Research Article

Induced Differentiation of Adipose-Derived Stem Cells into Neuron-Like Cells to Promote Regeneration after Sciatic Nerve Injury

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Abstract | In this study, sixteen healthy adult rats were used to evaluate the effects of inducing differentiation of adipose-derived mesenchymal stromal cells (AD-MSCs) into neuron-like cells on sciatic nerve regeneration. All rats that underwent left axonotmesis (crush) injury were subjected to compression with hemostatic forceps for 60 seconds under aseptic conditions and general anesthesia. The animals were randomly divided into two groups. In the control group, 10 mL of PBS buffer was administered perineurally to treat the defect. In the MSC-treated group, 5×10^6 AD-MSCs in suspension were transplanted perineurally using a Hamilton microsyringe. Immunophenotypic analysis showed that the MSCs were negative for the hematopoietic marker CD34 but positive for the mesenchymal stem cell marker CD90, confirming their mesenchymal identity. By day 8 post-induction, the cells exhibited neural-like morphology characterized by cell body shrinkage, elongated bipolar or multipolar projections, and, in some cases, pyramidal-shaped neuronal forms. Histopathological evaluation at day 112 revealed that the MSC-treated group displayed active Schwann cells, robust myelination, minimal scar tissue formation, proper axonal orientation, and notable angiogenesis. In contrast, the saline-treated group exhibited vacuolation at the injury site, excessive collagen deposition in the peri- and epineurium, and moderate adhesions around the anastomosed region. These findings demonstrate the strong differentiation capacity of locally implanted AD-MSCs and their potential to promote nerve regeneration and functional recovery following sciatic nerve injury.

Keywords | Sciatic nerve, Neural stem cells differentiation, Nerve regeneration, Rat

Received | September 15, 2025; **Accepted** | November 27, 2025; **Published** | March 04, 2026
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Citation | Al-Ameri SHA, Zedan ZK, Al-Maliki NS, Hasan AF (2026). Induced differentiation of adipose-derived stem cells into neuron-like cells to promote regeneration after sciatic nerve injury. J. Anim. Health Prod. 14(2): 428-434.
DOI | https://dx.doi.org/10.17582/journal.jahp/2026/14.2.428.434
ISSN (Online) | 2308-2801

INTRODUCTION

Adipose-derived stem cells (ASCs) are adult multipotent cells with strong homing abilities, immunomodulatory properties, and the capacity to promote tissue repair (Bourin *et al*., 2013). Their clinical application is also more ethically acceptable compared to embryonic stem cells, as their collection is minimally invasive and free from major moral concerns (Dominici *et al*., 2006; Yaseen *et al*., 2025). Adipose tissue is an excellent source of autologous mesenchymal stem cells (MSCs) and can be harvested more easily than bone marrow–derived stem cells, with fewer complications for the donor. This advantage is partly due to their lack of MHC-II expression and their low levels of MHC-I proteins, reducing the risk of immune rejection (Zuk *et al*., 2001).

Furthermore, several studies (Liqing *et al.*, 2011; Bajek *et al.*, 2017; Obaid *et al.*, 2025; Bourin *et al.*, 2013) have highlighted the growing interest in adipose-derived stem cells for regenerative medicine. These studies emphasize the need to optimize isolation techniques, characterization standards, culture conditions, and differentiation protocols to ensure genetic stability, reproducible therapeutic effects, and overall safety of ASC-based therapies. Bone marrow stromal cells have also been shown to differentiate into Schwann cell–like cells capable of stimulating peripheral nerve regeneration. Several studies further demonstrate that these cells can migrate to the dorsal root ganglia and spinal cord (Rodriguez *et al.*, 2019; Obaid *et al.*, 2020) and can form microglial and neuroectodermal cell lineages (Brayfield *et al.*, 2010; Yaseen *et al.*, 2024).

Therefore, the objective of this study was to examine the effect of transplanting neuron-like stem cells on the regeneration of sciatic nerve neurotmesis in rats.

MATERIALS AND METHODS

Experimental design

The study was conducted with the ethical approval of the Ethics Committee of the Biotechnology Research Center, Al-Nahrain University, under decision number E.B.B.16 dated 2.1.2024. The experimental investigation included sixteen adult male rats, aged 8–12 weeks and weighing approximately ±20 g (Essa *et al.*, 2020).

In the control group, 10 mL of PBS buffer was administered perineurally to treat the defect. In the MSC-treated group, rats received a perineural transplantation of 5×10^6 cells. All animals were housed in the central animal care facility and provided with free access to food and water throughout the study period. On the 112th postoperative day, all animals from both groups were euthanized for histological evaluation of sciatic nerve regeneration.

