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Long-term Hyperbaric Oxygen Treatment Enhances Nerve Regeneration and Remyelination in a Rat Sciatic Nerve Graft Model

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Background: Peripheral nerve injuries often lead to inconsistent outcomes due to the complexity of nerve regeneration. Hyperbaric oxygen therapy (HBOT) has been proposed to enhance regeneration by improving oxygenation, promoting angiogenesis, and facilitating cellular repair processes. This study evaluated the effects of long-term HBOT on axonal regeneration, remyelination, and functional recovery in a rat model of sciatic nerve injury repaired with autologous nerve grafts.
Methods: Forty-two adult male Wistar rats were divided into control (surgery only) and treatment (surgery plus HBOT) groups. HBOT (2 atmospheres absolute, 100% oxygen, 1 h/d, 5 d/wk) was administered for 8 weeks. Functional recovery was assessed using the sciatic functional index, whereas regeneration was evaluated with electrophysiological tests, histological analysis, and immunohistochemistry at postoperative days (PODs) 14, 35, and 90.
Results: HBOT enhanced axonal regeneration and remyelination in the middle and distal segments of the graft by POD 35. Motor neuron preservation was significantly higher in the treatment group, approaching levels of uninjured nerves by POD 90. Electrophysiological analyses revealed earlier and more consistent reinnervation in the HBOT group, with improved normalized compound muscle action potential amplitudes at POD 60. However, functional recovery assessed by the sciatic functional index showed no significant differences between groups, likely due to autotomy and the lack of physiotherapy.
Conclusions: Long-term HBOT accelerates axonal regeneration and remyelination in a rat model of peripheral nerve injury and grafting. These findings suggest that HBOT is a promising adjunctive therapy for complex nerve injuries, with potential clinical applications in reconstructive nerve surgery. *(Plast Reconstr Surg Glob Open 2025;13:e7039; doi: 10.1097/GOX.0000000000007039; Published online 29 August 2025.)*

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INTRODUCTION

Unresolved peripheral nerve injuries have significant functional and psychosocial consequences. Surgical repair is often required when a nerve injury results in a continuity defect or loss of function that cannot be recovered with nonsurgical treatment. In severe cases, nerve repair or grafting is necessary to restore continuity between the proximal and distal portions of the nerve, especially when there is irreversible damage or loss of nerve substance.[1]

Disclosure statements are at the end of this article, following the correspondence information.

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Donor nerve grafts are harvested from expendable sensory nerves, such as the sural nerve, and are placed in a reversed orientation as interpositional grafts. These harvested nerve grafts undergo Wallerian degeneration, providing mechanical guidance and a supportive structure for regenerating axons.[2] Autologous nerve grafts meet the criteria for an ideal nerve conduit, offering a permissive and stimulating scaffold that includes Schwann cell basal laminae, neurotrophic factors, and adhesion molecules.[3] In specific clinical scenarios, including upper limb and brachial plexus reconstruction, facial nerve reconstruction, and face and limb allotransplantation, long nerve grafts are often required.[4] Although effective, long grafts have inconsistent outcomes due to poor vascularization and delayed regeneration.[5–7]

Hyperbaric oxygen therapy (HBOT) was first introduced in 1966 for use in plastic surgery[8] and has since been applied to numerous surgical conditions.[9] HBOT has shown promise in enhancing nerve repair via improved oxygenation, reduced edema, and enhanced cellular viability.[10–15] Previous animal studies have primarily focused on short-term HBOT protocols, lasting a few days to weeks, in models of crush injuries or direct nerve repair.[16–24] However, its long-term effects in graft models remain underexplored. This study evaluates the impact of extended HBOT on axonal regeneration and functional recovery following sciatic nerve grafting in rats.

Takeaways

Question: Can long-term hyperbaric oxygen therapy (HBOT) enhance peripheral nerve regeneration and functional recovery following sciatic nerve injury repaired with autologous nerve grafts?

Findings: This study demonstrated that a long-term HBOT protocol (8 wk) significantly improved axonal regeneration, remyelination, and motor neuron preservation in a rat sciatic nerve graft model. Functional recovery was assessed through gait analysis, electrophysiological testing, and histological evaluation, with the HBOT group showing superior regenerative outcomes compared with controls.

Meaning: Long-term HBOT is a promising adjunctive therapy for complex peripheral nerve injuries, with the potential to improve outcomes in nerve reconstruction procedures.

METHODS

Surgical Procedure

Adult male Wistar rats (10–14 wk old) were used for the study. The rats were anesthetized using Cepetor (0.4 mg/100 g) and ketamine (60 mg/100 g). The left sciatic nerve was exposed and carefully separated from the thigh and gluteal muscles. A sharp transection was performed, and a 10-mm nerve segment was inverted to act as an autologous nerve graft. Immediate end-to-end coaptations were performed using nonabsorbable 10-0 sutures (Figs. 1, 2). (**See Video [online]**, which displays how the left sciatic nerve was sharply transected. A nerve segment of 10 mm was inverted so that it could act as a nerve graft.) Coaptation of the nerve fascicles was carried out to ensure that all fascicles were preserved within the epineural sac. Wounds were closed in layers. A total of 42 rats underwent the surgical procedure. They were randomly divided into 2 groups of 21 rats each. The treatment group received HBOT following surgery, whereas the control group underwent the surgical procedure without additional treatment.

Hyperbaric Oxygen Therapy

Rats in the treatment group were subjected to HBOT daily in an animal hyperbaric chamber. The protocol mirrored clinical practices for central nerve damage,[25] consisting of 1-hour sessions of 2 atmospheres absolute with 100% oxygen, administered 5 days a week for 8 weeks, totaling 40 sessions. The control group did not receive any additional treatment after surgery.

