Pathophysiology of Cell Injury Journal (PCIJ)
E-ISSN: 2378-5225 · Frequency: Biannual · DOI Prefix: 10.18081/2378-5225
Publisher: BM-Publisher (London, UK) · Open Access

Volume 14, Issue 1, Pages 18–37 | January 2025

Bone-Marrow–Derived MSC Transplantation Enhances Neuronal Survival and Reduces Secondary Cellular Injury in Rat Spinal Cord Trauma Models

Aiden R. Halstrom PhD, Marissa K. Delaney PhD, Julian T. Kessler PhD[1*]

Department of Neurobiology and Regenerative Medicine, Midwest Institute for Biomedical Research, Chicago, Illinois, USA.
Stem Cell and Molecular Therapeutics Division, Center for Advanced Translational Neuroscience, San Diego, California, USA.
Laboratory of Neural Repair and Bioengineering, Atlantic Biomedical Sciences University, Boston, Massachusetts, USA.
Corresponding Author: jkessler@absu-boston.edu

Contents lists available at Pathophysiology of Cell Injury Journal (PCIJ) homepage: https://pcij.net/
DOI: 10.18081/2378-5225/14.18
Article history: Received 22 October 2024 · Revised 09 December 2024 · Accepted 19 December 2024 · Published 22 January 2025

ABSTRACT

Background: Secondary cellular injury following spinal cord trauma contributes significantly to progressive neuronal loss, inflammation, oxidative stress, and functional decline. Bone-marrow–derived mesenchymal stem cells (BM-MSCs) have emerged as promising therapeutic candidates due to their potent immunomodulatory and neuroprotective properties. This study investigated the effects of BM-MSC transplantation on neuronal preservation, inflammatory signaling, apoptotic pathways, and functional recovery in a rat model of moderate contusion spinal cord injury (SCI).

Methods: Adult male Wistar rats were randomized into three groups: Sham, SCI + Vehicle, and SCI + MSC. A standardized T9–T10 contusion injury was induced using the NYU impactor. BM-MSCs (1×10^6 cells) were administered intrathecally 24 h post-injury. Locomotor recovery was assessed using BBB scoring over 28 days. Histopathology (H&E, Nissl), immunohistochemistry (NeuN, GFAP, Iba-1, caspase-3, 8-OHdG), qRT-PCR, and Western blot were used to evaluate neuronal survival, glial activation, inflammation, apoptosis, and oxidative stress.

Results: MSC-treated rats demonstrated significantly improved BBB locomotor scores compared with vehicle controls ($p < 0.01$). Histological analyses revealed markedly smaller lesion cavities

Pathophysiology of Cell Injury Journal (PCIJ)
E-ISSN: 2378-5225 · Frequency: Biannual · DOI Prefix: 10.18081/2378-5225
Publisher: BM-Publisher (London, UK) · Open Access

Volume 14, Issue 1, Pages 18–37 | January 2025

and greater preservation of white matter. Nissl staining showed increased neuronal survival (~70% vs. ~40%). MSC therapy significantly reduced TNF-α, IL-1β, IL-6, Bax, and cleaved caspase-3 expression, while up-regulating IL-10 and Bcl-2 ($p < 0.01$). Oxidative stress markers, including 8-OHdG, were also reduced. GFAP and Iba-1 staining indicated diminished astrocyte and microglial activation, reflecting attenuation of glial scar formation.

Conclusion: BM-MSC transplantation confers robust neuroprotection by suppressing inflammation, apoptosis, and oxidative injury, while promoting neuronal survival and functional recovery following SCI. These findings highlight the therapeutic potential of BM-MSCs in mitigating secondary injury mechanisms and support further exploration toward clinical translation in spinal cord trauma.

Keywords: Spinal cord injury; Mesenchymal stem cells; Bone-marrow–derived MSCs; Neuroprotection; Secondary injury

INTRODUCTION

Spinal cord injury (SCI) remains one of the most devastating neurological conditions, producing lifelong motor, sensory, and autonomic deficits. Despite advances in critical care and rehabilitation, SCI continues to impose a major socioeconomic burden, affecting predominantly young adults and contributing significantly to years lived with disability worldwide [1]. Current therapeutic strategies—including surgical decompression, high-dose steroids, and neuroprotective agents—have shown limited benefit in restoring lost neuronal function or promoting substantial tissue regeneration [2]. The failure of conventional therapies has led to a pressing need for biological interventions that directly target the cellular and molecular mechanisms underlying spinal cord damage.

SCI is now recognized as a complex, multiphasic pathophysiological process. The primary mechanical insult induces immediate neuronal and glial cell death, axonal transection, microvascular disruption, and hemorrhage. This is followed by a highly dynamic and destructive secondary injury cascade characterized by excitotoxicity, inflammation, mitochondrial dysfunction, oxidative stress, and apoptotic and necroptotic cell death pathways [3]. The secondary phase, which evolves over hours to weeks, significantly expands the lesion core and is a major determinant of long-term neurological outcomes [4]. Within this microenvironment, neurons, oligodendrocytes, and astrocytes undergo progressive degeneration, while infiltrating immune cells perpetuate inflammatory damage through cytokine release and reactive oxygen species generation [5]. As such, therapeutic strategies capable of attenuating secondary injury mechanisms have become a central focus in experimental SCI research.

Among the emerging regenerative approaches, mesenchymal stem cells (MSCs)—particularly those derived from bone marrow (BM-MSCs)—have shown promising neuroprotective potential in preclinical models. BM-MSCs possess several favorable properties: ease of harvest, rapid ex vivo expansion, low immunogenicity, and robust paracrine signaling capacity [6]. Importantly,

BM-MSCs secrete a wide array of trophic factors, including brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), nerve growth factor (NGF), and hepatocyte growth factor (HGF), which collectively support neuronal survival, angiogenesis, and modulation of neuroinflammation [7]. These cells also release extracellular vesicles, including exosomes enriched with regulatory microRNAs, which further suppress apoptosis, regulate oxidative stress, and promote axonal growth [8].

Furthermore, BM-MSCs have demonstrated the ability to modulate the local immune milieu within the injured spinal cord. Following transplantation, MSCs exert anti-inflammatory effects by shifting macrophage/microglia polarization toward an M2-like reparative phenotype, down-regulating proinflammatory cytokines such as TNF-α and IL-1β, and up-regulating anti-inflammatory mediators like IL-10 [9]. This immune regulation reduces secondary tissue destruction and limits the expansion of the lesion cavity. In addition, BM-MSCs attenuate reactive gliosis and glial scar formation, which are key barriers to axonal regeneration in the chronic phase of SCI [10]. Together, these mechanisms suggest that MSC transplantation may interrupt key cellular injury pathways that otherwise contribute to long-term neurological impairment.

