. The thin motor branches to the semitendinous, semimembranous, and biceps muscles were also sacrificed to ensure the proper release of sciatic nerve. A 1-cm long nerve segment was measured and resected 1–2 mm proximal to the bifurcation point. This free segment was used as a nerve graft to reconstruct the same defect, without rotating or turning the graft upside down. The reattachment of the graft to its original position was performed by placing six epineural sutures with 10-0 Ethilon (Johnson & Johnson – Ethicon Endo-Surgery, Inc., Cincinnati, OH, USA).

In Groups 2 and 4, a long epineural sheet, approximately 6 mm, was peeled off circumferentially from the mid-area of the nerve graft to reveal the fascicle area, which was surrounded only by the perineural layer that maintains the blood-brain barrier. In Groups 3 and 4, 0.5 mL of PRP was injected into the surgical field. After 10 weeks, electrophysiological studies of the sciatic nerves were performed under general anesthesia. Muscle and nerve biopsies were then taken, and the rats were sacrificed. Details on the surgical protocols are shown in Figure 2.



**Figure 2.** Exploration and dissection of rat sciatic nerve to release it from surrounding tissue. (A) Arising muscles after skin incision. (B) Exploration of nerve after separating semitendinous and semimembranous muscles. (C) Releasing of nerve from surrounding soft tissue. (D) In order to release sciatic nerve completely the thin motor branch to semitendinous and semimembranous muscles was sacrificed. (E) A 1-cm long nerve segment was measured and resected from 1–2 mm proximal to bifurcation point. (F) Reconstruction of defect with resected segment. (G) Resection of epineural layer from graft under magnification of microscope 25-gauge needle is used to incise epineurium. (H) Dissection and resection of epineurium from the central part of the graft. (K) Appearance after epineural resection.

## Electrophysiological assessments

The Biopac MP-30 system (BIOPAC Systems, Inc., Goleta, CA, USA) was used for electromyography (EMG) recordings under general anesthesia. The EMG recordings were obtained from both the proximal and distal levels of the nerves for three times by giving stimulus which produced supramaximal muscle response with 1 Hz frequency and 0.05 m/sn duration. The EMG data were processed using the Biopac Student Lab Pro software, version 3.6.7 (BIOPAC Systems, Inc.) program.

## Histomorphologic assessments

### Muscle weight ratios

The whole gastrocnemius muscles of the posterior extremities were excised. The wet muscle weights from the operated left and nonoperated right sides were weighed using a digital scale (BP4100S; Sartorius, Göttingen, Germany).

### Histology

Histologic assessments were undertaken of slices from the middle side and distal to the nerve graft. It was assumed that there were no differences between proximal sides. Histological preparations were stained with toluidine blue and anti-S-100. In all the groups, the numbers of immune-

reactive Schwann cells around myelin rings in the middle region of the nerve grafts were counted and then analyzed statistically.

### Measurement of the thickness of perineural fibrosis

In each group, the Image-Pro Express 4.5 (Media Cybernetics, Inc., Rockville, MD, USA) program was used to measure the thicknesses of the perineural layers in the middle regions of the grafts and the level of fibrosis covering these layers in the histological specimens and then analyzed statistically.