Evaluation of Regeneration

Functional motor recovery of the sciatic nerve was assessed preoperatively and postoperatively through sciatic functional index (SFI), electromyography (EMG), histology, and morphometric analysis. To monitor regeneration, 5 rats from each group were euthanized at postoperative days (PODs) 14 and 35 as intermediate

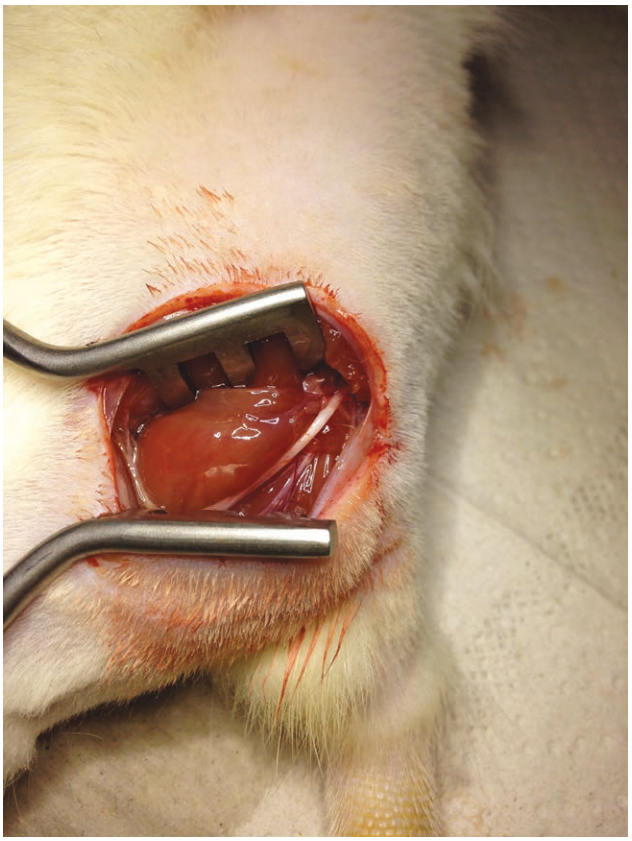

Fig. 1. The left sciatic nerve was exposed and carefully separated from the thigh and gluteal muscles.

checkpoints, whereas 11 rats from each group were followed up for comprehensive evaluation across a 90-day period.

Gait Analysis for Motor Performance

Gait was assessed on a standardized trail. Hind limbs were dipped in nontoxic ink, and the rats were made to walk along the track, leaving footprints on paper. Measurements included print length (PL), toe spread (TS), and intermediary toe spread (IT) on both the treated and untreated hind limbs (E for treated, N for untreated limb, ergo EPL and NPL and so on). The SFI was calculated using the modified formula by Bain et al[26]:

$$\mathrm{SFI} = -38.3 * \frac{(\mathrm{EPL} - \mathrm{NPL})}{\mathrm{NPL}} + 109 * \frac{(\mathrm{ETS} - \mathrm{NTS})}{\mathrm{NTS}} + 13.3 * \frac{(\mathrm{EIT} - \mathrm{NIT})}{\mathrm{NIT}} - 8.8.$$

An SFI of 0 represents normal function, whereas −100 indicates complete impairment. Rats were evaluated presurgery and at 7, 30, 60, and 90 days postreconstruction.

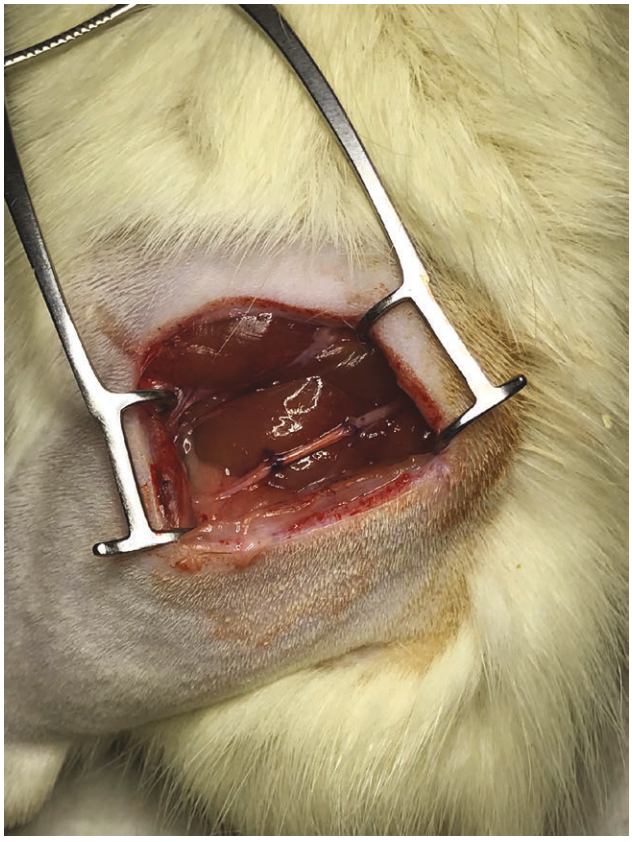

Fig. 2. Immediately thereafter, end-to-end coaptation was performed using 2–3 nonabsorbable 10-0 sutures.

Electromyographic Analysis

Electrophysiological tests were conducted using a Dantec "Keypoint" workstation to assess muscle reinnervation. Nerve conduction tests were performed preoperatively and at PODs 20, 30, 60, and 90.[27] EMG was not performed on rats with active autotomy wounds. For control values, the contralateral (right) untreated limb was tested. Under general anesthesia, the sciatic nerve was stimulated with a single electric pulse (100 μs duration, supramaximal intensity), and compound muscle action potentials (CMAPs) were recorded from the plantar muscles. Recording needles were placed based on anatomical landmarks. Electric stimuli (100 μs duration) were delivered at progressively increasing intensities (starting at 1 mA and increasing in 2 mA increments) until a maximal CMAP amplitude was achieved. Nerve stimulation elicited 2 distinct waves: an M wave (short latency) and an H wave (mid-latency). For each test, the highest amplitude recordings of the M waves were analyzed. The M wave latency (ms) was measured from stimulus artifact to wave onset, and its amplitude (mV) was measured from baseline to peak. M wave amplitudes were normalized to the contralateral intact limb to assess muscle reinnervation (Fig. 3).[28,29]

Histological Assessment of the Nerve

At designated time points, rats were euthanized, and the entire nerve graft, including the proximal and distal coaptation sites, was harvested and fixed in 4% paraformaldehyde. Sciatic nerve samples were divided into 3 segments: proximal to the graft (proximal to the first coaptation), the middle of the graft, and distal to the graft (distal to the second coaptation).

