

Domain-Resolved Concordance of Gelatin Sponge and OM–MSC-Loaded Gelatin Sponge After Complete T10 Transection

Seyed Asad Alireza^{1,*}, Seyed Alireza Taghavi¹

¹ Gastroenterohepatology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

*Correspondence: asadalir@sums.ac.ir

ABSTRACT

Complete spinal cord injury (SCI) simultaneously alters hindlimb function, tissue inflammation, lesion structure, and inflammasome-associated cell-death signaling. These outcomes are measured on incompatible scales, which makes direct numerical pooling inappropriate. The study determined how consistently acellular gelatin sponge (GS) and GS carrying olfactory mucosal mesenchymal stromal cells (GS+OM–MSC) satisfied a common favorable-response criterion across locomotor recovery, inflammatory tissue injury, and pyroptotic signaling after complete T10 transection. Fourteen endpoints from a four-group rat experiment were fixed before calculation of the domain-resolved concordance index (DRCI). An endpoint received a value of 1 when the treatment-versus-SCI comparison was significant at $p < 0.05$ and changed in the favorable biological direction; otherwise it received 0. Functional recovery contained BBB scores at days 14 and 28 and day-28 stride length; inflammatory tissue injury contained LDH, IL-1 β , and TNF- α ; pyroptotic signaling contained NLRP3, pro-caspase-1, cleaved caspase-1 at 22 and 20 kDa, GSDMD-FL, GSDMD-N, IL-1 β , and IL-18. Domain breadth was the responsive proportion within each domain, and DRCI was the equally weighted mean of the three breadth values. GS produced breadth values of 0.333, 1.000, and 0.500 for function, inflammatory injury, and pyroptotic signaling, yielding DRCI=0.611. GS+OM–MSC produced 1.000, 1.000, and 0.875, yielding DRCI=0.958. BBB scores at days 14 and 28, pro-caspase-1, western-blot IL-1 β , and IL-18 met the criterion under GS+OM–MSC but not under GS. Endpoint weighting gave 0.571 and 0.929, and omission of any one domain preserved the numerical ordering. GS was biologically active across all three inflammatory endpoints and selected functional and pyroptotic endpoints. OM–MSC loading was associated with complete functional coverage and broader coverage of the NLRP3–caspase-1–GSDMD pathway while retaining the GS-associated inflammatory response. DRCI quantifies response breadth rather than effect magnitude and does not establish causal mediation.

KEYWORDS: spinal cord injury, olfactory mucosal mesenchymal stromal cells, gelatin sponge, pyroptosis, neuroinflammation, locomotor recovery, domain-resolved concordance index

1 Introduction

Ahuja et al. reviewed traumatic SCI as a sequence beginning with primary mechanical disruption and extending into secondary degeneration, repair, and regenerative responses [1]. Hu et al. further summarized the molecular mechanisms of SCI, including excitotoxic, oxidative, inflammatory, mitochondrial, and cell-death pathways that evolve after the initial lesion [2]. These processes evolve over

different time scales and are captured using distinct assays. A treatment can reduce inflammatory mediators without restoring coordinated hindlimb movement, improve a gait variable while inflammasome signaling remains active, or preserve tissue structure without producing a corresponding behavioral change. Interpretation therefore requires attention to how treatment responses are distributed across biological levels rather than reliance on a single

readout.

A multi-level view is especially important in SCI because the measured variables do not represent interchangeable versions of the same outcome. Locomotor scores summarize integrated behavior, cytokines and LDH reflect components of tissue injury and inflammation, histology describes spatial preservation, and inflammasome-related proteins indicate activity at selected molecular positions. Improvement in one layer can precede, follow, or occur without a detectable change in another. A treatment may therefore have a biologically coherent effect even when not every endpoint crosses the same inferential threshold, while a striking change in one assay should not automatically be generalized to the entire lesion response. The central interpretive question is therefore not simply whether an individual marker changes, but how broadly favorable responses extend across function, tissue injury, and intracellular inflammatory signaling.

The distinction between breadth and magnitude is also important. An intervention that produces moderate but statistically supported changes in several biologically distinct outcomes has a different response profile from an intervention producing a very large change in one molecular marker but little change elsewhere. Neither pattern can be reduced to a single raw-scale average because the underlying measurements use different units and ranges. A domain-based summary can therefore be useful when it is kept subordinate to the original endpoint-level evidence and when its meaning is explicitly restricted to response coverage rather than treatment potency.

Lukovic et al. described complete rat spinal-cord transection as a stringent model for translational cell-transplantation studies because recovery is minimally attributable to spared descending fibers [3]. Li et al. showed, however, that locomotor improvement after complete SCI can involve mechanisms such as endogenous neuronal relay formation in addition to direct motor-axon regeneration, emphasizing the complexity of functional interpretation [4]. Hindlimb recovery is commonly assessed with the Basso–Beattie–Bresnahan (BBB) scale, which Basso et al. developed as a sensitive and reliable open-field locomotor rating system for rats [5]. BBB score alone cannot describe gait geometry, inflammatory burden, ultrastructural injury, or molecular activation. In the

complete T10 model considered here, behavioral measurements were therefore interpreted together with LDH and cytokine concentrations, histology, ultrastructure, inflammasome-related proteins, donor-cell localization, and organ histology.

Inflammation after SCI has both damaging and reparative components. Orr and Gensel reviewed the interaction between inflammatory responses, glial scarring, and therapeutic attempts to modify these processes after SCI [6]. Bradbury and Burnside emphasized that the glial scar is biologically heterogeneous and that effective repair strategies must move beyond treating it as a uniformly inhibitory structure [7]. David and Kroner detailed the diverse phenotypic and functional responses of microglia and macrophages after SCI, including roles in debris clearance, cytokine signaling, tissue damage, and repair [8]. Biomaterial implantation can further modify this environment. Li et al. reviewed how biomaterial-mediated control of the lesion microenvironment can influence SCI repair [9]. Wang et al. specifically discussed biomaterial strategies for reversing adverse immune microenvironments after SCI [10]. Chen et al. summarized regenerative biomaterial strategies designed to support neural repair after SCI [11]. Ma et al. focused on functional biomaterials that modulate pathological features of the SCI microenvironment [12]. Zheng et al. experimentally demonstrated that scaffold stiffness can alter the foreign-body reaction, tissue stiffness, angiogenesis, and neuroregeneration after SCI [13]. Consequently, gelatin sponge cannot be assumed to function only as a passive carrier.

The inflammatory environment also changes over time. Hu et al. described the evolving molecular cascade from acute secondary injury to later reparative and remodeling processes after SCI [2]. Orr and Gensel emphasized that inflammatory and glial responses change across the post-injury period rather than remaining biologically static [6]. Bradbury and Burnside discussed the temporal evolution of scar-associated cellular and extracellular-matrix responses during spinal-cord repair [7]. David and Kroner likewise showed that microglial and macrophage phenotypes vary with injury stage and local signals [8]. A day-28 measurement therefore represents the accumulated result of several earlier events rather than an isolated late inflammatory state. This temporal layering is relevant to the present endpoint panel: LDH, cytokines, histology, locomotor

behavior, and pyroptosis-associated proteins can each retain information from different phases of lesion evolution. Their simultaneous favorable direction is more informative biologically than treating each result as an unrelated assay outcome.

Biomaterial placement adds another layer to this interaction. Sáenz et al. provided a foundational overview showing that biomaterial performance depends on material composition, structure, processing, and interaction with the biological environment [59]. Li et al. emphasized that biomaterials can be designed to regulate the local SCI microenvironment rather than merely occupy the lesion cavity [9]. Wang et al. discussed how biomaterials can influence adverse immune conditions within injured spinal tissue [10]. Chen et al. reviewed regenerative scaffold strategies that integrate structural support with biological modulation [11]. Ma et al. described functional biomaterials intended to counter pathological features of the SCI microenvironment [12]. Zheng et al. demonstrated that scaffold stiffness itself can modify foreign-body reactions, vascularization, and neuroregenerative outcomes [13]. Suzuki et al. reviewed current scaffold concepts for regenerative SCI therapy, including the importance of scaffold architecture and host integration [14]. Feng et al. summarized applications of biomaterials in SCI and highlighted how material properties affect repair-related cellular responses [15]. Cao et al. identified design criteria for functional SCI scaffolds, including physical, structural, and biological requirements [16]. A porous sponge can therefore change the physical continuity of the transection gap, provide surfaces for cell adhesion and host-cell infiltration, and influence the local distribution of soluble mediators even in the absence of transplanted cells. For this reason, the acellular GS group is not merely a technical control for implantation. It is a treatment condition whose own biological activity must be resolved before any additional response under GS+OM-MSC is attributed to the cellular component.

Pyroptosis is particularly relevant because it links danger sensing to membrane disruption and inflammatory cytokine release. Swanson et al. reviewed the molecular activation and regulation of the NLRP3 inflammasome and its role in inflammatory signaling [17]. Shi et al. described pyroptosis as a gasdermin-mediated form of

programmed necrotic cell death [18]. Sborgi et al. demonstrated that GSDMD forms membrane pores during pyroptotic cell death [19]. Liu et al. independently showed that inflammasome-activated GSDMD causes pyroptosis through membrane-pore formation [20]. Within SCI, Xu et al. linked TLR4-associated signaling to microglial pyroptosis after injury [21]. Jiang et al. reported that modulation of pyroptosis through topoisomerase-1 inhibition was associated with improved recovery after SCI [22]. Gu et al. reviewed the broader evidence connecting pyroptosis with SCI pathogenesis and emerging therapeutic approaches [23]. NLRP3, pro-caspase-1, cleaved caspase-1, GSDMD-FL, GSDMD-N, IL-1 β , and IL-18 therefore occupy different positions in a biologically connected sequence. Nevertheless, pathway association does not by itself establish that pyroptosis suppression causes locomotor improvement.

