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Effects of leukocyte-platelets rich fibrin and nanoparticles on the healing of hemisected sciatic nerve in dogs: Experimental study

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Abstract

The regeneration of peripheral nerves, especially the sciatic nerve, remains limited, making clinical management difficult and outcomes of routine surgical techniques often unsatisfactory. Emerging bioactive materials, including Leukocyte–Platelet Rich Fibrin (L-PRF) and Nano–Magnesium Oxide (NMO), offer new therapeutic possibilities. The primary objective of this research was to conduct a comparative assessment of the regenerative capabilities of L-PRF and NMO in enhancing both functional recovery and structural repair of hemisected sciatic nerves in dogs. Twenty-seven adult male dogs were randomly allocated into three groups (n = 9): control (untreated), L-PRF, and NMO (treated). Following standardized surgical hemitransection of the sciatic nerve, the bioactive materials were locally applied at the site of incision according to the respective experimental groups. Clinical observations, macroscopic assessments, histopathological examination, and immunohistochemical analysis for collagen type IV were performed at 7, 30, and 60 days postoperatively. The NMO group achieved the fastest and most complete limb function recovery, with histology showing enhanced remyelination, organized collagen deposition, and reduced inflammation. Immunohistochemistry indicated early, strong upregulation of collagen type IV, suggesting accelerated matrix remodeling and nerve organization. The L-PRF group also improved sciatic nerve regeneration over the control, though to a lesser extent than NMO. This study provides compelling evidence that both NMO and L-PRF significantly enhance peripheral nerve repair compared to the control, with NMO exhibiting the most pronounced improvement in both structural and functional recovery among all groups. These findings underscore the therapeutic potential of NMO and L-PRF as adjunctive strategies in peripheral nerve repair.

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Introduction

The lumbosacral plexus is constituted by the convergence of the ventral rami of the spinal nerves from the fourth lumbar to the second sacral segments (1). This plexus is considered the main component of the femoral, obturator and sciatic nerve as well as the tibial and peroneal nerves (2). The largest peripheral nerve, the sciatic nerve, is naturally protected against external trauma due to its deep location near bony structures and beneath thick muscle layers (3). Nonetheless trauma or injury of the peripheral nerves especially the sciatic nerve is not uncommon in dogs. There have been numerous cases of sciatic nerve injuries reported in dogs, conjunct with pelvis and femur fractures, as well as with penetrating and deep wounds or iatrogenic causes. In addition, sciatic nerve damage resulting from age or some diseases (4). Various surgical approaches have been employed in the treatment of peripheral nerve damages, including nerve autografting, direct end-to-end coaptation of the damaged nerve ends, and the application of artificial nerve guidance conduits. Despite the use of these advanced techniques, the outcomes of peripheral nerve regeneration

remain suboptimal (5). Recent studies shown the local application of bioactive materials at the site of peripheral nerve injury has yielded satisfactory results in nerve healing and almost complete restoration of function (6).

Platelet-Rich Fibrin is a biologically functional active substance which has excellent results in tissue regeneration with improvement the healing of injured soft and hard tissues (7). Platelet-rich fibrin contains more than 1500 bioactive factors counting growth factors, cytokines, chemokines and enzymes (8). There are four main different methods used to obtain concentrated platelets. These different methods produce concentrated platelets in different specifications modulated by the presence of leukocytes and texture or solidity of the fibrin which formed; they are Pure-Platelet Rich Plasma, Leukocyte-Platelet Rich Plasma, Pure-Platelet Rich Fibrin, and Leukocyte-Platelet Rich Fibrin (L-PRF) (9). L-PRF is considered the second generation of concentrated platelets that does not require adding of an anticoagulant or other chemical substances to the fresh collected peripheral blood sample. In addition to that, the procedure of L-PRF considered as a simplest, fastest method and least expensive with production of sufficient quantities of platelets rich fibrin (10). The components of L-PRF matrix are approximately 50% of leukocytes and about 97% platelets of the original blood (11). A distinctive feature of L-PRF is its sustained and progressive release of growth factors and cytokines, which facilitate angiogenesis and encourage mesenchymal stem cells to multiply and specialize within the damaged tissue. Several researches have shown that the adding of L-PRF at the site of injured peripheral nerves greatly helps in enhancing and accelerating its healing (12).

On the other hand, nanomaterials are defined as a materials having a size less than 100 nanometers. Nanomaterials have unique properties that made it's used in various life sectors, including agriculture, environmental sciences, medicine and cosmetic medicine (13). Nanotechnology has found applications across various medical disciplines, particularly in the diagnosis and treatment of certain diseases, as well as in the field of regenerating tissues and accelerating their healing, moreover using nanotechnology in aesthetics industry, dentistry, also in manufacture of various medicines and delivering them to the required tissues in the body (14). Recently, researches have reported that the nanomaterials possess an ability to improve or accelerate healing of soft and hard tissue injuries (15). Due to their nanoscale dimensions, nanomaterials play a crucial role in tissue repair by enhancing the activity of growth factors and anti-inflammatory agents, and by influencing cellular interactions and tissue penetration (16). Nowadays they are used in regeneration and enhancing healing of peripheral nerves injuries (17). One of these materials is Nano-Magnesium Oxide (NMO) which has many distinct properties and characteristics, which makes it one of the materials that support the results of scientific research in various fields of medicine. NMO is characterized by high biological activity, biocompatibility, biodegradability, in addition to being non-toxic material and also has antibacterial and anti-cancer properties (18).

Immunohistochemistry examination arises from the broad range of specific antibodies available, enabling the identification of axons, Schwann cells, macrophages, fibroblasts, vascular structures, and various other elements associated with nerve repair (19). Collagen is considered as a structural unit protein presents in various body tissues. Every tissue in the body contains a basement membrane which is an integral part to support it, and this basement membrane is mainly composed of collagen type IV (20). Collagen type IV acts as a scaffold for integrity and cohesion of cells, as well as for interaction between the cells. collagen IV is responsible for cells adjacency, migration, as well as their differentiation, proliferation, and survival, within the tissue (21). This study aimed to evaluate and compare the effects of L-PRF and NMO on the healing process of a hemitransected sciatic nerve in dogs, based on clinical observations, histopathological findings, and immunohistochemical analysis.

Materials and methods

Ethical Approval

The study protocol was reviewed and approved by the Ethics Committee of the College of Veterinary Medicine, University of Mosul, approval No. UM.VET.2024.067, with official confirmation granted on March 28, 2024.