In vitro study

Isolation and cultivation of AD-MSCs

AD-MSCs were isolated following the method described by Zedan and Al-Ameri (2023). The cells were prepared in the Tissue Culture Unit, Department of Biotechnology, Al-Nahrain University. Briefly, under anesthesia, subcutaneous adipose tissue was aseptically excised from the experimental rat (Figure 1). The tissue was thoroughly washed in phosphate-buffered saline (PBS) and then minced into small fragments. It was digested with collagenase type I (1 mg/mL; Gibco, USA) for 1 hour at 37°C.

After digestion, the suspension was filtered and cultured. The medium was replaced after 72 hours, and non-adherent cells were discarded. When the culture reached confluence on day 12, the monolayer was rinsed twice with 2 mL of PBS (pH 7.2). A solution of 0.20% trypsin–0.02% EDTA (Sigma, USA) was added and incubated for 2 minutes to detach the cells. DMEM supplemented with 10% FBS was added to neutralize trypsin, and the detached cells were gently aspirated. The cell suspension was transferred to sterile tubes and centrifuged at 2000 rpm for 5 minutes.

The resulting pellet was resuspended in 1 mL of DMEM. The cell count was determined using a hemocytometer to obtain a final concentration of 5×10^6 AD-MSCs.

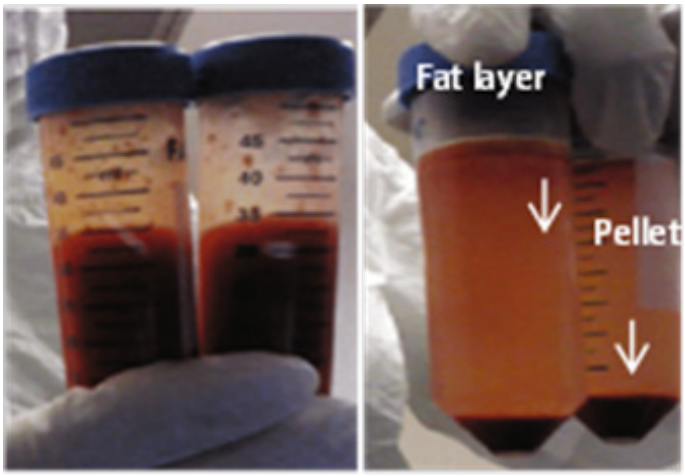

Figure 1: The initial step of isolating adipose-derived mesenchymal stromal cells from rats.

Immunohistochemistry analysis of MSCs

After MSCs were suspended in DMEM growth medium and dissociated using trypsin–PBS, the cells were re-cultured in multi-well tissue culture slides. Eight wells were seeded in DMEM supplemented with 15% FBS, and the plates were incubated at 37 °C to allow the cells to form a monolayer within 1–3 days. The medium was then removed, and the cells were fixed with 4% paraformaldehyde for 10 minutes at room temperature (20–25 °C) in a humid chamber.

Following fixation, the cells were washed with PBS and incubated with 1% hydrogen peroxide for 10–15 minutes to block endogenous peroxidase activity. To minimize background staining, 1.5% blocking serum was applied for 1 hour. The cells were then rinsed with PBS (three times, 5 minutes each).

Next, the samples were incubated with 125 µL of diluted primary antibody and then washed again three times with PBS. This was followed by incubation with 1.2 mL of biotinylated secondary antibody for 30 minutes. After washing, 650 µL of ABC reagent was added to the wells, followed by additional PBS rinsing.

For visualization, the cells were treated with peroxidase

substrate (three drops) for approximately 10 minutes, or until adequate stain intensity was achieved, and then rinsed with purified water. The samples were counterstained with hematoxylin for 5–10 seconds and washed again.

Finally, one to two drops of permanent mounting medium were applied, and the slides were examined under a light microscope at 40× and 100× magnification (Bucan *et al.*, 2019).

Neurogenic differentiation

Neuronal induction of AD-MSCs was performed using a modified protocol based on published methods (Ying *et al.*, 2012). After 24 hours of incubation, the culture medium was removed and replaced with serum-free MEM containing 2 mM β-mercaptoethanol (BME) for 1 hour. The medium was then discarded, and the cells were washed with serum-free MEM.