Tissue samples were embedded in paraffin, sectioned at 5 μm, and prepared for immunofluorescence staining. Sections were stained with anti–choline acetyltransferase antibody (a motor neuron marker) and neurofilament (NF) antibody (a general neuronal marker), whereas 4',6-diamidino-2-phenylindole (DAPI) was used for nuclear staining. Stains were analyzed separately to evaluate the ratio of intact motor fibers to general neuronal fibers. Fluorescent microscopy (Eclipse Ni-U; Nikon) and ImageJ software (National Institutes of Health) were used for quantitative analysis. For myelin labeling, sections were deparaffinized, boiled in sodium citrate buffer, and stained with anti–myelin basic protein and anti-neurofilament heavy antibodies, alongside DAPI. Fluorescent images were captured with a panoramic digital slide scanner (3DHISTECH) and analyzed using ImageJ. Morphometric data were quantified by blinded pathologists.

Statistical Analysis

Statistical analyses were performed using SAS for Windows (version 9.4). Categorical variables were expressed as relative frequencies and compared using Pearson χ^2 or Fisher exact tests. Continuous variables were analyzed with *t* tests for normal distributions or Wilcoxon tests for nonnormal distributions. Univariate analysis was conducted to compare groups across time points. Due

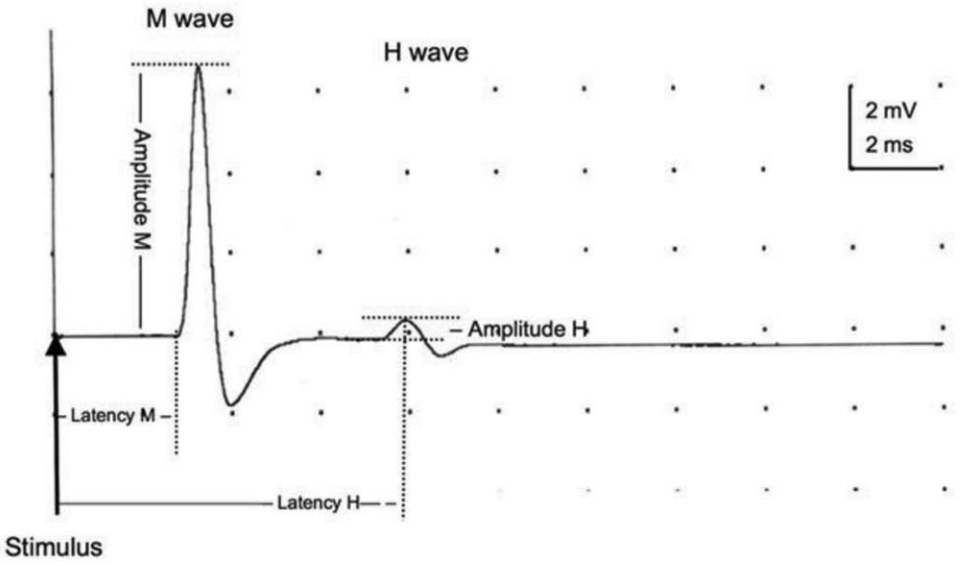

Fig. 3. In motor nerve conduction tests in the rat, nerve stimulation elicits 2 waves: a first M wave of short latency and a reflex H wave of mid-latency.

to the small sample size, data were analyzed using Mann-Whitney *U* tests and reported as medians with interquartile ranges. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

Preoperative Evaluation

Functional and electrophysiological analyses showed no significant differences between the control and treatment groups before surgery.

Gait Analysis for Motor Performance

Gait analysis showed progressive improvement in SFI postsurgery, with HBOT-treated rats demonstrating consistently better, though not statistically significant, scores. A known challenge after sciatic nerve transection in rodents is "autotomy," where the animals scratch and bite the anesthetic limb, sometimes resulting in toe amputations. This behavior interferes with SFI measurements, as the analysis relies on intact footprints. Despite efforts to reduce rates,[30,31] autotomy remained high (45%) with no group difference ($P = 0.545$).

The incidence remained high, affecting 45% of rats (control: 52.6%, treatment: 38.1%; $\chi^2 = 0.37$, $P = 0.545$), with no significant difference between groups. This rate aligns with values reported in the literature for this rat strain. All rats were housed in standard cages and were not subjected to physical activity resembling physiotherapy during the study.

Electromyographic Analysis

Preoperatively, there was no statistical difference between the 2 groups regarding M wave amplitude. The mean amplitude in the treatment group was 7380.2 μV (SD 2300) compared with 7340.8 μV (SD 2598.3) in the treatment group ($P = 0.92$). The left-to-right limb amplitude ratio was also similar (with a mean ratio of 1.1 [SD 0.4] in the treatment group compared with 1.05 [SD 0.3] in the control group, $P = 0.57$).

On POD 20, EMG was performed on 16 rats in the control group and 12 rats in the treatment group. All rats in the treatment group exhibited recordable M waves with small amplitudes and long latencies in their CMAPs. In contrast, only 6 rats (37.5%) in the control group showed recordable M waves (Fisher exact test, $P < 0.001$). On POD 30, EMG was conducted on 14 rats in the control group and 15 rats in the treatment group. By this point, all rats in the treatment group had recordable M waves, whereas only 9 rats (64.3%) in the control group showed recordable M waves (Fisher exact test, $P < 0.041$). Group differences in amplitude, latency, and duration were not significant.