Experimental Design

The experiment was designed to compare the effect of both L-PRF and NMO on the healing of partial sciatic nerve transection in dogs. Twenty-seven adult male dogs, weighing between 12-26 ±0.88 kg and aged between 18-30 ±0.72 months. The animals were maintained in the animal facility of the College of Veterinary Medicine, University of Mosul. They were randomly assigned into three equal groups, each consisting of nine animals: the first group served as the control (C); the second group received L-PRF; and the third group was treated with NMO. The experimental follow-up extended over a two-month period, during which tissue specimens from all experimental groups were systematically harvested at 7, 30, and 60 days postoperatively for comprehensive histopathological and immunohistochemical analyses**.**

Preparation of L-PRF

Prior to the surgical procedure in the animals of the second group, 10 ml of jugular vein blood was drawn and placed in a plain test tube free of anticoagulant agents. The blood sample was immediately subjected to centrifugation at 2700 rpm (equivalent to 408 g) for a duration of 12 minutes. Subsequently, the fibrin clot was extracted from the tube, and the red blood cells were carefully trimmed away using sterile

surgical scissors. The isolated fibrin was then applied locally to the site of the sciatic nerve hemitransection (11).

Preparation of NMO

In the third group (NMO), nanoparticles composed of magnesium oxide were utilized. Manufactured by US Research Nanomaterials, Inc., with a reported purity of 99% on average particle size (APS) 20nm with specific surface area (SSA) 60 m^2/g". A sensitive balance was used to weigh 100 micrograms of NMO particles which were dissolved in 5 ml of distilled water. Then take 2 ml of this suspension were taken, equivalent to 40 micrograms of NMO, were injected directly at the sciatic hemitransected site (22).

Surgical procedure

Following the induction of general anesthesia in all animals, which included in this study with ketamine and xylazine at respective doses of 10 mg/kg and 3 mg/kg, which were administered via intramuscular injection, and Atropine sulfate was administered intramuscularly at a dose of 0.04 mg/kg about 10 minutes prior (23). The aseptic surgical technique was used to make a 10 cm skin incision at the lateral side of the thigh area. The sciatic nerve, located along the posterior side of the femur, was exposed by carefully bluntly dissecting the thigh muscles. At the mid-thigh level, the sciatic nerve was undergone a standardized transverse hemitransection, using a surgical scalpel after careful isolation from the surrounding musculature (Figure 1). The nerve was then left unsutured. After that, the muscles were sutured with Vicryl suture No. 1/0 and the skin was closed with Silk suture No. 1. In the first group (C group), the sciatic nerve was left untreated following hemitransection, without application of any bioactive substances. In the second group L-PRF, the fibrin was applied at the site of the sciatic nerve hemitransection. In the third group NMO, nanoparticles were administered at the hemitransected site of the sciatic nerve.

Histopathological and Immunohistochemical Preparation Methods

Histopathological examinations were performed on biopsies collected from the three groups included in this study at three different time points. Biopsies were obtained from the site of sciatic nerve hemitransection at the 7^{th}, 30^{th}, and 60^{th} day post-surgery. An estimated 1 cm segment of the sciatic nerve was removed at the hemitransection site, and the proximal and distal nerve stumps were then approximated and sutured with 3/0 nylon suture material. Postoperatively, the animal received appropriate care and treatment until full recovery. Biopsy samples of the sciatic nerve were preserved in 10% neutral buffered formalin, processed through paraffin embedding, and then stained with hematoxylin and eosin for histopathological examination (24). Immunohistochemical staining for collagen type IV was carried out using a rabbit polyclonal anti-collagen IV antibody (Sigma-Aldrich, C1926, USA), which has been previously reported to exhibit cross-reactivity with canine tissues. Sample preparation, including deparaffinization, antigen retrieval, and blocking procedures, was performed according to the protocol described by Abed *et al*. (25). Visualization of immunoreactivity was achieved using a chromogenic detection system, and the stained sections were examined microscopically to assess collagen IV expression.

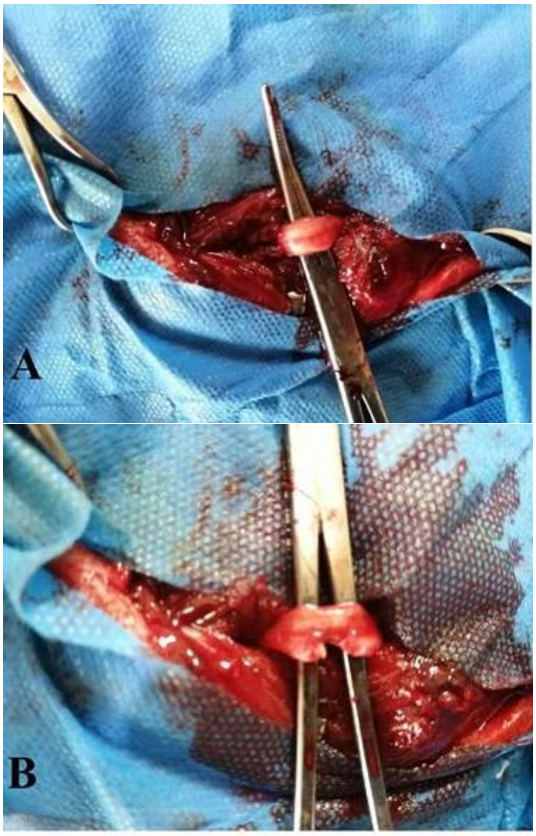

Figure 1: Intraoperative exposure and experimental transection of sciatic nerve in a canine model. (A) Macroscopic view of the intact sciatic nerve prior to surgical intervention. (B) Sciatic nerve following standardized hemitransection at the mid-thigh level, illustrating the created defect for subsequent evaluation of nerve regeneration.

Results

Clinical observations

The research spanned two months, throughout which the experimental animals were consistently observed. All animals included in the study exhibited signs of inflammation at the surgical site (lateral aspect of the thigh), which resolved within 7 to 10 days postoperatively. During the first postoperative week, all animals in the study groups exhibited pronounced lameness, marked by a failure to support weight on the injured limb or to properly place it on

the surface. Animals in the control group (C) revealed persistent lameness characterized by continuous non-weight-bearing of the affected limb from the first postoperative week. However, the severity of lameness gradually diminished over time, with partial weight-bearing observed by the end of the eighth week. In contrast, animals in the second group (L-PRF) began to bear weight partially on the affected limb by the end of fifth postoperative week, exhibiting only mild lameness that persisted through the remainder of the study period. In the third group (NMO), animals initiated weight-bearing on the affected limb by the fourth postoperative week, although gait instability and episodes of stumbling were still evident during ambulation. By the eighth week, gait patterns had substantially improved, with near-complete restoration of weight-bearing on the injured limb.

