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Review

Reprogramming the Oxidative Stress Microenvironment after Spinal Cord Injury with Exosome Mimetics and Biomimetic Nanofibrous Hydrogels

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Abstract: Oxidative stress links vascular disruption, mitochondrial dysfunction, inflammation, and neural-cell death after spinal cord injury. Extracellular vesicles, artificially generated exosome mimetics, and biomimetic nanofibrous hydrogels offer complementary strategies for modifying this environment, but their evidence bases are uneven and their functions are often conflated. This narrative review examines the biological rationale, representative preclinical studies, material design principles, and translational limitations of these approaches. Particular attention is given to the distinction between secreted vesicles and mechanically produced mimetics, the contribution of matrix architecture beyond passive encapsulation, and the evidence required to connect local delivery with redox regulation and neuroprotection. Published studies support vesicle-mediated modulation of inflammatory and vascular responses, peptide-scaffold support for neural repair, and improved therapeutic delivery through selected hydrogel systems. Direct mimetic and hydrogel studies are emerging, yet findings from different cargos and matrices cannot establish the effectiveness of an untested combined formulation. Reduced fluorescent oxidant signals, increased antioxidant-associated proteins, and improved locomotion provide complementary observations rather than interchangeable proof of mechanism. The strongest development strategy integrates product identity, functional potency, release behavior, cell-specific target engagement, and durable neurological outcomes. Progress toward clinical translation depends on reproducible manufacturing, appropriate component controls, clinically relevant delivery windows, and robust evidence that sustained local exposure remains both biologically active and safe for patients.

Keywords: spinal cord injury; oxidative stress; extracellular vesicles; exosome mimetics; hydrogels; neuroprotection

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1. Introduction

Spinal cord injury (SCI) impairs neural connectivity and triggers a secondary cascade that may extend tissue damage following the initial mechanical trauma. Vascular dysfunction, altered ionic homeostasis, inflammatory cell infiltration, and degeneration of neurons and glial cells interact across distinct temporal phases. Their relative contributions depend on injury severity, anatomical location, and the viability of surrounding tissue [1]. Current understanding of SCI pathophysiology therefore highlights the interplay among multiple biological processes rather than attributing pathology to a single molecular abnormality. Oxidative stress represents a promising focus for therapeutic development because it intersects several of these pathways; however, effective strategies must sustain tissue functionality within this dynamically evolving biological context.

Reactive oxygen species (ROS) serve physiological roles in redox signaling under controlled conditions, whereas excessive or spatially dysregulated oxidant generation can cause damage to lipids, proteins, and nucleic acids [2]. In spinal tissue after injury, mitochondrial impairment and inflammatory oxidant production can mutually exacerbate one another. Iron-mediated lipid peroxidation constitutes an additional mechanism contributing to cellular loss. Accordingly, the therapeutic aim is more accurately described as restoration of physiological redox equilibrium rather than global suppression of ROS. Effective neuroprotection also necessitates concurrent support for vascular perfusion, plasma membrane integrity, and the structural and biochemical milieu encountered by surviving axons.

Extracellular vesicles (EVs) are of growing interest due to their capacity to influence recipient cells through membrane-associated and intraluminal cargo [3, 4]. Mechanically derived vesicle mimetics provide alternative manufacturing approaches that may enhance scalability, while hydrogels enable localized delivery and offer a supportive biomimetic matrix. Nanofibrous scaffolds add further design versatility by enabling precise spatial organization and presentation of bioactive signals. Although these modalities appear complementary in principle, independent demonstration of individual functions does not establish that their integration yields enhanced neuroprotective outcomes. For instance, a scaffold retaining a fluorescent tracer may not maintain functional vesicle cargo integrity; similarly, behavioral improvement observed in preclinical models may reflect preservation of microvascular function rather than targeted antioxidant delivery to neurons.

This review addresses a focused question: what empirical evidence supports the integration of exosome mimetics with biomimetic nanofibrous hydrogels to regulate the oxidative-stress microenvironment after SCI, and what key knowledge gaps remain? It uses naturally secreted EVs as the primary biological reference, examines methods for mimetic generation and targeting fidelity, and assesses scaffold-specific matrix functions alongside redox mechanisms. Emphasis is placed on clarifying methodological distinctions across studies and evaluating the generalizability of reported findings [5]. No original animal experiments, cell-based assays, quantitative efficacy metrics, or transcriptomic data are presented. The central proposition is that delivery efficiency, biological activity, and functional neurological outcomes must be rigorously linked within a unified formulation before claims of comprehensive microenvironmental reprogramming can be substantiated.

2. Review Scope and Terminology

This is a focused narrative review, not a systematic review or meta-analysis. Targeted searches of PubMed-indexed and publisher records were conducted through 17 September 2026 using combinations of spinal cord injury, oxidative stress, ferroptosis, extracellular vesicles, exosome mimetics, peptide hydrogels, nanofibers, and local delivery. Landmark mechanistic studies were considered alongside recent applications in spinal cord injury and established reporting guidance [6, 7]. Sources were selected for their relevance to the biological or material link under discussion. Differences across injury models, treatment routes, preparations, and outcome definitions precluded meaningful pooled effect estimation. The resulting selection should not be interpreted as exhaustive coverage of every therapeutic formulation.