By POD 60, all rats in both groups exhibited recordable M waves. The mean amplitude in the treatment group was 222.2 μV (SD 142.9) compared with 217.4 μV (SD 138) in the control group, with differences approaching but not reaching statistical significance ($P = 0.07$). However, when amplitudes were normalized using the ratio of injured-to-contralateral limbs for each rat, the treatment group demonstrated significantly better results (with a mean ratio of 0.04 [SD 0.02] compared with 0.035 [SD 0.03] in the control group, $P = 0.01$). This ratio reduced variability caused by differences in the absolute number of axons between rats by providing a relative measure, comparing each rat's injured limb to its own uninjured limb. By POD 90, no significant differences were found between the 2 groups.

Histological Assessment

At various time points post graft transfer, animals were euthanized, and histological sections were prepared to evaluate axonal regeneration (Fig. 4). On POD 14, the

number of nerve fibers decreased distally from the transection site, as expected. At this early stage, group sizes were small, and the variability in nerve fiber counts precluded statistical significance for both general nerve fibers and motor neurons in the middle and distal segments. However, immunofluorescence analysis using anti–myelin basic protein antibody (marking myelin debris) showed a trend toward reduced myelin debris in the treatment group compared with the control group (Figs. 5–7), suggesting that hyperbaric oxygen conditions may enhance the clearance of cell debris, a critical step in peripheral nerve regeneration.[32] On POD 35, the treatment group exhibited a greater number of general nerve fibers in both the middle and distal segments, comparable to the numbers in the proximal segments. In the control group, the number of nerve fibers in the middle and distal segments remained significantly lower than in the proximal segment (middle-to-proximal ratio: P = 0.014; distal-to-proximal ratio: P = 0.021), indicating enhanced regeneration in the treatment group (Fig. 8). (**See figure, Supplemental Digital Content 1**, which displays the motor fiber percentage on POD 35: the treatment group showed a higher percentage of motor fibers, **https://links.lww.com/PRSGO/E254**.) Additionally, immunofluorescence analysis revealed significantly increased remyelination in the treatment group in the middle (1.65-fold, P = 0.026) and distal (3.3-fold, P = 0.0079) segments (Figs. 9–11).

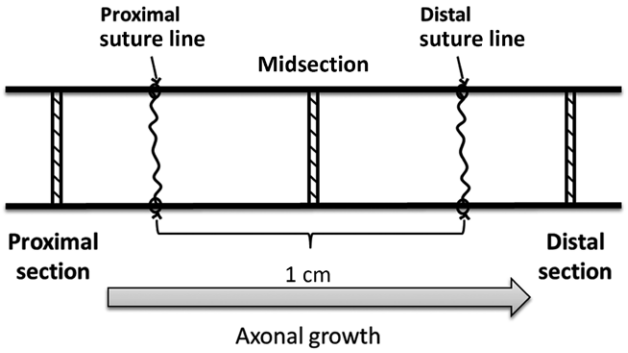

Fig. 4. Immunohistochemistry scheme—histology sections were prepared from 3 different segments. The first segment was taken 5 mm proximal to the nerve graft, the second from the middle of the nerve graft, and the third, 5 mm distal to the nerve graft.

Due to variability in nerve fiber counts across rats, the percentage of motor neurons among all neurons—a more consistent measure—was used to compare nerve regeneration.[32,33] On POD 90, the treatment group showed significantly higher percentages of motor fibers in the middle and distal segments compared with the control group, with percentages in the treatment group approximating those in normal control nerves (Fig. 12). (**See figure, Supplemental Digital Content 2**, which displays the motor fiber percentage on POD 90, showing a normal percentage in the middle and distal sections in the treatment group, **https://links.lww.com/PRSGO/E255**.) Regarding myelination, no differences were observed between groups on POD 90.

DISCUSSION

Nerve regeneration after injury is unpredictable due to the complex healing process. Factors such as hypoxia,