Mesenchymal stromal cells (MSCs) can influence injured tissue through several complementary mechanisms. Galipeau and Sensébé reviewed the therapeutic opportunities and translational challenges of MSCs, including their paracrine and immunomodulatory properties [24]. Wang et al. summarized evidence that MSC-derived exosomes can contribute to SCI repair [25]. Dutta et al. reviewed extracellular vesicles as mediators of SCI pathobiology and as potential therapeutic agents [26]. Kim et al. discussed the therapeutic potential of both MSCs and MSC-derived extracellular vesicles for SCI [27]. Zeng reviewed current progress in multipotent MSC-based therapies for SCI and emphasized their multimodal biological actions [28]. For olfactory mucosa-derived MSCs specifically, Ge et al. characterized a secretome containing multiple potentially therapeutic factors [29]. Huang et al. showed that hypoxia-preconditioned olfactory mucosa MSCs can suppress microglial pyroptotic and apoptotic death in a cerebral ischemia/reperfusion model [30]. Lindsay et al. reviewed olfactory mucosa-derived cells as part of multi-target strategies for central-nervous-system repair [31]. Scaffold support may further modify these cell effects. Lv et al. reported that biomaterial-supported MSC transplantation can enhance cell-cell communication in SCI [32]. Jeon et al. synthesized evidence on associations between neural stem/progenitor cells and biomaterials in SCI therapies [33]. Blando et al. reviewed the therapeutic rationale for MSC-enriched

scaffolds in SCI [34]. Guo et al. placed cell transplantation, tissue engineering, and extracellular-vesicle approaches within a common framework for spinal-cord repair [35]. Zhang et al. found in a Bayesian network meta-analysis that scaffold-supported bone-marrow stem-cell strategies can improve motor recovery in rat SCI models [36]. Ma et al. reviewed how biomaterials can reinforce MSC transplantation for SCI repair [37]. Finally, Wang et al. documented substantial heterogeneity across rat MSC-SCI studies in cell source, delivery strategy, and outcomes [38]. A separate network meta-analysis by Wang et al. similarly emphasized variability across MSC treatment protocols and experimental designs [39].

These sources of heterogeneity make local cell presentation particularly relevant. Lv et al. showed that biomaterial-supported MSC delivery can alter cell-cell communication within the SCI treatment environment [32]. Blando et al. reviewed how scaffold enrichment can influence MSC survival, retention, and therapeutic behavior after SCI [34]. Guo et al. emphasized that cell delivery method and tissue-engineering context shape how transplanted cells interact with injured spinal tissue [35]. Ma et al. likewise described biomaterials as active supports that can improve MSC retention and functional integration [37]. In addition to direct cellular effects, Brennan et al. reviewed biomaterials functionalized with MSC-secreted extracellular vesicles and soluble factors as regenerative systems [40]. Prado-Yupanqui et al. reviewed the regenerative potential of the MSC secretome and highlighted cell-free signaling mechanisms [41]. Kim et al. demonstrated that three-dimensional spheroid culture can increase exosome secretion from MSCs [42]. Holkar et al. showed that physicochemical conditions in three-dimensional culture can modify secretion of osteogenic and angiogenic extracellular vesicles [43]. Wang et al. summarized the role of MSC-derived exosomes in SCI treatment [25]. Dutta et al. described extracellular-vesicle signaling as an emerging component of SCI pathobiology and therapy [26]. Kim et al. further integrated evidence for MSCs and MSC-derived extracellular vesicles as complementary SCI interventions [27]. The observed response of a scaffold-supported construct may therefore reflect host response to the material, donor-cell persistence, extracellular-matrix deposition, and soluble or vesicular signaling rather than a single mechanism.

Gelatin sponge combines porosity, biodegradability, clinical familiarity, and simple handling. Ye et al. described the characteristics and clinical potential of a cellularly modified gelatin sponge, supporting gelatin as a practical cell-compatible biomaterial [44]. Zeng et al. showed that a biocompatible gelatin sponge scaffold promoted robust tissue remodeling after SCI in a non-human primate model [45]. Other scaffold systems illustrate additional design possibilities. Fan et al. used an exosome-loaded electroconductive hydrogel to promote tissue repair through immunoregulation and myelinated axon growth [46]. Papa et al. reported that MSCs encapsulated in a biomimetic hydrogel could preserve cytoarchitecture and support functional recovery while releasing CCL2 locally [47]. Yao et al. demonstrated that hierarchically aligned fibrin nanofiber hydrogel accelerated axonal regrowth and locomotor recovery in rat SCI [48]. Zhang et al. reviewed the combined use of exosomes and biomaterials for SCI treatment [49]. Haratizadeh et al. reviewed contemporary biomaterial and cell-based strategies after SCI [50]. Khavandegar et al. systematically reviewed neuroregenerative and neuroprotective effects of bioengineered scaffolds across preclinical and early clinical SCI studies [51]. Liao et al. summarized recent progress in biomaterials for spinal-cord repair [52]. Xu et al. reviewed tissue-engineering approaches for SCI repair [53]. Liu et al. developed a microenvironment-responsive injectable conductive hydrogel for SCI repair [54]. Wang et al. reported a multifunctional conductive stem-cell-delivery hydrogel combined with low-frequency pulsed electromagnetic fields [55]. Suzuki et al. reviewed the broader conceptual basis of biomaterial scaffolds in regenerative SCI therapy [14]. Feng et al. summarized current applications of biomaterials across SCI repair strategies [15]. Cao et al. outlined design criteria and therapeutic strategies for functional SCI scaffolds [16]. These approaches differ in complexity, but all reinforce the need to distinguish the biological contribution of the carrier from that of the cellular component.

The four-group complete T10 experiment described by Li et al. [56] provides this distinction because untreated SCI, acellular GS, and GS+OM-MSC were evaluated under the same lesion conditions. The analysis focuses on 14 directionally interpretable endpoints divided into three domains: functional recovery, inflammatory tissue injury, and pyroptotic

signaling. The DRCI records the proportion of endpoints meeting a common favorable-response rule within each domain and then gives the three domains equal weight. This prevents the eight-protein pyroptosis panel from dominating the integrated value solely because it contains more measurements than the three-endpoint functional and inflammatory domains.

The organization of the endpoint set also reflects differences in biological scale. The functional domain asks whether treatment-related changes are detectable in whole-animal hindlimb performance. The inflammatory tissue-injury domain asks whether biochemical signs of membrane damage and pro-inflammatory cytokine burden move in a favorable direction. The pyroptotic domain asks whether the response extends across several positions in an inflammasome-associated sequence, from NLRP3 and caspase-1 states to GSDMD cleavage and mature inflammatory cytokine-associated readouts. Equal domain weighting does not imply that these domains are equally important for every biological purpose; rather, it ensures that the numerical summary is not determined by how many assays happened to be reported within one domain.

Accordingly, the integrated value is descriptive and conditional on the selected endpoint set. It should be interpreted together with the endpoint audit, the original assay directions, and the explicit distinction between direct group comparisons and differences in significance status. This is particularly important when sample sizes are small, because a non-significant comparison can reflect limited precision rather than absence of a biological effect. The DRCI does not remove this limitation; it makes the location of threshold-crossing responses transparent.

Three questions are addressed. First, does OM-MSC loading increase the number of functional endpoints that satisfy the treatment-versus-SCI response criterion beyond the pattern observed with GS? Second, does GS+OM-MSC extend criterion-positive responses to additional positions in the NLRP3-caspase-1-GSDMD sequence while retaining the inflammatory response observed with GS? Third, does the numerical treatment ordering remain unchanged when equal-domain weighting is replaced by endpoint weighting or when any one domain is omitted? These questions concern response coverage. A significant comparison with SCI in one treatment

group and a non-significant comparison with SCI in another do not, by themselves, constitute a direct statistical test between the two treatments [57].

2 Materials and Methods

2.1 Experimental design and treatment groups

The DRCI was applied to the complete T10 transection experiment described by Li et al. [56]. The present work is a structured re-analysis of previously reported experimental evidence rather than a new animal experiment. Comparable re-analysis strategies have been used in other experimental contexts to address secondary questions while retaining the boundaries of the source dataset [61]. Forty 8-week-old female Sprague-Dawley rats weighing 200–220 g were allocated to sham operation, untreated SCI, GS, and GS+OM-MSC groups ($n = 10$ initially per group). Animals were housed at $22 \pm 1^\circ\text{C}$ under a 12-h light/12-h dark cycle. After intraperitoneal sodium pentobarbital anesthesia at 50 mg/kg, the T10 lamina was exposed. Sham animals underwent laminectomy without transection. In the SCI, GS, and GS+OM-MSC groups, a 3-mm segment of spinal cord was removed with ophthalmic scissors. The defect was left untreated in the SCI group and filled immediately with acellular GS or GS carrying OM-MSCs in the two treatment groups. Penicillin was administered at 2×10^6 U/kg/day for 3 days, and bladders were manually expressed twice daily until function returned. Follow-up continued for 28 days [56].

Human OM-MSCs were obtained from superior nasal turbinate tissue and expanded in DMEM/F12 containing 10% fetal bovine serum at 37°C and 5% CO_2 . Flow cytometry showed greater than 98% purity, with CD73, CD90, and CD105 expression and absence of CD34 and CD45; adipogenic and osteogenic differentiation were demonstrated by Oil Red O and Alizarin Red staining. A $15 \times 15 \times 5$ mm gelatin sponge was washed in PBS, incubated overnight in 0.75 mg/L poly-L-lysine, washed again, and inoculated with 1×10^6 OM-MSCs in 1 mL complete medium. After 2 h of attachment, 2 mL complete medium was added. The sponge contained pores of approximately 50–300 μm , and the 15-day culture interval was selected because cell proliferation remained high while the increase in dead cells was still limited [56].

BBB locomotor scoring was performed on

postoperative days 0, 7, 14, 21, and 28 by two blinded investigators. Day-28 footprint analysis used a 600-mm-long, 100-mm-wide runway. Tissue assessments included ELISA measurement of LDH, IL-1 β , and TNF- α ; western blotting for pyroptosis-associated proteins; NLRP3 and caspase-1 immunofluorescence; transmission electron microscopy; H&E and Nissl staining; GFP-based OM-MSc localization; and histology of brain, heart, spleen, lung, liver, and kidney [56]. The DRCI calculation required no additional animal or human procedures.

The day-28 endpoint configuration places the integrated analysis at a time when acute surgical effects have evolved into a subacute repair environment while measurable locomotor recovery is already present. This timing is useful for cross-domain comparison because behavioral, biochemical, structural, and cell-localization observations are available within the same overall follow-up period. At the same time, it does not imply that all measured changes arose at day 28. BBB scoring at earlier time points contributes temporal functional information, whereas the terminal tissue assays summarize the lesion state after four weeks of treatment exposure. The DRCI therefore combines endpoints that are biologically related but not temporally identical, and the interpretation is response breadth across the study period rather than synchronicity of all effects.

The principal comparison for every binary code was treatment versus untreated SCI. Sham animals define the uninjured reference condition, but they were not used to determine whether GS or GS+OM-MSc received a response value of 1. This choice keeps the scoring question focused on whether each treatment produced a favorable change relative to the injured untreated state. It also avoids treating incomplete normalization to sham as equivalent to treatment failure, which would answer a different biological question.