Terminology is consequential [8, 9]. EV denotes a naturally released membrane-bound particle; exosome implies an endosomal origin that size or a few common markers cannot establish. Artificial cell-derived vesicles created by extrusion or other physical methods should be distinguished from secreted EVs, even when termed exosome mimetics in prior literature. Here, 'mimetic' identifies an engineered preparation rather than certifying biological equivalence. Similarly, a hydrogel is not necessarily nanofibrous, and a hydrated porous structure does not by itself demonstrate extracellular-matrix mimicry.

Three levels of evidence are distinguished throughout: direct findings in a spinal cord injury model; enabling evidence from relevant cell or material systems; and design inferences requiring experimental validation. This distinction is especially important for neurotrophic scaffolds without vesicles, mimetics tested outside the nervous system, and omics atlases generated without therapeutic application. Publication status is recorded for accepted manuscripts available online. Publication status should not be equated with independent replication; a sophisticated formulation likewise does not guarantee that a finding has been reproduced [10].

3. THE Oxidative Stress Microenvironment After Spinal CORD Injury

3.1. Vascular Disruption and Mitochondrial Stress

Mechanical injury impairs blood flow and compromises the integrity of vascular barriers, leading to altered delivery of oxygen and metabolic substrates while permitting exposure of neural tissue to inflammatory mediators and blood-derived components. Mitochondrial dysfunction subsequently links insufficient energy production with increased reactive oxygen species generation, thereby compromising essential cellular functions such as ion homeostasis and plasma membrane stability. The resulting pathological changes exhibit regional heterogeneity: a central lesion zone with extensive structural compromise coexists with adjacent tissue showing partial functional preservation, presenting distinct biological contexts for intervention [11, 12]. A therapeutic strategy aimed at protecting viable cells must therefore be assessed based on its actual biodistribution and accessibility to target tissue, rather than assuming uniform recoverability across the entire injured spinal segment.

Time-resolved analyses of mitochondrial respiration and oxidative damage markers during the initial 24 hours following contusive spinal cord injury support an early association between bioenergetic collapse and oxidative injury [13, 14]. Such dynamic profiling underscores why single time-point measurements may overlook the critical window during which tissue susceptibility emerges. However, isolated mitochondrial assays cannot comprehensively identify all intracellular sources of reactive oxygen species or confirm subcellular delivery of vesicle-based therapeutics to mitochondria.

This pathophysiological context also reframes the interpretation of antioxidant effects. A reduction in oxidant-sensitive readouts may arise from improved microvascular perfusion, attenuated immune cell infiltration, upregulated endogenous antioxidant defenses, or changes in overall cell viability—each implying distinct mechanistic targets and optimal therapeutic timing. Microvascular protection may secondarily promote neuronal redox homeostasis, whereas neuron-targeted interventions may prove ineffective if local perfusion remains insufficient [15]. Consequently, adopting a neurovascular perspective—consistent with frameworks used in ferroptosis research—provides a more comprehensive framework for evaluating delivery systems whose benefits extend beyond neuronal populations. This approach necessitates concurrent assessment of endothelial barrier function, tissue oxygenation status, and neural survival outcomes.

3.2. Lipid Peroxidation and Ferroptosis

Ferroptosis provides a mechanistic link between iron availability, lipid oxidation, and regulated cell death. Foundational studies established its distinction from conventional apoptotic death and identified glutathione peroxidase 4 (GPX4) as a key protective factor. These studies define the biological process but do not directly establish its contribution to spinal cord injury. Direct evidence from spinal cord injury models is required before extrapolating this mechanism from other cellular or disease contexts.

A rat contusion model study using the ferroptosis inhibitor SRS16-86 reported improvements in tissue integrity and functional outcomes, along with corresponding changes in ferroptosis-associated biomarkers. Such pharmacological studies elevate ferroptosis beyond a descriptive label for oxidized tissue, although considerations regarding compound specificity and dependence on the injury model remain important

[16]. In a vesicle–hydrogel study, increased GPX4 expression or decreased lipid-peroxidation products would be consistent with mitigation of ferroptotic stress. More definitive attribution requires demonstration that the ferroptotic pathway is actively engaged and that modulation of the proposed protective mechanism alters the observed therapeutic effect. General reductions in apoptosis or overall reactive oxygen species levels cannot substitute for this specific mechanistic validation.

The vascular compartment has also been investigated directly [13, 17]. A study tested liproxstatin-1 in a rat contusion spinal cord injury model and assessed blood–spinal cord barrier integrity alongside complementary experiments in cultured endothelial cells. The findings associate ferroptosis-related injury with vascular dysfunction and extend the mechanistic rationale beyond neuronal protection. Effects on other cell types could not be ruled out, and the isolated endothelial culture system does not fully recapitulate the complexity of the spinal neurovascular unit. The study supports further investigation of a vascular mechanism rather than serving as a universal explanation for antioxidant-based approaches.

