Basic Medical Research

# Adipose-derived mesenchymal stem cells enhance regeneration in a chronic peripheral sciatic nerve injury Sprague-Dawley rat model

Wahyu Widodo[1,2,3], Ismail Hadisoebroto Dilogo[2], Achmad Fauzi Kamal[2], Radiana Dhewayani Antarianto[4,5], Puspita Eka Wuyung[6], Nurjati Chairani Siregar[6], Fitri Octaviana[7], Aria Kekalih[8], Heri Suroto[9], Dina Aprilya[3], Anissa Feby Canintika[2]



pISSN: 0853-1773 • eISSN: 2252-8083
https://doi.org/10.13181/mji.oa.257780
**Med J Indones. 2025;34:141–5**

**Received:** September 07, 2024
**Accepted:** May 20, 2025

**Authors' affiliations:**
*[1]Doctoral Program in Medical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, [2]Department of Orthopaedic and Traumatology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia, [3]Department of Orthopaedic and Traumatology, Fatmawati Hospital, Jakarta, Indonesia, [4]Department of Histology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, [5]Stem Cell and Tissue Engineering, Indonesian Medical Education and Research Institute (IMERI), Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, [6]Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia, [7]Department of Neurology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia, [8]Department of Community Medicine, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, [9]Department of Orthopaedic and Traumatology, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Hospital, Surabaya, Indonesia*

**Corresponding author:**
Wahyu Widodo
Department of Orthopaedics and Traumatology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jalan Pangeran Diponegoro No. 71, Kenari, Senen, Central Jakarta 10430, DK Jakarta, Indonesia
Tel/Fax: +62-21-3155996/+62-21-3929655
**E-mail:** wahyuwidodo.orthofkui@gmail.com

**ABSTRACT**

**BACKGROUND** Obtaining optimal functional outcomes in patients with chronic peripheral nerve injuries (PNIs) remains challenging due to the limited regeneration of the neuromuscular junction (NMJ). Adipose-derived mesenchymal stem cells (AD-MSCs) can differentiate into Schwann-like cells, secrete neurotrophic factors, and recruit native Schwann cells. This study aimed to analyze the effects of AD-MSCs on functional outcomes in a chronic PNI model.

**METHODS** An *in vivo* study was performed using 20 male Sprague-Dawley rats with chronic PNI of the right sciatic nerve. Rats were divided into two groups: the AD-MSC group (n = 10), receiving human AD-MSC injections in the NMJ; and the control group (n = 10), receiving normal saline injections. Walking track analysis and electrophysiological assessments were performed 8 weeks after the nerve repair. Postmortem gastrocnemius muscle weights and immunohistochemical examinations were also performed.

**RESULTS** The AD-MSCs showed significantly larger fiber diameters than the control group (45.54 [13.1] *versus* 35.46 [6.64]; $p = 0.011$). While clinical, electrophysiological, and gastrocnemius muscle weight data suggested a trend toward improved outcomes in the AD-MSCs group, the differences were not statistically significant.

**CONCLUSIONS** AD-MSC implantation may enhance nerve regeneration, as demonstrated by a better outcome profile in the AD-MSCs group.

**KEYWORDS** animal model, mesenchymal stem cells, sciatic neuropathy

Chronic peripheral nerve injuries (PNIs) significantly affect patients' quality of life and ability to perform their daily activities.[1,2] Although surgical intervention is the standard treatment, obtaining optimal functional outcomes remains challenging despite advances in microsurgery.[3] This is due to the limited regenerative capability of the peripheral nervous system, which has a growth rate of 1 mm/day.[4,5]

Stem cells are precursor cells capable of differentiating into various cell lineages.[6] Several studies have analyzed their potential for nerve regeneration, with neural stem cells being an excellent source for nerve and neuromuscular junction (NMJ) regeneration; however, they are difficult to obtain and pose a risk of carcinogenicity.[7–9] In contrast, adipose-derived mesenchymal stem cells (AD-MSCs) are easier to harvest and have low immunogenicity and donor morbidity. AD-MSCs can differentiate into Schwann cells, secrete neurotrophic factors, and recruit native Schwann cells.[10–12] Furthermore, they have been shown to enhance functional recovery in animal models.[13] This study aimed to analyze the effects of AD-MSCs on regeneration in a chronic PNI model.