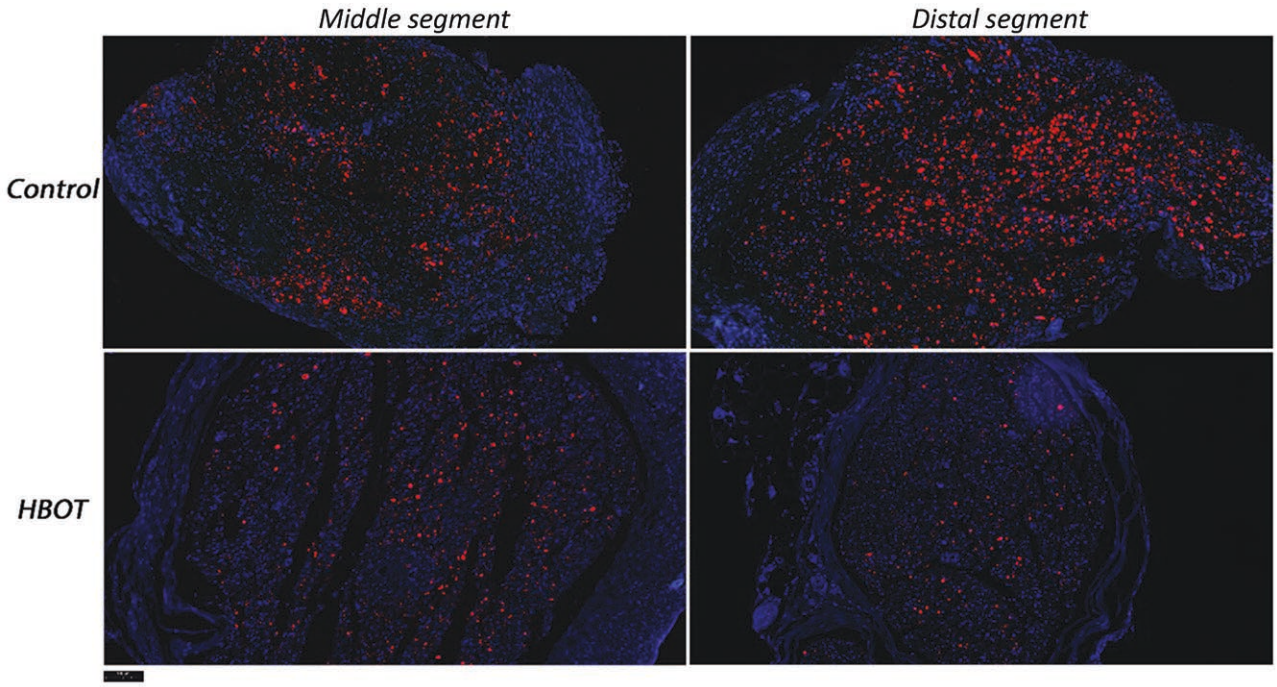

Fig. 5. HBOT enhanced myelin debris clearance and remyelination in the distal and middle injured nerve segments. Myelin was labeled with myelin basic protein (red), whereas cell nuclei were labeled with DAPI (blue).

delayed debris clearance, and poor angiogenesis impair axonal growth. Trauma disrupts blood flow, causing local hypoxia and creating an environment unfavorable for regeneration. Our findings demonstrate that long-term HBOT enhances axonal regeneration and remyelination in a rat model of sciatic nerve grafting. HBOT provides enhanced oxygenation, which has been shown to promote tissue repair and accelerate wound healing by stimulating angiogenesis and decreasing edema.[34,35] By improving oxygen delivery to ischemic tissues, HBOT supports the survival of critical cells, including Schwann cells and endothelial cells, and facilitates axonal regeneration.[36] This study introduces several key differences compared with prior research. Unlike most previous studies, which used short-term HBOT regimens lasting only a few days or weeks, this study implemented an 8-week treatment protocol (40 sessions), reflecting a more clinically relevant duration for nerve regeneration. This prolonged exposure to hyperoxia likely contributed to sustained benefits in nerve fiber preservation and remyelination over time. Consistent with prior studies,[24,37] our findings show that HBOT accelerates axonal regrowth and improves nerve recovery, particularly during the early and intermediate stages of healing. On POD 14, faster clearance of myelin debris and cellular waste was observed in the HBOT group compared with controls, a crucial step for Schwann cell activation and axonal regeneration. By POD 35, significant

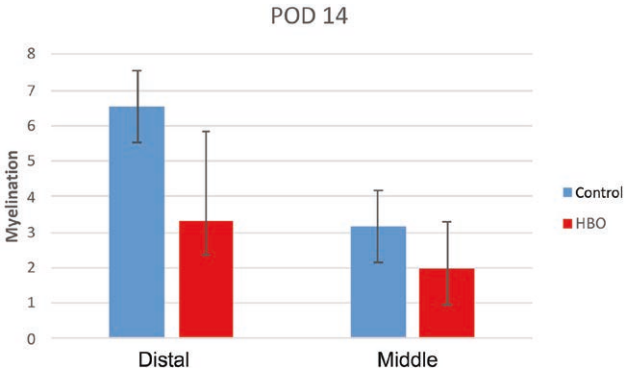

Fig. 6. Graph depicting the amount of myelin (as assessed by myelin basic protein signal coverage area) in the distal and middle segments of HBO-treated and control groups at POD 14 (N = 4,3, respectively). HBO, hyperbaric oxygen.