3.3. Antioxidant Responses and Inflammatory Cell States

Nuclear factor erythroid 2-related factor 2 (Nrf2) coordinates a broad stress-response program that has attracted attention in spinal cord injury research. The literature describes its relationship to antioxidant defense and injury responses. Nevertheless, increased abundance of Nrf2-associated proteins does not by itself demonstrate beneficial pathway activation in the relevant cell population. Protein localization, downstream transcription, redox state, and the timing of sampling all affect interpretation. Heme oxygenase 1 (HO-1) also participates in heme metabolism, so its expression cannot be treated as an isolated universal measure of recovery [17].

Direct perturbation evidence was supplied through comparison of wild-type and Nrf2-deficient mice after compression spinal cord injury. Sulforaphane-associated protection differed with Nrf2 status, supporting pathway involvement in that model. A whole-animal genetic comparison constitutes stronger evidence than a correlated marker change, but it does not identify Nrf2 as the necessary mediator of every vesicle treatment. Cell-specific contributions and the actions of a multicomponent formulation remain separate questions [18, 19].

Inflammatory cells further complicate a simple 'less inflammation is better' narrative. Microglia have been shown to contribute to a protective scar response after experimental spinal cord injury; depletion impaired tissue organization and worsened outcomes. This finding does not negate damaging inflammatory activity [20]. It demonstrates that cell identity, location, and function matter. A treatment that suppresses every activated microglial response may remove containment or repair functions together with injurious signals. Accordingly, changes in a few inflammatory markers should be interpreted in the context of tissue preservation and the phase of injury.

3.4. Spatial and Temporal Heterogeneity

Single-cell profiling has resolved diverse cellular responses following spinal cord injury and revealed that macrophage populations do not conform to a simple binary M1/M2 classification. Integrated single-cell and spatial atlases further elucidate the cellular and anatomical organization of injured spinal tissue. These resources enable more precise interpretation of redox-associated gene expression: a shift in bulk-tissue signatures may reflect changes in cellular composition rather than functional recovery within individual cell types [21].

For biomaterials, this implies that a uniform delivery depot interfaces with a spatially and temporally heterogeneous pathological environment. Early intervention may prioritize tissue preservation, whereas later-stage treatment must address persistent inflammation, extracellular matrix remodeling, and neural circuit repair. Optimal vesicle retention duration cannot be determined solely from maximal achievable release kinetics. An effective delivery system must align with dynamic biological requirements while

permitting safe, adaptive evolution of its structure and cargo [22]. Figure 1 summarizes this relationship between injury processes, material functions, and evaluation outcomes.

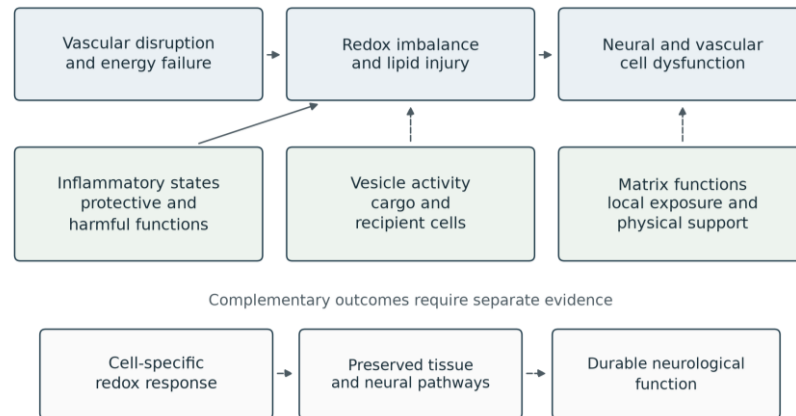


Figure 1. Conceptual Relationships among Oxidative Injury, Vesicle Activity, and Hydrogel Functions After SCI. The Diagram Is an Original Synthesis of Redox and Cellular Evidence [6,10,12], Not an Experimental Result. Dashed Connections Indicate Context-Dependent or Incompletely Established Links.

4. Extracellular Vesicles and Exosome Mimetics

4.1. Biological Activity of Secreted Vesicle Preparations

Mesenchymal stromal cell (MSC)-derived extracellular vesicles (EVs) serve as an important benchmark for the biological activity that engineered mimetics aim to retain or enhance [23]. Human umbilical cord MSC EV preparations have been shown to modulate inflammatory responses and improve multiple recovery parameters in mouse models of spinal cord contusion injury. Dose-dependent effects were observed, indicating that biological origin alone does not determine therapeutic effectiveness. Administration occurred very early post-injury; therefore, these findings do not establish efficacy under conditions reflecting typical clinical delays in delivery. While an anti-inflammatory effect may plausibly contribute to secondary redox protection, it does not constitute direct evidence of reactive oxygen species (ROS) scavenging.

Delivery to recipient cells represents a separate consideration. Intravenously administered MSC EVs have been detected in the injured spinal cord and found to associate with CD206-positive macrophages, suggesting interaction with lesion-associated immune populations. However, this localization does not demonstrate functional reprogramming of macrophage phenotypes, nor does it confirm therapeutic benefit. Trafficking, cellular response, and functional recovery must be evaluated as distinct endpoints, even when collectively described as mechanistic targets [17].

