

REVIEW ARTICLE

Neural stem cell-derived exosomes as a regenerative strategy for traumatic brain injury

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Abstract

Traumatic brain injury (TBI) is a leading cause of death and long-term disability worldwide, and current management rarely restores higher-order neurological function. Neural stem cell (NSC) transplantation offers regenerative potential but is constrained by poor graft survival, immune rejection, tumorigenic risk, and challenges in crossing the blood–brain barrier (BBB). These limitations have driven interest in exosome-based cell-free strategies. Human NSC-derived exosomes (hNSC-Exos) can cross the BBB and deliver neurotrophic factors and microRNAs to injured neural circuits, providing a biologically aligned vector for TBI treatment. This narrative review synthesizes evidence on hNSC-Exos as regenerative agents for TBI. We summarize their neurotrophic and neuroprotective properties and key mechanisms in preclinical models, including signal transducer and activator of transcription 3-mediated microglial polarization, phosphatase and tensin homolog-induced kinase 1/Parkin-dependent mitophagy, phosphoinositide 3-kinase/protein kinase B anti-apoptotic signaling, and extracellular signal-regulated kinase 1/2-driven synaptic plasticity, alongside effects on angiogenesis and BBB restoration. This review examines major translational barriers: donor and culture-related heterogeneity, the lack of standardized, good manufacturing practice-compatible isolation and characterization methods, limited biodistribution and dosing data, and the absence of early clinical trials in TBI. Finally, the review outlines priorities in exosome engineering, intranasal and other minimally invasive delivery routes, and biomarker-based potency assays to advance hNSC-Exos toward clinically actionable, cell-free regenerative therapies for TBI.

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1. Introduction

Traumatic brain injury (TBI) is a major cause of death and disability worldwide. Recurrent head trauma and the resultant long-lasting neurological complications contribute substantially to morbidity, loss of independence, and healthcare expenditures. Recent global burden estimates highlight an alarming rise in TBI-related disability, with millions of people living with TBI and tens of millions of new cases reported each year.¹

From a pathological perspective, TBI can be understood as an injury that first causes a focal mechanical trauma, followed by a protracted insult involving multilayered mechanisms of secondary brain injury, including excitotoxicity, oxidative stress, blood–brain barrier (BBB) disruption, neuroinflammation, and delayed cell death. These mechanisms cause a secondary, progressive loss of brain tissue and permanently disrupt neural circuits. As the adult central nervous system has a limited capacity for self-repair, clinical approaches that simply stabilize patients in the acute phase rarely restore lost structure or higher-order function. This therapeutic gap has motivated intensive work on regenerative strategies capable of reconstituting neural tissue and promoting circuit-level recovery.

Their capacity for self-renewal and lineage-specific differentiation into neurons, astrocytes, and oligodendrocytes underpins the regenerative potential of neural stem cells (NSCs). However, NSC transplantation has so far failed to fully realize this potential because of low survival and engraftment in the hostile post-injury environment, immune rejection, potential tumorigenesis, and the complexities of cell delivery across the BBB. All of these factors, individually and collectively, have stymied clinical progress. This has led researchers to focus on the paracrine actions of stem cells, rather than on cell-replacement therapies.²

Among promising cell-free effectors of stem cell-mediated repair, exosomes have gained increasing clinical and translational attention. Exosomes are nanometer-sized, lipid-bilayer microvesicles that contain and transport proteins, lipids, and a variety of RNAs, including microRNA (miRNA). They can circulate systemically, are biologically stable in body fluids, have low immunogenicity, and can cross the BBB to reach the injured brain, making them favorable therapeutic vectors for central nervous system disorders. Preclinical studies on exosomes derived from cells, particularly mesenchymal stromal cells, have shown promise in TBI models. These include the suppression of neuroinflammation, reduction of apoptosis, increased angiogenesis, and improved functional outcomes, thereby validating the exosome-based, cell-free therapeutic approach.³

Among exosomes derived from NSCs, human NSC (hNSC)-derived exosomes (hNSC-Exos) have attracted particular interest as practical treatment candidates. Because NSCs share developmental and anatomical affinities with brain tissue, hNSC-Exos are likely to be enriched in pro-neurogenic and neurotrophic cargo that supports repair processes. Mechanistic studies have identified specific miRNAs within hNSC-Exos that regulate NSC fate and neuronal maturation. For example, miR-9 within hNSC-Exos promotes NSC differentiation

and maturation into neurons by repressing *Hes1*, providing a direct molecular link among exosomal cargo, neural cell lineage commitment, and neural circuitry repair.⁴

Another frequently observed mediator in exosomal biology is miR-21, which has gained recognition for its neuroprotective and anti-inflammatory properties across numerous studies of cell-derived extracellular vesicles (EVs). In models of experimental brain injury, exosomal delivery of miR-21 promotes neuronal survival and activates pro-survival signaling pathways (e.g., phosphatase and tensin homolog [PTEN]/protein kinase B [AKT]), demonstrating that a single exosomal miRNA can exert multiple protective effects on damaged neural tissue. While there have been numerous studies on miR-21 that utilize MSC- or neuron-derived exosomes, they serve a broader purpose by exemplifying the mechanisms that hNSC-Exos are most likely to employ and by suggesting specific engineering approaches (cargo enrichment or loading) to enhance therapeutic potency.⁵

Despite promising preclinical data, critical deficiencies still hinder the clinical application of hNSC-Exos for TBI. These deficiencies include mechanistic gaps (e.g., which miRNAs and proteins are most relevant across different injury contexts), the absence of standardized, quality-controlled isolation protocols, limited biodistribution and dosing data, and a paucity of evidence beyond rodent models. A focused and timely synthesis of the hNSC-Exos literature can integrate diverse molecular and functional mechanisms (including miRNA-mediated effects) with translational barriers, thereby helping to prioritize standardization efforts and move hNSC-Exos from preclinical promise toward clinical investigation.