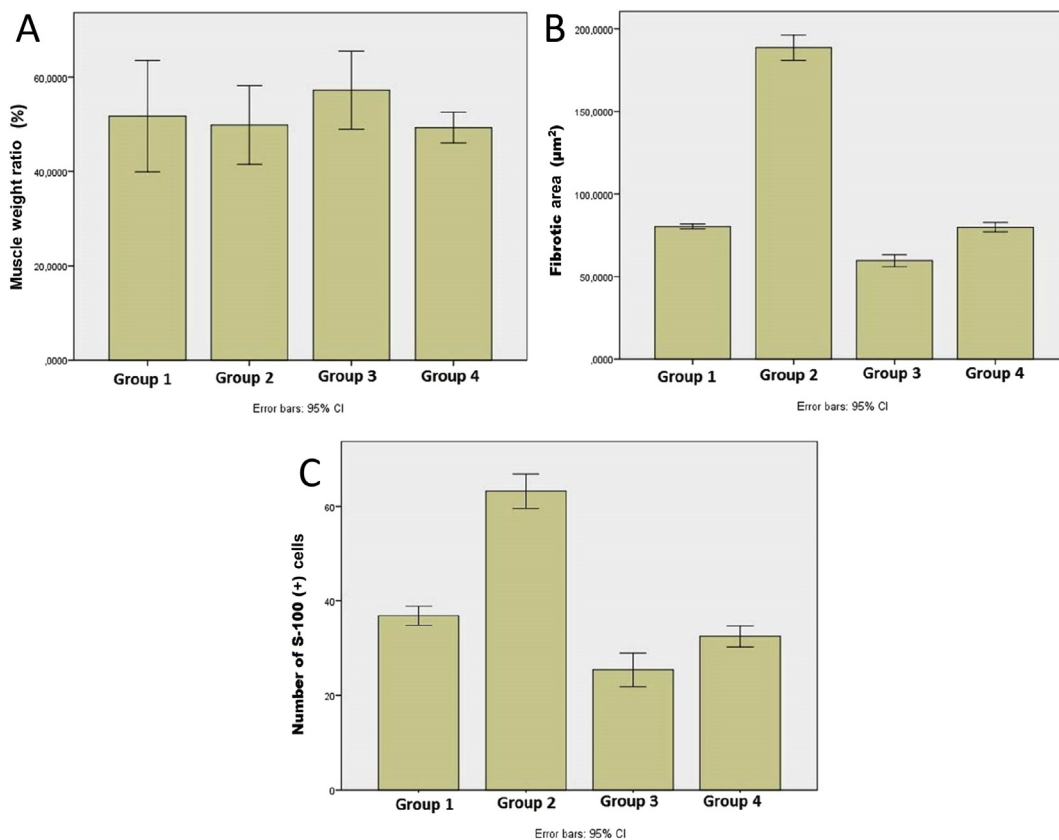
## Results

### Muscle morphometrics

There was no significant difference in the morphometrical parameters of the groups according to the Kruskal–Wallis test. Similarly, there was no statistically significant difference between the muscle weight ratios of any of the groups (Figure 3A).

### Electrophysiology

Table 1 shows the average compound muscle action potential amplitude, which was correlated with axonal regeneration, values of the groups. Group 3 showed better axonal



**Figure 3.** (A) Comparison of groups muscle weight ratios. (B) Statistical analysis of Perineural fibrosis of the groups ( $p < 0.001$ ). (C) Statistical analysis of S-100 (+) Schwann cell counts of the groups ( $p < 0.001$ ).



**Table 1** Average compound muscle action potential (CMAP) amplitude results of the groups obtained by electromyography evaluation.

Groups	CMAP amplitude values (mV)
Normal right posterior legs	10.2 ± 0.4
Group 1 left posterior legs	0.3 ± 0.1
Group 2 left posterior legs	0.1 ± 0.07*
Group 3 left posterior legs	0.9 ± 0.2**
Group 4 left posterior legs	0.4 ± 0.1 ***

\*  $p < 0.05$  Groups 1 and 2.

\*\*  $p < 0.05$  Groups 1 and 3.

\*\*\*  $p > 0.05$  Groups 1 and 4.

regeneration properties than the control group (Group 1). In all the groups, there was no statistically significant difference in axonal regeneration of the nonoperated right sides. However, there was a significant difference in axonal regeneration between the operated sides of Groups 2 and 3 ( $p < 0.05$  and  $p < 0.001$ , respectively) as compared to Group 1. There was no difference in axonal regeneration between Groups 1 and 4 ( $p > 0.05$ ; Table 1).

According to these results, the level of axonal regeneration was much higher in Group 3 (PRP group) than in Group 1. In Group 2, the epineural sheet resection had a negative effect on axonal regeneration when compared with that in Group 1.