For the induction phase, serum-free MEM supplemented with 1 mM retinoic acid (Gibco, USA), 10 ng/mL nerve growth factor (NGF) (Gibco, USA), and 0.1 ng/mL sonic hedgehog (Shh) (Gibco, USA) was added. Cells were incubated at 37°C for four days under these conditions.

As a negative control, a parallel set of MSCs was cultured under the same environmental conditions but without induction factors. All cultures were maintained in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C, and the medium was replaced every two days.

Assessment of neurogenic differentiation

Histological examination was performed after 21 days. The pellets were fixed in 4% buffered formalin at 4 °C for short-term fixation, then dehydrated in ethanol and embedded in paraffin. Sections with a thickness of 4 µm were cut from the paraffin blocks, deparaffinized with xylene, and rehydrated with tap water. The obtained sections were stained with hematoxylin and eosin (H&E) to visualize cell morphology.

In vivo animal study

Surgical procedure of sciatic nerve injury

Each mouse was anesthetized with a combination of drugs at a dose of 75 mg/kg. The left rump was prepared aseptically. A longitudinal incision was made on the posterolateral thigh, approximately 1 cm caudolateral to the greater trochanter, exposing about 33% of the distal femur. The subcutaneous tissue and fascia lata were carefully dissected. Through this skin entry point, the cranial portion of the biceps femoris and the posterior portion of the semitendinosus muscles were separated using blunt dissection with Mayo scissors to expose the sciatic nerve and isolate it from the surrounding tissues.

As previously described, a hemostatic forceps was applied for 60 seconds to induce an axonotmesis (crush) injury (Liu *et al.*, 2020). Using a Hamilton microsyringe, 10^6 cells in suspension were transplanted perineurally into the MSC site. After transplantation, the musculature and skin were sutured. The mouse was then allowed to recover in a clean enclosure on a 37 °C warming pad. Antimicrobials (gentamicin, 100 mg/kg, i.m.) were administered daily for 7 days post-injury.

Histological investigation of sciatic nerve repair

The samples underwent standard histological procedures on the 12th day after surgery. Tissue sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope for histological alterations (Luna, 1992).

RESULTS

Morphology of bone marrow-derived mesenchymal stem cells

Small cell colonies were observed in the experimental culture 48–72 hours after seeding. The cells were round and flattened, and they began to attach to the plastic surface of the culture flask in their initial state (Figure 2A). After ten days, a monolayer of adherent cells was formed, during which the colonies and individual cells expanded, and the cells gradually increased in size and interconnected. After 12 days of growth at passage 1 (P1), the cells exhibited a large, flattened, spindle-shaped, and slightly irregular morphology (Figure 2B). Two days after subculture at passage 2 (P2), the AD-MSCs showed a high proportion of spindle-shaped cells that aligned along their longitudinal axis. By five days post-subculture at passage 3 (P3), the AD-MSCs displayed a heterogeneous morphology, including large, flattened, spindle-shaped, round, and polygonal cells (Figure 2C).

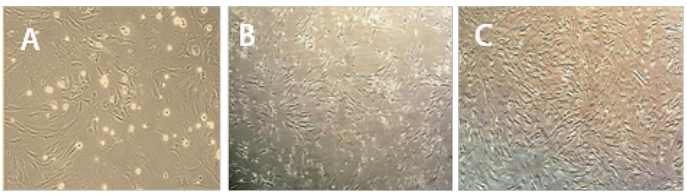

Figure 2: AD-MSCs in primary culture growing as a monolayer under in vitro conditions, observed with an inverted microscope. Scale bar: 100 µm.

Immunophenotypic analysis of MSCs

Immunocytochemical staining was used to rapidly identify MSCs from a mixed cell suspension. This analysis revealed that most cells expressed the characteristic MSC markers (Figure 3A). In contrast, the cells were negative for CD34 and were distinctly counterstained blue with Harris hematoxylin (Figure 3B).

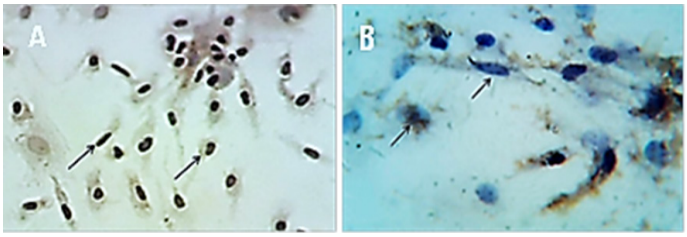

Figure 3: Inverted microscope images of AD-MSCs at passage 3 showing immunophenotypic analysis. (A) Cells exhibit a positive response for CD90, stained brown. (B) Cells show a negative response for CD34, counterstained blue with H&E. Scale bar = 100 µm.