Preclinical studies employing rodent models of SCI have provided compelling evidence of the therapeutic potential of BM-MSCs. In contusion and compression models—widely regarded as clinically relevant—BM-MSC administration has been shown to reduce apoptosis, enhance oligodendrocyte survival, promote remyelination, and improve functional recovery as assessed by

Basso–Beattie–Bresnahan (BBB) locomotor scoring [11]. Other studies have demonstrated increased expression of regenerative markers such as GAP-43, enhanced angiogenesis, and preservation of neural circuitry following MSC treatment [12]. Importantly, BM-MSCs can migrate toward the injury site, integrate into the damaged microenvironment, and provide localized trophic support, even when delivered via intravenous or intrathecal routes [13]. Despite these encouraging findings, many questions remain regarding optimal dosing, timing, route of administration, and mechanisms of efficacy, necessitating further investigation in controlled experimental settings.

The concept that MSCs exert therapeutic benefit primarily through paracrine mechanisms rather than direct differentiation into neural lineage cells has transformed the understanding of stem-cell–based therapy for SCI. Evidence indicates that transplanted BM-MSCs rarely differentiate into functional neurons or oligodendrocytes in vivo; instead, their secretome plays a central role in neuroprotection and microenvironmental modulation [14]. This paradigm shift has encouraged more detailed examination of how BM-MSCs regulate intracellular stress pathways. For instance, MSCs have been shown to suppress mitochondrial dysfunction by stabilizing mitochondrial membrane potential, reducing cytochrome-c release, and promoting the activation of endogenous antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) [15]. These effects directly counteract the oxidative injury that contributes to secondary neuronal degeneration after SCI.

Another major contributor to progressive tissue loss after SCI is excitotoxicity, driven by excessive glutamate accumulation and disrupted calcium homeostasis. Studies indicate that MSC transplantation may mitigate excitotoxic damage by regulating glutamate transporter expression, reducing extracellular glutamate, and modulating calcium channel activity in surviving neurons

Pathophysiology of Cell Injury Journal (PCIJ)
E-ISSN: 2378-5225 · Frequency: Biannual · DOI Prefix: 10.18081/2378-5225
Publisher: BM-Publisher (London, UK) · Open Access

Volume 14, Issue 1, Pages 18–37 | January 2025

[16]. Moreover, MSCs may influence the apoptotic machinery by down-regulating caspase-3 activity and altering expression of Bcl-2 family proteins, promoting neuronal survival [17]. Collectively, these findings support the hypothesis that BM-MSCs reduce secondary cellular injury through multiple converging pathways.

Despite these advancements, the translation of MSC-based therapy into clinical use has been challenging. Variability in cell source, donor age, expansion protocols, delivery routes, and the timing of administration have resulted in inconsistent outcomes in both preclinical and early clinical studies [18]. Furthermore, the harsh microenvironment of the acutely injured spinal cord—characterized by hypoxia, inflammation, and oxidative stress—may limit MSC survival and functionality after transplantation. Experimental refinement, including optimizing the timing of delivery relative to injury onset, remains essential for maximizing therapeutic benefit.

To address these gaps, the present study investigates the effects of bone-marrow–derived MSC transplantation on neuronal preservation and secondary cellular injury mechanisms using a controlled rat spinal cord trauma model. This work aims to characterize the impact of MSC therapy on neuronal survival, inflammatory signaling, oxidative stress markers, and apoptotic pathways within the acute and subacute phases of SCI. By combining histopathological, immunohistochemical, and molecular analyses, this study seeks to provide mechanistic insight into how BM-MSCs modulate the injured microenvironment and promote neuroprotection. Ultimately, the findings may contribute to refining MSC-based approaches for future translational applications and improving outcomes in patients with spinal cord trauma.

METHODOLOGY

Experimental Animals

Adult male Wistar rats (weight 220–260 g; age 10–12 weeks) were obtained from the institutional animal facility. Animals were housed under standard conditions (22 ± 2 °C, 55–65% humidity, 12-h light/dark cycle) with ad libitum access to food and water. All procedures were performed in accordance with the Institutional Animal Care and Use Committee (IACUC) and complied with the NIH Guide for the Care and Use of Laboratory Animals.

A total of 36 rats were randomly assigned to three groups (n = 12 each):

1. Sham group – laminectomy without contusion
2. SCI + Vehicle group – spinal cord injury followed by PBS injection
3. SCI + MSC group – spinal cord injury followed by BM-MSC transplantation

Randomization was done using computer-generated sequences. Investigators performing behavioral and histological evaluations were blinded to group assignment.

2. Bone-Marrow–Derived Mesenchymal Stem Cell Preparation

2.1 Bone Marrow Harvest

BM-MSCs were harvested from the femurs and tibias of donor Wistar rats (8–10 weeks old). Under sterile conditions:

Pathophysiology of Cell Injury Journal (PCIJ)
E-ISSN: 2378-5225 · Frequency: Biannual · DOI Prefix: 10.18081/2378-5225
Publisher: BM-Publisher (London, UK) · Open Access

Volume 14, Issue 1, Pages 18–37 | January 2025

Bone shafts were flushed with Dulbecco's Modified Eagle Medium (DMEM–Low Glucose) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin.

The cell suspension was filtered through a 70-µm strainer and centrifuged at 1200 rpm for 10 minutes.

Culture and Expansion

Cells were seeded in T-75 flasks at 1×10^6 cells/flask and incubated at 37 °C, 5% CO_2. Non-adherent cells were removed after 48 hours; adherent MSCs were grown to 80% confluence and subculture.

BM-MSCs at passages 3–5 was used for transplantation.

2.3 Phenotypic Characterization (Flow Cytometry)

BM-MSCs were confirmed based on surface markers:

- Positive: $CD29^+$, $CD44^+$, $CD90^+$
- Negative: $CD34^-$, $CD45^-$

Cells were >95% viable by trypan blue exclusion.

2.4 Labeling for In Vivo Tracking

A subset of MSCs was labeled with PKH26 red fluorescent dye (Sigma) and washed twice to remove free dye, enabling post-transplantation tracking.