## Histology

### Graft area

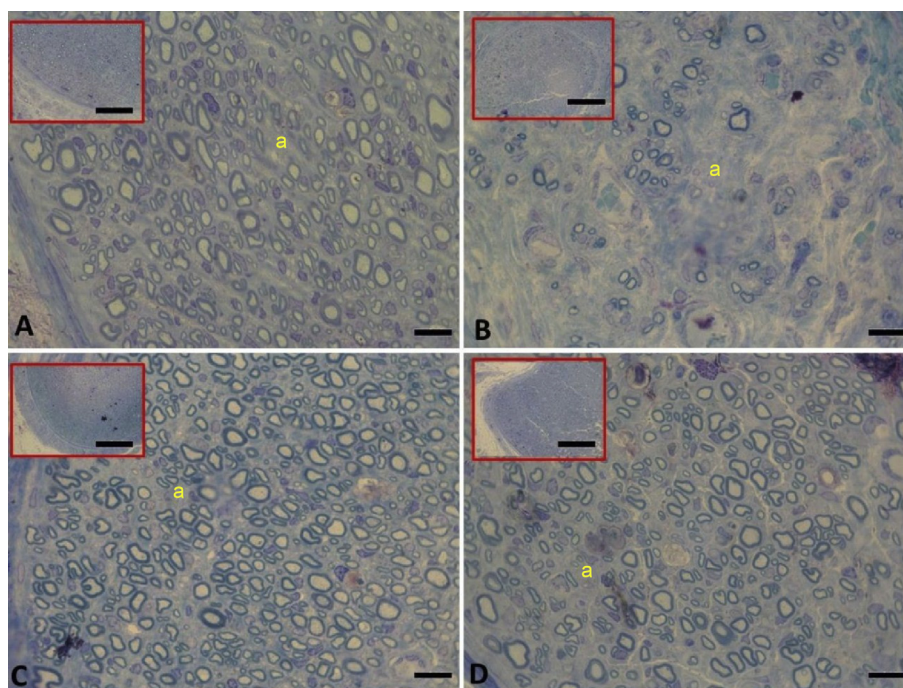
In Group 1, conformational deterioration and separation of small, medium, and larger myelinated rings were observed.

In addition, the endoneurium and perineurium showed minimal thickening, and the axons were rarely blurred. Of all the groups, Group 2 showed the highest level of conformational deterioration, separation of myelinated fibers, and axonal degeneration. In Group 3, the endoneurium resembled normal sciatic nerve histology, with most preserved axonal configuration. Although the perineurium was thicker than normal, it was not as thick as in Group 2. In addition, the endothelium of the vessels was apparent as a sign of good neovascularization. In Group 4, the histological findings were similar to those in Group 1, with big myelinated axons preserved more than Group 2 (Figures 4 and 5). Interestingly, in Groups 3 and 4, metachromatic mast cells, which are thought to play an important role in the nerve-healing process, were observed close to or just around the vessels, with more of these cells observed in Group 3 (Figure 5).

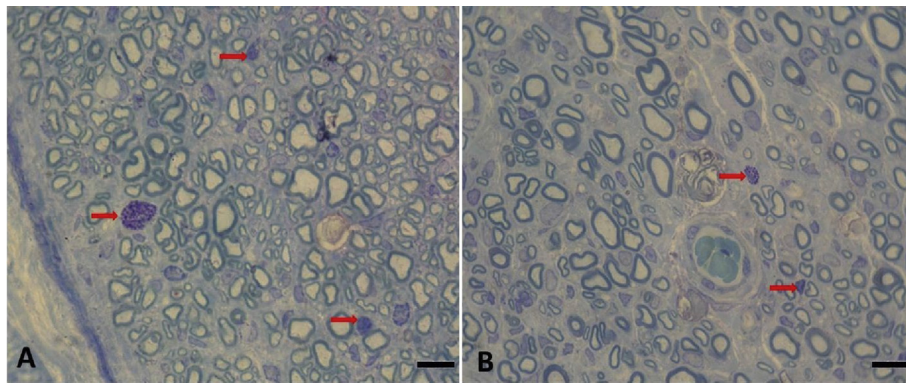
### Anti-S-100 staining

In Group 1, more positive immune reactivity to the S-100 dye was observed in the peripheral areas than in the central regions where the myelinated rings were blurred. In Group 2, both the central and peripheral regions showed very strong immune reactivity, and cytoplasmic hypertrophy of Schwann cells was particularly apparent in small- and medium-sized myelinated rings. Histological findings of Group 3 were the most similar to normal peripheral nerve morphology. In Group 3, central staining reactivity was statistically significant when compared with that in Group 1. In Group 4, central and peripheral S-100 reactivity were similar, with the normal nerve structure (Figure 6).

