International Journal of Nanomedicine

Dovepress
Taylor & Francis Group

Open Access Full Text Article

REVIEW

Exosome-Based Diagnostics and Cell-Free Therapeutics for Traumatic Brain Injury: From Mechanisms to Bedside

Meiqi Chen[1,2,*], Chenyang Gu[1,2,*], Yishen Lai[3], Yuqi Lin[2,4], Yifan Xu[2,4], Xiaoyin Li[2], Xuyi Chen[2]

[1]Integrative Medical Center, Tianjin University, Tianjin, 300072, People's Republic of China; [2]Research Department, Characteristic Medical Center of Chinese People's Armed Police Force, Tianjin, 300162, People's Republic of China; [3]Rehabilitation Department, Qingyuan Hospital of Traditional Chinese Medicine, Qingyuan, Guangdong, 511500, People's Republic of China; [4]School of Traditional Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin, 301617, People's Republic of China

*These authors contributed equally to this work

Correspondence: Xuyi Chen, Characteristic Medical Center of Chinese People's Armed Police Force, 220 Chenglin Road, Tianjin, 300162, People's Republic of China, Email chenxuyi1979@126.com

Abstract: Traumatic brain injury (TBI) is one of the leading neurological disorders worldwide. The complexity of its pathological mechanisms and substantial interindividual variability pose considerable challenges to conventional diagnostic and therapeutic approaches. Exosomes, a subtype of extracellular vesicles, have attracted growing interest due to their excellent biocompatibility and ability to cross the blood–brain barrier, demonstrating considerable potential in TBI diagnosis and treatment. This review focuses on the application of exosomes in the field of TBI, clarifying the pathophysiological mechanisms by which exosomes regulate inflammation, neuronal repair, vascular changes and cognitive function after TBI, and discussing their value as novel biomarkers in the early diagnosis and prognosis assessment of TBI. Subsequently, we summarize the application of exosome tissue engineering in TBI, comb through the preclinical translational basis of exosomes, and analyze the current challenges including standardization of isolation procedures, safety and long-term efficacy. In summary, exosomes provide a novel paradigm for cell-free therapy and precision diagnosis of TBI, and further addressing translational bottlenecks will enable them to exert greater advantages.