This review synthesizes current evidence on exosomes derived from NSCs, with a particular focus on hNSC-Exos as cell-free therapeutics for TBI. We first outline epidemiological data underscoring the need for regenerative approaches, then summarize the biological properties of exosomes and the molecular cargo of NSC-derived exosomes, including validated mediators, such as miR-9 and miR-21. We next review preclinical evidence of therapeutic benefit and discuss key barriers to translation into clinical testing. Our goal is to highlight areas with the strongest support, identify critical knowledge gaps, and propose a framework for experimental and policy advances that could enable the safe and effective clinical development of hNSC-Exos for TBI.

2. Overview of exosomes and NSCs

2.1. NSCs and their regenerative potential

NSCs are highly specialized multipotent cells that can self-renew and differentiate into neurons, astrocytes, and

oligodendrocytes, making them central to brain development and repair. They are prevalent during embryogenesis and persist into adulthood.⁶ During embryonic development, radial glial NSCs undergo highly regulated symmetric and asymmetric divisions that generate the complex structures of the central nervous system.⁷ In adults, NSCs are mainly located in the subventricular zone of the lateral ventricles and the dentate gyrus of the hippocampus. Although they remain relatively quiescent under baseline conditions, they contribute to neuroplasticity, learning and memory, and the response to injury, aging, and disease.⁶ These processes are controlled by complex interactions among intrinsic transcription factors (e.g., sex-determining region Y-box 2 [SOX2] and B lymphoma Moloney Murine leukemia virus insertion region 1 homolog), epigenetic processes, and niche-derived extrinsic signals, such as growth factors, neurotransmitters, and components of the extracellular matrix. Such regulation is vital to avoid premature depletion, inappropriate differentiation, or tumorigenesis.⁷ Recent research has shown that, in addition to their cellular functions, NSCs also release exosomes, small EVs enriched with proteins, lipids, and RNAs, further expanding their capacity to modulate neural repair.²

2.2. Exosomes as mediators of intercellular communication

Exosomes are nanoscale vesicles, typically 40–100 nm in diameter, surrounded by a phospholipid bilayer and secreted by almost all mammalian cells. The term “exosome” was first used in 1987, when Johnstone *et al.* described these vesicles in sheep reticulocytes. Since then, exosomes have attracted growing scientific attention for their roles in communication and in modifying disease processes.^{2,8–10} These vesicles serve as vehicles of lipids, proteins, and nucleic acids, thus enabling long-range communication between cells and modulating immune and metabolic responses.^{11–14} Exosomes are increasingly recognized for their potential use in diagnostics and therapeutic delivery due to their impact on key cellular physiological processes, such as apoptosis, inflammation, and angiogenesis.^{12,14–16} hNSC-Exos are particularly advantageous for treating TBI. In contrast to stem cell transplantation, hNSC-Exos can cross the BBB and deliver neuroprotective factors to modify local and remote cellular environments without the risks of immune rejection or tumor formation.^{2,17–19} These properties make hNSC-Exos promising candidates for non-cellular regenerative therapies to repair neural injury.

2.3. NSC-derived exosomes as cell-free therapeutic agents

Exosomes share fundamental structural and communication features, including those derived from

hNSCs. However, hNSC-Exos differ from generic exosomes in clinically meaningful ways, particularly in TBI, where targeted delivery of neurotrophic and anti-inflammatory molecules is essential.

Unlike exosomes derived from non-neural cell types, hNSC-Exos are enriched with neurotrophic factors, anti-inflammatory mediators, and pro-neurogenic miRNAs, which enhance their ability to cross the BBB and support repair of injured neural tissue.^{11,12,20,21} These characteristics also confer a lower immunogenic risk compared to stem cell transplantation.^{2,17,18,22} For these reasons, hNSC-Exos represent a more suitable and biologically aligned therapeutic vector for TBI than generic exosomes.^{23,24} The main differences between generic exosomes and hNSC-Exos are presented in Table 1.

A transformative, cell-free approach to neuroregeneration emerges from integrating stem cell biology with exosome research. NSCs contribute to neural repair not only through direct differentiation but also through exosome-mediated paracrine signaling. As endogenous nanoscale messengers, hNSC-Exos carry cell-specific therapeutic cargo while avoiding major translational barriers of cellular therapy, including immune rejection and tumorigenesis.

Table 1. Comparison between general exosomes and human neural stem cell-derived exosomes

Characteristics	General exosomes	hNSC-Exos
Origin	Secreted by most mammalian cells	Secreted specifically by hNSCs
Content	Lipids, proteins, RNA (varies by cell type)	Enriched with neurotrophic factors, anti-inflammatory, and neuroprotective molecules
Functions	General intercellular communication, immune signaling, and metabolic regulation	Promote neurogenesis, synaptic plasticity, and reduce neuroinflammation
BBB penetration	Variable and often limited	Efficiently cross the BBB
Therapeutic use	Broad research applications, less cell-specific	Strong potential for treating neurological diseases, especially TBI
Immunogenicity	Possible depending on source and purity	Lower risk than direct stem cell therapy
Clinical potential in TBI	Still under exploration	Strong potential; demonstrated ability to promote repair and functional recovery

Abbreviations: BBB: Blood–brain barrier; hNSC: Human neural stem cells; hNSC-Exos: Human neural stem cell-derived exosomes; RNA: Ribonucleic acid; TBI: Traumatic brain injury.

In particular, hNSC-Exos are neuroprotective, anti-inflammatory, capable of crossing the BBB, and able to modulate damaged neural microenvironments, positioning them as a compelling bridge between fundamental neurobiology and translational regenerative medicine for TBI.