Cargo engineering provides more direct evidence that the biological properties of EV preparations can be deliberately modified. EVs secreted from MSCs genetically modified to overexpress miR-133b have yielded improved outcomes related to neuronal survival and axonal growth in experimental spinal cord injury models. Similarly, EVs derived from MSCs engineered to express miR-126 have been used to examine effects on vascular remodeling and neural repair. These findings support the functional tunability of cargo-engineered EVs, yet they do not equate mechanically synthesized mimetics with naturally secreted EVs. Increases in axonal or vascular markers require validation within appropriate anatomical and functional contexts before being interpreted as evidence of newly formed neural connections or restored tissue perfusion [24].

4.2. Redox Mechanisms and the Limits of Pathway Attribution

A study examined small extracellular vesicles derived from mesenchymal stromal cells cultured under hypoxic conditions, using a rat spinal cord contusion model and hydrogen peroxide-injured rat pheochromocytoma-derived PC12 cells. The results linked antioxidant and anti-apoptotic effects to sirtuin 1 (SIRT1)-associated Nrf2/HO-1 signaling.

Inhibition of SIRT1 diminished protective effects in the cellular model, offering stronger mechanistic insight than changes in protein expression alone [25]. However, this modulation was not applied in the injured animal, and the *in vivo* experimental groups did not include a control group receiving vesicles from normoxically cultured cells. Thus, the data confirm biological activity of the tested vesicle preparation but do not establish that hypoxic conditioning confers superior efficacy *in vivo*.

This case highlights a common interpretive challenge. Vesicle preparations comprise numerous molecular constituents and may influence multiple recipient cell types. A signaling pathway may be activated as a downstream consequence of improved cellular health rather than serving as the primary effector mechanism. Conversely, a pathway shown to contribute meaningfully in a simplified cellular system may account for only a subset of the therapeutic effect observed in the complex, multicellular environment of a spinal cord lesion. Conclusions should therefore specify the experimental level at which mechanistic support is obtained. Demonstrating essential involvement across the full therapeutic context—encompassing the same vesicle cargo, target cells, and physiological setting—requires consistent evidence bridging *in vitro*, *ex vivo*, and *in vivo* levels.

4.3. Manufacturing Mimetics and Engineering Tissue Access

Exosome mimetics encompass diverse fabrication strategies. Serial extrusion of source cells has been used to generate drug-carrying membrane nanovesicles, achieving higher production yield compared with naturally secreted exosomes in that system. This approach was applied in a tumor-targeting context, establishing a scalable manufacturing precedent rather than providing evidence of efficacy in spinal cord injury. The key insight is that membrane-based carriers can be produced through controlled physical processes. Neither the observed yield advantage nor the activity of a chemotherapeutic cargo confirms preservation of the endogenous neuroprotective functions associated with mesenchymal stromal cell–derived extracellular vesicles.

Direct studies in spinal cord injury nevertheless indicate that artificial nanovesicles can exert therapeutic effects. One study combined source-cell preconditioning with iron oxide nanoparticles and magnetic guidance to enhance delivery, reporting improvements in vascular integrity, inflammation modulation, and functional recovery. Another approach fused macrophage membranes with mesenchymal stromal cells prior to extrusion, yielding nanovesicles with enhanced lesion accumulation and recovery-associated outcomes in a mouse compression injury model [26]. Both support the engineering of mimetic delivery systems, yet their multicomponent designs necessitate careful attribution of mechanism. Magnetic targeting, cargo composition, and membrane-derived recognition properties may each contribute distinctively. Neither investigation employed a nanofibrous hydrogel or isolated a general antioxidant property common across all mimetic platforms.

An alternative production route involves using isolated membrane sheets. Ectosome-mimetic vesicles with engineered cargo anchoring have been generated and shown to exhibit lung-directed biodistribution in murine models. Although not conducted in spinal cord injury, this work is instructive because it treats organ-specific distribution as a quantifiable product characteristic rather than an assumed benefit. A preparation that is both scalable and biologically active may still accumulate predominantly outside the spinal lesion. Engineering frameworks further distinguish cell-derived, synthetic, and hybrid vesicle strategies, emphasizing that composition and delivery behavior must be evaluated independently (As shown in Table 1).

Table 1. Vesicle Categories Relevant to the Review. Production Route and Biological Origin Determine Which Comparisons Are Justified.

Category	Representative evidence	Principal advantage examined	Main unresolved comparison
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Secreted MSC EVs	Sun et al. [15]	Endogenous immunomodulatory activity	Effective dose and source dependence
Engineered secreted EVs	Li et al. [17]; Huang et al. [18]	Modified therapeutic cargo	Contribution of engineering versus baseline EV activity
Whole-cell-derived mimetics	Lee et al. [21]; Kim et al. [22]	Production and lesion targeting	Equivalence to secreted EVs and cargo-specific potency
Isolated-membrane mimetics	Liu et al. [23]	Defined membrane and cargo engineering	Applicability of non-SCI distribution findings

4.4. Potency Matters More Than Particle Abundance

A higher particle yield can improve manufacturing feasibility without increasing the number of effective therapeutic doses. Particle counts may include structures with varying cargo, membrane orientation, or integrity. Total protein measurements may also reflect nonvesicular material. Loading efficiency, stability, and reproducible production have been identified as interrelated delivery challenges. Comparisons should therefore report both physical recovery and functional activity in a biologically relevant assay [27]. For a redox-directed spinal cord injury product, that assay must distinguish intact, active preparations from damaged or inactive ones, rather than merely confirming the presence of nanoscale particles.