The structure in Figure 1 keeps the biological measurements and the integrated calculation distinct. Individual-animal covariance was not available for the DRCI, so the integrated score is interpreted as endpoint coverage rather than as a multivariate treatment-effect estimate.

2.2 Assay sample sizes and endpoint verification

Initial allocation was $n = 10$ per group, whereas the analyses contributing to the DRCI used smaller assay-specific samples. The BBB/gait and ELISA panels reported $N = 4$, and the western-blot panel reported $N = 3$ [56]. For footprint analysis, the Methods described random selection of five surviving rats per group, whereas the corresponding figure caption stated $N = 4$. The scored stride-length endpoint therefore uses the figure-level $N = 4$, with the discrepancy retained in Table 1 rather than resolved by assumption.

The statistical procedures comprised Student's t tests or ANOVA with Bonferroni-corrected post hoc testing, with $p < 0.05$ considered significant and each experiment repeated at least three times [56]. Response codes followed the stated pairwise significance and biological direction. When exact p values were not given, the significance status shown in the text or figure annotation was used without estimating numerical values from plotted symbols.

Assay-specific sample size was retained because the inferential unit for each response code is the comparison actually reported for that assay. The initial group allocation of ten animals does not justify assigning $N = 10$ to analyses performed on three or four biological samples, and repeated technical measurements or repeated experimental runs do not increase the number of independent animals contributing to a comparison. This distinction is important in a threshold-based summary: the precision of the reported significance status depends on the biological sample used in the corresponding assay, not on the nominal size of the original allocation.

No attempt was made to reconstruct unreported animal-level observations from bar heights, error bars, or microscopy fields. Such reconstruction would create artificial precision and could alter the binary response assignment. Where the report contained an internal discrepancy, as for the footprint sample size, the discrepancy was documented explicitly. This audit-oriented treatment of reporting uncertainty is preferable to silently harmonizing inconsistent values because the integrated score is intended to remain traceable to the stated endpoint evidence.

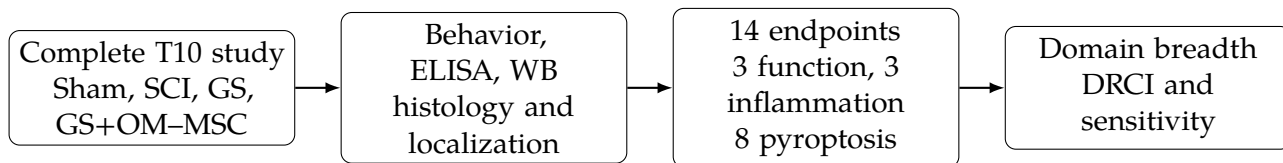


Figure 1. Study structure and DRCI calculation.

2.3 Domain definitions and response coding

The 14 endpoints were fixed before the DRCI values were calculated. The endpoint set was not prospectively registered. Functional recovery contained BBB scores at days 14 and 28 and average stride length at day 28, for which higher values were favorable. Inflammatory tissue injury contained LDH, IL-1 β by ELISA, and TNF- α by ELISA, with lower values favorable. Pyroptotic signaling contained NLRP3, pro-caspase-1, cleaved caspase-1 at 22 and 20 kDa, GSDMD-FL, GSDMD-N, IL-1 β by western blot, and IL-18 by western blot; lower abundance was favorable.

For treatment g and endpoint e , the response indicator was

$$I_{ge} = \begin{cases} 1, & \text{if the treatment-versus-SCI comparison} \\ & \text{is significant at } p < 0.05 \text{ and changes} \\ & \text{in the favorable biological direction,} \\ 0, & \text{otherwise.} \end{cases} \quad (1)$$

The binary indicator records whether each endpoint crosses the defined inferential boundary; it does not grade p values or estimate effect magnitude. A difference between one significant treatment-versus-SCI comparison and one non-significant comparison is not, by itself, evidence of a significant difference between the two treatments [57]. Direct GS-versus-GS+OM-MSC statements are therefore restricted to comparisons explicitly reported for those groups.

IL-1 β appears twice because the ELISA measurement belonged to the tissue inflammatory panel, whereas western-blot IL-1 β was measured together with NLRP3, caspase-1, GSDMD, and IL-18. The two entries therefore represent different assay contexts but not independent biological events. NLRP3 and caspase-1 immunofluorescence were kept outside the score because the same targets were already represented by western blot.

Endpoint inclusion was deliberately conservative. Histological appearance, ultrastructure, GFP localization, and organ histology were used as supporting biological observations because their reported form did not provide the same type of explicit pairwise significance information for a uniform 0/1 rule. Including visually interpreted outcomes in the numerator while applying statistical thresholds to the biochemical and behavioral outcomes would have mixed different evidentiary standards. Likewise, immunofluorescence for proteins already represented by western blot was not given an additional vote, which limits duplication of the same target within the pyroptotic domain.

The selected functional time points were also restricted to comparisons that contributed clear treatment-versus-SCI information. Earlier BBB observations remain important for the time course, but the scoring set emphasizes day 14 and day 28, when divergence of recovery is more interpretable after the immediate post-transection floor effect. Stride length supplies a complementary gait measure at day 28. The resulting functional domain is intentionally small, so each endpoint has substantial influence on its breadth value; this is why endpoint-level results remain necessary beside the integrated score.

2.4 Domain breadth and integrated concordance

Let E_d denote the endpoints assigned to domain d , with $n_d = |E_d|$. Domain response breadth was calculated as

$$B_{gd} = \frac{1}{n_d} \sum_{e \in E_d} I_{ge}. \quad (2)$$

Because the domains contain 3, 3, and 8 endpoints, the primary integrated value gave the three domains equal weight:

$$\text{DRCI}_g = \frac{B_{g,\text{function}} + B_{g,\text{inflammation}} + B_{g,\text{pyroptosis}}}{3}. \quad (3)$$

An endpoint-weighted score (EWS) was calculated as

$$\text{EWS}_g = \frac{1}{14} \sum_{e=1}^{14} I_{ge}, \quad (4)$$

and the integrated value was recalculated after omitting function, inflammation, and pyroptotic signaling in turn. These calculations assess whether the numerical ordering depends on equal domain weighting or is driven by a single domain. They do not estimate the probability that one treatment is superior. The coding table and calculation script are supplied with the manuscript.

Equal-domain weighting and endpoint weighting answer slightly different descriptive questions. Equal-domain weighting asks whether each major biological level shows broad response without allowing the largest panel to dominate. Endpoint weighting asks what fraction of all coded observations are positive irrespective of domain membership. Agreement between the two calculations indicates that the numerical pattern is not created solely by the choice of domain normalization. The leave-one-domain-out calculations provide a further check by asking whether the same ordering persists when the functional, inflammatory, or pyroptotic component is removed completely.

Because the same animals can contribute information to more than one endpoint and several molecular measurements belong to one signaling sequence, the resulting scores are not treated as sums of independent Bernoulli trials. No binomial confidence interval or conventional hypothesis test is attached to DRCI. Doing so would incorrectly assume independence among repeated behavioral measures, cytokines, and pathway proteins. The integrated values are therefore reported as deterministic summaries of the coded evidence available from the experiment.

The endpoint audit in Table 1 distinguishes initial group allocation from the assay sample size used for each scored endpoint. It also records the explicit evidence supporting each 0/1 assignment. The footprint inconsistency is visible rather than silently reconciled, and no individual-level values are inferred beyond the reported analyses.

3 Results

3.1 Cell-scaffold preparation defined the implantation state

The gelatin sponge had a loose porous architecture with pores of approximately 50–300 μm . OM–MSCs entered the internal spaces and became broadly distributed through the material. During the first 15 days, cellular coverage increased and laminin and fibronectin staining increased within the cell-loaded sponge, indicating formation of a cell-associated extracellular matrix [56].

Culture duration changed cell viability and material appearance. Conventional two-dimensional OM–MSC culture reached its highest CCK-8 signal around day 10, whereas cells within gelatin sponge reached their highest signal around day 15. Live/dead staining showed increasing cell death after day 15, and 30-day culture showed fragmented nuclei and a more uneven surface-weighted cellular distribution. The sponge became progressively smaller and more translucent, with edge rounding after approximately 10 days and a jelly-like appearance after approximately 20 days. These observations support the 15-day preparation used for implantation because it combined strong proliferation with less cell loss than later culture intervals [56].

The preparation observations are relevant to interpretation of the in vivo response because they indicate that implantation occurred after cells had already interacted with the three-dimensional material for an extended culture interval. The construct was therefore not simply a sponge wetted with a freshly prepared cell suspension. Cell penetration, increasing matrix-associated staining, and the different proliferation time course relative to conventional culture indicate that the cells experienced a distinct three-dimensional environment before implantation. These features provide context for the day-28 localization and tissue findings without establishing which specific cell product or matrix component mediated the subsequent response.

The progressive change in sponge appearance during prolonged culture also cautions against treating material state as fixed. Gelatin is biodegradable, and interaction with cells can alter local matrix deposition, hydration, and degradation. The in vitro appearance does not provide a quantitative degradation rate in vivo, but it helps explain why residual material at day 28 should be interpreted as a dynamic tissue–material interface rather than an unchanged implant. This

Table 1. Endpoint coding and assay details.

Domain	Endpoint	N	GS	GS+OM-MSC	Coding evidence
Functional	BBB, day 14	4	0	1	GS vs SCI ns; GS+OM-MSC vs SCI sig.
Functional	BBB, day 28	4	0	1	GS vs SCI ns; GS+OM-MSC vs SCI sig.; direct GS+OM-MSC vs GS sig.
Functional	Stride length, day 28	4	1	1	Both treatments sig. vs SCI; GS vs GS+OM-MSC ns.
Inflammatory	LDH, ELISA	4	1	1	Lower than SCI; significant for both treatments.
Inflammatory	IL-1 β , ELISA	4	1	1	Lower than SCI; significant for both treatments.
Inflammatory	TNF- α , ELISA	4	1	1	Lower than SCI; significant for both treatments.
Pyroptotic	NLRP3, WB	3	1	1	Lower than SCI; significant for both treatments.
Pyroptotic	Pro-caspase-1, WB	3	0	1	GS ns; GS+OM-MSC significantly lower than SCI.
Pyroptotic	Cleaved caspase-1, 22 kDa, WB	3	1	1	Lower than SCI; significant for both treatments.
Pyroptotic	Cleaved caspase-1, 20 kDa, WB	3	1	1	Lower than SCI; significant for both treatments.
Pyroptotic	GSDMD-FL, WB	3	0	0	Neither treatment met the response criterion.
Pyroptotic	GSDMD-N, WB	3	1	1	Lower than SCI; significant for both treatments.
Pyroptotic	IL-1 β , WB	3	0	1	GS ns; GS+OM-MSC significantly lower than SCI.
Pyroptotic	IL-18, WB	3	0	1	GS ns; GS+OM-MSC significantly lower than SCI.

distinction becomes important when comparing acellular GS with GS+OM-MSC, because the presence of cells can modify both the biological environment and the physical evolution of the construct.