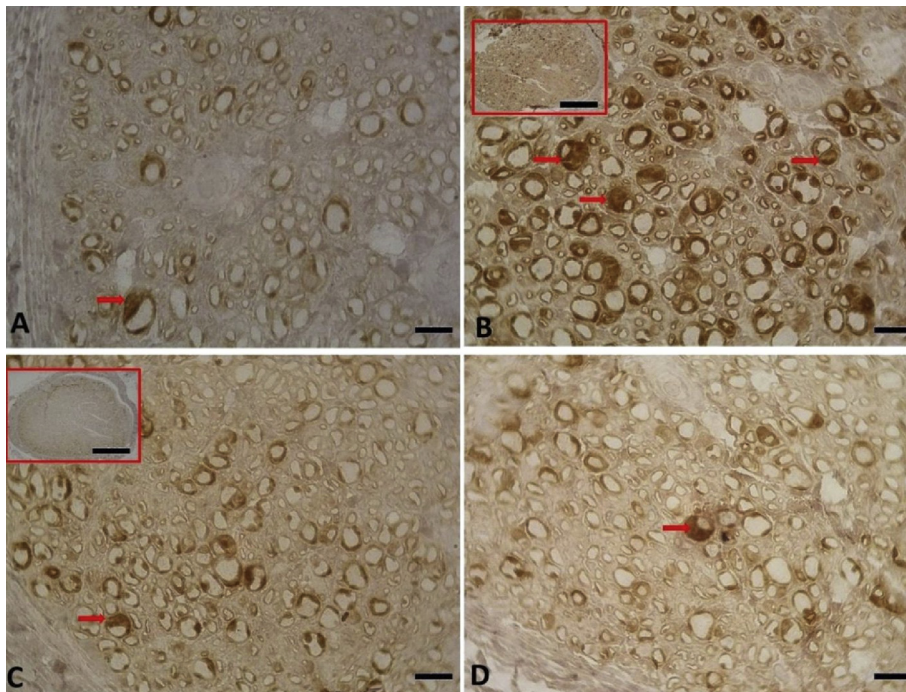
Due to the immune-reactive Schwann cell counts, the axonal degeneration in Group 3 was better compared to the Group 1 ( $p < 0.001$ ). Although the severe degeneration findings in Group 4 were relatively better than Group 2



**Figure 4.** Toluidine blue staining ( $\times 100$  magnification; small frames are  $\times 20$  magnification. Scale bar = 250  $\mu\text{m}$ ). (A) Sciatic nerve of Group 1. (B) Sciatic nerve of Group 2. (C) Sciatic nerve of Group 3. (D) Sciatic nerve of Group 4. <sup>a</sup> Endoneurium.



**Figure 5.** Toluidine blue staining. (A) Sciatic nerve of Group 3. (B) Sciatic nerve of Group 4. Red arrows indicate mast cells;  $\times 100$  magnification, scale bar =  $50 \mu\text{m}$ .



**Figure 6.** Anti S100 staining. (A) Sciatic nerve of Group 1. (B) Sciatic nerve of Group 2. (C) Sciatic nerve of Group 3. (D) Sciatic nerve of Group 4. Red arrows indicate Schwann cells with hypertrophic cytoplasm;  $\times 100$  magnification, scale bar =  $50 \mu\text{m}$ ; small frames are  $\times 20$  magnification, scale bar =  $250 \mu\text{m}$ .

( $p < 0.05$ ), the histological findings both in these groups were negatively affected by the partial resection of epineurium [Group 2 compared with Group 1 ( $p < 0.001$ ) and Group 4 with Group 3 ( $p < 0.001$ ); [Figure 3C](#)]. In consequence of these findings, PRP had benefits on nerve healing but the resection of epineurium did not.