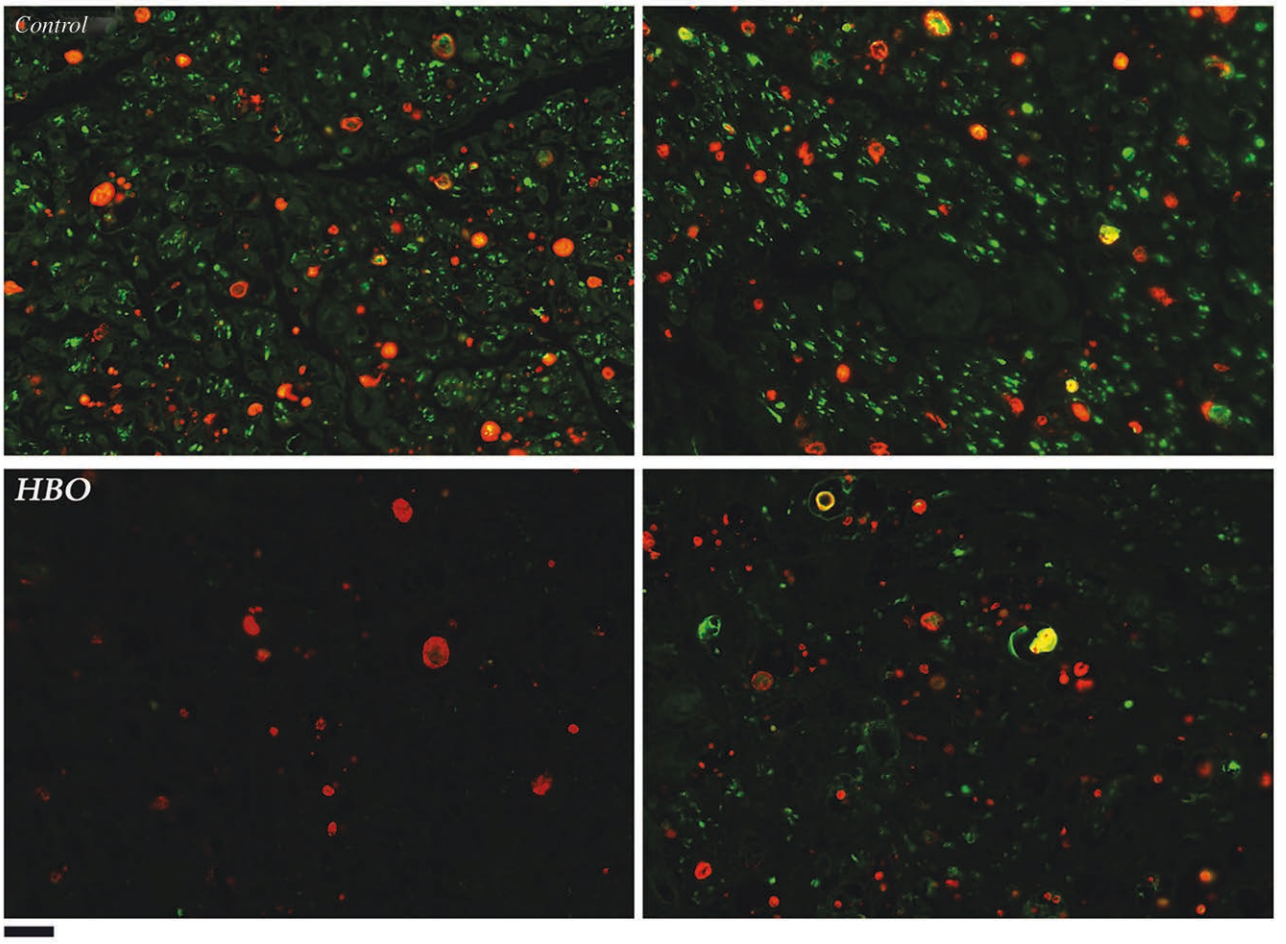

Fig. 7. Sciatic nerve cross sections of distal nerve segments from 2 control and 2 HBOT-treated rats on POD 14 labeled with anti–NFH (green, axonal marker) and anti–myelin basic protein (red, myelin marker), showing increased clearance of both axonal and myelin debris in HBO-treated rats. HBO, hyperbaric oxygen; NFH, neurofilament heavy.

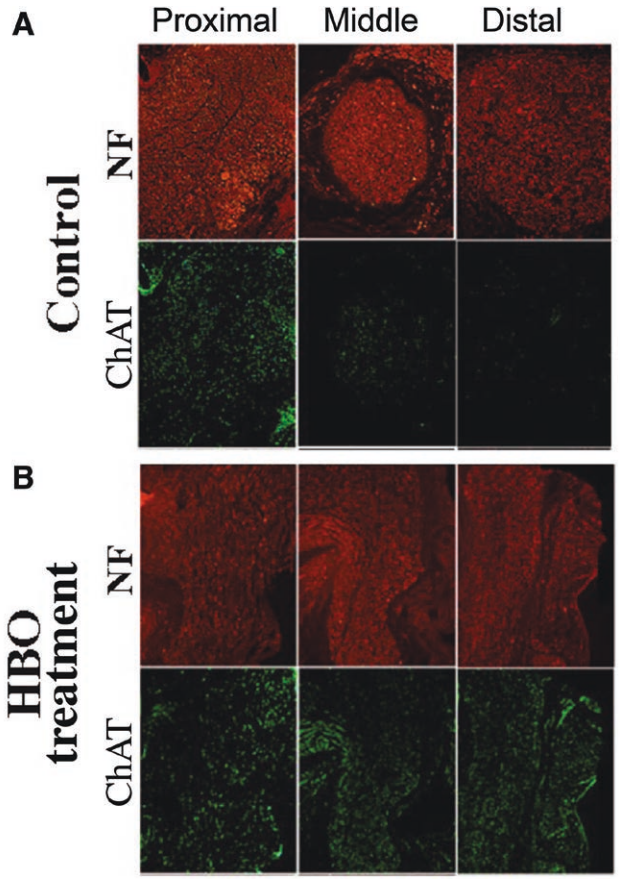

Fig. 8. Sample picture for histology results on POD 35. A, Control group. B, Hyperbaric oxygen group. General nerve fibers were labeled with NF (red), whereas motor nerve fibers were labeled with ChAT (green). The proximal sections showed similar numbers of motor and general neuronal fibers, with similar motor nerve percentages. The treatment group showed higher numbers of both general and motor neurons, as well as a higher percentage of motor fibers in the medial and distal sections. ChAT, choline acetyltransferase.

improvements in axonal regrowth and remyelination were noted in the treatment group, particularly in the middle and distal nerve segments. These findings suggest that HBOT not only facilitates the initial stages of regeneration but also enhances the pace of repair processes over time. Interestingly, by POD 90, no significant differences in remyelination were observed between the groups, suggesting that axon growth, rather than remyelination, may be the rate-limiting factor in this model. The significantly higher proportion of motor neurons in the HBOT group at POD 90 highlights the potential long-term benefits of this therapy in preserving motor function. Although many prior studies have examined direct nerve repair or crush injuries, our study specifically evaluates nerve regeneration through an interpositional autograft. This distinction is critical. Unlike direct nerve coaptation, where axonal regeneration occurs more reliably, long nerve grafts present greater challenges due to delayed revascularization, prolonged denervation of target muscles, and a higher risk of axonal misdirection. These factors contribute to worse functional outcomes in clinical scenarios requiring long grafts. HBOT's potential role in improving oxygenation and enhancing Schwann cell function may be particularly valuable for long nerve grafts, making this study highly relevant for reconstructive nerve surgery.

Our study provides a comprehensive, time-course evaluation of nerve regeneration, incorporating histological analysis, immunohistochemical markers (choline acetyltransferase for motor neurons, NF for total axons), and electrophysiological assessments (CMAPs). These analyses allowed us to track progressive changes during 90 days, providing a more detailed picture of HBOT's long-term effects compared with prior reports that focused on single time-point assessments.

Histology confirmed regeneration, but SFI gains were less pronounced. This may reflect the limitations of SFI in capturing subtle improvements or the impact of high autotomy rates (45%), which interfered with reliable functional measurements. Additionally, the lack of structured physiotherapy in the experimental design likely limited functional recovery.

Electrophysiological studies, however, revealed a clearer benefit of HBOT. Early improvements in M wave recordings were observed in the treatment group, with all treated rats showing recordable M waves by POD 30, compared with only 64.3% in the control group. Normalizing CMAP amplitudes to the contralateral limb reduced interindividual variability and highlighted significant improvements in the HBOT group on POD 60. These findings reinforce the utility of electrophysiological measures in assessing functional recovery in nerve regeneration studies.