Mimetics additionally require careful consideration of which cellular components are retained during production. Cell lysis and membrane isolation methods selectively enrich different constituents, while subsequent purification and storage steps may further alter the final preparation. These differences are not inherently problematic; they define distinct product profiles. The central question is whether composition and function remain sufficiently consistent to support predictable clinical performance [28]. Claims regarding immunogenicity, neural targeting, or safety advantages cannot be generalized across all mimetic formulations.

5. Biomimetic Nanofibrous Hydrogels

5.1. Architecture and Presentation of Biological Signals

Hydrogels can occupy irregular spaces and retain water, but their biological functions depend on more than these shared properties [29]. In a nanofibrous system, the molecular organization of the network affects how adhesion motifs are presented and how cells contact the material. Dense presentation of the laminin-derived IKVAV sequence on self-assembled peptide-amphiphile nanofibers was shown to influence neural progenitor differentiation and neurite development. This establishes a principle of nanoscale signal presentation, not proof that every IKVAV-containing formulation will behave similarly.

An IKVAV-presenting nanofiber system was subsequently tested in injured mouse spinal cord, with reported effects on tissue preservation, axonal extension, and behavioral recovery. More recent work linked variation in supramolecular motion within bioactive scaffolds to differences in signaling and repair-associated outcomes. These studies make architecture and molecular dynamics relevant design variables. They do not establish a redox mechanism or a vesicle-delivery benefit, but they show why an apparently identical biochemical motif can have different effects when its physical presentation changes.

A direct example of an RADA16–IKVAV-containing scaffold is a hybrid nanofiber gel combining the designer peptide with silk fibroin and delivering neurotrophin-3 in a rat transection model. It supports the feasibility of this peptide-containing architecture for spinal repair. Its release and biological properties belong to a specific composite carrying a soluble neurotrophic factor; they cannot be assigned unchanged to RADA16–IKVAV

alone or to a formulation carrying membrane vesicles. Replacing the cargo can alter structural formation, diffusion, cell interaction, and degradation [21].

5.2. Retention and Release of Biologically Active Vesicles

A hydrogel may reduce rapid dispersion through physical confinement, adhesive interactions, or reversible association with vesicle surfaces. Sustained local delivery of human mesenchymal stromal cell–derived extracellular vesicle preparations immobilized in an adhesive hydrogel has been reported, accompanied by improvements in the injured spinal environment and recovery-related outcomes. This provides direct evidence for local vesicle retention, though it does not establish that prolonged retention is universally beneficial or that the matrix possesses a peptide-nanofiber architecture [25].

Release must be interpreted in relation to the specific entity being measured: a retained membrane dye, vesicle-associated protein, intact particle, or functional cargo each represent distinct readouts. Binding that prevents early washout may simultaneously hinder transfer to recipient cells. Similarly, matrix degradation may release intact vesicles, damaged membrane fragments, or previously adsorbed soluble material [30]. An informative release study therefore integrates physical measurements with assessment of the biological activity of the recovered fraction—a requirement arising from the conceptual distinction between delivery and functional potency, not an indication that hydrogels inherently compromise vesicle integrity.

Nanofibrous architecture may support neural attachment while functioning as a reservoir, but these roles must be analytically separated. A matrix could enhance neurite extension via ligand presentation even if its vesicle release profile remains unaltered. Conversely, improved retention might augment vesicle-mediated effects independently of any direct neural-adhesion benefit. Comparisons among free vesicles, unloaded scaffolds, and combined formulations constitute essential baseline assessments; however, additional matched material controls are required to isolate the contribution of fiber organization—distinct from confounding variables such as stiffness, surface chemistry, or loading density.

5.3. Matrix Chemistry and Responsive Delivery

Some materials can modulate the redox environment independently of their encapsulated cargo. A conductive hydrogel composed of silk fibroin, black phosphorus, and glycyrrhizic acid demonstrated favorable outcomes in experimental spinal injury models. This supports the concept of a therapeutically active matrix. However, it introduces an attribution challenge: conductivity, reactive species scavenging, and pharmacological activity coexist within the same construct. The presence of a nanomaterial should not be interpreted as evidence of a nanofibrous scaffold or vesicle-mediated delivery mechanism.

Responsive systems aim to couple cargo release with lesion-associated stimuli [31]. In principle, oxidant-sensitive chemical linkages can enhance cargo availability under elevated oxidative conditions. Their functional utility depends on the interplay among the triggering stimulus, local exposure kinetics, and biological demand. If degradation proceeds too rapidly, responsiveness may devolve into uncontrolled burst release; if the material consumes or alters the trigger, release may decelerate before therapeutic needs are fully met. These behaviors are design-dependent and must be evaluated under physiologically relevant exposure conditions—not inferred from single accelerated degradation assays.