Gross examination of sciatic nerve

Gross examination of the hemitransection sciatic nerve site was performed at the 7th, 30th, and 60th day postoperatively, coinciding with the nerve biopsy collection periods for subsequent histopathological and immunohistochemical analysis. In the control group, gross examination at 7th day postoperatively revealed severe inflammation at the injured site, characterized by visible fluid accumulation and significant swelling. At the 30th day postoperatively, pronounced adhesions were observed between the hemitransection sciatic nerve and the surrounding thigh musculature. By 60th day postoperatively, while the adhesions appeared less severe upon gross inspection, noticeable thickening was evident at the location of the nerve damage. In the L-PRF group, gross examination at the 7th day postoperatively revealed clear signs of inflammation, though less severe in comparison with the control group. At 30th day postoperatively, moderate adhesions were observed between the sciatic nerve and the surrounding musculature. By 60th day postoperatively, all signs of inflammation had resolved, and no adhesions were noted. However, the site of nerve injury remained subtly detectable. In the NMO group, gross examination at the 7th day postoperatively revealed signs of inflammation at the surgical site, with swelling observed in the region of the injured sciatic nerve. By 30th day postoperatively, the surgical site showed minimal signs of inflammation and closely resembled normal tissue. At this stage, the sciatic nerve exhibited no visible swelling or palpable thickening at the site of injury. By 60th day postoperatively, it became increasingly difficult to distinguish the site of nerve injury, which appeared nearly identical to its normal state.

Histopathological examination

Histopathological examination at the 7th day post-operation revealed distinct inflammatory responses among the groups. In the control group (C), the inflammatory reaction was characterized by vascular congestion, marked infiltration of inflammatory cells, minimal Schwann cell proliferation, and limited neovascularization (Figure 2-C). In contrast, both the L-PRF and NMO groups exhibited comparable histopathological features, including moderate infiltration of inflammatory cells, moderate Schwann cell proliferation, vascular congestion, and mild formation of new capillaries (Figure 2-L-PRF; Figure 2-NMO).

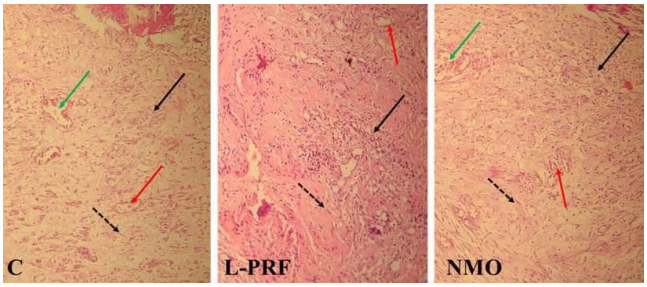

Figure 2: Microscopic examination of the first period at 7 days post-hemitransection of sciatic nerve in the experimental dogs. C: Control group, shows vascular congestion (green arrow), marked infiltration of inflammatory cells (black arrow), minimal proliferation of Schwann cells (black dotted arrow), and limited formation of new capillaries (red arrow). L-PRF; and NMO: show moderate infiltration of inflammatory cells, (black arrow), moderate Schwan cell proliferation (black dotted arrow), vascular congestion (green arrow), and mild new capillary formation (red arrow), H&E stain, 100X.

Histopathological evaluation at the 30th day post-operation revealed notable differences among the study groups. Within the control group, there was evident proliferation of Schwann cells, wavy nerve fibers, and axonal vacuolation (Figure 3-A), accompanied by inflammatory cells infiltration, severe vascular congestion, and disorganized collagen fibers (Figure 3-B). The L-PRF group exhibited enhanced regenerative activity compared to the control group. Sections revealed marked Schwann cells proliferation, and moderate inflammatory cell infiltration, along with the presence of edema (Figure 4-A). Additionally, wavy nerve fibers, severe formation of new capillaries, and disorganized collagen structures were observed, along with evident alterations in the architecture of the epineurium (Figure 4-B). These features suggest active, though still disorganized, reparative processes. In the NMO group, histological sections showed signs of nerve fiber remyelination, characterized by densely packed eosinophilic nerve bundles and prominent proliferation of Schwann cells organized into endoneurial tubes. Although inflammatory cells were still present, their infiltration appeared less pronounced compared to the control group (Figure 5-A). Moreover, collagen fibers were notably better organized, and only mild hemorrhage was evident in the surrounding tissue (Figure 5-B).

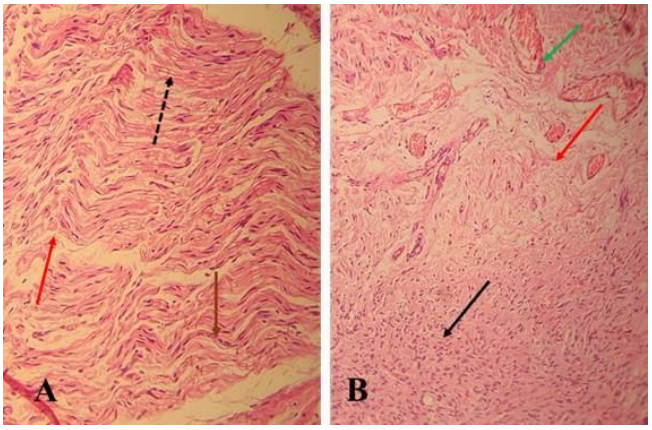

Figure 3: Microscopic examination of the second period at 30th day post-hemitransection of sciatic nerve in the dogs of control group. A: Shows proliferation of Schwann cells as endoneurial tubes (brown arrow), wavy nerve fiber (red arrow) and vacuolation of nerve axonal (black dot arrow). B: Shows infiltration of inflammatory cells (black arrow) and severe vascular congestion (green arrow), disorganized collagen fibers (red arrow). H&E stain, 100X.