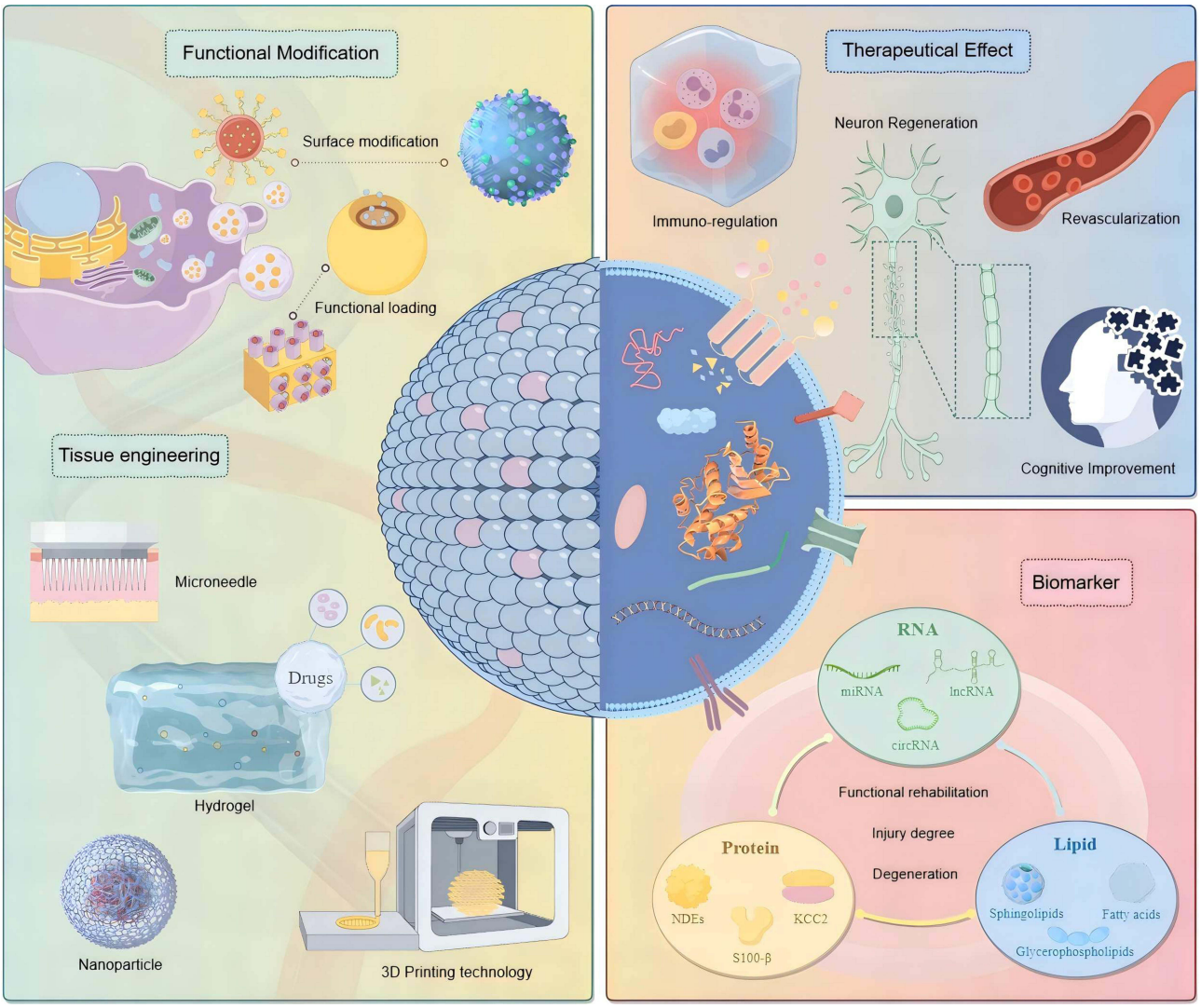
Keywords: exosome, traumatic brain injury, biomarker, tissue engineering, cell-free therapy

Background

Traumatic Brain Injury (TBI) is a serious neurological injury, which is one of the main factors in disability and death worldwide. According to epidemiological data, approximately 50 million people globally are affected by TBI annually.[1,2] In China, more than 125,000 new cases are reported each year,[3] with a notably increasing incidence among young adults, seniors and athletes.[2] At present, there are no highly sensitive and specific diagnostic methods to identify the disease progression of TBI. Combining the Glasgow Coma Scale (GCS) with injury characteristics, TBI is classified as mild TBI (mTBI), moderate TBI or severe TBI (sTBI); the occurrence of two or more TBIs of any severity within 6 months to several years is defined as repetitive TBI (rTBI).[4] The clinical manifestations of TBI are complex and diverse, including primary mechanical injury and secondary processes such as neuroinflammation, apoptosis, and disruption of the blood-brain barrier (BBB).[5] The staging and manifestations of TBI are shown in Figure 1. Currently, TBI clinical methods focus on early treatment, decompressive craniectomy is often used to reduce cerebral edema in TBI patients.[6] However, surgical treatment is prone to complications such as infection, the effect of drug treatment is limited by the BBB and the short time window. Dynamic disease monitoring and neurological function reconstruction in the subacute and chronic phases are both major challenges in the diagnosis and treatment of TBI at present. To overcome these limitations, researchers have been actively pursuing more effective and reliable treatments for TBI.

https://doi.org/10.2147/IJN.S580440
Received: 11 November 2025
Accepted: 18 February 2026
Published: 16 March 2026