3. Metabolic and signaling pathways modulated by hNSC-Exos

This section details the key molecular pathways modulated by hNSC-Exos post-TBI, which involve core

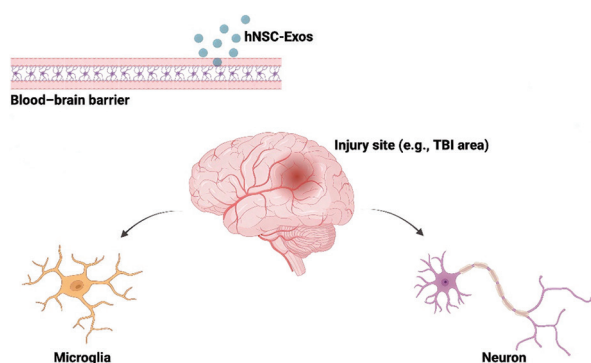


Figure 1. Mechanism of action of human neural stem cell-derived exosomes (hNSC-Exos) in traumatic brain injury (TBI). The diagram illustrates how hNSC-Exos traverse the blood-brain barrier following TBI and selectively target neurons and microglia, exerting neuroprotective and regenerative effects. Created with BioRender.com. Ng, N. (2025). <https://app.biorender.com/illustrations/68f8482acf4d49d321521ad3?slideId=6e491fca-56bd-436e-a0a8-28a344dce2d6>.

neuroprotective, anti-inflammatory, and neuroregenerative signaling mechanisms essential for neuronal survival and functional restoration. Figure 1 provides the mechanistic outline, while Table 2 summarizes these pathways alongside their therapeutic relevance.

4. Molecular mechanisms mediated by hNSC-Exos

The modulation of neural repair by hNSC-Exos occurs through multiple core signaling pathways, conferring potent neuroprotective and neuroregenerative effects after TBI. hNSC-Exos contain a diverse repertoire of bioactive molecules—including proteins, miRNAs, and neurotrophic factors—that enhance inflammatory resolution, maintain mitochondrial quality, protect neurons, and enhance synaptic plasticity. The following subsections describe the primary pathways altered by hNSC-Exos within the injured brain microenvironment.

4.1. Signal transducer and activator of transcription 3 (STAT3) pathway inhibition and microglial polarization

Neuroinflammation following TBI is reduced by hNSC-Exos, primarily through suppression of the STAT3 pathway. Reduced STAT3 phosphorylation diminishes expression of pro-inflammatory mediators, such as inducible nitric oxide synthase and tumor necrosis factor- α , thereby decreasing the M1 (pro-inflammatory) phenotype. Simultaneously, hNSC-Exos promote M2 (anti-inflammatory) polarization

Table 2. Key signaling and metabolic pathways modulated by human neural stem cell-derived exosomes in traumatic brain injury repair

Signaling pathway	Target cells/sites	Molecular actions	Therapeutic effect	References
STAT3	Microglia	<ul style="list-style-type: none"> • Inhibits STAT3 activation • $\downarrow$ct markers (TNF-α, iNOS) • M2 markers (Arg-1, CD206) 	<ul style="list-style-type: none"> • $\downarrow$Neuroinflammation • $\uparrow$Neurorepair 	2, 25, 26
PINK1/Parkin	Neurons	<ul style="list-style-type: none"> • $\uparrow$neuron and Parkin expression • Initiates mitophagy • Removes damaged mitochondria 	<ul style="list-style-type: none"> • $\downarrow$mitochondr stress • Restores mitochondrial functiown 	27-30
PI3K/AKT	Neurons	<ul style="list-style-type: none"> • Activates PI3K, resulting in AKT phosphorylation • $\downarrow$Bax, $\downarrow$ax, phoryla$\uparrow$ax, ph 	<ul style="list-style-type: none"> • Prevents neuronal apoptosis • Promotes cell survival 	31-33
ERK1/2 (MAPK)	Neurons	<ul style="list-style-type: none"> • Activates ERK1/2 cascade • $\uparrow$ascadeic proteins • $\uparrow$Neurite outgrowth 	<ul style="list-style-type: none"> • Enhances synaptic plasticity • Improves cognitive function 	31, 34-36
General pathways	Brain parenchyma, BBB	<ul style="list-style-type: none"> • Anti-inflammatory • Anti-apoptotic • Neuroregenerative 	<ul style="list-style-type: none"> • Facilitate BBB crossing • Deliver miRNAs, proteins, and neuroprotective molecules 	2, 22, 31

Notes: $\uparrow$ Upregulate; $\downarrow$ Downregulate.

Abbreviations: AKT: Protein kinase B; Arg-1: Arginase-1; Bax: Bcl-2 associated X protein; BBB: Blood-brain barrier; Bcl-2: B-cell lymphoma 2; CD206: Cluster of differentiation 206; ERK1/2: Extracellular signal-regulated kinase 1/2; hNSC: Human neural stem cell; iNOS: Inducible nitric oxide synthase; M1: Classically activated microglia; M2: Alternatively activated microglia; MAPK: Mitogen-activated protein kinase; PI3K: Phosphoinositide 3-kinase; PINK1: Phosphatase and tensin homolog-induced kinase 1; STAT3: Signal transducer and activator of transcription 3; TBI: Traumatic brain injury; TNF- α : Tumor necrosis factor alpha.

by enhancing the expression of arginase-1 (Arg-1) and cluster of differentiation (CD)206. This phenotypic shift toward a tissue-protective milieu supports neuroprotection, promotes cellular repair, and facilitates the regeneration of injured neural tissue.^{2,25,26}

4.2. Phosphatase and tensin homolog-induced kinase 1 (PINK1)/Parkin pathway activation and mitophagy regulation

Another key mechanism involves activation of the PINK1/Parkin pathway, which regulates mitophagy—the selective removal of damaged mitochondria. hNSC-Exos enhance neuronal expression of PINK1 and Parkin, promoting the sequestration and degradation of dysfunctional mitochondria. This process reduces reactive oxygen species (ROS), restores mitochondrial membrane potential, and prevents apoptotic signaling. By maintaining mitochondrial quality and cellular energy balance, hNSC-Exos protect neuronal integrity and function during ischemic stroke and TBI.^{27–30}