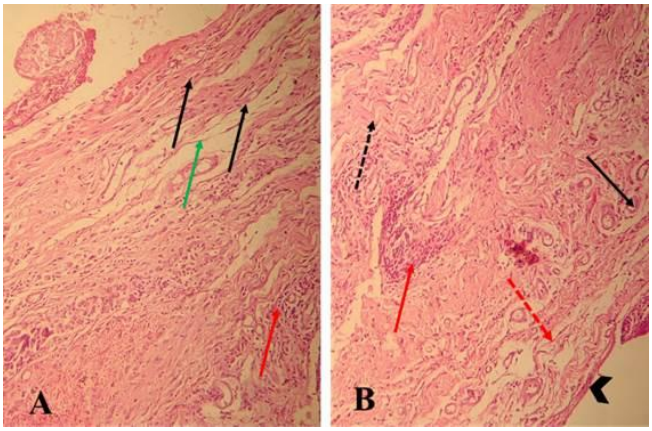

Figure 4: Microscopic examination of the second period at 30th day post-hemitransection of sciatic nerve in the dogs of L-PRF group. A: Exhibits marked Schwann cells proliferation (black arrow), infiltration of inflammatory cells (red arrow) and edema (green arrow). B: Shows wavy nerve fibers (black dot arrow), severe newly capillaries formation (black arrow), severe infiltration of inflammatory cells (red arrow), disorganized collagen (red dotted arrow), epineurium structure (black head arrow). H&E stain, 100X.

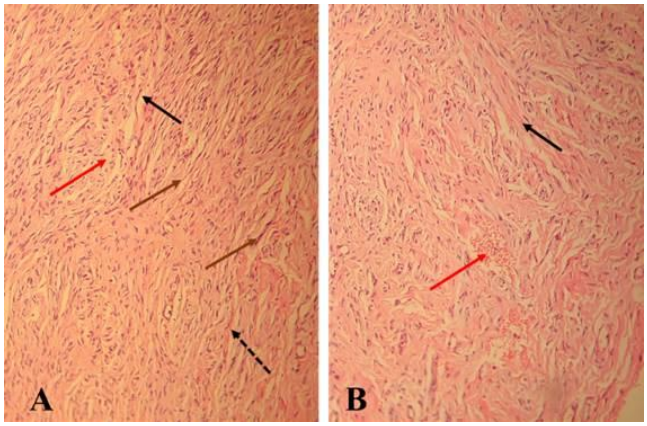

Figure 5: Microscopic examination of the second period at 30th day post-hemitransection of sciatic nerve in the dogs of NMO group. A: Shows nerve fiber remyelination (black arrow), densely packed eosinophilic nerve bundles (black dot arrow), sever proliferation of Schwann cells as endoneurial tubes (brown arrow) with infiltration of inflammatory cells (red arrow). B: Shows well organized collagen fibers (black arrow) and mild hemorrhage (red arrow). H&E stain, 100X.

At the 60th day post-surgical intervention, microscopic tissue analysis demonstrated notable variations in the extent of nerve regeneration across the experimental groups. Within the control group, microscopic analysis demonstrated the presence of axonal nerve fibers and wavy myelinated fibers (Figure 6-A). Additionally, there was marked proliferation of Schwann cells organized within endoneurial tubes, accompanied by notable inflammatory cells infiltration, vascular congestion, and interstitial edema (Figure 6-B). These features indicated a moderate degree of regenerative activity, but also suggested ongoing inflammatory response and incomplete tissue recovery. The L-PRF group exhibited histopathological evidence of improved neural regeneration relative to the control group. A slight increase in Schwann cells activity and emerging myelinated nerve fibers, with mild infiltration of inflammatory cells (Figure 7-A), along with detection of axonal nerve fibers were noted (Figure 7-B). However, regenerative changes remained limited, and signs of incomplete structural restoration persisted. In contrast, the NMO group demonstrated superior histopathological outcomes. Sections revealed presence of clearly defined axonal nerve fibers, minimal inflammatory cells infiltration, and only mild Schwann cells proliferation (Figure 8-A). Additionally, well-organized and continuous myelinated nerve fibers with a parallel orientation were observed (Figure 8-B), which further confirmed the advanced stage of neural regeneration in this group. Based on these histopathological findings, the NMO group showed the most significant and typical evidence of nerve repair and structural restoration at this postoperative period.

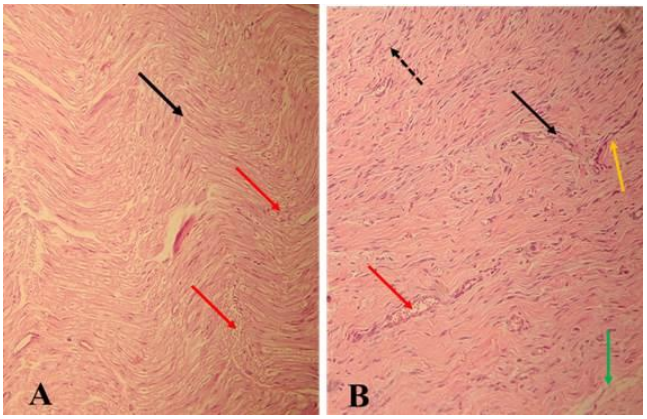

Figure 6: Microscopic examination of the third period at 60th day post-hemitransection of sciatic nerve in the dogs of the control group. A: Shows axonal nerve fibers (red arrow), wavy myelinated nerve fibers (black arrow). B: Show marked proliferation of Schwann cells (black dot arrow), arranged as endoneurial tubes (yellow arrow), infiltration of inflammatory cells (black arrow), congestion of a blood vessels (red arrow) and interstitial edema (green arrow). H&E stain, 100X.

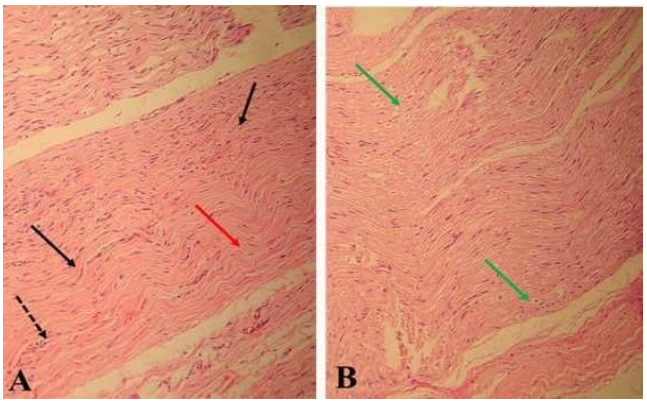

Figure 7: Microscopic examination of the third period at 60th day post-hemitransection of sciatic nerve in the dogs of the L-PRF group. A: Shows well continues myelinated nerve fibers (red arrow), mild proliferation of Schwan cells (black arrow), mild infiltration of inflammatory cells (black dot arrow). B: axonal nerve fibers (green arrow). H&E stain, 100X.