## METHODS

### Animal and experimental design

Twenty male Sprague-Dawley rats (8–12 weeks old, 150–250 g) were used in this study. The minimum sample size was not calculated in the pilot study. The rats were placed in cages and acclimatized for 1 week under controlled conditions (12-hour light/dark cycle, 18–21°C temperature, and 55% humidity) with *ad libitum* access to food and water. Each cage housed two rats. All procedures were approved by the Animal Care and Use Committee (ACUC) and conducted in compliance with the Animal Research: Reporting of In Vivo Experiments guidelines. Ethical clearance was obtained from the Ethics Committee of the Faculty of Medicine, Universitas Indonesia – Cipto Mangunkusumo Hospital (No: KET-708/UN2.F1/ETIK/PPM.00.02/2020).

### Stem cell preparation

AD-MSCs were obtained from the Stem Cells and Tissue Engineering Laboratory at the Faculty of Medicine, Universitas, Indonesia. They were isolated following the protocol previously reported by Pawitan et al,[14] then cultured at 37°C and 5% $CO_2$ using a complete medium consisting of alpha modification of Eagle's minimal essential medium, 1% penicillin-streptomycin, 1% amphotericin B, 1% GlutaMAX, 1% heparin, and 10% platelet concentrate. After reaching confluence, the cells were trypsinized using 3 ml *TrypLE Select* and incubated for 5 min until detachment, before 6 ml of complete medium was added. The cells were collected in tubes and centrifuged at 1,200 rpm for 10 min. After centrifugation, 1 ml of complete medium was added and the cells were resuspended.

### Study design and surgical procedure

The rats were randomly assigned to an experimental group (AD-MSC) and a control group (NaCl). A chronic PNI model was established by surgically transecting the right sciatic nerve following intraperitoneal administration of a mixture of ketamine and xylazine. An incision was made with the patient in the lateral decubitus position until the sciatic nerve was identified. The sciatic nerve was separated from the surrounding tissues using microsurgical instruments, transected 10 mm proximal to the tibial and peroneal nerve branches, and sutured to the surrounding muscles using an 8-0 nylon suture to prevent regeneration. The wound was irrigated and closed using a 4-0 vicryl suture. Intramuscular ceftriaxone injections (10 mg/24 hours, 0.1 ml) were administered to healthy thighs for 3 days to prevent infection.

After 3 weeks, the sciatic nerve was surgically reimplanted. Under anesthesia, an incision was made along the previous surgical scar and the proximal and distal sciatic nerve stumps were located. The free ends of the nerve stumps were cleaned until the nerve fascicles became visible, and the two stumps were sutured together using a 9-0 nylon suture. The treatment group received 2 million AD-MSCs injected into the NMJ of the gastrocnemius muscle, whereas the control group received 1 ml of 0.9% NaCl at the same site. The surgical wound was irrigated and closed by suturing with 4-0 polyglactin suture.

### Walking track analysis

Walking track analysis was performed before transection, 3 weeks after transection, and 8 weeks after surgical repair. Footprints were recorded using a plastic tank (80 × 40 × 25 cm), wood shavings, an acrylic tunnel, a digital camera, colored ink, and paper strips (8 × 100 cm). Two blinded examiners measured the heel-to-third toe distance (print length [PL]), first-to-fifth toe distance (toe spread [TS]), and second-to-fourth toe distance (intermediate toe spread [IT]). The sciatic functional index (SFI) was calculated using the following formula (Equation 1):

$$\mathrm{SFI} = \frac{-38.3(PL_E - PL_N)}{PL_N} + \frac{109.5(TS_E - TS_N)}{TS_N} + \frac{13.3(IT_E - IT_N)}{IT_N - 8.8} \quad (1)$$

E=experimental paw; N=normal paw; SFI=sciatic functional index

SFI values range from 0 (normal function) to –100 (total dysfunction).[15] Paw stride length (STL) was calculated by averaging the metatarsal base distance across three consecutive steps, whereas step length (SL) was measured as the distance between the footprints of both paws from the left to the right toe tip.

**Electrophysiology study**

An electrophysiological study was performed on both limbs 8 weeks after surgical repair under anesthesia. The sciatic nerve was identified through an incision and directly stimulated using electrodes placed 5 mm proximal and distal to the nerve junction, which was connected to a Neuro-MEP Stimulator (Neurosoft, Russia). A recording probe needle was subcutaneously inserted into the gastrocnemius muscle to measure the evoked amplitudes, compound muscle action potentials (CMAPs), and conduction blocks. A conduction block was defined as a ≥50% reduction in CMAP amplitude when stimulating the proximal *versus* the distal sciatic nerve.[16]

**Muscle weight and diameter measurement**

Eight weeks after the repair, the sciatic nerve and gastrocnemius muscle were harvested under anesthesia and fixed in 10% formaldehyde. The rats were euthanized under anesthesia, following the ACUC guidelines. The harvested gastrocnemius muscles from both limbs were weighed. Histomorphometric analysis was performed using confocal microscopy. Image processing was performed using ImageJ (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, USA) and the gastrocnemius muscle fiber diameter was analyzed using cross-sectional cuts.