4.3. Phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) pathway activation and neuronal survival

hNSC-Exos also activate the PI3K/AKT pathway, a central regulator of neuronal survival. Once internalized by injured neurons, hNSC-Exos stimulate PI3K activation, leading to AKT phosphorylation and downstream inhibition of pro-apoptotic mediators, such as B-cell lymphoma 2 (Bcl-2)-associated X protein (Bax) and caspase-3. Concurrently, anti-apoptotic Bcl-2 expression is upregulated. These combined actions promote neuronal survival, reduce apoptosis, and facilitate structural and functional recovery following TBI.^{31–33}

4.4. Extracellular signal-regulated kinase 1/2 (ERK1/2) pathway activation and synaptic plasticity

The ERK1/2 branch of the mitogen-activated protein kinase cascade is essential for neuronal growth and synaptic remodeling. hNSC-Exos stimulate ERK1/2 phosphorylation, increasing the expression of synaptic proteins, enhancing neurite outgrowth, and promoting neural network reorganization. This leads to improved synaptic plasticity and the restoration of cognitive and motor functions impaired by TBI. Sustained modulation of the ERK1/2 pathway by hNSC-Exos supports long-term neuroregeneration and functional enhancement.^{31,34–36}

4.5. Comparative appraisal of key signaling pathways

The available data indicate that the major pathways described above do not contribute equally to hNSC-Exos-

mediated repair after TBI. Among them, PI3K/AKT and PINK1/Parkin signaling pathways currently have the most direct support from preclinical models in which exosome administration reduces neuronal apoptosis, preserves mitochondrial function, and improves behavioral outcomes. In rodent TBI and related central nervous system injury models, exosome-enriched preparations were reported to activate AKT, downregulate Bax and caspase 3, and upregulate Bcl-2 in injured cortex or hippocampus, in parallel with improved neurological severity scores and reduced lesion volume.^{2,3,22,31,37,38} Similarly, activation of PINK1 and Parkin is associated with enhanced mitophagy, decreased ROS production, and restored mitochondrial membrane potential following administration of stem cell-derived exosomes.^{27,29,30,39} These convergent findings suggest that anti-apoptotic and mitochondrial quality control mechanisms represent core effectors of exosome-mediated neuroprotection in TBI and related disorders.

In contrast, modulation of ERK1/2 and STAT3 is supported by a heterogeneous body of evidence that spans multiple central nervous system injury paradigms. ERK1/2 activation has been associated with neurite outgrowth, synaptic protein upregulation, and angiogenic responses in models of TBI, ischemia, and spinal cord injury treated with NSC, mesenchymal stromal cell, or Schwann cell-derived exosomes, as well as in broader studies of neurological disease.^{31,34,35,40–43} In several of these reports, ERK1/2 is one of several concurrently activated cascades rather than a clearly isolated driver. Similarly, inhibition of STAT3 phosphorylation, with a shift from M1 to M2 microglial phenotypes, is a recurring observation in exosome-based interventions and in non-exosomal models of neuroinflammation, yet much of the mechanistic evidence derives from mesenchymal stromal cell exosomes or from inflammatory and ischemic settings rather than TBI-specific paradigms.^{2,25,26,44–46} As a result, the relative importance of ERK1/2 and STAT3 modulation for hNSC-Exos in TBI remains less firmly established than that of PI3K/AKT or PINK1/Parkin signaling pathways.

These pathways also operate on distinct but interacting cellular targets. STAT3 repression and associated M2 polarization predominantly shape the microglial and macrophage compartment and are therefore central to the resolution of neuroinflammation and modulation of glial scar formation.^{25,26,44,45,47} In contrast, PI3K/AKT and PINK1/Parkin signaling pathways are primarily engaged in neurons, where they regulate survival signaling, mitophagy, and mitochondrial quality control, and in some studies also influence oligodendrocyte and astrocyte resilience.^{27,29–31,39,48,49} ERK1/2 signaling pathway spans neurons and endothelial cells, linking synaptic remodeling with microvascular plasticity and thereby coupling

neuronal network reorganization to restoration of cerebral perfusion and BBB function.^{34,35,37,42,50-55} The temporal profile is likely to differ as well. Mitochondrial and anti-apoptotic pathways appear most prominent in the acute and early subacute phases after injury, whereas sustained ERK1/2 activation and microglial reprogramming may be more relevant to longer-term circuit reorganization and functional recovery.^{2,31,34,47,51}

An important limitation is that a substantial proportion of mechanistic work has been conducted with non-hNSC-Exos, including preparations derived from mesenchymal stromal cells, Schwann cells, induced pluripotent stem cell-derived NSCs, and brain endothelial cells, and in models of stroke, spinal cord injury, or generic neurotrauma rather than TBI.^{3,23,38,39,43,52,56,57} These studies provide a useful mechanistic framework but introduce uncertainty because exosomal cargo composition is highly source and context-dependent.^{17,18,31,58} It remains unclear to what extent hNSC-Exos reproduce the same balance of PI3K/AKT, PINK1/Parkin, ERK1/2, and STAT3 modulation when administered after TBI, and whether one or more of these pathways is necessary or sufficient for therapeutic benefit. Future work should therefore prioritize head-to-head comparisons of hNSC-Exos with other EV sources in standardized TBI models, integrate dose- and time-course designs that probe specific post-injury windows, and combine pathway-specific inhibition or genetic manipulation with functional and biomarker readouts.^{2,31,42,59,60} Such studies will be essential for distinguishing dominant mechanisms from ancillary ones and for rationally guiding the engineering of hNSC-Exos for maximal therapeutic potency.

5. Characterization and functional assessment of hNSC-Exos

5.1. *In vitro* isolation and characterization

In a previous study by Abedi *et al.*,²² hNSCs were cultured in Dulbecco's Modified Eagle Medium. hNSCs were isolated from the collected supernatant, and immunocytochemical staining was performed to confirm the NSC phenotype by assessing SOX2 and nestin expression. The isolated vesicles exhibited characteristic morphology and size distributions consistent with those of general exosomes, as verified by standard biophysical and imaging methods.