Peripheral nerve regeneration in humans is inherently more challenging than in rodents due to longer regeneration distances and slower axonal growth rates.[6,7] On average, human peripheral nerves regenerate at approximately 1 inch per month. Recent studies in patients treated with short-term HBOT after ulnar and median nerve injuries have shown promising results, with improved recovery compared with untreated patients.[38] Our findings suggest that extending HBOT to longer durations, as modeled in this study, may provide additional benefits for complex nerve injuries or cases requiring long grafts.

Initiating HBOT as early as possible after nerve repair is crucial, as shown in prior studies.[24,37,39] The improved axonal growth and remyelination observed in our study support the recommendation of long-term HBOT for patients with extensive nerve injuries. This therapy may be particularly beneficial in clinical scenarios such as facial nerve reconstruction, brachial plexus repair, and limb allotransplantation.

Although the exact mechanisms underlying the enhanced regeneration observed with HBOT were not directly investigated in this study, our findings suggest several potential pathways. Enhanced oxygenation likely improves macrophage activity, promoting faster clearance of degenerating axons and myelin debris. This, in turn, supports Schwann cell proliferation and the release of neurotrophic factors, facilitating axonal regrowth. The trend toward faster debris clearance observed in

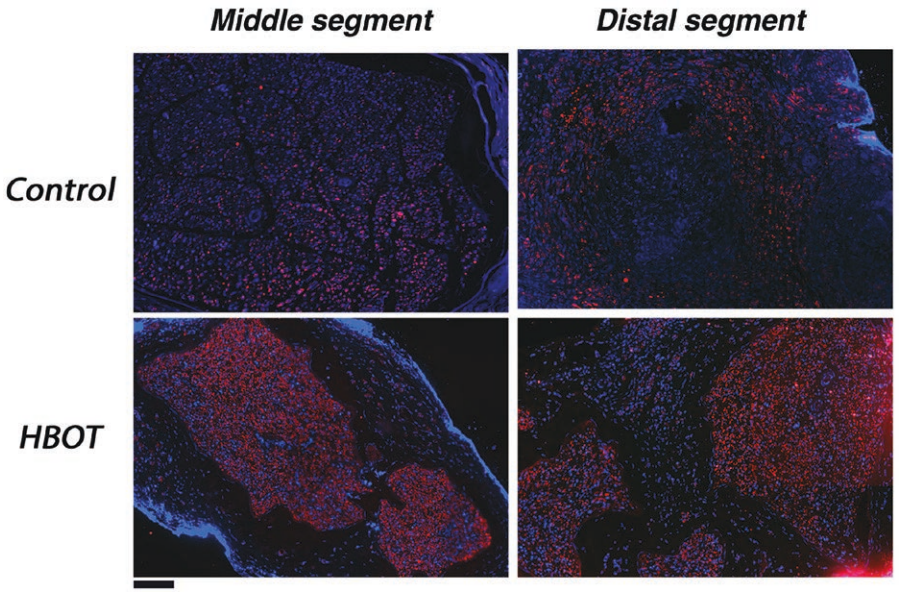

Fig. 9. Significantly more remyelination was observed in the HBO-treated group compared with the control in both the middle and distal segments. Myelin was labeled with myelin basic protein (red), whereas cell nuclei were labeled with DAPI (blue). HBO, hyperbaric oxygen.