3.2 Functional response coverage increased with OM-MSC loading

BBB scores were 21 before transection and fell to 0 immediately after complete SCI. At day 14, GS+OM-MSC produced a significant increase relative to untreated SCI, whereas GS did not meet the significance criterion. At day 28, GS+OM-MSC again differed significantly from SCI and also showed a reported direct difference from GS, while GS remained non-significant relative to SCI for the selected BBB comparison. Average stride length at day 28 was significantly greater than SCI under both GS and GS+OM-MSC, and the direct stride-length difference between the two treated groups was non-significant [56].

These three endpoints yielded functional breadth values of 0.333 for GS and 1.000 for GS+OM-MSC. GS met the criterion only for stride length, whereas GS+OM-MSC met it for stride length and both BBB time points. The day-14 contrast remains an endpoint-status difference rather than a direct between-treatment test; the day-28 BBB comparison is supported by the reported GS+OM-MSC-versus-GS result.

BBB score and stride length capture related but non-identical aspects of hindlimb performance. BBB scoring integrates joint movement, weight support, stepping, coordination, paw position, trunk stability, and tail position across its ordinal scale [5], whereas stride length is a spatial gait measure obtained during

runway traversal. A response in stride length can therefore occur without the same threshold-crossing pattern in BBB score. The GS profile illustrates this distinction: the day-28 gait measure met the favorable criterion even though the selected BBB comparisons did not. This is not an inconsistency; it shows that locomotor recovery has multiple measurable components and that a single behavioral endpoint would provide an incomplete description.

The GS+OM-MSC pattern is broader because all three selected functional observations met the criterion. Importantly, this breadth does not mean that function was fully normalized to sham, nor does it establish recovery of the original descending pathways severed by complete transection. The result is restricted to the measured behavioral outcomes. Lukovic et al. showed why complete transection is useful for limiting recovery attributable to spared descending pathways [3]. Li et al. further demonstrated that scaffold-facilitated locomotor improvement after complete SCI can involve endogenous neuronal relay formation as well as regenerative mechanisms [4]. The behavioral findings therefore support functional benefit without identifying a single anatomical substrate.

3.3 Both treatments covered all inflammatory tissue-injury endpoints

At day 28, untreated SCI tissue showed a central cavity, inflammatory-cell infiltration, and prominent vacuolation. Both treated groups showed milder disruption. ELISA measurements of LDH, IL-1 β , and TNF- α were lower after GS and GS+OM-MSC than after untreated SCI, and all six treatment-versus-SCI comparisons met the favorable-response criterion [56]. Inflammatory breadth was therefore 1.000 for both treatments.

The equal breadth values indicate complete criterion coverage, not identical effect magnitude. Once LDH, IL-1 β , and TNF- α all receive a value of 1, further numerical lowering cannot increase the breadth score beyond 1.000. Any difference in the size of the cytokine or LDH reductions must be interpreted from the assay values themselves rather than from DRCI.

The three inflammatory endpoints contribute complementary information despite sharing the same breadth result. LDH is associated with loss of membrane integrity and tissue injury, whereas IL-1 β and TNF- α are inflammatory mediators with different upstream controls and downstream effects. A favorable pattern across all three therefore indicates that the treatment-associated response is not confined to a single cytokine measurement. At the same time, these tissue measurements cannot distinguish whether the lower concentrations arose from reduced production per cell, fewer inflammatory cells, greater tissue preservation, altered release, or a combination of these processes.

Complete coverage under both treatments is also informative for the role of the carrier. If the cell-loaded construct alone had reduced the three inflammatory endpoints, the pattern could be interpreted primarily as a cell-associated effect. Instead, acellular GS already crossed the response threshold for LDH, IL-1 β , and TNF- α . The additional value of OM-MSC loading is therefore expressed more clearly in the functional and pyroptotic domains than in the binary inflammatory breadth, where both treatments have reached the ceiling of 1.000.

3.4 OM-MSC loading broadened criterion-positive responses across pyroptotic signaling

Transmission electron microscopy after untreated SCI showed neuronal membrane discontinuity, cytoplasmic vacuolation, enlarged mitochondria, swollen cristae, microglial membrane disruption, and mitochondrial swelling. These abnormalities were reduced after treatment, with the most preserved ultrastructure observed in GS+OM-MSC tissue [56].

NLRP3, cleaved caspase-1 at 22 kDa, cleaved caspase-1 at 20 kDa, and GSDMD-N were significantly lower than SCI under both treatments. Pro-caspase-1 met the criterion only under GS+OM-MSC. GSDMD-FL did not meet the criterion under either treatment. Western-blot IL-1 β and IL-18 met the criterion under GS+OM-MSC but not under GS [56]. Thus, four of

eight pyroptosis-associated endpoints were positive for GS and seven of eight for GS+OM-MSC, giving breadth values of 0.500 and 0.875.

The GSDMD pattern distinguishes precursor abundance from activation state. Shi et al. described GSDMD cleavage as a central event in gasdermin-mediated pyroptosis [18]. Sborgi et al. demonstrated that the cleaved GSDMD product forms membrane pores that execute pyroptotic cell death [19]. Liu et al. independently confirmed that inflammasome-activated GSDMD induces pyroptosis through membrane-pore formation [20]. Reduced GSDMD-N without a significant reduction in GSDMD-FL is therefore biologically coherent because activation can change the cleaved pore-forming fraction without requiring depletion of the full-length precursor pool. Pro-caspase-1 and the cleaved 22- and 20-kDa products likewise represent different positions in caspase-1 activation.

The pathway-level distribution is notable because the positive codes are not confined to one biochemical tier. Swanson et al. positioned NLRP3 as an upstream inflammasome sensor and regulatory platform [17]. Shi et al. described the downstream transition from inflammasome activation to gasdermin-mediated pyroptotic execution [18]. Sborgi et al. provided structural and functional evidence for GSDMD pore formation [19]. Liu et al. showed that inflammasome activation of GSDMD directly produces membrane pores during pyroptosis [20]. In the present endpoint panel, NLRP3 represents an upstream inflammasome component; pro-caspase-1 and cleaved caspase-1 describe precursor and activated protease states; GSDMD-N represents an execution-associated cleavage product; and IL-1 β and IL-18 represent inflammatory outputs linked to inflammasome activity. GS reached the criterion at NLRP3, both cleaved caspase-1 bands, and GSDMD-N, whereas GS+OM-MSC additionally reached pro-caspase-1 and both western-blot cytokine endpoints. The difference in breadth therefore reflects coverage across several positions in the sequence rather than accumulation of several measurements from only one protein.

GSDMD-FL is the sole pyroptotic endpoint that remains negative under both treatments. Retaining this negative result is important because it prevents the integrated summary from implying uniform suppression of every measured pathway component.

The pattern is more specific: cleavage-associated and cytokine-related readouts are broadly favorable, while the full-length GSDMD pool does not satisfy the defined criterion. Such mixed behavior is biologically plausible because activation can be regulated through processing and redistribution without requiring a large fall in total precursor abundance.

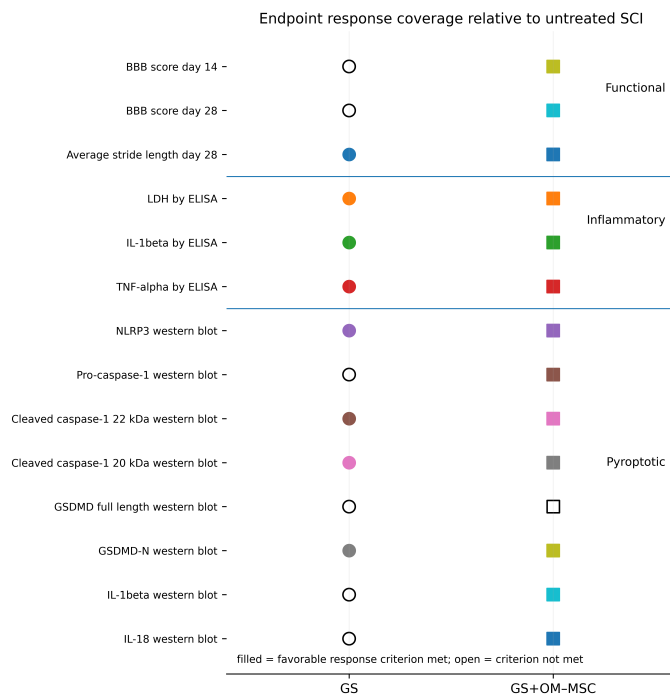


Figure 2. Endpoint response coverage.

The matrix in Figure 2 shows that every endpoint meeting the criterion under GS also meets it under GS+OM-MSC. Five additional endpoints are criterion-positive only under GS+OM-MSC: BBB days 14 and 28, pro-caspase-1, western-blot IL-1 β , and IL-18. This nested pattern demonstrates broader coverage but does not convert the five status differences into five direct between-treatment significance tests.

This nested pattern has an important descriptive implication. There is no endpoint for which GS is criterion-positive and GS+OM-MSC is criterion-negative. The cell-loaded condition therefore adds positive responses without losing any response already observed under the acellular carrier. Two of the additions are functional and three are pyroptosis-associated, while the inflammatory domain is already saturated under GS. This distribution identifies where the cell-loaded construct differs most clearly in response breadth: it extends the behavioral pattern and broadens molecular coverage

rather than creating a new inflammatory-domain response absent from the carrier alone.

3.5 Integrated concordance remained stable under alternative weighting

GS had functional, inflammatory, and pyroptotic breadth values of 0.333, 1.000, and 0.500, producing DRCI=0.611. GS+OM-MSC had corresponding values of 1.000, 1.000, and 0.875, producing DRCI=0.958. Direct endpoint weighting gave 8/14=0.571 for GS and 13/14=0.929 for GS+OM-MSC.

Table 2. Domain breadth and sensitivity results.