Immunohistochemistry Examination

In the context of peripheral nerve injuries, immunohistochemistry (IHC) stands out as a highly effective technique for evaluating the regeneration process. At the first time point at 7 days post-surgery, the immunohistochemical analysis of collagen type IV showed low expression levels in the control group (Figure 9), mild expression in the L-PRF group (Figure 10), and moderate immunoreactivity in the NMO group (Figure 11).

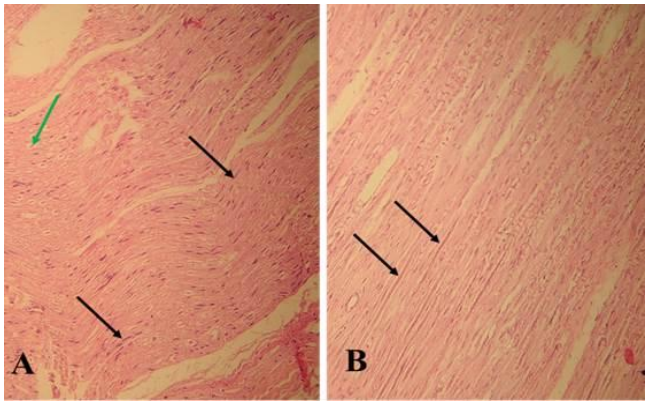

Figure 8: Microscopic examination of the third period at 60th day post-hemitransection of sciatic nerve in the dogs of the NMO group. A: mild proliferation of Schwan cells (black arrow), axonal myelinated nerve fibers (green arrow). B: Myelinated nerve fibers (black arrow). H&E stain, 100X.

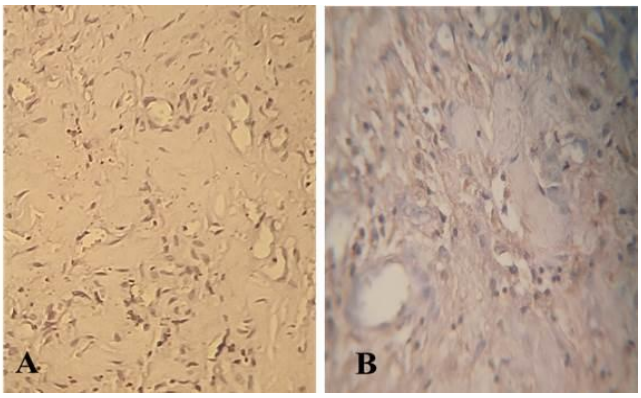

Figure 9: Immunohistochemical examination of collagen IV at 7th day post-hemitransection of sciatic nerve in the dogs of control group. A: negative immunoreactivity staining. B: weak immunoreactivity staining, 400X.

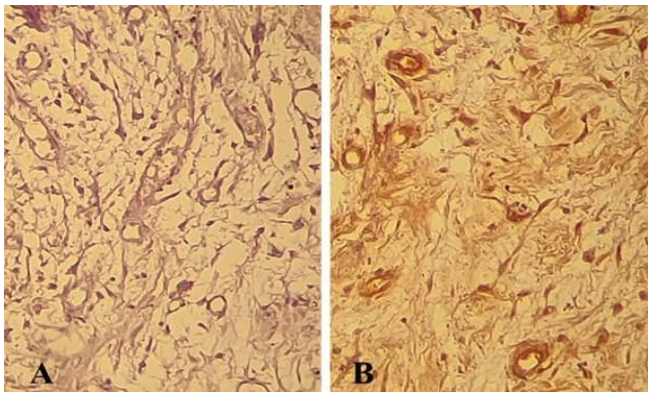

Figure 10: Immunohistochemical examination of collagen IV at 7th day post-hemitransection of sciatic nerve in the dogs of the L-PRF group. A: negative immunoreactivity staining. B: mild immunoreactivity staining, 400X.

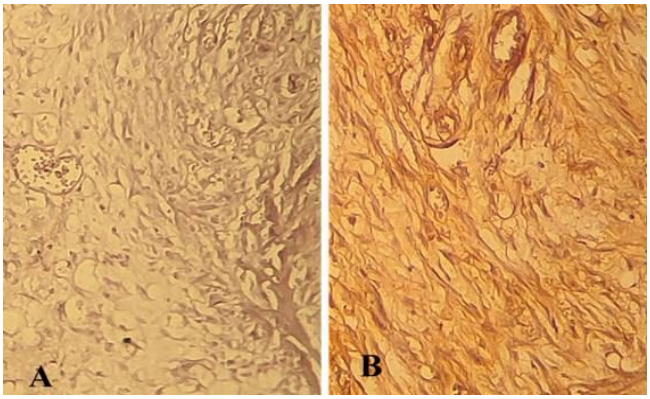

Figure 11: Immunohistochemical examination of collagen IV at 7th day post-hemitransection of sciatic nerve in the dogs of the NMO group. A: negative immunoreactivity staining. B: moderate immunoreactivity staining, 400X.

Immunohistochemical analysis of collagen type IV at the second time point at 30th day post-operation, demonstrated strong immunoreactivity in the control group (Figure 12) and markedly increased (stronger) immunoreactivity at L-PRF group (Figure 13). Notably, while NMO group exhibited intense (very strong) immunoreactivity (Figure 14), indicating a pronounced upregulation of collagen type IV expression.

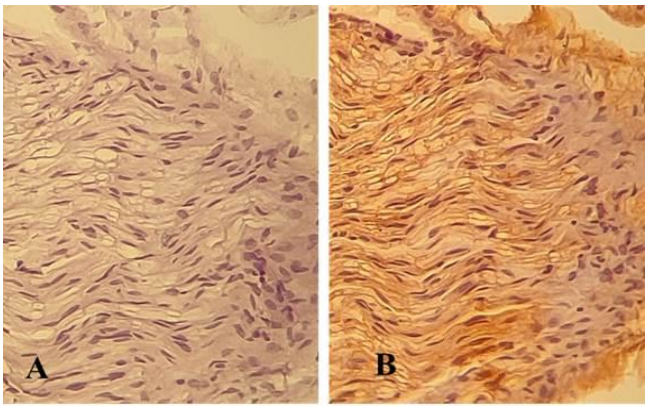

Figure 12: Immunohistochemical examination of collagen IV at 30th day post-hemitransection of sciatic nerve in the dogs of the control group. A: negative immunoreactivity staining. B: strong immunoreactivity staining, 400X.