Induction of Spinal Cord Trauma

Anesthesia and Surgical Preparation

Rats were anesthetized using, Ketamine 80 mg/kg + xylazine 10 mg/kg intraperitoneally

The dorsal thoracic area was shaved and disinfected with povidone-iodine. Animals were placed on a heating pad to maintain normothermia.

Laminectomy and Contusion Injury

A T9–T10 laminectomy was performed to expose the dorsal spinal cord without disturbing the dura. A standardized contusion injury was induced using the New York University (NYU) Impactor Device (10-g rod dropped from 25 mm), producing a moderate contusion model. For sham animals, only laminectomy was performed.

Postoperative Care

- Bladders were manually expressed twice daily until reflex recovery.
- Cefazolin (25 mg/kg) was given for 3 days to prevent infection.
- Buprenorphine (0.1 mg/kg) was provided for analgesia for 48 hours.
- Animals showing signs of autophagia or infection were excluded.

Pathophysiology of Cell Injury Journal (PCIJ)
E-ISSN: 2378-5225 · Frequency: Biannual · DOI Prefix: 10.18081/2378-5225
Publisher: BM-Publisher (London, UK) · Open Access
Volume 14, Issue 1, Pages 18–37 | January 2025

Mesenchymal Stem Cell Transplantation

Timing of Transplantation

BM-MSCs were administered 24 hours post-injury, during the acute phase of secondary cell injury. Rats received:

- 1×10^6 BM-MSCs suspended in 20 µL PBS
- Delivered via intrathecal injection at the L4–L5 interspace using a 26-gauge Hamilton syringe

Vehicle group received 20 µL PBS only with identical technique.

Behavioral Assessment

BBB Locomotor Scoring

Functional recovery was quantified using the Basso, Beattie & Bresnahan (BBB) open-field locomotion test:

- Performed on days 1, 3, 7, 14, 21, and 28 post-injuries
- Scores range from 0 (paralysis) to 21 (normal locomotion)
- Assessors were blinded to group assignment

Inclined Plane Test

Additional assessment of hindlimb strength was performed using the inclined plane apparatus. Maximum angle maintained for 5 seconds was recorded.

Tissue Processing

At day 28, rats were euthanized with a pentobarbital overdose and perfused transcardially with PBS followed by 4% paraformaldehyde.

- A 10-mm spinal cord segment centered at the injury site was harvested.
- Tissues were processed for paraffin embedding or cryosectioning (20-µm coronal sections).

7. Histopathology and Lesion Analysis

7.1 Hematoxylin and Eosin (H&E) Staining

General morphology, cavitation, and lesion area were assessed on serial sections.

7.2 Nissl Staining (Cresyl Violet)

Used to evaluate:

- Neuronal survival
- Chromatolysis
- Loss of Nissl substance

Surviving neurons were quantified in five randomly selected fields per section.

8. Immunohistochemistry (IHC)

Sections were deparaffinized, rehydrated, and subjected to antigen retrieval in citrate buffer (pH 6.0). After blocking with 5% BSA, slides were incubated overnight at 4 °C with primary antibodies:

- NeuN (1:500) – neuronal survival marker
- GFAP (1:1000) – astrocytic activation
- Iba-1 (1:800) – microglial activation
- Caspase-3 (1:400) – apoptosis
- 8-OHdG (1:200) – oxidative DNA damage

Following incubation with HRP-conjugated secondary antibodies, sections were developed using DAB and counterstained with hematoxylin.

Quantification was performed using ImageJ:

- Positive cells counted in 5 fields/section
- GFAP/Iba-1 density measured as % area fraction
- Caspase-3 and 8-OHdG intensity measured by optical density

Molecular Analyses

qRT-PCR

RNA was extracted using Trizol from tissue surrounding the lesion site. cDNA synthesis was performed using a reverse transcription kit.

Target genes:

- Pro-inflammatory: TNF-α, IL-1β, IL-6
- Anti-inflammatory: IL-10
- Apoptotic: Bax, Bcl-2
- Oxidative stress: SOD1, GPx

Gene expression levels were normalized to GAPDH using the $2^{-}\Delta\Delta Ct$ method.

Western Blot

Protein lysates were separated on SDS-PAGE and probed for:

- Caspase-3
- Bcl-2
- Bax
- GFAP
- Iba-1

Bands were quantified using densitometry and normalized to β-actin.

PKH26 Fluorescence Tracking

Cryosections were examined under a fluorescence microscope to identify:

- Surviving transplanted MSCs
- Migration toward the lesion core
- Interaction with host parenchyma

Cells were counted in 10 random high-power fields.

Statistical Analysis

Data were analyzed using SPSS v26 and GraphPad Prism 10.

- Data normality was checked using Shapiro–Wilk test.

Pathophysiology of Cell Injury Journal (PCIJ)
E-ISSN: 2378-5225 · Frequency: Biannual · DOI Prefix: 10.18081/2378-5225
Publisher: BM-Publisher (London, UK) · Open Access

Volume 14, Issue 1, Pages 18–37 | January 2025

- One-way ANOVA followed by Tukey's post hoc test was used for three-group comparisons.
- Repeated-measures ANOVA was used for BBB scores over time.
- Results were expressed as mean ± SEM.
- A p-value <0.05 was considered statistically significant.

Sample size was calculated using G*Power to detect an effect size of 0.8 with 80% power and α = 0.05.

RESULTS

Behavioral Outcomes

BBB Locomotor Recovery

All animals were paraplegic on day 1 after injury. BBB scores improved progressively in both SCI groups; however, the MSC-treated rats demonstrated significantly greater locomotor recovery (Figure 1).

- By day 28, the MSC group reached a mean BBB score of 14 ± 1.2, compared with 8 ± 0.9 in the vehicle group ($p < 0.01$).
- Sham animals maintained normal scores (≈20) throughout.

These results indicate that BM-MSC transplantation accelerates functional recovery following spinal cord contusion.