#### Distal to graft area

The distal areas of the nerves in Group 1 contained the highest number of large myelinated axons among all the groups. Among all the groups, the highest levels of conformational deterioration, degeneration, and blurring of axons were observed in the distal areas of the nerves in Group 2. In Group 3, most of the axons in the distal areas of

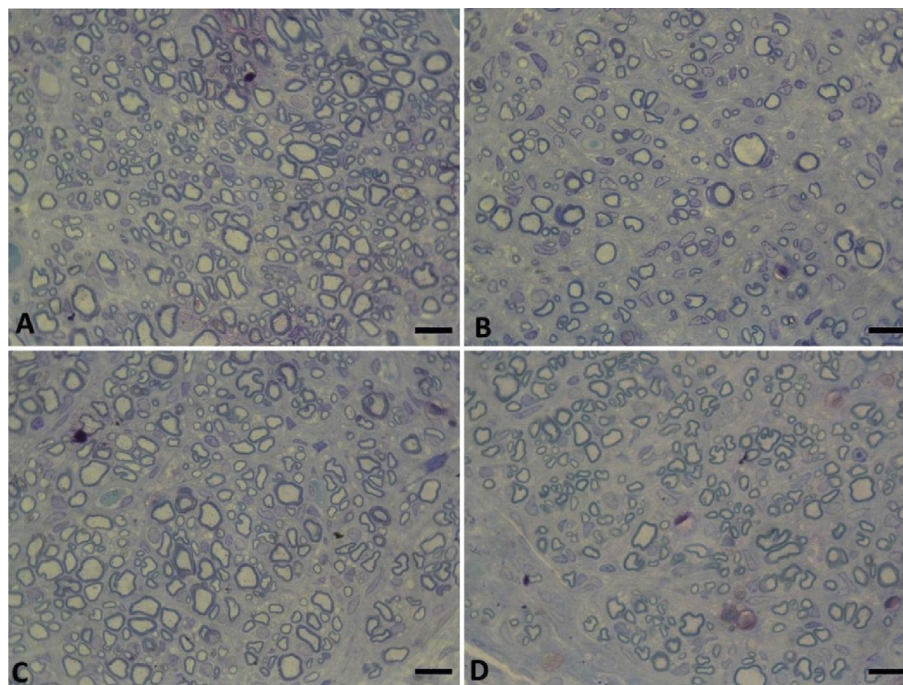
the nerves showed myelination, with minimal conformational deterioration, and blurring. In addition, endoneural and perineural thickness were relatively increased in Group 3. In Group 4, there were fewer large myelinated axons in the distal areas of the nerves as compared to Group 1 ([Figure 7](#)).

There were increased numbers of mast cells close to the vessels in Groups 3 and 4. This was observed both in the graft and in the distal nerve. Compared to Group 3, the numbers of mast cells were reduced in Group 4.

#### Perineural fibrosis

Less fibrosis developed in the PRP groups than the non-PRP groups (Group 1 compared with Group 3,  $p < 0.05$ ; and





**Figure 7.** Toluidine blue staining. (A) The distal parts of the sciatic nerves of Group 1. (B) The distal parts of the sciatic nerves of Group 2. (C) The distal parts of the sciatic nerves of Group 3. (D) The distal parts of the sciatic nerves of Group 4.  $\times 100$  magnification, scale bar = 50  $\mu\text{m}$ .

Group 2 compared with Group 4,  $p < 0.05$ ). In Groups 2 and 4, in which some of the epineural layers were resected, the increase in fibrosis was statistically significant then unresected groups (Group 1 compared with Group 2,  $p < 0.05$ ; and Group 3 compared with Group 4,  $p < 0.05$ ). The fibrosis thickness was greatest in Group 2, in which only the central part of the graft epineurium was resected, and PRP was not applied to the surgical site (Figure 3B).

## Discussion

Artificial biological substances and a variety of tissues other than nervous tissue and nerve allografts have been widely used in experimental studies of nerve defect reconstruction in the literature [1,3,4]. However, their clinical usage is still limited, and nerve autografts remain the gold standard method for the reconstruction of nerve defects [1–6].

In this experiment, excised nerve segments were used as nerve grafts in the same area. The nerve grafts were not inverted or rotated because our aim was to investigate PRP's effect on standard method rather than comparing the effects of changes in the topographic anatomy of grafts. Therefore, the topography of the fascicles was considered to be same in all the groups.

The use of active substances in nerve regeneration can have different effects on healing in primary nerve repair models and nerve defect models. For example, according to a study by Welch et al. [18] on the use of trophic factors in primary repair and nerve defect models, when the nerve stumps were tightly stacked together, the effects of mechanical factors become more dominant than those of trophic factors in the nerve-healing process. However, both

studies of primary repair models and studies of crush injury models reported that PRP had positive effects on nerve regeneration [12,13].