5.4. Distinct Forms of Structural Support

Living-cell constructs exhibit additional functionalities beyond vesicle storage. A bioactive nanofiber hydrogel incorporating insulin-like growth factor 1 was employed to support neural stem cells, while also examining paracrine signaling associated with vesicles and extracellular vesicle delivery from the gel matrix. This work links scaffold-based structural support with intercellular communication; however, the multifaceted treatment design does not isolate or confirm an antioxidant mechanism mimicking

mesenchymal stromal cells. Cell viability, growth-factor-mediated signaling, and vesicle-related activity represent interrelated yet experimentally separable contributions.

Neural stem cell-laden hydrogel microfibers reinforced with electrospun nanosheets have been developed to provide spatial guidance and lesion filling during repair. Such organized architectures—characterized by micron-scale fibers, viable cellular payloads, and composite chemical composition—differ fundamentally from self-assembling peptide gels loaded with vesicles. Maintaining conceptual clarity around these distinctions ensures that the term 'fibrous hydrogel' does not obscure the distinct biological challenges each system is designed to address. Within this review, such studies serve as structural references and comparative benchmarks rather than equivalent realizations of a single therapeutic platform.

6. Integrated Delivery Systems and the Strength of Evidence

6.1. From Complementary Components to Combined Treatment

Integrated formulations yield the most robust evidence when their component functions are evaluated within a single injury model. A study combined mesenchymal stromal cell-derived extracellular vesicles with a conductive porous hydrogel and assessed immune responses, neural differentiation, myelinated axon metrics, and functional recovery. The hydrogel matrix alone elicited an unfavorable early inflammatory response, rendering the unloaded control especially informative. These results support complementary roles for the material scaffold and vesicular cargo, while underscoring that a delivery carrier cannot be presumed biologically inert.

Another platform integrated a nanofiber scaffold, hyaluronic-acid hydrogel, Schwann-cell-derived extracellular vesicles, and methylprednisolone, yielding outcomes related to inflammation modulation and neural protection in a rat spinal cord injury model. Here, the corticosteroid functions as an active pharmacological agent, the vesicles originate from Schwann cells, and the nanofiber-hyaluronic-acid composite differs fundamentally from self-assembling peptide matrices [32]. Surface-based application may circumvent direct lesion penetration while still requiring surgical exposure. Such distinctions critically constrain the generalizability of design principles to exosome-mimetic nanofibrous systems.

6.2. Redox-Responsive EV and Mimetic Systems

A study integrated secreted extracellular vesicles derived from three-dimensional human umbilical cord mesenchymal stromal cell cultures, a corticosteroid formulation, surface modifications, and incorporation into a phenylboronic-acid-containing hyaluronic-acid/tannic-acid hydrogel. Responsive release was evaluated alongside oxidative damage, inflammatory, and neural functional outcomes in a rat spinal cord transection model. The integrated formulation demonstrated therapeutic efficacy; however, observed protein-expression changes alone do not confirm the essential role of the Nrf2/HO-1 pathway in mediating its benefits. These engineered secreted vesicles must be distinguished from mechanically produced mimetics and should not be reclassified as such.

Another study employed physically extruded nanovesicles from human umbilical cord mesenchymal stromal cells embedded in a reactive oxygen species-responsive polyvinyl-alcohol hydrogel featuring a boronic ester linker. Local administration was assessed in a rat complete spinal cord transection model, with endpoints including oxidative stress markers, inflammatory parameters, and locomotor recovery. This work provides direct evidence of combined use of synthetic mimetics and redox-responsive matrices. The hydrogel matrix differs from self-assembling peptide systems; limited vesicle characterization in the primary report and a short observational period restrict mechanistic insight and translational inference [33, 34]. Publication status is explicitly noted; independent validation is required regardless of that status.

6.3. What an Integrated Mechanism Would Establish

The existing literature collectively supports convergence, yet distinct types of convergence must be carefully differentiated. For instance, one study may indicate that a scaffold maintains cargo exposure; another, that a vesicle modulates an immune response; and a third, that a peptide enhances neuronal attachment. While such findings support a hypothesis of integration, they do not determine the release profile, redox activity, or functional outcome of the fully assembled product. Table 2 therefore delineates evidence boundaries rather than ranking platforms by apparent efficacy across methodologically incompatible models.

Table 2. Selected Integrated or Closely Related SCI Platforms. The Comparison Emphasizes Actual Components and the Limit of Transfer to an Exosome-Mimetic Nanofibrous Formulation.

Study	Tested platform	Evidence contribution	Important boundary
Feng et al. [29]	RADA16–IKVAV, silk fibroin, neurotrophin-3	Functionalized nanofiber scaffold	No vesicle cargo
Fan et al. [34]	MSC EVs and conductive porous gel	Combined immune and neural effects	Matrix chemistry differs from peptide fibers
Zhu et al. [35]	Schwann EVs, steroid, fiber–hydrogel patch	Local composite delivery	Multiple active components
Cao et al. [36]	Engineered secreted EVs and responsive HA gel	Redox-responsive delivery	Neither mechanical mimetics nor RADA16

A robust integrated mechanism would explicitly link the characterized identity of the vesicle preparation to active local exposure, a specific recipient-cell response, preservation of tissue integrity, and sustained functional performance. Claims of synergy necessitate demonstrable interaction beyond the sum of individual component effects, assessed within a clearly defined comparative framework. Although a combination surpassing either constituent alone indicates a practical advantage, it does not inherently confirm supra-additive effects. Additive contributions may still deliver substantial value—provided the increased manufacturing and delivery complexity is consistently offset by reproducible functional benefit [35].