Measure	GS	GS+OM-MSC
Functional breadth	0.333	1.000
Inflammatory breadth	1.000	1.000
Pyroptotic breadth	0.500	0.875
DRCI, equal domain weights	0.611	0.958
Endpoint-weighted score	0.571	0.929
Without functional domain	0.750	0.938
Without inflammatory domain	0.417	0.938
Without pyroptotic domain	0.667	1.000

The values in Table 2 show that equal domain weighting is not responsible for the numerical ordering. Removing function gives 0.750 for GS and 0.938 for GS+OM-MSC; removing inflammation gives 0.417 and 0.938; removing pyroptotic signaling gives 0.667 and 1.000. The smallest separation occurs when function is removed, whereas the largest occurs when inflammation is removed because inflammation is the only domain with identical complete coverage.

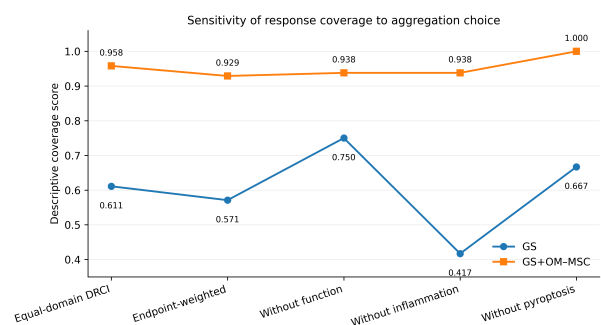


Figure 3. Weighting sensitivity.

The trajectories in Figure 3 remain ordered in the same direction for all five calculations. Thus, the observed ordering is not attributable solely to the eight-endpoint pyroptosis panel, the shared complete inflammatory response, or the three functional endpoints.

The absolute separation between the two integrated values is 0.347 under equal-domain weighting and 0.358 under endpoint weighting. When one domain is removed, the separation is 0.188 without function, 0.521 without inflammation, and 0.333 without pyroptotic signaling. The smaller separation after removing function reflects the importance of the 1/3 versus 3/3 functional contrast, whereas the larger separation after removing inflammation reflects removal of the only domain in which the two treatments have identical complete breadth. These differences are arithmetic descriptions of the coded pattern, not effect sizes with sampling distributions.

The sensitivity calculations also show why both domain-level and endpoint-level displays are necessary. A single integrated number can conceal the fact that the treatments are identical in inflammatory breadth but differ in function and pyroptotic coverage. Conversely, inspecting only individual endpoints can make it difficult to see that the positive GS responses form a subset of the GS+OM-MSC responses. Table 2, Figure 2, and Figure 3 therefore provide complementary views of the same endpoint structure.

3.6 Tissue preservation, scaffold appearance, donor-cell persistence, and organ histology

H&E staining showed extensive cavitation and vacuolation after untreated SCI, with milder lesions after GS and GS+OM-MSC. Nissl staining showed very few Nissl bodies after untreated SCI, a small number of neuronal cell bodies after GS, and larger and more numerous neuronal cell bodies after GS+OM-MSC [56]. These structural observations were not assigned DRCI votes because the available comparisons were predominantly image based.

Residual gelatin material was visible in GS spinal-cord sections at day 28, whereas conspicuous residual material was not observed in GS+OM-MSC sections. Because scaffold mass, degradation products, and quantitative histomorphometry were not measured, this difference cannot establish a degradation rate or mechanism. GFP-labeled OM-MSCs remained detectable at the lesion site at day 28, supporting local persistence over the observation period. Histology of brain, heart, spleen, lung, liver, and kidney did not show overt structural abnormalities at day 28 [56]. These findings define short-term tissue compatibility and localization but do not establish chronic biodistribution, tumorigenicity, neuropathic pain risk, immune neutrality, or long-term material safety.

The structural observations nonetheless add biological context to the scored endpoints. Reduced cavitation and vacuolation, greater preservation of Nissl-positive neuronal cell bodies, and improved ultrastructural appearance are directionally compatible with lower LDH and inflammatory mediators. The strongest structural preservation in the cell-loaded condition is also consistent with its broader functional and pyroptotic response. Because the histological observations were not incorporated into the numerical score, this agreement is supportive rather than mechanically responsible for the higher DRCI.

Donor-cell persistence at the lesion is similarly relevant without proving mechanism. GFP-positive cells at day 28 establish that at least a detectable population remained locally associated with the injured cord during the observation period. Persistence creates an opportunity for continued paracrine, vesicular, matrix-related, or contact-dependent signaling, but localization alone cannot determine which of these processes occurred or whether the detected cells were sufficient to account for the measured functional changes. The organ histology provides a limited short-term safety observation, not a comprehensive systemic safety assessment.

4 Discussion

4.1 Gelatin sponge was an active treatment component

The GS group met 8 of the 14 favorable-response criteria: day-28 stride length; LDH, IL-1 β , and TNF- α by ELISA; NLRP3; cleaved caspase-1 at 22 and 20 kDa; and GSDMD-N. Li et al. reviewed how biomaterials can actively modulate the SCI microenvironment [9]. Wang et al. described biomaterial strategies for reversing adverse immune conditions after SCI [10]. Chen et al. summarized regenerative biomaterial strategies that combine structural and biological functions [11]. Ma et al. reviewed functional biomaterials designed to modulate pathological SCI microenvironments [12]. Haratizadeh et al. discussed biomaterial and cell-based approaches as interacting components of SCI therapy [50]. Khavandegar et al. synthesized preclinical and early clinical evidence for neuroregenerative scaffold effects [51]. Liao et al. summarized recent biomaterial approaches to spinal-cord repair [52]. Xu et al. reviewed tissue-engineering strategies that use scaffold

properties to shape repair [53]. Zheng et al. experimentally showed that scaffold stiffness alters foreign-body response, angiogenesis, and neuroregeneration [13]. Suzuki et al. reviewed scaffold concepts relevant to regenerative SCI therapy [14]. Feng et al. described multiple ways in which biomaterials can affect cellular and tissue responses after SCI [15]. Cao et al. identified structural and biological design criteria for functional SCI scaffolds [16]. For gelatin specifically, Ye et al. characterized a cellularly modified gelatin sponge as a clinically relevant biomaterial platform [44]. Zeng et al. demonstrated substantial tissue remodeling with gelatin sponge in a non-human primate SCI model [45]. Taken together, this literature supports interpreting GS as a biologically active comparator rather than as a neutral carrier.

This point constrains attribution to OM-MSC loading. The GS+OM-MSC condition retained every criterion-positive endpoint observed with GS and added BBB days 14 and 28, pro-caspase-1, western-blot IL-1 β , and IL-18. These additional positive codes identify where the response pattern widened; they do not establish that each GS+OM-MSC effect is statistically larger than the corresponding GS effect. Only the day-28 BBB comparison was explicitly reported as significantly different between the two treated groups [56].

The carrier-associated pattern has practical interpretive importance. In a scaffold-supported cell therapy, the relevant comparison is not simply treatment versus no treatment; it is the extent to which cell loading changes a response already modified by the implanted material. Here, GS alone reaches the favorable threshold in all three inflammatory endpoints and half of the pyroptosis panel. This indicates that the lesion microenvironment encountered by OM-MSCs in the combined treatment is already being altered by the scaffold. The biological effect of the combined construct should therefore be understood as an interaction between a responsive host-material interface and the transplanted cells rather than as an isolated cell effect added to an inert support.

This interpretation is consistent with broader biomaterial evidence. Wang et al. emphasized that biomaterials can reshape adverse immune microenvironments after SCI [10]. Chen et al. reviewed regenerative strategies in which scaffold

architecture contributes to cellular and tissue repair [11]. Ma et al. described how functional biomaterials can modulate the pathological SCI microenvironment [12]. Zheng et al. experimentally demonstrated that scaffold stiffness influences foreign-body response, tissue stiffness, angiogenesis, and neuroregeneration [13]. Suzuki et al. reviewed the importance of material architecture and host integration in SCI scaffold therapy [14]. Feng et al. summarized how material characteristics can affect multiple biological processes relevant to SCI repair [15]. Cao et al. formalized design criteria linking scaffold properties to therapeutic objectives [16]. The present data do not identify which property of the gelatin sponge is responsible for its response profile, but the acellular group demonstrates that material-associated activity is large enough to be visible across several endpoint classes.

4.2 OM-MSC loading widened functional and pyroptotic coverage

Functional breadth increased from 0.333 to 1.000 because GS+OM-MSC met the BBB criteria at days 14 and 28 while retaining the stride-length response shared with GS. Lukovic et al. emphasized that complete transection is valuable precisely because it minimizes interpretation based on spared descending fibers [3]. Li et al. nevertheless showed that scaffold-facilitated locomotor improvement after complete SCI may involve endogenous neuronal relay formation as well as axonal regeneration [4]. The agreement between BBB scoring and day-28 gait therefore provides complementary behavioral evidence, but the present complete-transection model does not identify which neural circuit accounts for the locomotor change.

Pyroptotic breadth increased from 0.500 to 0.875. Both treatments covered NLRP3, the two activated caspase-1 fragments, and GSDMD-N; GS+OM-MSC additionally covered pro-caspase-1, IL-1 β , and IL-18. Xu et al. linked microglial pyroptosis with SCI through a TLR4-associated signaling mechanism [21]. Jiang et al. reported improved SCI recovery when pyroptosis was therapeutically modulated through topoisomerase-1 inhibition [22]. Gu et al. reviewed pyroptosis as a broader pathogenic and therapeutic target in SCI [23]. These studies support the biological relevance of the NLRP3-caspase-1-GSDMD axis, while the tissue-level assays used here cannot assign the signal to neurons, microglia, astrocytes,

infiltrating macrophages, or another specific cell population.

The absence of a significant GSDMD-FL response in either treatment does not contradict the GSDMD-N result. Shi et al. explained that pyroptosis depends on proteolytic activation of gasdermins rather than simple depletion of their full-length precursors [18]. Sborgi et al. demonstrated that the cleaved GSDMD fragment forms membrane pores [19]. Liu et al. independently confirmed that inflammasome-activated GSDMD drives pyroptosis by creating membrane pores [20]. Keeping the two species separate therefore preserves a biologically meaningful distinction between precursor abundance and pore-forming activation.

The relationship between behavioral and molecular breadth is also informative. The cell-loaded construct adds two functional responses and three pyroptosis-associated responses, so the broader behavioral pattern is accompanied by broader molecular coverage rather than appearing as an isolated gait effect. This cross-domain correspondence strengthens biological coherence, but it should not be interpreted as proof that the additional molecular responses caused the additional functional responses. A shared upstream effect—for example, improved tissue preservation or altered inflammatory-cell behavior—could produce both patterns without a simple one-directional causal chain.