To improve healing, the graft tissue must be revascularized. Some studies claim that neovascularization from the evolving soft tissue bed was the most important mechanism in the restoration of blood flow in such grafts [19,20]. Another study suggested that revascularization might occur via bidirectional inosculation, which favored proximal vascular growth [21]. However, a study by Best et al. [22] found no evidence of peripheral neovascularization or dependence on the graft bed as a source of revascularization in conventional grafts.

In addition to revascularization, diffusion plays a role in graft survival, with thinner grafts surviving more often than thicker grafts due to better diffusion ability. The lack of diffusion in thick nerve grafts has been attributed to central necrosis [8]. We speculated that the elimination or reduction of barriers that blocked diffusion might promote healing. Thus, in the present study, in two of the experimental groups (Groups 2 and 4), we excised some of the epineural sheet in the middle parts of the grafts to enhance surface diffusion.

Many studies have reported that PRP had positive effects on the nerve-healing process [10–13]. These effects were attributed to nerve-healing growth factors and promoters secreted from activated platelets (platelet derived growth factor, fibroblast growth factor, vascular endothelial growth factor, and insulin like growth factor I) [14–17].

If recombinant equivalents of these factors are applied locally just once, their effects will not be exerted for a sufficient period to induce healing. To enhance their activity, they must be applied a number of times or applied by

some additional methods (e.g., fibrin sealant clots, foams, organic/synthetic tubes, and osmotic pumps) that guarantee a long secretion time. However, the aforementioned formulations are all associated with various risks such as infection and foreign body reactions [23–26]. By contrast, one study demonstrated that PRP exerted its effects for a long period (about 4 weeks) without any other method [9].

In the present study, the histology of the grafted area in Group 3 was thought to be most similar to that of normal peripheral nerve histology, and we concluded that this contributed to the effects of PRP. Although PRP showed some benefit in Group 4 (partially peeled graft group), the same peeling procedure of the epineural layer caused severe degeneration in Group 2. These positive findings were related to the protective effects of PRP.

Although the Schwann cell count was lowest in Group 3, it was closest to normal. Most of the cells in Group 2 showed neuroma development, with large numbers of Schwann cells, especially around thin and moderate myelinated fibers.

Epineural scar formation during nerve repair prevents axonal growth, in addition to causing adhesions of nerve, which is thought to precipitate traction injury and ischemia during movements [27]. In the present study, PRP had beneficial effects when the groups were compared with respect to epineural fibrosis of the graft. However, PRP did not ameliorate the negative effects of the epineural peeling procedure (Groups 2 and 4).

The PRP treatment resulted in increased mast cell numbers in Groups 3 and 4, with the increase more significant in Group 3. Kotulska et al. [28] suggested that there was a relationship between mast cells and neuroma formation. Based on the findings of the present study, we speculate that mast cells may be associated with revascularization and nerve regeneration rather than neuroma formation and that the increase in the mast cell population was due to enrichment with PRP-related factors in the microenvironment.

Electrophysiological assessments are frequently used to evaluate the regeneration of peripheral nerves. The distance between negative and positive peaks in compound muscle action potential curves (p-p distance), which is obtained by submaximal stimulation, is referred to as the amplitude [29]. The amplitude provides information about the number of motor fibers evoked by a stimulus, synchronization of their responses, and the number of the innervated motor units. Electrophysiological findings were compatible with histological ones. PRP had a positive effect in Group 3 ( $p < 0.001$ ) and the epineural peeling procedure had a negative effect in Group 2 ( $p < 0.05$ ) when compared to Group 1.

In conclusion, although many factors, including neurotropic factors, are known to affect nerve regeneration, the impacts of these factors remain unclear. Compared to recombinant growth factor, PRP has a number of advantages, including being easy to obtain, quick to apply, and cost effective. The present study confirmed that PRP had positive effects on nerve regeneration and healing, but there are limited studies in the literature about nerve healing with PRP. This is a novel study in that the healing effects of PRP over the nerve graft were studied for the first time. These positive results were probably due to the growth

factors in PRP. To shed more light on the effects of PRP on nerve regeneration, more studies are needed.

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