**Statistical analysis**

Bivariate numerical analyses were performed using unpaired *t*-tests or Mann-Whitney *U* tests. Normally distributed data are presented as mean (standard deviation), whereas non-normally distributed data are presented as median (min–max). Statistical significance was set at $p<0.05$.

## RESULTS

**Walking track analysis**

Walking track analyses at baseline, 3 weeks after denervation, and 8 weeks after surgical repair showed no significant differences between the groups (Table 1). However, at Week 8, the AD-MSC-treated group exhibited higher median SFI, STL, and SL values (mm) than the control group.

**Electrophysiological outcome**

The AD-MSC-treated group exhibited higher median CMAP amplitudes than the control group, although the difference was not statistically significant (Table 2). Conduction was observed in all samples except for two rats in the AD-MSC group.

**Muscle weight and diameter**

Following electrophysiological analysis, an incision was made over the scar, with no signs of surgical wound infection. The gastrocnemius muscle

**Table 1.** Comparison of walking track analysis in various of time

| Parameter | | AD-MSCs (n = 10) | Control (n = 10) | *p* |
|---|---|---|---|---|
| SL (mm) | Baseline | 46.91 (16.071) | 46.63 (17.31) | 0.97* |
| | 3 weeks after denervation | 59.18 (9.57) | 58.21 (6.86) | 0.796* |
| | 8 weeks after surgical repair | 52.83 (39–98.5) | 54.15 (33.67–72) | 0.940† |
| STL (mm) | Baseline | 82.31 (27.14) | 87.81 (21.9) | 0.624* |
| | 3 weeks after denervation | 99.75 (15.18) | 105.83 (17.81) | 0.423* |
| | 8 weeks after surgical repair | 92.18 (12.1) | 101.12 (19.42) | 0.232* |
| SFI | Baseline | −3.28 (10.77) | 92.18 (12.1) | 0.281* |
| | 3 weeks after denervation | −44.96 (11.55) | −50.57 (12.37) | 0.308* |
| | 8 weeks after surgical repair | −45.54 (30.14) | −54.95 (32.4) | 0.51* |

AD-MSCs=adipose-derived mesenchymal stem cells; SD=standard deviation; SFI=sciatic function index; SL=step length; STL=stride length
Data are presented as mean (SD), except for the SL value on 8 weeks after surgical repair (median [min–max])
*Unpaired *t*-test; †Mann–Whitney *U* test

**Table 2.** Comparison of peak CMAP amplitude recorded on proximal and distal to the nerve junction

| Limb | Sciatic stimulation | Median (min–max) | | *p** |
|---|---|---|---|---|
| | | AD-MSCs (n = 10) | Control (n = 10) | |
| CMAP (mV) | | | | |
| Left (normal) | Proximal | 21.15 (12.6–30.8) | 15.85 (6.8–29.2) | 0.093 |
| | Distal | 23.95 (11.5–31.7) | 20.8 (10.1–30.1) | 0.453 |
| Right (experimental) | Proximal | 5.45 (1.2–14.8) | 5.2 (1.9–10.8) | 0.876 |
| | Distal | 5.65 (3.3–14.8) | 4.7 (1.4–12.5) | 0.607 |

AD-MSCs=adipose-derived mesenchymal stem cells; CMAP=compound muscle action potential
*Unpaired *t*-test

**Table 3**. Comparison of gastrocnemius muscle weight and diameter 8 weeks after surgical repair

| Limb | AD-MSCs (n = 10), mean (SD) | Control (n = 10), mean (SD) | *p** |
|---|---|---|---|
| Muscle weight (g) | | | |
| Left (normal) | 1.69 (0.517) | 1.60 (0.382) | 0.664 |
| Right (experimental) | 1.21 (0.094) | 1.12 (0.262) | 0.507 |
| Muscle fiber diameter (μm) | 45.54 (13.1) | 35.46 (6.64) | **0.011** |

AD-MSCs=adipose-derived mesenchymal stem cells; SD=standard deviation
*Unpaired *t*-test

mass was higher in the AD-MSC group than in the control group, although the difference was not statistically significant (Table 3). Additionally, muscle fiber diameter was significantly larger ($p = 0.011$) than that in the control group.