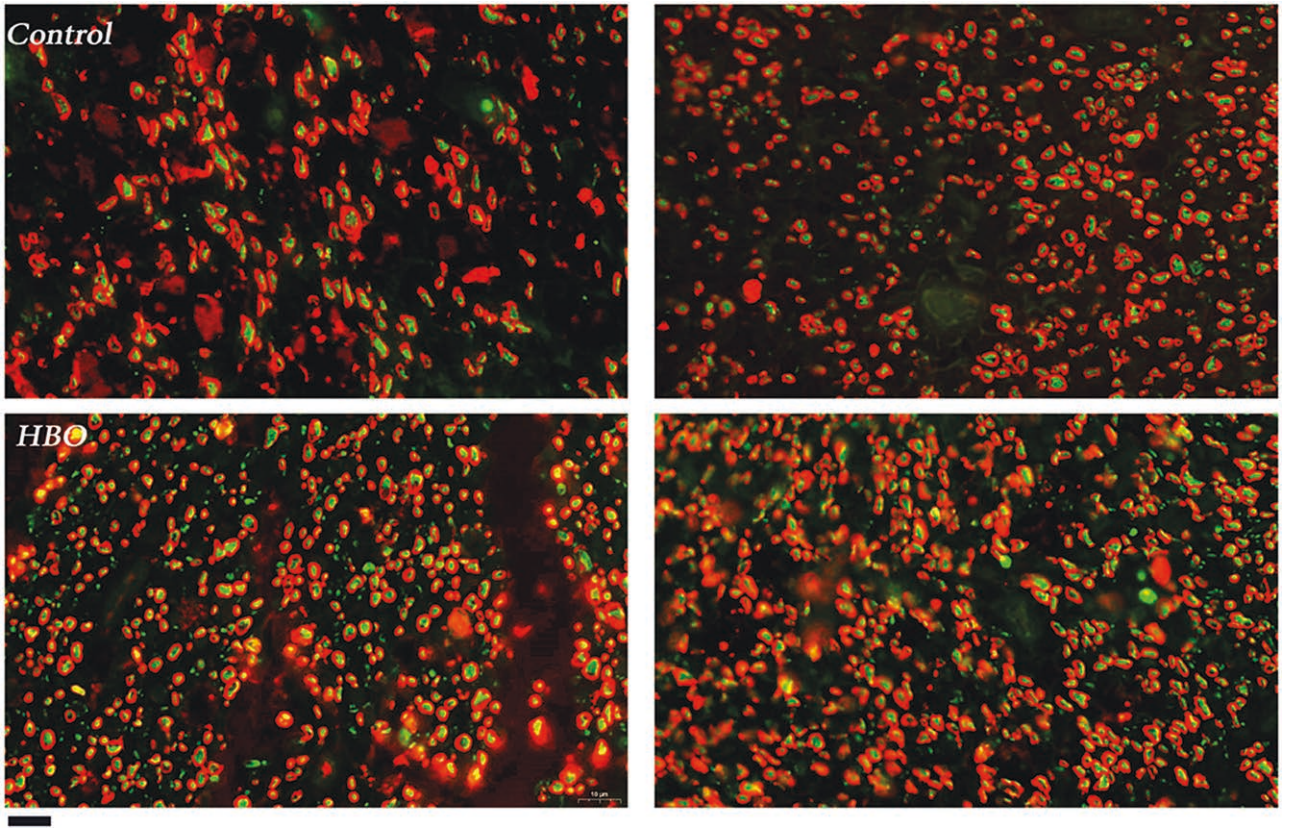

Fig. 10. Sections of distal nerve segments from 2 control and 2 HBOT-treated rats on POD 35 labeled with anti–NFH (green, axonal marker) and anti–myelin basic protein (red, myelin marker), showing increased myelination in HBO-treated rats. HBO, hyperbaric oxygen; NFH, neurofilament heavy.

the HBOT group at POD 14 likely contributed to the improved regeneration noted at later time points. This study has several limitations. The high rate of autotomy limited the reliability of functional assessments, highlighting the need for alternative behavioral measures or strategies to mitigate autotomy. The lack of physiotherapy

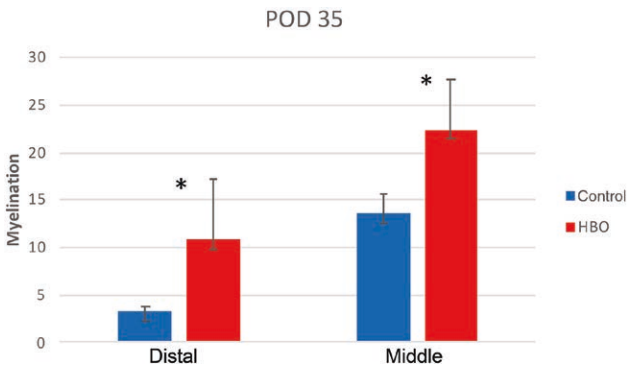

Fig. 11. A graph depicting the amount of myelin (assessed by myelin basic protein signal coverage area) in the distal and medial segments of the HBO-treated and control groups on POD 35 (N = 5 per group). Asterisk (*) shows increased remyelination in the treatment group in both the middle and distal segments of the nerve graft. HBO, hyperbaric oxygen.

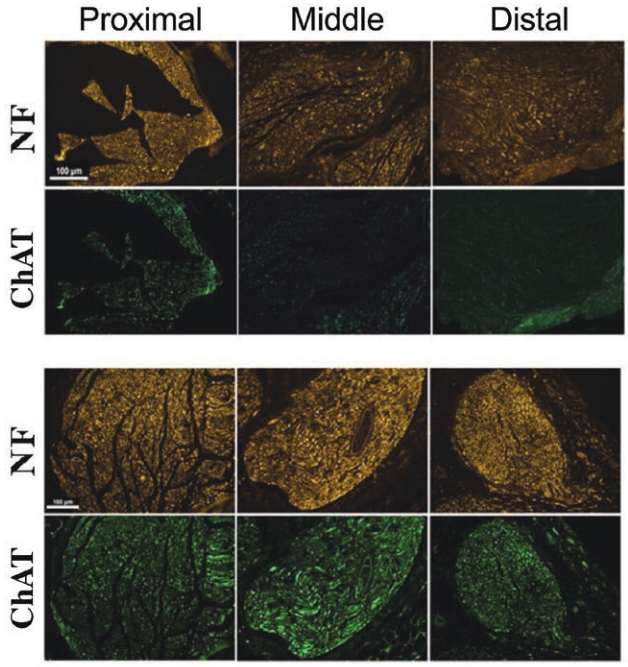

Fig. 12. Sample picture for histology results on POD 90: general nerve fibers were labeled with NF (yellow), whereas motor nerve fibers were labeled with ChAT (green). The treatment group showed higher numbers of both general and motor neurons, as well as a higher percentage of motor fibers. ChAT, choline acetyltransferase.

or exercise in the study design may have also restricted functional recovery, despite the observed histological and electrophysiological improvements. Furthermore, the relatively small sample size at certain time points reduced statistical power for some analyses. Future studies should explore combination therapies that integrate HBOT with physiotherapy or pharmacological agents to optimize functional outcomes. Investigating the effects of HBOT in larger animal models and eventually in human clinical trials will be essential to confirm its therapeutic potential. HBOT has become more accessible in recent years, both globally and in Israel. At our hospital, a hyperbaric chamber is available, allowing us to explore its potential role in nerve regeneration. However, we recognize that, at present, HBOT for peripheral nerve injuries remains an off-label use and comes with challenges, including significant financial burden and a lengthy treatment protocol, which can be demanding for patients. Although our current animal model study provides encouraging preliminary results, it is premature to recommend HBOT as a standard clinical protocol for nerve injuries. Further clinical evidence is necessary before HBOT can be integrated into routine medical practice for this indication.

CONCLUSIONS

Our findings demonstrate that long-term HBOT significantly enhances axonal regeneration, remyelination, and motor neuron preservation in a rat model of sciatic nerve injury and grafting. These results highlight the potential of HBOT as a valuable adjunctive therapy for complex peripheral nerve injuries. Clinical translation of these findings, particularly in patients undergoing facial nerve reconstruction or similar procedures, could pave the way for improved outcomes in nerve repair and recovery.

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DISCLOSURES

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ETHICAL APPROVAL

The authors declare that they have conformed to the Declaration of Helsinki (local institutional review board no. 32-11-16).

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