5.2. *In vivo* functional assessment

For *in vivo* functional assessment, Abedi *et al.*²² utilized male Wistar rats to establish a moderate TBI model. Cortical injury was induced in the rats by removing the dura mater. Both hNSC- and hNSC-Exos-treated rats demonstrated progressive improvements in neurological function, with the hNSC-Exos-treated cohort exhibiting significant

recovery by week four. Quantitative real-time polymerase chain reaction (qRT-PCR) confirmed upregulation of stemness-associated markers, such as SOX2 and nestin, while glial fibrillary acidic protein immunohistochemistry verified astrocyte activation in response to cortical injury.²²

5.3. Characterization methods

Several characterization methods, such as transmission electron microscopy (TEM), scanning electron microscopy (SEM), and dynamic light scattering (DLS), were employed by Abedi *et al.*²² to characterize the vesicular morphology and size range of hNSC-Exos. TEM was used to visualize membrane-bound vesicles, SEM to reveal spherical ultrastructures, and DLS to indicate a monodisperse population consistent with exosomal dimensions. Gene expression profiling by qRT-PCR further supported neurogenic activity associated with hNSC-Exos. Nevertheless, characterization was limited by the absence of exosomal surface marker validation (CD9, CD63, CD81) and incomplete profiling of the molecular cargo.²²

5.4. Reliability, reproducibility, and sources of variability

Although the polyethylene glycol (PEG)-based isolation method demonstrates procedural consistency, it carries a known risk of co-precipitating protein contaminants, potentially compromising the purity of hNSC-Exos.^{2,58} Variations in sample handling and processing may also alter vesicle structure and functional integrity. While electron microscopy characterization provides useful information, TEM is susceptible to structural distortion due to electron beam exposure, which limits absolute confidence in morphological fidelity.⁵⁸ Small sample size and short observation period (<28 days) reduce reproducibility and statistical power, underscoring the importance of independent replication and longer-term studies.⁶¹

To validate the biological relevance of hNSC-Exos, both *in vitro* and *in vivo* assessments were conducted, as summarized in Table 3.

Beyond analytical limitations, the therapeutic consistency of hNSC-Exos is strongly influenced by donor heterogeneity. Differences in NSC source (fetal, adult, or induced pluripotent stem cell-derived), donor age, genetic background, and disease status can all alter exosomal protein and miRNA cargo, immunomodulatory profiles, and yield.^{8,10,17,31,58} For example, passage-dependent drift in NSC phenotype and epigenetic state may shift expression of neurotrophic and anti-inflammatory mediators within released vesicles, thereby changing potency even when isolation methods remain unchanged. To reduce this source of variability, translational programs will likely

Table 3. *In vitro* and *in vivo* assessment of human neural stem cell-derived exosomes

Parameter	<i>In vitro</i>	<i>In vivo</i>
Model/system	Cultured hNSCs in DMEM supplemented with B27, N2, EGF, FBS, antibiotics	Male Wistar rats with a moderate TBI
Treatment method	PEG-based isolation	Exosome treatment administered post-injury
Validation method	SOX2, nestin immunostaining confirmed stemness	mNSS assessed neurological recovery
Verification method	TEM, SEM, and DLS confirmed nanoscale vesicular structures	GFAP staining for astrocyte activation
Functional indicators	Gene expression profiling (SOX2, nestin upregulation)	Improved neurological recovery by week four
Limitations	Lacked surface marker validation (CD9, CD63, CD81)	Small sample size and limited observation period

Abbreviations: B27/N2: Nutrient supplements; CD: Cluster of differentiation; DLS: Dynamic light scattering; DMEM: Dulbecco's Modified Eagle Medium; EGF: Epidermal growth factor; FBS: Fetal bovine serum; GFAP: Glial fibrillary acidic protein; hNSC: Human neural stem cell; mNSS: Modified neurological severity score; PEG: Polyethylene glycol; SEM: Scanning electron microscopy; SOX2: Sex-determining region Y-box 2; TBI: Traumatic brain injury; TEM: Transmission electron microscopy.

require well-characterized master cell banks, strict donor eligibility criteria, and routine phenotypic and omics-based characterization of parental hNSCs.^{10,17,31}

Culture conditions represent a second major determinant of exosome composition and function. Serum-containing media can introduce bovine-derived vesicle contaminants and confound downstream analyses, whereas serum-free or exosome-depleted supplements reduce this risk but may affect NSC viability and secretory profiles.^{8,10-12} Oxygen tension, cell seeding density, and passage number also modulate EV yield and cargo; hypoxic or inflammatory “priming” can enrich vesicles in specific miRNAs and cytokine regulators but may simultaneously increase batch-to-batch variability if not tightly controlled.^{10,31,58} The transition from small-scale two-dimensional flasks to three-dimensional culture systems or bioreactors further alters shear stress, nutrient gradients, and vesicle release kinetics, which can be harnessed to increase productivity but must be linked to predefined critical quality attributes to avoid uncontrolled heterogeneity.^{10,14,17,31}

Post-production storage and handling introduce additional variability. Although exosomes are relatively stable at low temperatures, variation in storage conditions, such as storing samples at -20°C instead of -80°C , prolonged refrigeration, or repeated freeze-thaw cycles, can cause vesicle aggregation, alter particle size, reduce cellular uptake, and lead to the loss of bioactivity.^{11,12,58} Stability

can be improved by minimizing freeze-thaw events, using cryoprotectants, and exploring lyophilization-based formulations that preserve membrane integrity and cargo function, but these approaches require rigorous stability-indicating assays.^{3,10,31} In the context of hNSC-Exos for TBI, inconsistent donor selection, culture conditions, or storage protocols are expected to cause substantial variability in key functional outcomes, including microglial polarization, mitochondrial protection, and neurobehavioral improvement. Establishing potency assays that quantify defined readouts will therefore be essential to link process parameters to therapeutic performance and to support reproducible clinical translation.^{2,17,22,31,61}