## DISCUSSION

Stem cells represent a potential cellular therapy for PNI.[17,18] This study highlighted the potential of AD-MSCs in the treatment of chronic PNI, demonstrating their role in increasing NMJ regeneration and subsequently affecting nerve conduction, muscle regeneration, and functional outcomes. In our chronic PNI rat model, the AD-MSC group exhibited higher CMAP amplitudes than the control group, although the difference was not statistically significant. This may be due to incomplete restoration of NMJ function, resulting in minimal differences in muscle depolarization. A study by Vannucci et al[19] in rats with an acute sciatic nerve transection model showed that CMAP amplitude recovery reached normal levels (83%) at 16 weeks after reinnervation but was only 23% and 40% at Weeks 3 and 4, respectively, in the normal leg. These findings suggest that a longer follow-up period is needed to observe significant improvements in CMAPs after AD-MSC treatment.

Weighing the gastrocnemius muscle is one way to assess sciatic NMJ regeneration. Muscle mass increases as the tibialis posterior nerve branches regenerate, whereas transection causes significant atrophy, making the muscle lighter than that in healthy limbs. In this study, the AD-MSC group had a greater muscle mass than the control group at 8 weeks post-reinnervation, indicating regenerative changes. Additional observation periods could enhance muscle regeneration and increase muscle activity, resulting in significant weight differences. Mohammadi et al[20] observed a significant increase in muscle mass 12 weeks after reinnervation with bone marrow stromal cells in an acute nerve injury model. Given the use of a chronic PNI model, an extended follow-up may be needed.

The primary objective of nerve reconstruction is functional improvement. Walking track analysis was used to assess sciatic nerve motor function. The AD-MSC group showed higher SFI, SL, and STL scores than the control group, although the differences were not statistically significant. As chronic PNI involves significant structural damage to the NMJ, a longer evaluation period may have resulted in greater differences. This is because recovery from chronic nerve injuries is longer than recovery from acute nerve injuries.[21] Mohammadi et al[20] observed functional improvements in rat sciatic nerve regeneration 12 weeks after nerve repair and bone marrow-derived stromal cell injection.

Most studies on peripheral nerve regeneration have focused on acute injury models.[8,22,23] AD-MSCs offer an easily accessible, minimally invasive, and autologous stem cell source that lowers the risk of tissue rejection.[24] The application of this method

was straightforward, involving AD-MSC injections at the NMJ, which can be performed using a minimally invasive technique. Improving NMJ regeneration may allow nerve repair in cases of chronic injury and delay the need for challenging reconstruction procedures. Although the clinical, electrophysiological, and muscle weight data indicated better outcomes in the AD-MSC group, the differences were not statistically significant.

This study has several limitations. Single-time electrophysiological examinations make it challenging to obtain data on muscle regeneration. Repeated assessments, such as nerve stimulation, were not conducted, which could have accounted for factors influencing regeneration. Post-surgical repair examinations could have helped differentiate whether the changes were due to AD-MSC-driven muscle regeneration or prevention of degeneration. Additionally, the sample size may have limited the statistical significance because of insufficient data. Future studies should include larger sample sizes to determine whether the observed improvements are statistically significant. Considering the chronic nature of PNI, a longer follow-up period may yield more conclusive results. In conclusion, AD-MSC implantation in the NMJ may delay end-organ degeneration and enhance regeneration, as shown by the improved morphometric profile in the AD-MSC group.

**Conflict of Interest**

The authors affirm no conflict of interest in this study.

**Acknowledgment**

The authors would like to thank the Faculty of Medicine, Universitas Indonesia for their support in the completion of this study.

**Funding Sources**

None.