OM-MSCs have several plausible routes of action. Ge et al. characterized the olfactory-mucosa MSC secretome and identified multiple potentially therapeutic components [29]. Huang et al. showed that hypoxia-preconditioned olfactory-mucosa MSCs can suppress microglial pyroptotic and apoptotic death in an ischemia/reperfusion model [30]. Brennan et al. reviewed regenerative biomaterials functionalized with MSC-derived extracellular vesicles and soluble factors [40]. Prado-Yupanqui et al. summarized the broader regenerative potential of MSC secretomes in cell-free therapy [41]. Wang et al. reviewed evidence for MSC-derived exosomes in SCI treatment [25]. Lindsay et al. discussed olfactory mucosa-derived cells as multi-target tools for central-nervous-system repair [31]. Dutta et al. reviewed extracellular vesicles as both mediators and therapeutic candidates in SCI [26]. Kim et al. described the therapeutic potential of MSCs and MSC-derived extracellular vesicles for SCI [27]. Wang

et al. reported that olfactory-mucosa MSC transplantation can promote SCI repair through microglial polarization [58]. Zeng reviewed the broader therapeutic mechanisms and future prospects of MSC-based SCI treatment [28]. The present endpoint set cannot rank these possibilities; it shows only that cell loading is associated with a more extensive favorable pattern across functional and inflammasome-related observations than is seen with the scaffold alone.

4.3 Inflammatory and structural findings strengthen biological coherence without proving mediation

Both treatments met all three inflammatory criteria, and both showed less severe tissue disruption than untreated SCI. The GS+OM-MSC condition also showed the clearest preservation of neuronal cell bodies and the widest functional and pyroptotic coverage. These observations occur in the same treatment condition and therefore support cross-domain concordance.

Importantly, concordance should not be interpreted as mediation. Molecular measurements were not collected as a sufficiently dense longitudinal series from the same animals, and no selective NLRP3, caspase-1, or GSDMD perturbation was used to establish a causal sequence. Reduced inflammasome-associated proteins may contribute to tissue preservation and locomotor recovery, but the experiment does not demonstrate that pyroptosis suppression is the mechanism that produced BBB improvement.

The distinction matters because the molecular panel is measured at the tissue level. NLRP3, caspase-1, GSDMD, IL-1 β , and IL-18 can be influenced by several resident and infiltrating cell populations. A lower tissue signal could result from reduced activation within a cell type, a change in the abundance of that cell type, preservation of tissue composition, or combinations of these factors. Without cell-resolved quantification and temporal coupling to behavior, the molecular results should be described as pathway-associated evidence rather than a cell-specific mechanistic demonstration.

The structural data are subject to a related limitation. H&E, Nissl staining, and electron microscopy provide strong anatomical context, but qualitative preservation is not equivalent to a quantified mediator of locomotor recovery. The complementary use of

biochemical and histopathological outcomes is also seen in other rat injury models, where biochemical markers and tissue architecture provide related but non-interchangeable evidence of treatment response [60]. Their value here is concordant direction: the treatments that reduce tissue-injury and inflammatory markers also show less severe structural disruption, and the cell-loaded condition shows the broadest overall pattern. This correspondence strengthens the internal biological narrative while preserving appropriate limits on causal language.

4.4 DRCI separates response breadth from effect magnitude

The DRCI addresses a deliberately narrow quantitative question: what proportion of selected endpoints satisfy a favorable, statistically supported treatment-versus-SCI criterion within each biological domain? Equal weighting prevents the eight pyroptosis measurements from contributing more than half of the integrated value simply because that pathway was sampled more densely. The endpoint-weighted and leave-one-domain-out calculations show that the numerical ordering persists when the aggregation rule changes.

Binary coding inevitably sacrifices information about effect magnitude and statistical precision. An endpoint with $p = 0.049$ and one with a much smaller p value both receive 1 when the direction is favorable, while a biologically relevant but imprecisely estimated change receives 0. The DRCI also lacks a confidence interval because individual-animal multivariate observations and cross-endpoint covariance were not available for the integrated calculation. It should therefore be read beside effect magnitudes and uncertainty estimates rather than as a substitute for them.

A further limitation is the ceiling created by binary breadth. Once every endpoint in a domain is positive, that domain cannot distinguish a modest response from a very large one. The inflammatory domain demonstrates this directly: GS and GS+OM-MSC both score 1.000 even though the underlying assay values may not be numerically identical. Conversely, in the pyroptotic domain, one additional threshold-crossing endpoint changes breadth by 0.125 because eight endpoints are present, whereas one additional response in the three-endpoint functional domain changes breadth by 0.333. These properties are mathematically transparent and reinforce the need

to interpret the score as coverage rather than magnitude.

Threshold dependence is another consequence of the binary rule. A reported p value just below 0.05 changes the code from 0 to 1, whereas a value just above 0.05 leaves the code at 0 even if the point estimate is similar. The analysis does not claim that this threshold transforms biological reality. Instead, it uses one explicit rule consistently so that readers can trace how the integrated values arise. The endpoint audit is therefore as important as the final DRCI numbers.

Dependence among endpoints is also unavoidable. BBB days 14 and 28 are longitudinally related; NLRP3, caspase-1, GSDMD, IL-1 β , and IL-18 belong to one signaling sequence; and IL-1 β appears in both ELISA and western-blot form. Equal domain weighting corrects unequal endpoint counts but does not make the measurements statistically independent. DRCI consequently represents coverage across selected observations, not the number of independent biological processes altered by treatment.

This dependence also explains why conventional inferential statistics were not applied to the integrated score. Treating the 14 codes as independent observations would overstate the effective information because several arise from related time points or members of the same pathway. In addition, the score is derived from already tested endpoint comparisons rather than from raw multivariate animal-level data. The most defensible use is therefore descriptive: to expose how criterion-positive responses are distributed and to test whether that distribution is robust to simple alternative aggregation rules.

The same logic limits comparisons with future studies. A DRCI calculated from another experiment would only be directly comparable if the endpoint definitions, domains, response criterion, and direction rules were matched. Adding more proteins to one domain or choosing different behavioral time points would change the denominator and potentially the score. The present values should therefore be treated as a concise summary of this particular endpoint set, not as a universal scale of SCI treatment efficacy.

4.5 Assay reporting defines the precision of the DRCI codes

Initial allocation of 10 animals per group should not be confused with the smaller assay samples used in

the scored comparisons. BBB/gait and ELISA panels used $N = 4$, whereas western blots used $N = 3$ [56]. The footprint Methods described five surviving animals selected per group but the corresponding figure caption stated $N = 4$; the coding table therefore uses the figure-level value and records the inconsistency explicitly.

Exact p values were not assigned when only significance symbols or categorical statements were available. The binary codes therefore inherit the precision of the reported pairwise comparisons. The accompanying CSV contains all 14 endpoint assignments and the calculation script reproduces the domain breadth, DRCI, endpoint-weighted, and leave-one-domain-out values directly from those assignments.

The small assay samples also affect interpretation of the zero codes. A zero means that the reported comparison did not satisfy both significance and favorable direction under the predefined rule; it does not prove equality with untreated SCI. This is particularly important for GS at BBB days 14 and 28 and for several western-blot endpoints. The DRCI intentionally preserves that reported inferential status rather than replacing it with a qualitative judgment based on the visual direction of bars or images.

Conversely, a positive code does not guarantee a large or clinically meaningful difference. The binary rule is useful because it is reproducible from the available report, but it cannot replace confidence intervals, standardized effect sizes, or animal-level trajectories. These limitations are not defects that can be removed by adding more arithmetic to the same summarized data; they are properties of the available evidence and define the appropriate scope of the integrated result.

4.6 Translational limits of the cell–scaffold construct

The construct combined human OM–MSCs with a rat complete-transection lesion. Cross-species immune interactions can affect donor-cell persistence, host inflammation, and secretory behavior, so the xenogeneic setting is directly relevant to interpretation of the inflammatory and pyroptotic endpoints [56]. The 28-day observation period also captures only subacute tissue remodeling. Chronic scaffold degradation, late cell migration, persistent survival, spasticity, pain, autonomic recovery, delayed immune responses, and tumorigenicity were not determined.

The lesion model is another boundary. Lukovic et al. described complete transection as a model that minimizes recovery attributable to spared descending fibers [3]. Li et al. showed that even in complete SCI, scaffold-facilitated recovery can involve endogenous relay mechanisms, underscoring differences from typical human lesion biology [4]. Haratizadeh et al. reviewed how biomaterial and cell-based SCI therapies are shaped by lesion context [50]. Khavandegar et al. highlighted variation in lesion model and treatment context across scaffold studies [51]. Liao et al. described how biomaterial performance depends on the pathological and structural features of the injured cord [52]. Xu et al. similarly emphasized that tissue-engineering strategies must be matched to the lesion environment [53]. Zheng et al. demonstrated experimentally that local mechanical properties such as scaffold stiffness alter host response and neuroregeneration [13]. Suzuki et al. reviewed the dependence of scaffold therapy on material design and regenerative context [14]. Feng et al. summarized the diverse interactions between biomaterials and the SCI microenvironment [15]. Cao et al. linked scaffold design criteria directly to the therapeutic requirements of SCI repair [16]. The DRCI values therefore describe the measured 28-day response pattern in female rats with a complete T10 defect and should not be generalized directly to chronic or anatomically incomplete human SCI.

Translation is further limited by scale and lesion composition. A 3-mm experimental transection creates a sharply defined gap that can be filled immediately with a prepared implant. Human SCI more commonly contains irregular zones of hemorrhage, necrosis, spared tracts, cystic evolution, and variable rostrocaudal extension, and treatment frequently occurs after the initial injury rather than at the moment of lesion creation. These differences can alter material placement, cell retention, diffusion distances, immune exposure, and the neural substrates available for functional recovery. Evidence of local donor-cell persistence and short-term organ histological compatibility in the rat model is therefore encouraging but cannot be equated with clinical feasibility or long-term safety.

The use of human cells in rats introduces an additional interpretive tension. It provides information about the behavior of the intended human-derived cell type within injured spinal tissue,

but it also creates xenogeneic interactions that differ from autologous or allogeneic human transplantation. Immune modulation is itself one of the proposed actions of MSCs, so species mismatch can influence the same inflammatory outcomes used to judge treatment response. The broad response under GS+OM-MSC should consequently be understood within the specific host-donor context of the experiment.