6. Mechanisms of hNSC-Exos therapy in TBI

TBI initiates a biphasic cascade comprising primary mechanical damage followed by secondary biochemical, oxidative, and inflammatory insults.⁶¹ The neurovascular unit comprises neurons, astrocytes, microglia, endothelial cells, and vascular smooth muscle cells, with astrocytes playing a central regulatory role. Neuroinflammation is observed alongside oxidative stress and neuronal degeneration, while disruption of the BBB both contributes to and perpetuates this pathological cascade.⁶² hNSC-Exos modulate these processes through multimodal mechanisms that collectively promote neuroregeneration, neuroprotection, angiogenesis, and BBB repair.⁶³

6.1. Neurotrophic factor-mediated synaptic repair and axonal regeneration

hNSC-Exos are enriched with neurotrophic factors, including glial cell line-derived neurotrophic factor and brain-derived neurotrophic factor (BDNF), that promote synaptogenesis and collateral axonal sprouting.^{3,40,41,64} According to Xu *et al.*,⁶⁵ BDNF-conditioned exosomes are able to bind and activate the tropomyosin receptor kinase B, which initiates the downstream pathways of ERK1/2 and AKT, thus promoting neuronal survival and synaptic reconstruction in TBI rat models. Similarly, Schwann cell-derived exosomes containing miR-21 attenuate the activity of Ras homolog family member A, facilitating further axonal elongation and regeneration.

6.2. Neuroprotection and immunomodulation

Persistent ROS production is a major driver of neuronal apoptosis after TBI. In parallel, donor-related variability in hNSC-Exos can further influence mitochondrial function and resilience.^{47-49,66-68} hNSC-Exos enhance mitochondrial stability, upregulating antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase.^{47,48} Zhang *et al.*³⁹ demonstrated that hNSC-Exos activate the PINK1/Parkin signaling pathway,

facilitating mitophagy and selective removal of damaged mitochondria. When oxidative stress elevates PINK1 and Parkin expression, dysfunctional mitochondria are sequestered and degraded, thereby restoring bioenergetic homeostasis.^{39,69} These actions position hNSC-Exos as key regulators of mitochondrial quality control and neuronal resilience following TBI.

6.3. Anti-inflammatory effects

Exosomal miRNAs, such as miR-124-3p, miR-181b, and miR-103a, promote microglial polarization from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype. This shift increases production of anti-inflammatory mediators (interleukin [IL]-10, Arg-1) while suppressing pro-inflammatory cytokines (tumor necrosis factor- α , IL-1 β).^{44-46,70-74} Such miRNA-driven immunomodulation reduces glial scar formation, stabilizes the BBB, and enhances neural tissue repair. These processes (Figure 2) underscore the role of hNSC-Exos as paracrine

modulators of post-traumatic neuroinflammation.

6.4. Anti-apoptotic pathways

hNSC-Exos regulate neuronal apoptosis through intracellular survival pathways. miR-21, an exosomal cargo molecule, suppresses PTEN and programmed cell death 4 expression, thereby activating the PI3K/AKT signaling pathway and reducing caspase-3 activation.^{5,56,57,75} Gao *et al.*⁵ showed that these mechanisms contribute to the preservation of neuronal structure and improved behavioral recovery after TBI. The cumulative anti-apoptotic actions of hNSC-Exos help maintain neuronal viability during acute and subacute injury phases.

6.5. Angiogenesis and BBB repair

hNSC-Exos enhance angiogenesis by activating vascular endothelial growth factor-A signaling and the ERK1/2 pathway.^{37,42,43,76} These pathways stimulate endothelial cell