Histological analysis of MSCs

Changes in post-seeding morphology are shown in Figure 4. By the eighth day after seeding, the cells became smaller and more compact, forming bipolar or multipolar processes extending from the cell body. In some cells, a single long axon-like projection was observed, and certain neurons exhibited a pyramidal shape. Neural morphology was distinguished from typical cell morphology based on the refractive appearance of the cells, the presence of bipolar or multipolar neurite-like projections (with at least one projection equal to or longer than the soma), and the occasional presence of cellular extensions.

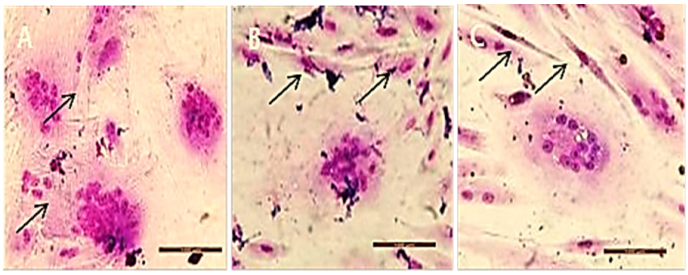

Figure 4: Inverted microscope images (H & E stain) of differentiated neurons derived from adipose tissue–derived MSCs. (A) Cells show bifurcation of processes. (B) Cells exhibit bipolar or multipolar neurite-like projections (C).

Histological evaluation of nerve regeneration

In the control group, the proximal stump showed relatively well-aligned regenerative nerve fibers, mild adhesion to surrounding tissue, vacuolated nerve fibers, collagen deposition (scar formation) in the perineurium and epineurium, and evidence of remyelination of regenerative nerve fibers (Figure 5A). Histological examination of the middle stump revealed a reduced number of nerve fibers, mild proliferation of Schwann cells, improved parallel alignment of nerve fibers, and the presence of fibrous tissue and inflammatory cells (Figure 5B). The distal stump displayed vacuolated, degenerated nerve fibers, minimal scar tissue, improved myelination, and inflammatory cell infiltration (Figure 5C).

In the MSC-treated group, the proximal stump demonstrated enhanced parallel alignment of nerve fibers, minimal residual endoneurial channels, robust remyelination of regenerative nerve fibers, and active Schwann cells (Figure 5D). The middle stump exhibited active Schwann cells, parallel arrangement of nerve fibers, formation of new blood vessels, remyelination of regenerative fibers, and few inflammatory cells (Figure 5E). In the distal stump, vacuolated degenerating nerve fibers were observed along with well-aligned regenerative nerve fibers and active Schwann cells (Figure 5F).

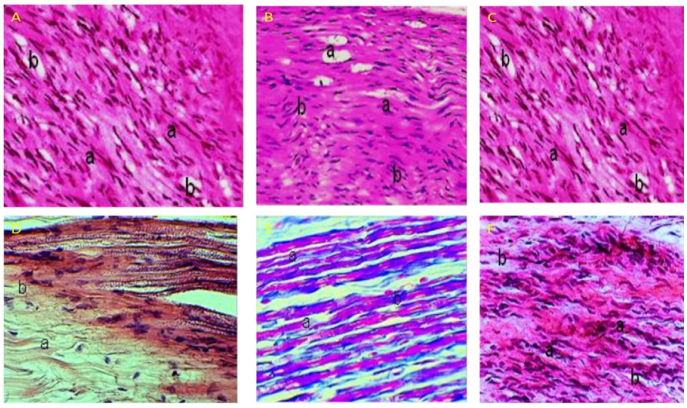

Figure 5: Representative histological sections at 112 days post-operation.
Control group proximal segment (A): Collagen fibers (a) and vacuolated nerve fibers (b) (H & E, 20×). Control group middle segment (B): Vacuolated nerve fibers (a) and intra-neural scar tissue (b) (H & E, 20×). Control group distal segment (C): Degenerated nerve fibers (a) with collagen fibers in the perineurium, epineurium, and intra-neural scar tissue (b) (H & E, 20×). MSCs group proximal segment (D): Myelinated axons (a) with normal orientation of nerve fibers (b) (H & E, 20×). MSCs group middle segment (E): Evidence of axonal regeneration (a) and Schwann cell proliferation near the lesion (b) (H & E, 20×). MSCs group distal segment (F): Myelinated axons (a) and regenerated nerve fibers (b) (H & E, 20×).