At the third postoperative time point (60th day), immunohistochemical staining for collagen type IV demonstrated intense (very strong) immunoreactivity in the control group (Figure 15), strong immunoreactivity in the L-PRF group (Figure 16), and moderate immunoreactivity in the NMO group (Figure 17). These findings highlight distinct variations in collagen type IV expression among the different experimental treatment groups.

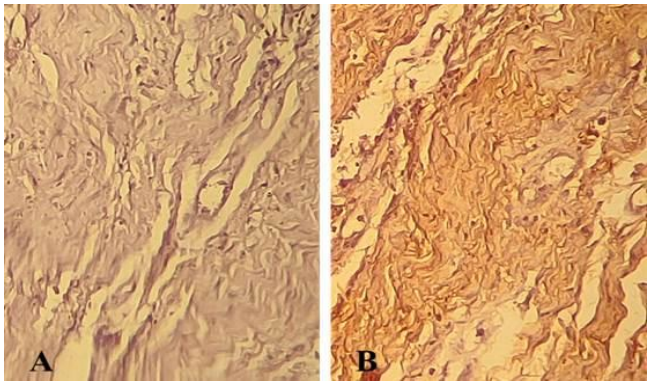

Figure 13: Immunohistochemical examination of collagen IV at 30th day post-hemitransection of sciatic nerve in the dogs of the L-PRF group. A: negative immunoreactivity staining. B: pronounced (stronger) immunoreactivity staining, 400X.

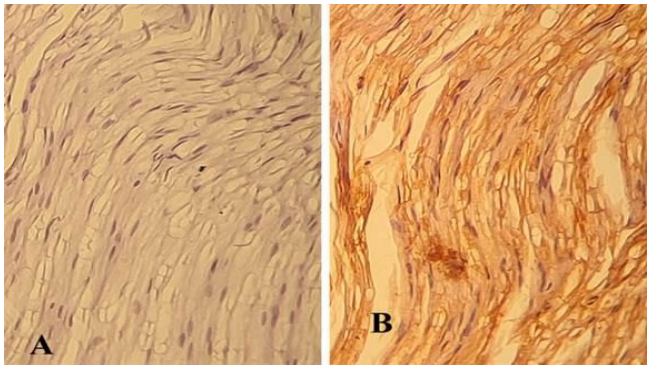

Figure 14: Immunohistochemical examination of collagen IV at 30th day post-hemitransection of sciatic nerve in the dogs of the NMO group. A: negative immunoreactivity staining. B: intense (very strong) immunoreactivity staining, 400X.

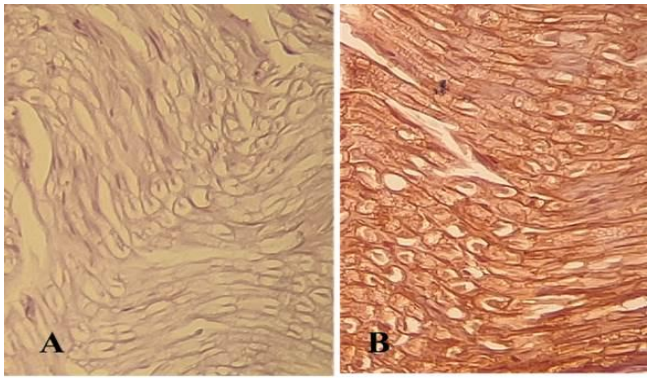

Figure 15: Immunohistochemical examination of collagen IV at 60th day post-hemitransection of sciatic nerve in the dogs of the control group. A: negative immunoreactivity staining. B: intense (very strong) immunoreactivity staining, 400X.

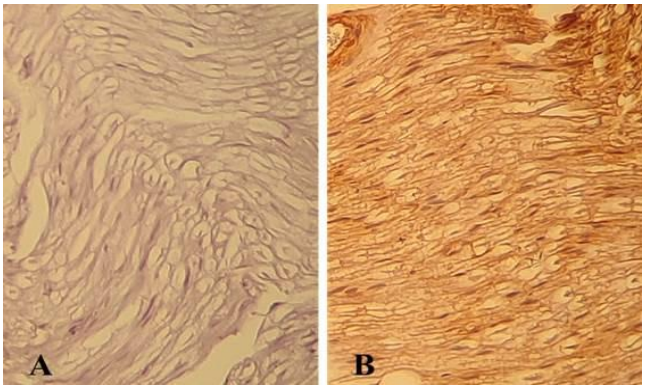

Figure 16: Immunohistochemical examination of collagen IV at 60th day post-hemitransection of sciatic nerve in the dogs of the L-PRF group. A: negative immunoreactivity staining. B: strong immunoreactivity staining, 400X.

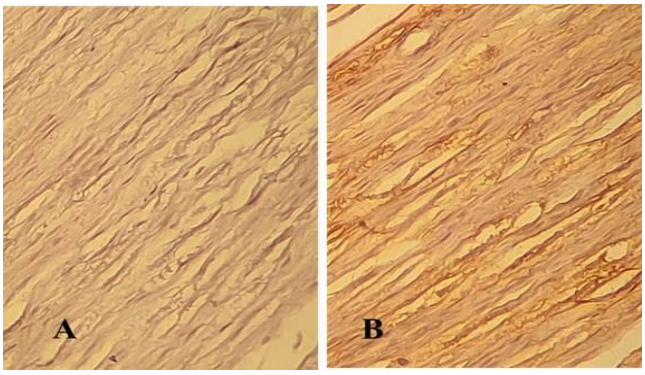

Figure 17: Immunohistochemical examination of collagen IV at 60th day post-hemitransection of sciatic nerve in the dogs of the NMO group. A: negative immunoreactivity staining. B: moderate immunoreactivity staining, 400X.

Discussion

The sciatic nerve is one of the largest nerves of the hind limb and is responsible for innervating of most of the hind limb muscles, particularly the muscles located at the back of the thigh, including the biceps femoris, semimembranosus, semitendinosus, and the ischial head of the adductor magnus. These muscles contribute to essential movements like knee flexion and hip adduction (26). Therefore, any injury to the sciatic nerve commonly results in pronounced pain and lameness in the affected limb. This observation is confirmed by other workers (4,27). Who emphasized that the lameness is a major clinical sign following sciatic nerve damage.