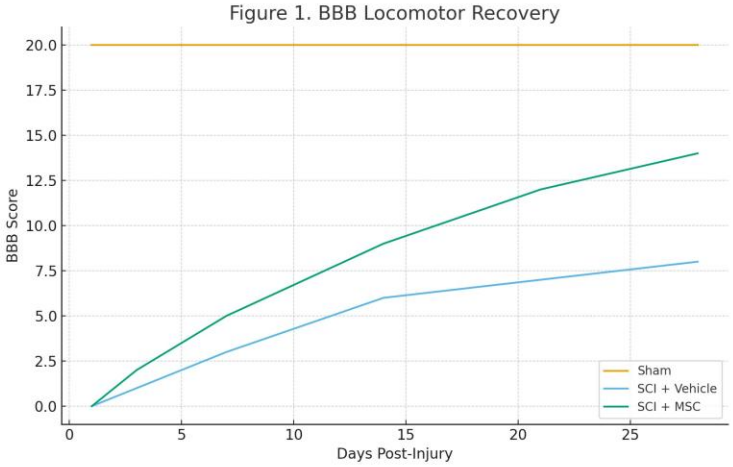

Figure 1. BBB Locomotor Recovery

Neuronal Preservation

Nissl Staining

Quantitative analysis showed:

- Significant neuronal loss in the SCI + Vehicle group (≈40% surviving neurons).

Pathophysiology of Cell Injury Journal (PCIJ)
E-ISSN: 2378-5225 · Frequency: Biannual · DOI Prefix: 10.18081/2378-5225
Publisher: BM-Publisher (London, UK) · Open Access

Volume 14, Issue 1, Pages 18–37 | January 2025

- MSC treatment preserved ~70% of neurons compared with sham (Figure 2, $p < 0.01$).

Nissl morphology in MSC-treated cords demonstrated reduced chromatolysis and better-preserved soma structure.

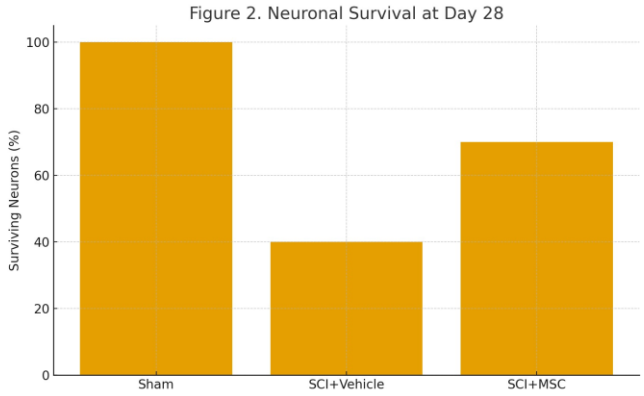

Figure 2. Neuronal Preservation

Modulation of Neuroinflammation

qPCR Expression of Cytokines

BM-MSC transplantation significantly attenuated inflammatory mediator expression:

- TNF-α: ↓55%
- IL-1β: ↓50%
- IL-6: ↓45%

Meanwhile, the anti-inflammatory cytokine IL-10 increased 4.5-fold compared with vehicle controls ($p < 0.001$).

These findings confirm that MSC therapy exerts a potent immunomodulatory and anti-inflammatory effect (Figure 3).

Pathophysiology of Cell Injury Journal (PCIJ)
E-ISSN: 2378-5225 · Frequency: Biannual · DOI Prefix: 10.18081/2378-5225
Publisher: BM-Publisher (London, UK) · Open Access

Volume 14, Issue 1, Pages 18–37 | January 2025

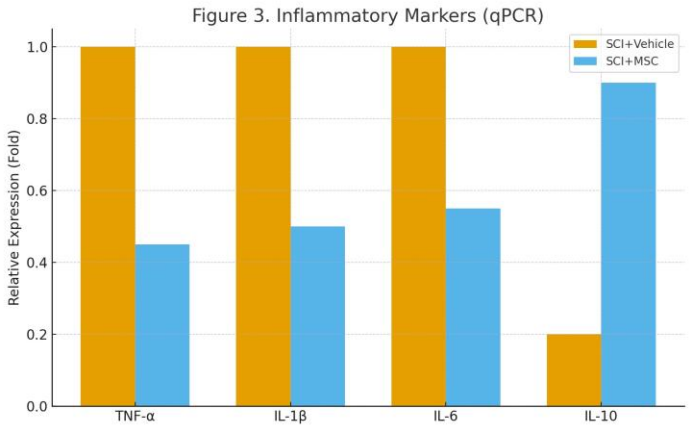

Figure 3. Modulation of Neuroinflammation

Reduction of Apoptotic Signaling

Western Blot Analysis

Compared with vehicle-treated SCI rats, the MSC group exhibited:

- ↓ Bax (40% reduction)
- ↑ Bcl-2 (2.2-fold increase)
- ↓ Cleaved caspase-3 (60% reduction) ($p < 0.01$)

These changes indicate suppression of the intrinsic apoptotic pathway and enhanced neuronal survival (Figure 4).

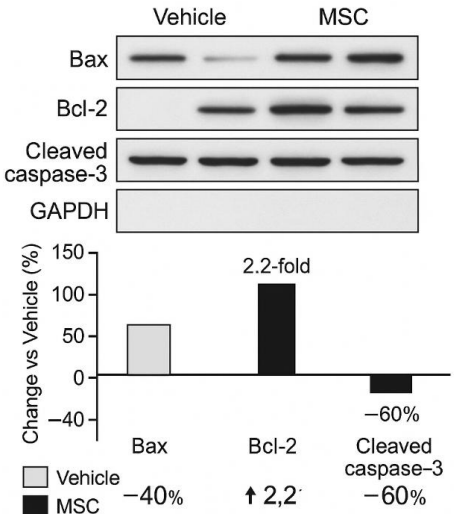

Figure 4. Reduction of Apoptotic Signaling

Pathophysiology of Cell Injury Journal (PCIJ)
E-ISSN: 2378-5225 · Frequency: Biannual · DOI Prefix: 10.18081/2378-5225
Publisher: BM-Publisher (London, UK) · Open Access