DISCUSSION

Transplantation of neural stem cells (NSCs) has been proposed as a potential therapeutic approach for neurodegenerative disorders (Phinney and Prockop, 2007). However, the need for immunosuppressive regimens in allogeneic transplantation and the limited availability of donor brain tissue pose significant challenges. These limitations could be overcome if NSCs were derived from clinically accessible autologous sources, such as adipose tissue or peripheral blood. Recently, mesenchymal stem cells (MSCs) have been shown to promote angiogenesis, neurogenesis, and the repair of injured tissues (Jang *et al.*, 2016; Obaid *et al.*, 2020; Kareem and Hussein, 2023).

In the present study, MSCs were characterized by their

morphology and confirmed by surface marker analysis, showing 98.9% positivity for CD90. This is consistent with previous reports (Fu *et al*., 2008) demonstrating that MSCs express characteristic antigens including CD29, CD44, CD73, CD90, CD105, and CD166, while lacking hematopoietic markers such as CD34, CD45, CD14, or CD11. Similarly, Marconi *et al*. (2012), Li *et al*. (2017), and Khoo *et al*. (2011) reported positive CD90 and negative CD34 expression in MSCs, consistent with our findings regarding homing receptor-mediated migration of hematopoietic and bone marrow stem cells.

For neural differentiation, a combination of growth factors and retinoic acid (RA) was employed. Growth factor-based differentiation has shown promising results in previous studies (Maden, 2007). However, chemical-based methods remain controversial due to the potential cytotoxic effects of these compounds on cell size and morphology (Montiel-Eulefi *et al*., 2012). RA, an active metabolite of vitamin A, is widely expressed in the nervous system during development and acts as a potent inducer of differentiation, with receptors and binding proteins throughout the brain, suggesting a critical role in neural development and function. These findings align with Varejão *et al*. (2001), who successfully used RA and other neural-inducing agents to generate neural-like cells.

Histomorphological analysis in this study revealed that transplantation of AD-MSCs enhanced the formation of nerve fibers and angiogenesis, suggesting a therapeutic role in nerve repair. These results are consistent with Altemeemi *et al*. (2021), who demonstrated that MSC implantation, either alone or in combination with guiding conduits, can improve peripheral nerve regeneration in cases of neurotmesis. Similarly, Cartarozzi *et al*. (2015) and Hameed *et al*. (2011) reported that stem cell therapy promotes neovascularization through multiple mechanisms, including differentiation of transplanted cells into endothelial cells and secretion of trophic or angiogenic factors (Zhang *et al*., 2017; Al-Maliki *et al*., 2025). Vascular endothelial growth factor (VEGF) is particularly important, influencing astrocytes, Schwann cells, microglia, and neural progenitors. VEGF can protect motor neurons from oxidative stress and hypoxia-induced apoptosis (Sarikcioglu *et al*., 2009; Zedan and Al-Ameri, 2022), stimulate axonal growth, and enhance the survival of neurons and satellite cells in dorsal root ganglia (Zachary, 2005).

Histological observations in the MSC-treated group showed minimal intraneural scarring, whereas the control group exhibited extensive collagen deposition. This suggests that axonal regeneration is influenced by a balance between regeneration and scar formation. Supporting this, studies by Varejão *et al*. (2004) and Sondell *et al*. (1999) demonstrated that interventions reducing scar formation, such as tissue plasminogen activator (tPA), enhance axonal regeneration and myelination in the peripheral nervous system. Collectively, these findings emphasize the potential of MSC therapy to promote neural repair while minimizing inhibitory effects of scar tissue.

CONCLUSIONS

Transplantation of neural-differentiated adipose-derived mesenchymal stromal cells significantly enhances axonal regeneration and limits scar formation following sciatic nerve crush injury in mice, highlighting their promising therapeutic potential for peripheral nerve repair.

ACKNOWLEDGEMENT

Thanks only to the authors for providing their time, effort and support for the manuscript.

NOVELTY STATEMENT

This study is the first to demonstrate that adipose-derived mesenchymal stromal cells (AD-MSCs), pre-stimulated for neural differentiation and angiogenesis, can effectively enhance sciatic nerve regeneration.

AUTHOR'S CONTRIBUTION

All authors contributed equally to the manuscript, including study design, manuscript writing, linguistic editing, and journal selection.

Funding sources

This study received no funding from any institution, university, or organization; all support was provided solely by the authors.

Generative AI and AI-assisted technology statement

No artificial intelligence tools were used in the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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