7. Mechanistic and Functional Evaluation

7.1. Separating Redox Measurements from Mechanistic Conclusions

Oxidative stress requires convergent assessment because commonly used readouts address distinct biological questions. Fluorescence from widely used probes is not uniquely specific to a single reactive species and can be influenced by probe chemistry. A lower signal after treatment therefore reflects the assay performance under its specific measurement conditions, not necessarily a quantitative reduction in total lesion reactive oxygen species. Materials that absorb, scatter, quench, or interact with assay reagents also require appropriate background controls [26]. These considerations are especially relevant when comparing chemically distinct hydrogels.

Lipid-damage products, glutathione status, mitochondrial function, and antioxidant enzyme activity provide complementary information. Concordance among these parameters strengthens interpretation, but combining them into an arbitrary composite score does not yield a validated biological endpoint. The same principle applies to protein markers: increased GPX4 abundance is distinct from preserved enzymatic activity, and higher Nrf2 expression is distinct from demonstrated pathway engagement. Mechanistic claims become more persuasive when orthogonal observations align with experimental perturbation or functional restoration evidence at the relevant biological level (As shown in Table 3).

Table 3. Interpretation of Common Evidence Used in Redox-Directed SCI Studies. These Distinctions Guide Literature Appraisal Rather Than Prescribe a New Experiment.

Readout	What it can support	What it cannot establish alone
Oxidant-sensitive fluorescence	Change in an oxidation-sensitive assay	Identity or absolute flux of all ROS
Lipid-damage products	Accumulated oxidative membrane injury	Ferroptosis as the exclusive death mechanism
Nrf2 or GPX4-associated measures	Antioxidant-response or defense status	Pathway necessity for treatment benefit
Vesicle-associated fluorescence	Distribution of the tracked label	Intact, functional cargo delivery
Transcriptomic stress signatures	Cell-state-associated expression	Direct ROS concentration or treatment efficacy
Locomotor rating scales	Behavioral recovery within a model	Causal redox mechanism or new circuit formation

7.2. Omics as Biological Context Rather Than Retrospective Validation

Single-cell and spatial datasets can identify populations and lesion regions in which a candidate mechanism is plausible. Publicly available atlases of this kind are valuable resources. Their public availability does not make them treatment-response datasets for formulations they were not designed to evaluate. A signature that separates injured from uninjured samples may reflect altered cell composition, inflammation, or general cellular stress. Classification performance alone would not demonstrate that a vesicle-loaded scaffold reverses those processes.

These atlases help compare biological contexts and identify questions about target cells and timing. Evidence of redox-state modification within a cell type should remain distinct from changes in that population's abundance. Spatial proximity between a marker and a lesion edge can guide interpretation but does not establish transfer of therapeutic cargo to those cells. This analytical restraint enhances the interpretability of omics data by aligning each observation with the specific conclusion it directly supports.

7.3. Tissue Preservation and Neurological Outcomes

The Basso–Beattie–Bresnahan scale provides a standardized method for assessing open-field locomotor function in rat models. Behavioral evaluation remains critical, as favorable molecular changes do not necessarily translate into functional improvement. Motor recovery may result from preservation of existing neural pathways, synaptic plasticity, compensatory mechanisms, or formation of new connections. However, axonal-marker staining combined with elevated locomotor scores alone cannot differentiate among these underlying processes. Complementary insights can be obtained through electrophysiological assessment, anatomical tracing, and carefully selected sensory or autonomic outcome measures. It is also essential to distinguish neuronal preservation from neuronal regeneration: although both may contribute to therapeutic benefit, they represent distinct biological phenomena. Figure 2 outlines these sequential evidential stages—from product characterization to durable functional benefit.

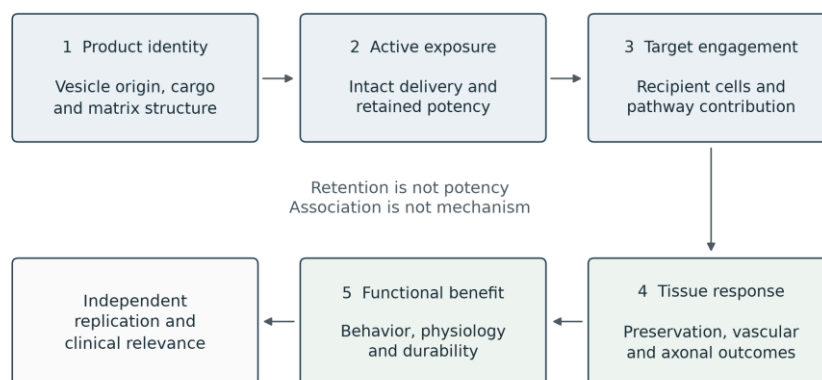


Figure 2. Evidence Connecting an Integrated Formulation to a Therapeutic Claim. Product Identity, Active Exposure, Mechanism, Tissue Response, and Function Are Separate Evidential Stages. The Sequence Is an Original Appraisal Framework Informed by Vesicle and Animal-Research Guidance [5,37]; Success at One Stage Does Not Establish the Next.