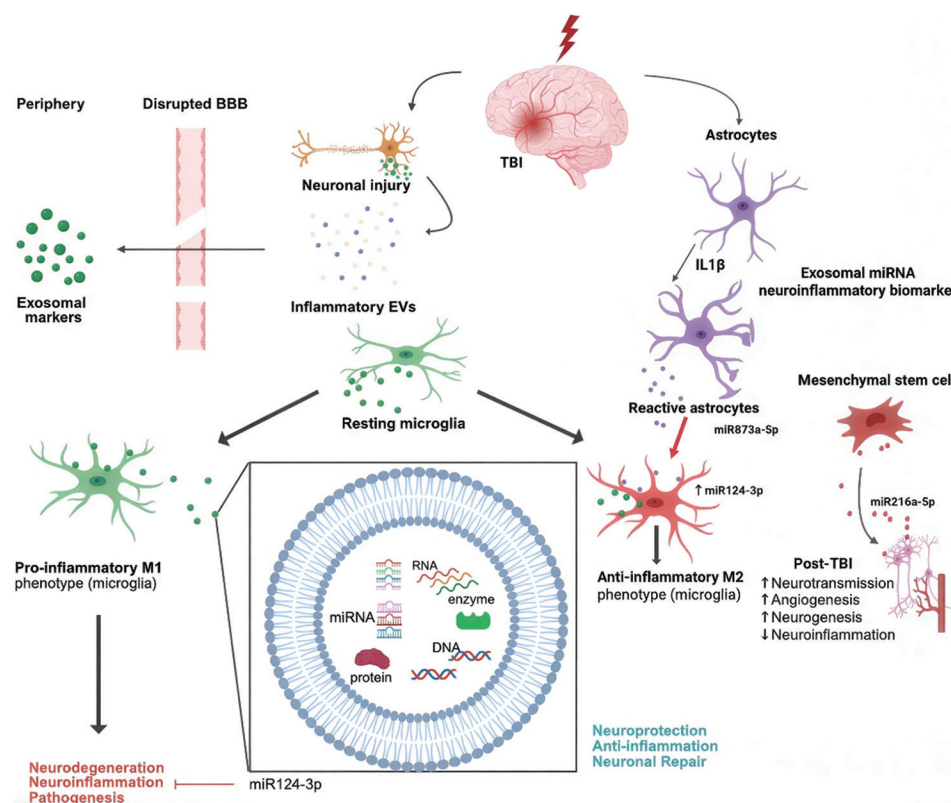


Figure 2. The microRNA (miRNA)-mediated modulation of microglial phenotypes following traumatic brain injury (TBI). After TBI, neuronal injury and blood–brain barrier (BBB) disruption lead to the release of inflammatory extracellular vesicles and exosomal markers into the periphery. Astrocyte activation triggers interleukin-1 β signaling, which promotes the release of exosomal miRNAs, such as miR-873a-5p and miR-124-3p, that regulate neuroinflammatory responses. Resting microglia can be polarized toward either the pro-inflammatory M1 phenotype, contributing to neurodegeneration and pathogenesis, or the anti-inflammatory M2 phenotype, which supports neuroprotection, repair, and angiogenesis. Mesenchymal stem cell-derived exosomes containing miRNAs, such as miR-216a-5p, further enhance neurogenesis and attenuate neuroinflammation in the post-TBI phase. Collectively, these miRNA-mediated pathways highlight the central role of exosomal communication in orchestrating microglial responses and neural recovery after brain injury. Created with BioRender. Ng, N. (2025). <https://app.biorender.com/illustrations/6904f6effe5453c5b79105ff?slideId=04babec9-3d6b-4a38-8040-04827d6b0e40>.

proliferation, migration, and microvascular remodeling, improving cerebral perfusion. Hydrogel-based delivery systems can prolong the release of hNSC-Exos, thereby improving motor recovery and enhancing neovascularization in TBI models.^{17,43,76} Additionally, hNSC-Exos promote BBB repair by activating astrocytic PI3K/AKT signaling pathway to upregulate tight junction proteins Zonula Occludens-1 and claudin-5, reducing vascular permeability and leukocyte infiltration.⁵⁰⁻⁵⁵ These coordinated angiogenic and barrier-restorative effects support structural and functional stabilization after traumatic injury.

7. Future perspectives

7.1. Translational pathways and routes of administration

The routes and modes of action of therapeutic interventions for TBI strongly influence their translational potential and clinical effectiveness. Of particular interest is intranasal (IN) delivery, which provides direct access to the central nervous system while bypassing the BBB and is particularly advantageous for therapeutics targeting secondary injury mechanisms, such as oxidative stress, inflammation, and neuronal apoptosis.

In a controlled cortical impact model, Brabazon *et al.*⁷⁷ demonstrated that IN insulin administration enhanced hippocampal glucose uptake, reduced lesion volume, and improved motor and memory performance, underscoring its neuroprotective and metabolic effects. Similarly, López-Preza *et al.*⁷⁸ reported that IN NeuroEPO, a non-erythropoietic erythropoietin derivative, improved sensorimotor function, reduced depressive-like behaviors, and suppressed oxidative stress markers, avoiding systemic erythropoietin-related side effects.

Further evidence supports IN delivery of neurotrophic agents. Manni *et al.*⁷⁹ reported that acute IN administration of nerve growth factor reduced neuroinflammation and astrogliosis, improving motor coordination in young rats after TBI. Li *et al.*⁸⁰ demonstrated that engineered EVs carrying therapeutic miRNAs reduced activation of nuclear factor kappa-light chain-enhancer of activated B cells and promoted neurorepair in rat and organoid models. Owjifard *et al.*⁸¹ also showed that IN administration of resveratrol decreased BBB disruption and inflammation in ischemic models, highlighting a mechanistic overlap with TBI.

These findings collectively reinforce the translational potential of IN delivery as a minimally invasive, rapidly acting, and central nervous system-targeted route. Nevertheless, pharmacokinetic optimization, anatomical

considerations, and cross-species dosing require further refinement before clinical application.

7.2. Comparison with other therapeutic modalities

Stem cell-based therapies have shown promise in improving functional recovery, reducing inflammation, and promoting neuroregeneration after TBI.⁸² However, issues such as immune rejection, limited engraftment, tumorigenic potential, and difficulty crossing the BBB continue to constrain their clinical translation.

In contrast, hNSC-Exos offer a cell-free therapeutic platform with enhanced safety and stability. These nanosized vesicles transport a repertoire of bioactive components—including proteins, RNAs, and miRNAs—that mediate immune modulation, neuroplasticity, and tissue remodeling.^{2,22} Unlike transplanted stem cells, hNSC-Exos are low-immunogenic, have no risks of uncontrolled proliferation, and readily cross the BBB.

While stem cell therapy has opened pathways for regenerative neuroscience, emerging evidence suggests that hNSC-Exos may be more practical, reproducible, and clinically translatable.³⁸ However, systematic research is needed to optimize controlled release, exosome engineering, and scalable manufacturing to fully realize their therapeutic potential.

7.3. Behavioral and neuropsychiatric considerations

Beyond structural and biochemical recovery, TBI frequently causes long-term behavioral and cognitive impairments that severely affect quality of life. Timmer *et al.*⁸³ reported that approximately 24% of individuals with TBI exhibited significant behavioral changes, including irritability, aggression, disinhibition, and impaired judgment. These symptoms complicate social integration and occupational rehabilitation.

Camilotto *et al.*,⁸⁴ using the Frontal Systems Behaviour Scale (FrSBe), identified apathy and executive dysfunction as the most common deficits, often co-occurring with depression and fatigue. Disinhibition, linked to impulsivity and inappropriate social behavior, appears to reflect injury to the orbitofrontal cortex.

Maresca *et al.*⁸⁵ documented increased aggression and antisocial behaviors in individuals with frontal or limbic lesions, particularly among those with early-age injuries or psychiatric comorbidities. Supporting clinical assessment, Niemeier *et al.*⁸⁶ demonstrated strong reliability of caregiver-reported FrSBe measures for detecting early post-TBI behavioral change. These findings emphasize the need for integrated neurorehabilitation strategies that combine biological repair (e.g., hNSC-Exos) with

behavioral and psychosocial interventions to optimize long-term functional outcomes.