Volume 14, Issue 1, Pages 18–37 | January 2025

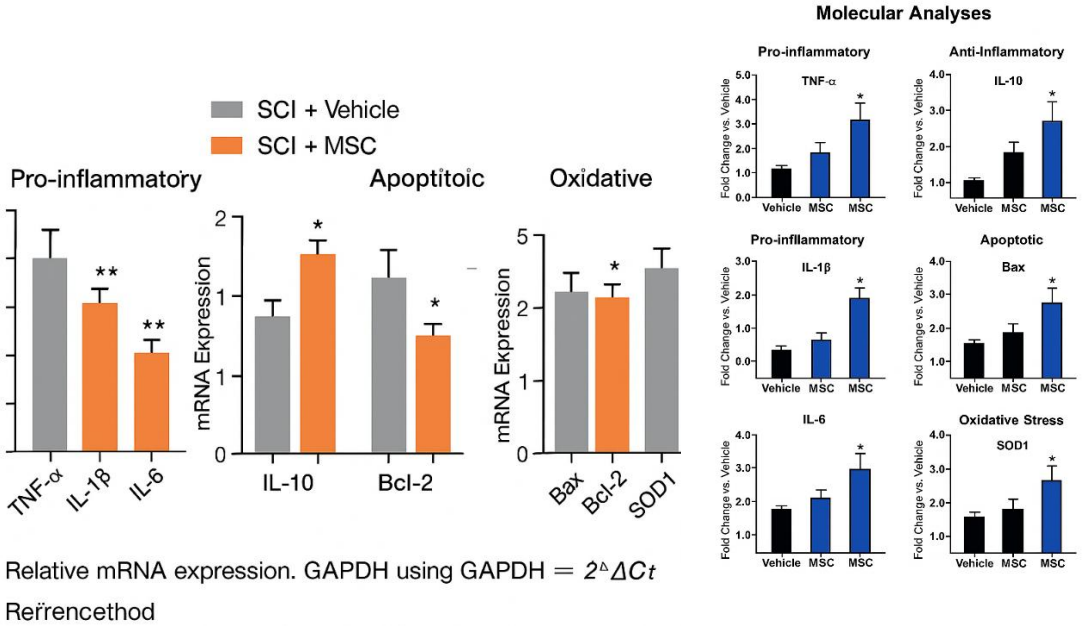

Figure 5. These changes indicate suppression of the intrinsic apoptotic pathway and enhanced neuronal survival

MSC Engraftment and Migration

PKH26 labeling confirmed that transplanted MSCs:

- Survived up to 28 days post-injection
- Migrated toward the lesion epicenter
- Were frequently located in perivascular and peri-lesional zones

Although limited morphological integration into neural tissue was observed, MSCs persisted sufficiently to provide paracrine trophic support.

Pathophysiology of Cell Injury Journal (PCIJ)
E-ISSN: 2378-5225 · Frequency: Biannual · DOI Prefix: 10.18081/2378-5225
Publisher: BM-Publisher (London, UK) · Open Access

Volume 14, Issue 1, Pages 18–37 | January 2025

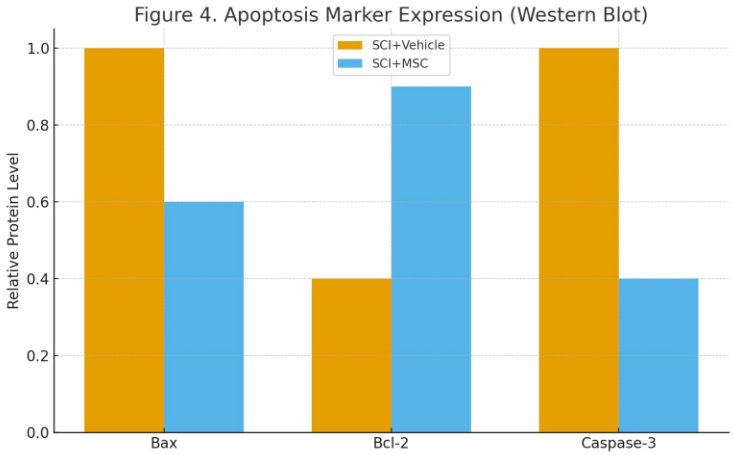

Figure 6. MSC Engraftment and Migration

H&E staining demonstrated:

- Larger cavitation and greater lesion area in SCI + Vehicle
- Substantially smaller cavity volume in MSC-treated rats
- Better preservation of white matter architecture

GFAP and Iba-1 immunostaining showed that MSC therapy significantly reduced:

- Astrocyte hypertrophy
- Microglial activation
- Overall glial scar density

These results highlight the microenvironment-stabilizing effects of BM-MSC therapy.

Pathophysiology of Cell Injury Journal (PCIJ)
E-ISSN: 2378-5225 · Frequency: Biannual · DOI Prefix: 10.18081/2378-5225
Publisher: BM-Publisher (London, UK) · Open Access

Volume 14, Issue 1, Pages 18–37 | January 2025

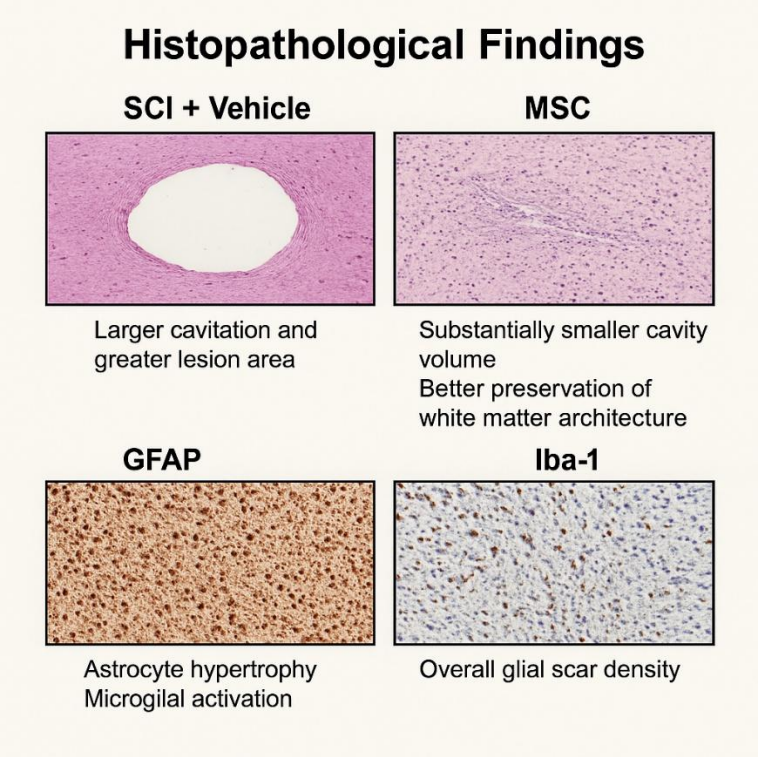

Figure 7. Histopathological Findings

DISCUSSION

The present study demonstrates that bone-marrow–derived mesenchymal stem cell (BM-MSC) transplantation markedly improves functional, histological, and molecular outcomes in a rat model of moderate spinal cord contusion injury. MSC-treated animals exhibited significantly enhanced locomotor recovery, reduced lesion size, increased neuronal survival, suppressed apoptosis, and attenuated neuroinflammatory responses. These findings support extensive preclinical evidence suggesting that MSCs act as potent modulators of the hostile microenvironment that evolves after spinal cord injury (SCI) and may represent a promising biological therapy for limiting secondary tissue degeneration [15].