OM-MSC activity may involve several mechanisms that cannot be separated in the present design. Ge et al. documented a biologically rich secretome from olfactory-mucosa MSCs [29]. Huang et al. demonstrated anti-pyroptotic and anti-apoptotic effects of preconditioned olfactory-mucosa MSCs on microglial cells in an ischemic model [30]. Brennan et al. reviewed the regenerative use of MSC-secreted extracellular vesicles and soluble factors delivered through biomaterials [40]. Prado-Yupanqui et al. reviewed secretome-mediated mechanisms in cell-free regenerative medicine [41]. Kim et al. showed that three-dimensional MSC culture can increase exosome secretion [42]. Holkar et al. demonstrated that three-dimensional physicochemical conditions can shape extracellular-vesicle output [43]. Wang et al. reviewed MSC-derived exosomes specifically in the context of SCI [25]. Lindsay et al. described olfactory mucosa-derived cells as multi-target contributors to CNS repair [31]. Dutta et al. highlighted extracellular-vesicle signaling as an emerging component of SCI therapy [26]. Kim et al. integrated evidence for both MSCs and MSC-derived extracellular vesicles in SCI treatment [27]. Wang et al. reported that olfactory-mucosa MSC transplantation can influence microglial polarization after SCI [58]. Zeng reviewed multiple trophic, immunomodulatory, and reparative mechanisms proposed for MSC therapy in SCI [28]. The absence of an OM-MSC-only group therefore prevents separation of intrinsic cell activity from scaffold-assisted retention and cell-matrix interaction. A factorial comparison of GS, OM-MSC suspension, GS+OM-MSC, and GS carrying cell-free OM-MSC products would be required to distinguish those contributions.

Within the available comparison, however, several conclusions are still supported without assigning a unique mechanism. First, the carrier has measurable activity of its own. Second, cell loading does not eliminate any criterion-positive response observed with the carrier. Third, the additional responses are

concentrated in functional recovery and in several positions of the pyroptotic panel. Fourth, the same treatment ordering is retained under equal-domain, endpoint-weighted, and leave-one-domain-out calculations. These features together make the broader GS+OM-MSC response unlikely to be an artifact of one isolated endpoint or one weighting choice, while still leaving the underlying causal pathway unresolved.

This distinction between robust description and unresolved mechanism is central to interpreting the study. The present evidence is strong enough to identify where response breadth expands, but not to state that a single molecular process explains the behavioral benefit. Maintaining that boundary avoids overextension of the molecular findings while preserving their value as part of a coherent cross-domain response pattern.

5 Conclusion

The three research questions are answered by the endpoint pattern. Functional coverage was 1/3 for GS and 3/3 for GS+OM-MSC; BBB days 14 and 28 were the additional criterion-positive functional endpoints under cell loading, while stride length was positive under both treatments. Inflammation showed complete 3/3 coverage under both conditions. Pyroptotic coverage was 4/8 for GS and 7/8 for GS+OM-MSC; pro-caspase-1, western-blot IL-1 β , and IL-18 were the additional positive endpoints under GS+OM-MSC, whereas GSDMD-FL remained non-responsive under both treatments. Equal-domain DRCI values were 0.611 and 0.958, endpoint-weighted values were 0.571 and 0.929, and the numerical ordering remained unchanged after removal of any one domain.

These results show that gelatin sponge itself contributes measurable biological activity after complete T10 SCI, particularly across inflammatory tissue injury and selected pyroptosis-associated proteins. OM-MSC loading is associated with a wider distribution of endpoints meeting the same treatment-versus-SCI criterion, extending the pattern to sustained BBB recovery and additional caspase/cytokine positions while retaining the scaffold-associated inflammatory response. The DRCI quantifies this breadth of concordant response; it does not measure treatment potency, prove statistical superiority for endpoints lacking a direct GS-versus-GS+OM-MSC test, or establish that

pyroptosis suppression caused locomotor recovery.

The response pattern also clarifies the biological division between the two treatment components. Acellular GS already produces complete inflammatory breadth, a stride-length response, and favorable changes in several activation-related pyroptosis markers. OM–MSC loading broadens that existing pattern rather than replacing it, with additional threshold-crossing responses in BBB recovery, pro-caspase-1, IL-1 β , and IL-18. The fact that no GS-positive endpoint becomes negative under GS+OM–MSC supports a nested response structure across the selected observations.

Accordingly, the paper's central question is answered at the level the evidence permits: under the same complete T10 lesion conditions, the cell-loaded gelatin sponge shows broader favorable response coverage across function and pyroptosis-associated signaling than the acellular sponge, while both treatments share complete coverage of the selected inflammatory tissue-injury endpoints. This conclusion remains descriptive rather than mechanistic and is confined to the reported 28-day rat experiment, the stated assay sample sizes, and the 14 endpoints used in the integrated calculation.

Declarations

Funding

This research received no external funding.

Conflicts of Interest

The author declares no conflicts of interest.

Data Availability

All data used in this study are included within the article and its accompanying materials. The integrated calculations are derived from the endpoint-level evidence described and cited in the manuscript.

Use of Artificial Intelligence

Artificial intelligence tools were used solely to assist with English-language editing and improvement of presentation. No AI tool was used to generate or alter the study data, perform the reported scientific analyses, or determine the scientific conclusions. The author reviewed and takes full responsibility for the final manuscript.

References

- [1] Ahuja, C. S., Nori, S., Tetreault, L., Wilson, J., Kwon, B., Harrop, J., et al. (2017). Traumatic spinal cord injury—repair and regeneration. *Neurosurgery*, 80, S9–S22.
- [2] Hu, X., Xu, W., Ren, Y., Wang, Z., He, X., Huang, R., et al. (2023). Spinal cord injury: Molecular mechanisms and therapeutic interventions. *Signal Transduction and Targeted Therapy*, 8, Article 245.
- [3] Lukovic, D., Moreno-Manzano, V., Lopez-Mocholi, E., Rodriguez-Jimenez, F. J., Jendelova, P., Sykova, E., et al. (2015). Complete rat spinal cord transection as a faithful model of spinal cord injury for translational cell transplantation. *Scientific Reports*, 5, Article 9640.
- [4] Li, X., Liu, D., Xiao, Z., Zhao, Y., Han, S., Chen, B., & Dai, J. (2019). Scaffold-facilitated locomotor improvement post complete spinal cord injury: Motor axon regeneration versus endogenous neuronal relay formation. *Biomaterials*, 197, 20–31.
- [5] Basso, D. M., Beattie, M. S., & Bresnahan, J. C. (1995). A sensitive and reliable locomotor rating scale for open field testing in rats. *Journal of Neurotrauma*, 12(1), 1–21.
- [6] Orr, M. B., & Gensel, J. C. (2018). Spinal cord injury scarring and inflammation: Therapies targeting glial and inflammatory responses. *Neurotherapeutics*, 15, 541–553.
- [7] Bradbury, E. J., & Burnside, E. R. (2019). Moving beyond the glial scar for spinal cord repair. *Nature Communications*, 10, Article 3879.
- [8] David, S., & Kroner, A. (2011). Repertoire of microglial and macrophage responses after spinal cord injury. *Nature Reviews Neuroscience*, 12(7), 388–399.
- [9] Li, Y., Zhang, Q., Liu, Z., Fu, C., & Ding, J. (2024). Microenvironments-modulated biomaterials enhance spinal cord injury therapy. *Advanced Functional Materials*, 34, Article 2403900.
- [10] Wang, H., Xia, Y., Li, B., Li, Y., & Fu, C. (2022). Reverse adverse immune microenvironments by biomaterials enhance the repair of spinal cord injury. *Frontiers in Bioengineering and Biotechnology*, 10, Article 812340.
- [11] Chen, K., Yu, W., Zheng, G., Xu, Z., Yang, C., Wang, Y., Yue, Z., Yuan, W., Hu, B., et al. (2024). Biomaterial-based regenerative therapeutic strategies for spinal cord injury. *NPG Asia Materials*, 16, Article 5.
- [12] Ma, D., Fu, C., Li, F., Ruan, R., Lin, Y., Li, X., Li, M., & Zhang, J. (2024). Functional biomaterials for modulating the dysfunctional pathological microenvironment of spinal cord injury. *Bioactive Materials*, 39, 521–543.
- [13] Zheng, Y., Nützl, M., Schackel, T., Chen, J., Weidner,