8. Translational Challenges and Research Priorities

8.1. Reproducibility Across the Complete Product

Translation requires a reproducible combination rather than a well-characterized carrier and an independently described cargo. Changes in source cells, conditioning, fabrication, purification, loading, or storage can alter performance. Vesicle guidance emphasizes identity, separation, characterization, and functional controls, while delivery research identifies loading and stability as additional obstacles. For an integrated product, specifications should include relevant matrix properties and the activity of vesicles after incorporation and release. A release test using fresh material cannot establish the performance of a stored final formulation.

The practical manufacturing endpoint is a consistent active dose, not the largest particle count. Batch comparisons should therefore connect physical measurements with a justified potency assay and demonstrate that the matrix does not invalidate the assay. A source change or scale-up that preserves particle size but changes biological activity should be treated as a material change to the product [36, 38]. These principles apply to both secreted extracellular vesicles and mimetics, although their sources of variability differ. Artificial production offers greater control over some steps without removing the need to characterize the final material.

8.2. Delivery Windows and Safety

Administration must be considered in the context of the clinical setting it is intended to serve. Direct placement can provide localized exposure but requires access to delicate spinal tissue; systemic delivery avoids intralaminar intervention while introducing challenges related to distribution and clearance. Treatment administered shortly after a controlled rodent injury does not demonstrate feasibility under conditions involving delays typical of clinical stabilization. Similarly, a construct designed to bridge a defined transection gap addresses a distinct anatomical scenario compared to an irregular contusion with residual neural tissue. These distinctions should inform development strategies rather than be obscured by the generic term SCI.

Safety evaluation must assess the complete formulation over a clinically relevant duration. Key considerations include local compression or edema, sustained inflammation, off-target dispersion, degradation byproducts, and unintended biological effects of incorporated therapeutic agents or nanoscale components. A material that enhances early cellular survival may still disrupt subsequent tissue remodeling. Conversely, overly rapid degradation may eliminate structural support before fulfilling its intended function. This balance is inherently formulation-dependent; descriptors such

as biomimetic, natural, or cell-free do not substitute for empirical evidence [39]. Claims of clinical readiness require comprehensive data extending beyond short-term cell viability assays and single-rodent efficacy studies.

8.3. More Discriminating Preclinical Evidence

The ARRIVE 2.0 guidelines specify essential reporting elements required to rigorously evaluate animal research, including study design, justification of sample size, randomization procedures, blinding methods, criteria for exclusions, and approaches to outcome analysis. In this field, animal-level replication must be clearly distinguished from multiple tissue sections or imaging fields derived from a single animal [37]. Similarly, repeated behavioral assessments do not constitute additional independent experimental units. Transparent reporting of animal deaths, missing outcome data, and use of replacement animals is critical for accurately interpreting apparent treatment effects rather than treating such information as administrative detail.

The most informative subsequent studies would target a specific unresolved question rather than incrementally expanding formulation complexity [27]. Key priorities include experimental comparisons that disentangle vesicle-mediated activity from the influence of matrix chemistry, direct evidence that cargo released from the construct retains biological functionality, and in vivo validation of whether a proposed redox mechanism meaningfully contributes to therapeutic benefit. Evaluating delayed intervention timing, extending follow-up duration, and replicating findings across distinct injury models would address fundamental translational questions that additional early biomarker profiling cannot resolve. Independent reproduction of a precisely defined preparation holds particular value, as complex interventions risk accumulating favorable endpoints without establishing a consistent, mechanistically grounded explanation for efficacy.

9. Conclusion

Exosome mimetics and biomimetic nanofibrous hydrogels represent a promising strategy for local neuroprotection following spinal cord injury, as they collectively address biological signaling, delivery persistence, and the physical microenvironment of surviving neural tissue. While individual functions—such as vesicle-mediated cargo delivery or hydrogel-mediated structural support—are increasingly well characterized, integrated vesicle–hydrogel systems remain under active investigation. Direct mimetic delivery and redox-responsive matrices have advanced into preclinical spinal cord injury models. However, evidence derived from disparate cargos, matrix formulations, and injury paradigms cannot predict the performance of an untested composite system.

Oxidative-stress modulation is most compelling when exposure metrics, cell-specific mechanisms, tissue preservation, and durable neurological outcomes converge. Reduced oxidation-sensitive fluorescence or elevated antioxidant-associated proteins are informative but insufficient alone to confirm sustained reprogramming of the local microenvironment. Future progress requires precise definition of the therapeutic product, mechanistic deconvolution of each active component, and evaluation under clinically relevant conditions. A rigorous alignment between material engineering design and biological validation provides a more robust foundation for translation than the assumption that increased structural complexity inherently enhances therapeutic efficacy.

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