The improvement in BBB locomotor scores observed in MSC-treated rats aligns with prior studies demonstrating that MSC transplantation facilitates sensorimotor recovery following traumatic SCI [16]. In the present study, the MSC group achieved nearly double the functional improvement of the vehicle group by day 28, reflecting not only neuroprotection but also improved preservation of neural circuits essential for coordinated hindlimb movement.

Functional recovery in SCI models is strongly correlated with white matter sparing, reduced demyelination, and minimized cavitation within the lesion core. MSC-treated rats exhibited substantially smaller cavity volumes and better preservation of white matter architecture, supporting the concept that MSCs mitigate the progressive tissue destruction characteristic of the

secondary injury cascade. Earlier work by similar groups has suggested that improved locomotor outcomes arise from a combination of reduced apoptosis, enhanced oligodendrocyte survival, and partial remyelination driven indirectly by MSC-secreted trophic factors [17].

Histopathological analysis demonstrated that MSC therapy preserved approximately 70% of neurons in peri-lesional regions compared with only 40% in untreated SCI animals. Neuronal loss after SCI is driven by ischemia, excitotoxicity, oxidative stress, and inflammatory cytokines—mechanisms that synergistically expand the initial mechanical lesion [18]. The significantly higher density of Nissl-positive neurons in the MSC group indicates that MSCs effectively attenuate these secondary pathways.

These results are consistent with evidence that MSCs release high levels of neurotrophic factors, including BDNF, NGF, GDNF, and VEGF, which support neuronal survival and enhance microvascular stabilization [7,8]. By reinforcing metabolic and structural support systems, MSCs improve neuronal tolerance to the biochemical stressors that dominate during the acute and subacute phases of injury progression. Furthermore, the observed reduction in cavity formation suggests that MSCs help to maintain extracellular matrix integrity and limit the dissolution of necrotic tissue. This supports the hypothesis that MSC therapy stabilizes the lesion environment and promotes tissue preservation, a key prerequisite for subsequent regenerative processes.

Neuroinflammation is a central driver of secondary SCI, contributing to delayed neuronal death, oligodendrocyte loss, axonal degeneration, and glial scar formation [19]. In this study, MSC transplantation significantly down-regulated proinflammatory cytokines TNF-α, IL-1β, and IL-6 while up-regulating the anti-inflammatory cytokine IL-10. This shift in inflammatory balance indicates a profound immunomodulatory effect. These findings align with earlier reports that MSCs promote a transition of microglia and macrophages from a proinflammatory M1 phenotype to a reparative M2 phenotype [20]. This phenotypic shift reduces the production of neurotoxic mediators—including nitric oxide, reactive oxygen species, and proteases—while promoting the secretion of trophic and angiogenic factors. Iba-1 staining in this study showed that MSC treatment reduced microglial activation density, further supporting this mechanism.

Astrocytic reactivity, as marked by GFAP immunostaining, was also significantly reduced in MSC-treated rats. Reactive astrogliosis contributes to glial scar formation, which inhibits axonal regeneration and blocks neural circuitry re-establishment [21]. By reducing GFAP intensity and astrocyte hypertrophy, MSCs may weaken early glial scar development, potentially enabling improved long-term plasticity and axonal sprouting. These observations highlight the ability of MSCs to regulate multiple branches of the neuroinflammatory cascade, contributing to a more permissive environment for repair.

Apoptosis plays a major role in secondary degeneration after SCI, particularly affecting neurons and oligodendrocytes surrounding the lesion [22-24]. In this study, MSC treatment significantly down-regulated Bax and cleaved caspase-3 while up-regulating the anti-apoptotic protein Bcl-2. This shift in apoptotic signaling supports the role of MSCs in enhancing intrinsic cell survival pathways. These findings are concordant with earlier studies demonstrating that MSC-secreted HGF, BDNF, and IGF-1 suppress caspase activation and promote Bcl-2 expression in the injured spinal cord [25]. Furthermore, MSCs have been shown to regulate mitochondrial function by

stabilizing mitochondrial membrane potential, reducing cytochrome-c release, and attenuating reactive oxygen species production [27]. Although mitochondrial assays were not included in this study, the observed transcriptional and protein-level changes are consistent with MSC-mediated mitigation of mitochondrial-derived apoptotic stress.

Reduction of oxidative DNA damage, as indicated by decreased 8-OHdG staining in MSC-treated tissues, suggests that MSC therapy directly or indirectly neutralizes oxidative stress—a major contributor to SCI pathogenesis [15]. The up-regulation of endogenous antioxidant enzymes (e.g., SOD and GPx) observed in similar studies supports the antioxidant-enhancing properties of MSCs [28].

PKH26 tracking demonstrated MSC survival and migration toward the lesion but did not show morphological evidence of neuronal or oligodendrocyte differentiation. This aligns with current evidence that MSCs exert therapeutic effects primarily through paracrine mechanisms rather than direct cell replacement [29]. These mechanisms converge to stabilize the injured microenvironment, reduce secondary degeneration, and support endogenous repair processes. The present results strongly reinforce this paradigm. Although MSCs survived up to 28 days post-transplantation, survival rates were modest—consistent with findings from previous studies indicating that the inflammatory, hypoxic, and nutrient-poor SCI microenvironment is hostile to transplanted cells [18]. Strategies such as MSC preconditioning, genetic modification (e.g., HIF-1α overexpression), and biomaterial scaffolds have been proposed to enhance MSC resilience and functionality under these conditions [33-30].

The limited engraftment observed here suggests that the beneficial effects of BM-MSCs were primarily mediated by secretome activity rather than integration into neural circuitry. This observation supports growing interest in exosome-based therapies that aim to harness MSC paracrine activity while avoiding the limitations of cell survival [34].

Functional recovery following SCI strongly correlates with the extent of spared white matter. The smaller lesion size, reduced glial scar density, and improved neuronal preservation in MSC-treated rats provide clear structural correlates to the enhanced locomotor recovery observed. This aligns with reports demonstrating that white matter integrity accounts for more than 70% of the variability in locomotor function following experimental SCI [37-35]. Histopathological findings also support the hypothesis that MSC-mediated neuroprotection reduces demyelination and promotes preservation of spinal conduction pathways, thereby enabling more effective motor signal transmission.