- N., Müller, R., & Puttagunta, R. (2025). Biomaterial scaffold stiffness influences the foreign body reaction, tissue stiffness, angiogenesis and neuroregeneration in spinal cord injury. *Bioactive Materials*, 46, 134–149.
- [14] Suzuki, H., Imajo, Y., Funaba, M., Ikeda, H., Nishida, N., & Sakai, T. (2023). Current concepts of biomaterial scaffolds and regenerative therapy for spinal cord injury. *International Journal of Molecular Sciences*, 24(3), Article 2528.
- [15] Feng, C., Deng, L., Yong, Y.-Y., Wu, J.-M., Qin, D.-L., Yu, L., et al. (2023). The application of biomaterials in spinal cord injury. *International Journal of Molecular Sciences*, 24(1), Article 816.
- [16] Cao, J., Wu, J., Mu, J., Feng, S., & Gao, J. (2021). The design criteria and therapeutic strategy of functional scaffolds for spinal cord injury repair. *Biomaterials Science*, 9, 4591–4606.
- [17] Swanson, K. V., Deng, M., & Ting, J. P.-Y. (2019). The NLRP3 inflammasome: Molecular activation and regulation to therapeutics. *Nature Reviews Immunology*, 19, 477–489.
- [18] Shi, J., Gao, W., & Shao, F. (2017). Pyroptosis: Gasdermin-mediated programmed necrotic cell death. *Trends in Biochemical Sciences*, 42, 245–254.
- [19] Sborgi, L., Rühl, S., Mulvihill, E., Pipercevic, J., Heilig, R., Stahlberg, H., et al. (2016). GSDMD membrane pore formation constitutes the mechanism of pyroptotic cell death. *The EMBO Journal*, 35, 1766–1778.
- [20] Liu, X., Zhang, Z., Ruan, J., Pan, Y., Magupalli, V. G., Wu, H., & Lieberman, J. (2016). Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. *Nature*, 535, 153–158.
- [21] Xu, S., Wang, J., Jiang, J., Song, J., Zhu, W., Zhang, F., et al. (2020). TLR4 promotes microglial pyroptosis via lncRNA-F630028O10Rik by activating PI3K/AKT pathway after spinal cord injury. *Cell Death & Disease*, 11, Article 693.
- [22] Jiang, W., He, F., Ding, G., & Wu, J. (2022). Topoisomerase 1 inhibition modulates pyroptosis to improve recovery after spinal cord injury. *The FASEB Journal*, 36, e22294.
- [23] Gu, G., Yu, H., Zou, H., Kou, W., Zhang, P., Gu, G., Lu, J., Shi, W., Chu, P., Zhang, Y., Sun, G., & Shang, J. (2024). Pyroptosis in spinal cord injury: Implications for pathogenesis and therapeutic approaches. *Frontiers in Bioscience-Landmark*, 29(6), Article 210.
- [24] Galipeau, J., & Sensébé, L. (2018). Mesenchymal stromal cells: Clinical challenges and therapeutic opportunities. *Cell Stem Cell*, 22, 824–833.
- [25] Wang, H., Zhao, C., Rong, Q., Cao, J., Chen, H., Li, R., Zhang, B., & Xu, P. (2024). The role of exosomes from mesenchymal stem cells in spinal cord injury: A systematic review. *International Journal of Stem Cells*, 17(3), 236–252.
- [26] Dutta, D., Khan, N., Wu, J., & Jay, S. M. (2021). Extracellular vesicles as an emerging frontier in spinal cord injury pathobiology and therapy. *Trends in Neurosciences*, 44(6), 492–506.
- [27] Kim, G.-U., Sung, S.-E., Kang, K.-K., Choi, J.-H., Lee, S., Sung, M., et al. (2021). Therapeutic potential of mesenchymal stem cells (MSCs) and MSC-derived extracellular vesicles for the treatment of spinal cord injury. *International Journal of Molecular Sciences*, 22(24), Article 13672.
- [28] Zeng, C.-W. (2023). Multipotent mesenchymal stem cell-based therapies for spinal cord injury: Current progress and future prospects. *Biology*, 12(5), Article 653.
- [29] Ge, L., Jiang, M., Duan, D., Wang, Z., Qi, L., Teng, X., et al. (2016). Secretome of olfactory mucosa mesenchymal stem cell, a multiple potential stem cell. *Stem Cells International*, 2016, Article 1243659.
- [30] Huang, Y., Hong, C.-G., Wang, X., Duan, R., Liu, J.-Y., He, J.-L., et al. (2020). Hypoxia-preconditioned olfactory mucosa mesenchymal stem cells abolish cerebral ischemia/reperfusion-induced pyroptosis and apoptotic death of microglial cells by activating HIF-1 α . *Aging*, 12, 10931–10950.
- [31] Lindsay, S. L., McCanney, G. A., Willison, A. G., & Barnett, S. C. (2020). Multi-target approaches to CNS repair: Olfactory mucosa-derived cells and heparan sulfates. *Nature Reviews Neurology*, 16(4), 229–240.
- [32] Lv, B., Zhang, X., Yuan, J., Chen, Y., Ding, H., Cao, X., et al. (2021). Biomaterial-supported MSC transplantation enhances cell–cell communication for spinal cord injury. *Stem Cell Research & Therapy*, 12, Article 36.
- [33] Jeon, J., Park, S. H., Choi, J., Han, S. M., Kim, H.-W., Shim, S. R., & Hyun, J. K. (2024). Association between neural stem/progenitor cells and biomaterials in spinal cord injury therapies: A systematic review and network meta-analysis. *Acta Biomaterialia*, 183, 50–60.
- [34] Blando, S., Anchesi, I., Mazzon, E., & Gugliandolo, A. (2022). Can a scaffold enriched with mesenchymal stem cells be a good treatment for spinal cord injury? *International Journal of Molecular Sciences*, 23(14), Article 7545.
- [35] Guo, S., Redenski, I., & Levenberg, S. (2021). Spinal cord repair: From cells and tissue engineering to extracellular vesicles. *Cells*, 10(8), Article 1872.
- [36] Zhang, D., Sun, Y., & Liu, W. (2023). Motor functional recovery efficacy of scaffolds with bone marrow stem cells in rat spinal cord injury: A Bayesian network meta-analysis. *Spinal Cord*, 61, 93–98.
- [37] Ma, T., Wu, J., Mu, J., & Gao, J. (2022). Biomaterials reinforced MSCs transplantation for spinal cord injury repair. *Asian Journal of Pharmaceutical Sciences*, 17(1), 4–19.

- [38] Wang, Y., Ding, Y., & Guo, C. (2024). Mesenchymal stem cells for the treatment of spinal cord injury in rat models: A systematic review and network meta-analysis. *Cell Transplantation*, 33, Article 09636897241262992.
- [39] Wang, R., Wang, Y., Yan, F., Sun, J., & Zhang, T. (2025). Assessment of mesenchymal stem cells for the treatment of spinal cord injury: A systematic review and network meta-analysis. *Frontiers in Cellular Neuroscience*, 19, Article 1532219.
- [40] Brennan, M. À., Layrolle, P., & Mooney, D. J. (2020). Biomaterials functionalized with MSC secreted extracellular vesicles and soluble factors for tissue regeneration. *Advanced Functional Materials*, 30, Article 1909125.
- [41] Prado-Yupanqui, J. W., Ramírez-Orrego, L., Cortez, D., Vera-Ponce, V. J., Chenet, S. M., Tejedo, J. R., et al. (2025). The hidden power of the secretome: Therapeutic potential on wound healing and cell-free regenerative medicine—a systematic review. *International Journal of Molecular Sciences*, 26, Article 1926.
- [42] Kim, M., Yun, H.-W., Park, D. Y., Choi, B. H., & Min, B.-H. (2018). Three-dimensional spheroid culture increases exosome secretion from mesenchymal stem cells. *Tissue Engineering and Regenerative Medicine*, 15, 427–436.
- [43] Holkar, K., Kale, V., & Ingavle, G. (2022). Well-orchestrated physico-chemical and biological factors for enhanced secretion of osteogenic and angiogenic extracellular vesicles by mesenchymal stem cells in a 3D culture format. *Biomaterials Science*, 10, 4458–4473.
- [44] Ye, D., Wu, S., Zhang, B., Hong, C., & Yang, L. (2021). Characteristics and clinical potential of a cellularly modified gelatin sponge. *Journal of Applied Biomaterials & Functional Materials*, 19, Article 22808000211035061.
- [45] Zeng, X., Wei, Q., Ye, J., Rao, J., Zheng, M., Ma, Y., et al. (2023). A biocompatible gelatin sponge scaffold confers robust tissue remodeling after spinal cord injury in a non-human primate model. *Biomaterials*, 299, Article 122161.
- [46] Fan, L., Liu, C., Chen, X., Zheng, L., Zou, Y., Wen, H., et al. (2022). Exosomes-loaded electroconductive hydrogel synergistically promotes tissue repair after spinal cord injury via immunoregulation and enhancement of myelinated axon growth. *Advanced Science*, 9, Article 2105586.
- [47] Papa, S., Vismara, I., Mariani, A., Barilani, M., Rimondo, S., De Paola, M., et al. (2018). Mesenchymal stem cells encapsulated into biomimetic hydrogel scaffold gradually release CCL2 chemokine in situ preserving cytoarchitecture and promoting functional recovery in spinal cord injury. *Journal of Controlled Release*, 278, 49–56.
- [48] Yao, S., Yu, S., Cao, Z., Yang, Y., Yu, X., Mao, H.-Q., et al. (2018). Hierarchically aligned fibrin nanofiber hydrogel accelerated axonal regrowth and locomotor function recovery in rat spinal cord injury. *International Journal of Nanomedicine*, 13, 2883–2895.
- [49] Zhang, X., Jiang, W., Lu, Y., Mao, T., Gu, Y., Ju, D., & Dong, C. (2023). Exosomes combined with biomaterials in the treatment of spinal cord injury. *Frontiers in Bioengineering and Biotechnology*, 11, Article 1077825.
- [50] Haratizadeh, S., Liu, H., Li, H., Adeli, M., & All, A. H. (2025). Biomaterials and cell-based therapy post spinal cord injury. *Journal of Translational Medicine*, 23, Article 1042.
- [51] Khavandegar, A., Safari Dehnavi, N., Ganau, M., Ramezani, Z., Khodadoust, E., Tabatabaei, M. S. H. Z., et al. (2025). Neuroregenerative and neuroprotective effects of bioengineered scaffolds in spinal cord injury: A systematic review of preclinical and early phase clinical studies. *Spinal Cord*, 63(9), 451–469.
- [52] Liao, Z., Bao, Q., Saijilahu, Chimedtsere, C., Tumurbaatar, K., & Saijilafu. (2025). Research progress on biomaterials for spinal cord repair. *International Journal of Nanomedicine*, 20, 1773–1787.
- [53] Xu, L., Zhou, S., Dai, X., Gu, X., & Ouyang, Z. (2025). Tissue engineering and spinal cord injury repair. *Engineering*, 46, 60–72.
- [54] Liu, Q., Wang, W., Yang, H., Wang, Y., Shi, Y., et al. (2024). Microenvironment-responsive injectable conductive hydrogel for spinal cord injury repair. *Advanced Functional Materials*, 34(46), Article 2406376.
- [55] Wang, C., Zhang, P., Pan, Y., Huang, C., Zhang, R., Yang, G., Zheng, J., Wan, C., & Wei, Z. (2024). Multifunctional conductive stem cell delivery hydrogel combined with low-frequency pulsed electromagnetic fields for spinal cord injury repair. *Materials & Design*, 246, Article 113356.
- [56] Li, W., Jiang, X., Lu, S., Lu, W., Ma, S., Zhuo, Y., et al. (2025). Olfactory mucosal mesenchymal stem cells delivered by gelatin sponge scaffolds promote functional recovery of spinal cord injury. *Frontiers in Bioengineering and Biotechnology*, 13, Article 1628758.
- [57] Gelman, A., & Stern, H. (2006). The difference between ‘significant’ and ‘not significant’ is not itself statistically significant. *The American Statistician*, 60(4), 328–331.
- [58] Wang, X., Hong, C.-G., Duan, R., Pang, Z.-L., Zhang, M.-N., Xie, H., et al. (2024). Transplantation of olfactory mucosa mesenchymal stromal cells repairs spinal cord injury by inducing microglial polarization. *Spinal Cord*, 62, 429–439.
- [59] Sáenz, A., Rivera-Muñoz, E., Brostow, W., & Castaño, V. M. (1999). Ceramic biomaterials: An introductory overview. *Journal of Materials Education*, 21(5–6), 297–306.

- [60] Khadum, I. W., Hameed, B. J., & Qasim, Q. A. (2026). Gastroprotective, hepatoprotective, and nephroprotective effects of α -lipoic acid against aspirin-induced toxicity in rats. *Revista Cultura Científica*, (24), 1007–1020.
- [61] Lu, Z., Jamil, A. A., Yichen, Y., & Zhang, J. (2026). The role of positive emotion in virtual forest interventions: Re-analyzing experimental data for stress relief in high-pressure academic contexts. *Revista Cultura Científica*, (24), 763–779.