In our study, the severity and duration of lameness varied among experimental groups, with the most severe lameness being in the first group (C) and the least severe and lasting for a shorter period of time in the third group (NMO), as the lameness in the second group (L-PRF) was less severe than in the first group. This could be due to the absence of bioactive material application following the sciatic nerve hemitransection in the control group, which resulted in clear lameness throughout the study period. In the NMO group, the magnesium oxide nanoparticles were applied at the hemitransection site, serving as biologically active agents. Researchers (28); confirmed that the magnesium has an efficient stimulating healing effect of peripheral nerves by modulating the inflammatory response. Furthermore, previous studies (29); reported that the magnesium oxide nanoparticles markedly enhance peripheral nerve regeneration, further supporting their therapeutic potential in nerve repair. As for the second group, the L-PRF group, the limping was less severe than in the control group. This may be due to presence of L-PRF, which is a biologically active substance applied at the hemitransection site of the sciatic nerve. This finding is consistent with the results of other researchers (30). Who reported that the application of platelet-rich fibrin to the site of sciatic nerve injury enhanced nerve regeneration and healing.

The histopathological assessment at 7th day following sciatic nerve hemitransection revealed distinct inflammatory and regenerative responses among the experimental groups, reflecting early-stage healing dynamics in peripheral nerve injury. In the control group, the presence of vascular congestion, extensive inflammatory cells infiltration, limited Schwann cells proliferation, and minimal neovascularization suggest a predominance of acute inflammatory processes with minimal regenerative activity. These findings align with the classical wound healing response where, in the absence of therapeutic intervention, the early phase post-injury is marked by cellular infiltration and tissue edema without substantial structural repair (31,32). In contrast, the L-PRF and NMO groups exhibited histopathological changes indicative of a modulated inflammatory response and early regenerative activity. The positive outcomes noted in the L-PRF group are likely linked to the bioactive characteristics of leukocyte- platelet rich fibrin, which serves as a matrix enriched with growth factors like PDGF, TGF-β, and VEGF. These molecules play key roles in enhancing blood vessel formation, regulating inflammatory responses, and stimulating Schwann cell activity (33,34). The inclusion of leukocytes in L-PRF may also aid in regulating the immune response by maintaining a balance between pro-inflammatory and anti-inflammatory cytokines. Similarly, the NMO group, treated with nanoparticle-based therapy, demonstrated comparable histopathological patterns. Nanomaterials are known to provide neuroprotective and anti-inflammatory benefits, frequently promoting cell proliferation and new blood vessel formation via controlled release systems and surface-driven interactions with surrounding tissues (35). The histological differences at this early postoperative time point suggest that both L-PRF and nanoparticle treatments contribute to an improved cellular microenvironment that facilitates early regenerative processes.

By the 30th day post-operation, the histopathological landscape in all groups had progressed beyond the acute inflammatory phase toward more established tissue remodeling and early regenerative events. In the control group, a moderate increase in Schwann cell numbers and the presence of wavy nerve fibers indicate the early stages of nerve regeneration. However, these were accompanied by persistent inflammatory cells infiltration, severe vascular congestion, and disorganized collagen deposition. Additionally, axonal vacuolation a hallmark of axonal degeneration or incomplete remyelination was observed. These findings are consistent with delayed or suboptimal nerve repair in untreated injuries, where sustained inflammation and collagen disarray may impair axonal regeneration and functional recovery, and this is in agreement with other workers (36). Who said that the lack of organized extracellular matrix (ECM) architecture and ongoing vascular congestion may further hinder nutrient delivery and cellular integration at the location of the injury. In the L-PRF-treated group, Schwann cell proliferation was more pronounced, indicating active engagement in axonal regeneration and endoneurial restructuring. The increase in Schwann cell activity and nerve fiber reorganization points to accelerated nerve healing relative to the control group and these findings support those of previous research (34,37). Who declared that the L-PRF stimulates regenerative responses through sustained release of growth factors and scaffold support. Notably, the NMO group displayed the most advanced histological evidence of regeneration, represented by the presence of densely packed eosinophilic nerve bundles and evidence of remyelination suggest effective axonal restoration. Proliferation of Schwann cells forming endoneurial tubes is a critical indicator of successful Wallerian degeneration resolution and subsequent axonal guidance and regeneration, these findings support those of previous research (38). Who indicated that the well-organized collagen fibers indicate restoration of ECM architecture, which provides structural integrity and supports further cellular migration and axonal extension. Compared to the other groups, the NMO group demonstrated a more mature regenerative response, possibly due to enhanced neuroprotective, anti-inflammatory, and matrix-organizing properties conferred by the nanomaterials (39).

At 60th day post-operation, the histopathological evaluation demonstrated varying degrees of nerve repair among the three experimental groups. In the control group, detection of axonal nerve fibers and wavy myelinated structures suggests the progression of endogenous repair mechanisms, and this agrees with (40). Who reported that the proliferation of Schwann cells within endoneurial tubes is a hallmark of axonal guidance and remyelination. However, the persistent presence of inflammatory cells, vascular congestion, and interstitial edema denotes an unresolved inflammatory response, which may hinder complete nerve regeneration and this coincides with other workers (41). Who mentioned that the chronic inflammation and tissue edema have been shown to impair axonal growth and promote fibrosis, compromising functional recovery. In contrast, the histopathological architecture in the L-PRF group showed significant improvement. The mild Schwann cells proliferation and appearance of nascent myelinated fibers indicates a supportive microenvironment, with cellular activity transitioning towards maintenance of the regenerated tissue. The presence of continuous, well-organized myelinated nerve fibers and minimal inflammatory cells infiltration reflect a more advanced stage of nerve regeneration. These outcomes align with previous findings reported by (42,43). Who confirmed the proposed benefits of L-PRF in promoting angiogenesis, reducing inflammation, and facilitating tissue remodeling via sustained release of bioactive molecules from concentration of leukocytes and platelets like PDGF, VEGF, and TGF-β. The NMO group demonstrated the most advanced histopathological regeneration. The presence of well-aligned, continuous myelinated fibers and clearly defined axonal profiles, coupled with minimal inflammatory cells infiltration, reflects a near-complete structural restoration. Magnesium-based nanoparticles are increasingly recognized for their neuroregenerative potential. Magnesium plays a vital role in regulating calcium channels, reducing oxidative stress, and modulating pro-inflammatory cytokine release (44). At the nanoscale, magnesium oxide exhibits enhanced cellular uptake and sustained ion release, promoting Schwann cell activity, axonal elongation, and myelination (45). The mild proliferation of Schwann cells observed in this group suggests a transition from the proliferative to the maturation phase of nerve healing, which aligns with the timeline of remyelination and axonal reorganization reported in recent peripheral nerve injury models (46).