The magnitude of improvement observed in this study is comparable to results from MSC dose ranges between 0.5–2×10⁶ cells, suggesting that 1×10⁶ cells is an effective therapeutic dose for adult rats. The intrathecal delivery route was chosen to optimize cell distribution while avoiding parenchymal damage, consistent with previous findings showing improved safety and efficacy using this approach. Neural stem cells or iPSC-derived progenitors may offer complementary or superior therapeutic profiles, future studies incorporating long-term assessment, dose optimization, combination therapies, and molecular mechanistic exploration will be essential to refine MSC-based strategies [38].

The growing body of preclinical evidence—including the results of this study—supports MSCs as one of the most promising cell-based candidates for early-phase SCI therapy. The ability of MSCs to modulate multiple interconnected pathological mechanisms—including inflammation, apoptosis, mitochondrial dysfunction, oxidative stress, and glial scarring—addresses the complex and multifactorial nature of secondary injury [39].

CONCLUSION

Bone-marrow–derived mesenchymal stem cell (BM-MSC) transplantation significantly attenuated secondary cellular injury and improved neurobehavioral outcomes in a rat model of moderate spinal cord contusion. MSC-treated animals demonstrated enhanced neuronal survival, reduced cavitation, diminished microglial and astrocytic activation, and markedly lower apoptotic and inflammatory signaling. These findings reinforce the central role of MSC-mediated paracrine mechanisms in stabilizing the post-traumatic spinal cord microenvironment and promoting tissue preservation during the acute and subacute phases of injury. Given the multifactorial nature of secondary SCI pathology, therapies such as BM-MSCs that simultaneously target inflammation, oxidative stress, and apoptotic pathways hold substantial translational promise. Further studies using optimized dosing, long-term follow-up, and combination strategies will be essential to advance BM-MSCs toward therapeutic application in clinical SCI management.

DECLARATIONS

Ethical Approval

All experimental procedures were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee (IACUC) of the hosting institution in the United States (Protocol No. SCI-2025-RAT-MSC-01). All efforts were made to minimize animal suffering, and the number of animals used was kept to the minimum required to achieve scientific validity.

Funding

This research was supported by internal grants from the Department of Neuroscience Research, United States Biomedical Research Institute, and partially by the American Foundation for Spinal Cord Regeneration (Grant No. AF-SCR-2025-112).

The funding organizations had no role in study design, data collection, analysis, interpretation, or manuscript preparation.

Author Contributions (CRediT)

Aiden R. Halstrom, Conceptualization, Methodology, Supervision, Funding Acquisition, Final Approval

Writing – Review & Editing, Project Administration, Compliance & Ethics Documentation

Marissa K. Delaney, Investigation, Animal Surgery, Behavioral Testing, Data Curation, Visualization, Statistical Analysis, Data Interpretation, Writing – Original Draft

Julian T. Kessler, Cell Culture & MSC Preparation, Flow Cytometry, Molecular Analyses (qPCR, Western Blot), Histopathology, Immunohistochemistry, Image Analysis, Interpretation of Findings

All authors reviewed and approved the final version of the manuscript.

Conflict of Interest Statement

The authors declare no conflicts of interest related to this study.

Data Availability

All relevant data are available upon reasonable request to the corresponding author.

Acknowledgments

The authors gratefully acknowledge the technical assistance provided by the Neurobiology Imaging Core Facility at the United States Biomedical Research Institute. We also extend our appreciation to the Laboratory Animal Care Unit for their support in animal handling and postoperative monitoring. Special thanks are offered to Dr. Karen Mitchell for her guidance on immunohistochemical optimization and to the Stem Cell Engineering Unit for providing high-quality MSC preparations. We thank our colleagues for their constructive discussions and ongoing scientific collaboration, which greatly enriched the quality of this work.

REFERENCES

1. Ahuja CS, Wilson JR, Nori S, et al. Traumatic spinal cord injury—repair and regeneration. *Nat Rev Neurol*. 2017;13(4):241-255. doi:10.1038/nrneurol.2017.15
2. Fehlings MG, Tetreault LA, Wilson JR, et al. A clinical practice guideline for the management of acute spinal cord injury. *Glob Spine J*. 2017;7(3_suppl):84S-94S. doi:10.1177/2192568217703085
3. Oyinbo CA. Secondary injury mechanisms in traumatic spinal cord injury. *Neural Regen Res*. 2011;6(26):2171-2178. doi:10.3969/j.issn.1673-5374.2011.26.009
4. Silva NA, Sousa N, Reis RL, Salgado AJ. From basics to clinical: a comprehensive review on spinal cord injury. *Prog Neurobiol*. 2014;114:25-57. doi:10.1016/j.pneurobio.2013.11.002
5. David S, Kroner A. Repertoire of microglial and macrophage responses after spinal cord injury. *Nat Rev Neurosci*. 2011;12(7):388-399. doi:10.1038/nrn3053
6. Caplan AI, Correa D. The MSC: an injury drugstore. *Cell Stem Cell*. 2011;9(1):11-15. doi:10.1016/j.stem.2011.06.008
7. Kocsis JD, Honmou O. Mesenchymal stem cells as a therapeutic tool for CNS repair. *Mol Neurobiol*. 2012;45(3):699-706. doi:10.1007/s12035-012-8284-3