## REFERENCES

1. Miller C, Peek AL, Power D, Heneghan NR. Psychological consequences of traumatic upper limb peripheral nerve injury: a systematic review. Hand Ther. 2016;22(1):35–45.
2. Wojtkiewicz DM, Saunders J, Domeshek L, Novak CB, Kaskutas V, Mackinnon SE. Social impact of peripheral nerve injuries. Hand (N Y). 2015;10(2):161–7.
3. Tan RE, Jeyaratnam S, Lim AY. Updates in peripheral nerve surgery of the upper extremity: diagnosis and treatment options. Ann Transl Med. 2023;11(11):391.
4. Slavin BR, Sarhane KA, von Guionneau N, Hanwright PJ, Qiu C, Mao HQ, et al. Insulin-like growth factor-1: a promising therapeutic target for peripheral nerve injury. Front Bioeng Biotechnol. 2021;9:695850.
5. Menorca RM, Fussell TS, Elfar JC. Nerve physiology: mechanisms of injury and recovery. Hand Clin. 2013;29(3):317–30.
6. Sugimura-Wakayama Y, Katagiri W, Osugi M, Kawai T, Ogata K, Sakaguchi K, et al. Peripheral nerve regeneration by secretomes of stem cells from human exfoliated deciduous teeth. Stem Cells Dev. 2015;24(22):2687–99.
7. Chen O, Wu M, Jiang L. The effect of hypoxic preconditioning on induced Schwann cells under hypoxic conditions. PLoS One. 2015;10(10):e0141201.
8. Li LK, Huang WC, Hsueh YY, Yamauchi K, Olivares N, Davila R, et al. Intramuscular delivery of neural crest stem cell spheroids enhances neuromuscular regeneration after denervation injury. Stem Cell Res Ther. 2022;13(1):205.
9. Fu X, Tong Z, Li Q, Niu Q, Zhang Z, Tong X, et al. Induction of adipose-derived stem cells into Schwann-like cells and observation of Schwann-like cell proliferation. Mol Med Rep. 2016;14(2):1187–93.
10. Georgiou M, Golding JP, Loughlin AJ, Kingham PJ, Phillips JB. Engineered neural tissue with aligned, differentiated adipose-derived stem cells promotes peripheral nerve regeneration across a critical sized defect in rat sciatic nerve. Biomaterials. 2015;37:242–51.
11. Tomita K, Madura T, Sakai Y, Yano K, Terenghi G, Hosokawa K. Glial differentiation of human adipose-derived stem cells: implications for cell-based transplantation therapy. Neuroscience. 2013;236:55–65.
12. Kolar MK, Kingham PJ. Regenerative effects of adipose-tissue-derived stem cells for treatment of peripheral nerve injuries. Biochem Soc Trans. 2014;42(3):697–701.
13. Yalçın MB, Bora ES, Erdoğan MA, Çakır A, Erbaş O. The effect of adipose-derived mesenchymal stem cells on peripheral nerve damage in a rodent model. J Clin Med. 2023;12(19):6411.
14. Pawitan JA, Liem IK, Suryani D, Bustami A, Puryoko RY. Simple lipoaspirate washing using a coffee filter. Asian Biomed. 2013;7(3):333–8.
15. Bain JR, Mackinnon SE, Hunter DA. Functional evaluation of complete sciatic, peroneal, and posterior tibial nerve lesions in the rat. Plast Reconstr Surg. 1989;83(1):129–38.
16. American Association of Electrodiagnostic Medicine; Olney RK. Guidelines in electrodiagnostic medicine. Consensus criteria for the diagnosis of partial conduction block. Muscle Nerve Suppl. 1999;8:S225–9.
17. Jiang L, Jones S, Jia X. Stem cell transplantation for peripheral nerve regeneration: current options and opportunities. Int J Mol Sci. 2017;18(1):94.
18. Heine W, Conant K, Griffin JW, Höke A. Transplanted neural stem cells promote axonal regeneration through chronically denervated peripheral nerves. Exp Neurol. 2004;189(2):231–40.
19. Vannucci B, Santosa KB, Keane AM, Jablonka-Shariff A, Lu CY, Yan Y, et al. What is normal? Neuromuscular junction reinnervation after nerve injury. Muscle Nerve. 2019;60(5):604–12.
20. Mohammadi R, Vahabzadeh B, Amini K. Sciatic nerve regeneration induced by transplantation of in vitro bone marrow stromal cells into an inside-out artery graft in rat. J Craniomaxillofac Surg. 2014;42(7):1389–96.
21. Sulaiman W, Gordon T. Neurobiology of peripheral nerve injury, regeneration, andfunctional recovery: from bench top research to bedside application. Ochsner J. 2013;13(1):100–8.
22. Sumarwoto T, Suroto H, Mahyudin F, Utomo DN, Romaniyanto, Tinduh D, et al. Role of adipose mesenchymal stem cells and secretome in peripheral nerve regeneration. Ann Med Surg (Lond). 2021;67:102482.
23. Kappos EA, Baenziger-Sieber P, Tremp M, Engels PE, Thommen S, Sprenger L, et al. Epineural adipose-derived stem cell injection in a sciatic rodent model. Brain Behav. 2018;8(7):e01027.
24. Pikuła M, Marek-Trzonkowska N, Wardowska A, Renkielska A, Trzonkowski P. Adipose tissue-derived stem cells in clinical applications. Expert Opin Biol Ther. 2013;13(10):1357–70.