7.4. Perspectives and translational challenges

hNSC-Exos represent a promising cell-free strategy for TBI repair, delivering neuroregenerative, angiogenic, and anti-inflammatory molecules across the BBB.² Engineered exosomes enriched with miR-124, miR-21, or miR-210 further enhance neuroprotection and neurogenesis.⁶⁰

However, significant challenges remain. Standardizing exosome isolation and purification is the most pressing issue, as yield, purity, and functional consistency vary across methods.² Equally important, donor selection, NSC culture parameters (including serum use, oxygen tension, and culture method), and storage conditions (temperature, duration, and freeze-thaw history) each exert substantial effects on hNSC-Exos cargo composition and bioactivity, and therefore must be treated as critical process parameters rather than technical details.^{8,10-12,17,31} Storage instability, cargo heterogeneity, and the absence of good manufacturing practice (GMP)-compliant large-scale production further impede therapeutic readiness.

Additionally, there is limited information on human safety, biodistribution, pharmacodynamics, and immunogenicity, contrasting sharply with robust preclinical results.⁶⁰ Addressing these gaps requires integrating: (i) exosome surface engineering, (ii) targeted delivery technologies, (iii) high-throughput omics profiling, and (iv) harmonized manufacturing standards, alongside well-designed early-phase clinical trials. These efforts are essential to advance hNSC-Exos from experimental therapy toward clinical application.

7.5. Regulatory, manufacturing, biodistribution, and clinical trial considerations for hNSC-Exos

The anticipated clinical translation of hNSC-Exos requires a coordinated framework that spans regulatory classification, GMP-compliant manufacturing, *in vivo* pharmacology, and clinically meaningful trial designs. Current regulatory guidance for EV therapeutics remains fragmented across jurisdictions, and most agencies have not yet formalized EV-specific approval pathways. In the United States and Europe, exosome-based therapies are generally considered biologics or advanced therapy medicinal products, requiring rigorous demonstration of identity, purity, potency, stability, and safety, together with well-defined critical quality attributes.^{10,11,58} A major barrier is the absence of universally accepted standards for exosome characterization, particularly regarding surface markers, cargo profiling, and functional potency assays,

which complicates regulatory evaluation and between-study comparability.

Scaling hNSC-Exos to GMP production introduces additional upstream and downstream challenges. Upstream variables include donor eligibility, cell banking strategies, xeno-free culture systems, bioreactor optimization, and minimization of batch-to-batch heterogeneity.^{8,10,12,17,31} Downstream purification remains a key technical bottleneck; although ultrafiltration, size-exclusion chromatography, and tangential flow filtration offer higher purity than PEG precipitation, their adoption in large-scale workflows requires integration with closed-system GMP processes and validated removal of process-related impurities.^{10,11,58} Furthermore, establishing stability-indicating assays that capture freeze-thaw sensitivity, aggregation, and cargo degradation is essential to support storage, transport, and long-term shelf-life claims.^{3,10,31}

Pharmacokinetic and biodistribution profiles of hNSC-Exos also remain insufficiently defined. Although exosomes can cross the BBB and exhibit selective tropism in animal models, quantitative biodistribution studies using labelled EVs reveal variable uptake across peripheral organs, rapid hepatic clearance, and dose-dependent accumulation kinetics.⁵⁰⁻⁵⁵ These uncertainties limit the establishment of human-equivalent dosing strategies and raise safety concerns about off-target immunomodulation or interactions with the tumor microenvironment. More standardized *in vivo* imaging methods (e.g., near-infrared dyes, membrane-integrated reporters) and quantitative assays are needed to map organ-level kinetics, persistence, and clearance.

Early-phase clinical trial design should prioritize safety, feasibility, and biomarker-driven endpoints. Dose-escalation Phase I trials may include IN or intravenous routes, with careful monitoring of infusion reactions, immunogenicity, cytokine responses, and neurological status. Functional potency biomarkers, such as plasma or cerebrospinal fluid exosomal miRNA signatures, neuroinflammatory mediators, or magnetic resonance imaging-based BBB permeability, may help establish pharmacodynamic signals. Given the heterogeneity of TBI, stratification by injury severity and post-injury timing is critical. Adaptive Phase II trials incorporating imaging, digital neurocognitive assessments, and long-term behavioral follow-up may offer more sensitive readouts than traditional neurological scores alone. Ultimately, harmonizing regulatory expectations, manufacturing scalability, and robust pharmacological data will be pivotal for advancing hNSC-Exos from experimental therapy toward clinically actionable interventions for TBI.

8. Conclusion

hNSC-Exos represent a promising cell-free therapeutic strategy for TBI. Unlike transplanted stem cells, hNSC-Exos can modulate neuroregeneration, inflammation, and apoptosis while avoiding the risks of immunogenicity, cellular instability, and limited BBB penetration. Their ability to activate multiple molecular pathways, including PI3K/AKT, STAT3, ERK1/2, and PINK1/Parkin, highlights their broad neuroprotective and reparative potential.

Although preclinical studies demonstrate substantial therapeutic promise, significant barriers to clinical translation remain, particularly regarding standardized production methods, consistency of exosomal cargo, large-scale manufacturing, and long-term safety. In addition, the behavioral and neuropsychiatric consequences of TBI, including disinhibition, apathy, and executive dysfunction, persist as major challenges and underscore the need to combine biological repair with structured behavioral and cognitive rehabilitation.

Future research should prioritize large-scale, well-designed clinical studies; improved IN or other minimally invasive delivery methods; comprehensive exosome characterization; and multidimensional neurobehavioral evaluation. With continued scientific progress, hNSC-Exos have strong potential to transform the clinical management of TBI and support the development of effective regenerative therapies.

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Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

This manuscript is a narrative review and does not involve primary data collection from human participants. Hence, ethics approval and consent to participate were not required.

Consent for publication

Not applicable.

Availability of data

This is a review article and does not contain original datasets. Sources of all referenced studies are publicly available and properly cited within the manuscript.

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