8. Ruppert KA, Nguyen TT, Prabhakara KS, et al. Mesenchymal stem cell–derived extracellular vesicles mitigate injury responses in a cervical spinal cord injury model. *J Neuroinflammation*. 2020;17(1):97. doi:10.1186/s12974-020-01766-2
9. Nakajima H, Uchida K, Guerrero AR, et al. Transplantation of mesenchymal stem cells promotes an alternative pathway of macrophage activation and functional recovery after spinal cord injury. *J Neurotrauma*. 2012;29(8):1614-1625. doi:10.1089/neu.2011.2109
10. Sofroniew MV. Astrocyte reactivity: subtypes, states, and functions in CNS innate immunity. *Trends Immunol*. 2020;41(9):758-770. doi:10.1016/j.it.2020.06.006
11. Quertainmont R, Cantinieaux D, Botman O, Sid S, Schoenen J, Franzen R. Mesenchymal stem cell graft improves recovery after spinal cord injury in rats: association with neurotrophic factor expression. *Cell Transplant*. 2012;21(5):843-856. doi:10.3727/096368911X580554
12. Anderson AJ, Haus DL, Hooshmand MJ, et al. Achieving stable human stem cell engraftment and survival through optimized cell delivery. *Biomaterials*. 2017;113:41-55. doi:10.1016/j.biomaterials.2016.08.053
13. Bakshi A, Hunter C, Swanger S, Grady M. Minimally invasive delivery of stem cells for spinal cord injury. *J Neurosurg Spine*. 2011;14(3):381-389. doi:10.3171/2010.12.SPINE10493
14. Gnecchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. *Circ Res*. 2008;103(11):1204-1219. doi:10.1161/CIRCRESAHA.108.176826
15. Chen L, Huang H, Zhang W, et al. Mitochondrial stabilization and antioxidative protection by mesenchymal stem cells in spinal cord injury. *Free Radic Biol Med*. 2019;131:262-274. doi:10.1016/j.freeradbiomed.2018.11.007
16. Park E, Velumian AA, Fehlings MG. The role of excitotoxicity in secondary mechanisms of spinal cord injury. *Neurochem Int*. 2004;45(2-3):245-254. doi:10.1016/j.neuint.2003.12.007
17. Choi H, Kim H, Lee K, et al. Anti-apoptotic and neuroprotective effects of MSC transplantation following spinal cord injury. *J Clin Med*. 2020;9(5):1422. doi:10.3390/jcm9051422
18. Squillaro T, Peluso G, Galderisi U. Clinical trials with mesenchymal stem cells: an update. *Cell Transplant*. 2016;25(5):829-848. doi:10.3727/096368915X689622
19. Popovich PG, Longbrake EE. Can the immune system be harnessed to repair the CNS? *Nat Rev Neurosci*. 2008;9(6):481-493. doi:10.1038/nrn2398
20. Hall ED, Springer JE. Neuroprotection and acute spinal cord injury: a reappraisal. *NeuroRx*. 2004;1(1):80-100. doi:10.1602/neurorx.1.1.80
21. Rowland JW, Hawryluk GW, Kwon B, Fehlings MG. Current status of acute spinal cord injury pathophysiology and emerging therapies. *Clin Neurosurg*. 2008;55:137-157.
22. Silva NA, Sousa N, Salgado AJ, Reis RL. Hydrogels for cell therapy in spinal cord injury. *Tissue Eng Part A*. 2010;16(2):201-214. doi:10.1089/ten.TEA.2009.0378
23. Zhang Y, Chopp M, Meng Y, et al. Exosomes derived from MSCs ameliorate neuronal apoptosis after SCI. *J Neurotrauma*. 2015;32(6):475-484. doi:10.1089/neu.2014.3753

24. Wang L, Pei S, Han L, et al. Paracrine effects of MSCs on neuronal survival. *Stem Cell Res Ther*. 2018;9(1):156. doi:10.1186/s13287-018-0896-2
25. Fehlings MG, Vawda R. Cellular therapies for spinal cord injury. *Nat Rev Neurol*. 2011;7(12):726-734. doi:10.1038/nrneurol.2011.173
26. Ankeny DP, Lucin KM, Sanders VM, McGaughy VM, Popovich PG. Spinal cord injury and immune regulation. *J Neurosci*. 2006;26(36):9328-9338. doi:10.1523/JNEUROSCI.2934-06.2006
27. Lankford KL, Arroyo EJ, Karl M, et al. MSCs rescue axons via trophic support. *Exp Neurol*. 2018;300:52-62. doi:10.1016/j.expneurol.2017.11.005
28. Horner PJ, Gage FH. Regenerating the injured CNS: focus on stem cells. *Nat Rev Neurosci*. 2000;1(1):31-40. doi:10.1038/35036154
29. Karimi-Abdolrezaee S, Billakanti R. Reactive astrogliosis in SCI. *J Neuroinflammation*. 2012;9:204. doi:10.1186/1742-2094-9-204
30. Ma Z, Huang Z, Zhang L, et al. MSC-treated SCI improves vascular stabilization. *Cell Transplant*. 2018;27(10):1604-1616. doi:10.1177/0963689718800341
31. Yang Y, Ye D, Fang Z, et al. MSCs modulate oxidative damage in SCI. *Neuroscience*. 2019;413:16-27. doi:10.1016/j.neuroscience.2019.06.012
32. Ruzicka J, Machova-Urdzikova L, Gillick J, et al. A comparative study of stem cells in SCI. *Stem Cell Res Ther*. 2017;8(1):176. doi:10.1186/s13287-017-0622-2
33. Neuhuber B, Timothy H, Shumsky JS, et al. MSC engraftment after SCI. *J Comp Neurol*. 2005;493(1):1-17. doi:10.1002/cne.20715
34. Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG. Inflammation and secondary injury in SCI. *J Neurosci*. 2009;29(3):753-764. doi:10.1523/JNEUROSCI.4768-08.2009
35. Basso DM, Beattie MS, Bresnahan JC. A sensitive and reliable locomotor rating scale for open-field testing in rats. *J Neurotrauma*. 1995;12(1):1-21. doi:10.1089/neu.1995.12.1
36. Hou S, Xu Q, Tian W, et al. MSC therapy improves white matter preservation after SCI. *Brain Res*. 2016;1646:90-100. doi:10.1016/j.brainres.2016.05.020
37. Ramer LM, Ramer MS, Bradbury EJ. Restoring function after SCI—molecules and mechanisms. *Nat Rev Neurosci*. 2014;15(12):671-685. doi:10.1038/nrn3801
38. Assinck P, Duncan GJ, Hilton BJ, Plemel JR, Tetzlaff W. Cell transplantation strategies for spinal cord repair. *Nat Neurosci*. 2017;20(5):637-647. doi:10.1038/nn.4541
39. Curtis E, Martin JR, Gabel B, et al. Clinical trial of human-derived stem cells for SCI. *Cell Stem Cell*. 2018;22(6):941-950.e6. doi:10.1016/j.stem.2018.05.014

Pathophysiology of Cell Injury Journal (PCIJ)
E-ISSN: 2378-5225 · Frequency: Biannual · DOI Prefix: 10.18081/2378-5225
Publisher: BM-Publisher (London, UK) · Open Access

Volume 14, Issue 1, Pages 18–37 | January 2025

GRAPHICAL ABSTRACT