The immunohistochemical evaluation of collagen type IV at three distinct post-operative time points (7th, 30th, and 60th day) revealed dynamic patterns of expression across the control, L-PRF, and NMO groups. Type IV collagen is an essential structural element of the basal lamina, which is vital for the repair and maintenance of the structural integrity of peripheral nerves after injury. Its expression serves as a key indicator of extracellular matrix (ECM) remodeling and angiogenic activity during nerve repair (47).

At the early phase of regeneration 7th day, the control group exhibited low levels of collagen type IV expression. likely reflecting the initial phase of Wallerian degeneration and limited matrix deposition at this stage (48). In contrast, the L-PRF group exhibited mild expression, suggesting an early stimulatory effect of growth factors and cytokines released from leukocyte and platelet contained within the L-PRF matrix, which may have initiated angiogenesis and ECM reorganization (49). Notably, the NMO group demonstrated moderate immunoreactivity, indicating that nano-magnesium oxide may have a more pronounced effect on early matrix remodeling, potentially due to its bioactive

properties and role in modulating cellular oxidative stress and macrophage polarization (50). By day 30, collagen type IV expression was markedly upregulated in all groups, with the control group showing strong reactivity, likely due to the natural course of nerve regeneration where Schwann cells and fibroblasts contribute to ECM remodeling and basal lamina reconstruction (51). The L-PRF group exhibited even stronger immunoreactivity, consistent with findings that L-PRF enhances the proliferation of Schwann cells and neovascularization, which are critical for nerve bridge formation and guidance of regenerating axons (52). The NMO group presented the highest intensity of collagen IV expression, suggesting that nano-magnesium oxide may not only support structural regeneration but also influence ECM deposition through enhanced fibroblast activation and integrin-mediated signaling pathways (28). Interestingly, by day 60, collagen IV expression patterns diverged among the groups. The control group maintained intense expression, which might reflect a prolonged phase of matrix deposition and scar maturation. In the L-PRF group, collagen IV levels were slightly reduced but remained strongly expressed, indicating a possibly more regulated and mature matrix environment supportive of functional recovery (53,54). However, in the NMO group, a moderate decline in expression was noted, suggesting that the earlier intense stimulation may have led to an accelerated transition to tissue remodeling and maturation, with reduced the need for sustained ECM component expression at this stage. This trend may imply that nano-magnesium oxide facilitates an earlier shift from inflammatory to regenerative phases (39). In summary, immunohistochemical findings of Collagen IV expression demonstrate that both L-PRF and NMO influence ECM remodeling during peripheral nerve regeneration. L-PRF facilitates sustained matrix activity via growth factor release, whereas NMO induces a pronounced early response, likely through oxidative stress attenuation, immunomodulation, and angiogenic activation, suggesting a superior healing profile.

Conclusions

This study demonstrates that both L-PRF and NMO significantly enhanced peripheral nerve regeneration following sciatic nerve hemisection in a canine model. However, NMO showed superior outcomes in remyelination, inflammation reduction, and early collagen type IV expression. These findings support that NMO is a promising adjunctive biomaterial for improving outcomes in peripheral nerve repair.

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Conflict of interest

The authors affirm that there are no anticipated conflicts of interest related to this study.

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تأثير الخلايا البيضاء لليفين الغني بالصفيحات الدموية و صغائر الجسيمات على شفاء القطع الجزئي للعصب الوركي في الكلاب: دراسة تجريبية

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الخلاصة

لا يزال تجدد الأعصاب الطرفية، وخصوصًا العصب الوركي، محدودًا، مما يجعل التعامل السريري لاصابات العصب الوركي صعبًا، وغالبًا ما تكون نتائج التقنيات الجراحية الروتينية غير مُرضية. ان توفّر المواد النشطة والفعالة بيولوجيا، بما في ذلك الليفين الغني بالصفيحات الدموية والكريات البيضاء (L-PRF) وأوكسيد المغنيسيوم النانوي (NMO)، اتاح إمكانيات علاجية جديدة. الهدف الرئيسي من هذا البحث هو إجراء تقييم مقارن للقدرات التجديدية لكل من الليفين الغني بالصفيحات الدموية والكريات البيضاء (L-PRF) و أوكسيد المغنيسيوم النانوي (NMO) في تعزيز كل من الشفاء الوظيفي والإصلاح البنيوي للعصب الوركي المقطوعة جزئيًا في الكلاب. تم توزيع سبعة وعشرين كلبًا بالغًا من الذكور عشوائيًا إلى ثلاث مجموعات (n = 9): مجموعة ضابطة (غير معالجة)، ومجموعة معالجة بـ L-PRF، ومجموعة معالجة بـ NMO. وبعد إجراء قطع نصفي جراحي موحد للعصب الوركي، تم اضافة المواد النشطة بيلوجيا موضعيًا في موقع االقطع النصفي للعصب الوركي و وفقًا للمجموعة التجريبية التابعة لها. تم ملاحظة التغييرات السريرية، والتغييرات العيانية، والفحوصات النسجية المرضية، والتحليل المناعي الكيميائي للكولاجين من النوع الرابع في الأيام ٧ و٣٠ و٦٠ بعد الجراحة. حققت مجموعة NMO أسرع وأكمل تعافٍ وظيفي للطرف، حيث أظهرت الفحوصات النسيجية تعزيزًا في إعادة تشكيل الغمد النخاعي، وترسيبًا منظمًا للكولاجين، وانخفاضًا في درجة الالتهاب. وأظهر التحليل المناعي الكيميائي زيادة مبكرة وقوية في التعبير عن الكولاجين من النوع الرابع، مما يشير إلى تسارع في إعادة تشكيل المصفوفة وتنظيم بنية العصب. وايضا أظهرت مجموعة L-PRF تحسنًا في تجدد العصب الوركي مقارنةً بالمجموعة الضابطة، وإن كان ذلك بدرجة أقل من مجموعة NMO. اظهرت هذه الدراسة دليلًا قويًا على أن كلًا من أوكسيد المغنيسيوم النانوي والليفين الغني بالصفيحات الدموية والكريات البيضاء، يعززان بشكل ملحوظ إصلاح الأعصاب الطرفية مقارنةً بالمجموعة الضابطة، حيث أظهرت مجموعة NMO تحسن اكثر وضوحًا في كل من التعافي البنيوي والوظيفي من بقية مجاميع الدراسة. تؤكد هذه النتائج الإمكانات العلاجية لكل من أوكسيد المغنيسيوم النانوي (NMO) والفيبرين الغني بالصفيحات الدموية والكريات البيضاء (L-PRF) كاستراتيجيات مساندة في إصلاح الأعصاب الطرفية.