Graphical Abstract



In 1983, Harding et al first observed small vesicles secreted by certain cells in reticulocytes,[7] and these vesicles were later termed "exosomes" by Rose Johnstone.[8] Exosomes are nanoscale vesicles secreted by various cells through exocytosis, typically measuring 30–150 nm in diameter. They carry a variety of biological molecules such as proteins, lipids, and small RNAs,[9–12] serving as important tools for intercellular communication.[13] In the nervous system, exosomes are secreted by neural cells including neurons, neural stem cells (NSCs), astrocytes and microglia.[14,15] They participate in biological processes such as neural development and inflammation regulation.[16,17] More and more evidence indicates that differentially expressed exosomes in TBI patients, suggesting their potential use in monitoring disease progression, injury diagnosis, and therapeutic development.[18–21] As an endogenous biological carrier, exosomes have good biocompatibility and immune escape ability. Using exosomes as delivery vehicles to carry neurotrophic and anti-inflammatory factors can reduce the inflammatory response after TBI, protect the BBB, and promote neural repair at the lesion site.[22–24] Compared with traditional surgical or pharmacological interventions, exosome-based cell-free therapy offers the advantages of minimal invasiveness and high targeting specificity, showing great potential in the treatment and diagnosis of TBI. However, it is undeniable that research on the application of exosomes in TBI remains at an early stage.

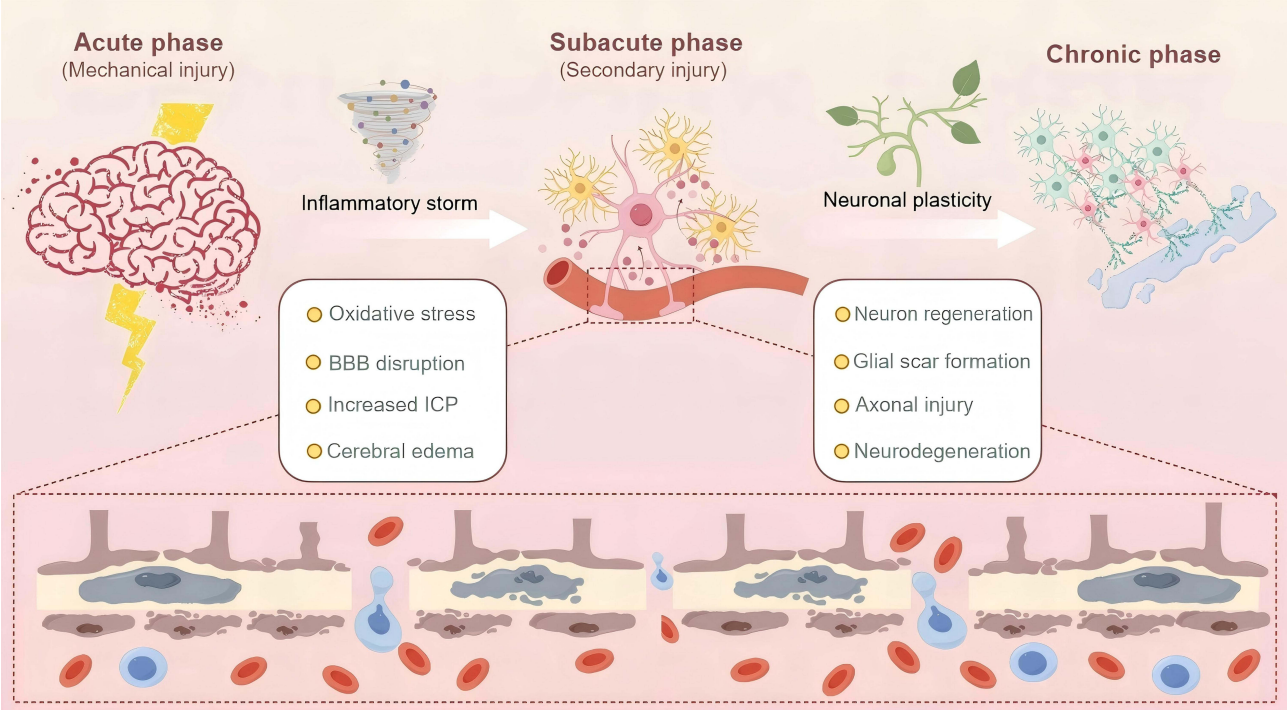


Figure 1 Phases and manifestations of TBI Injury. The acute phase is characterized by primary injury and BBB disruption. The subacute phase sees an inflammatory surge alongside the initiation of neural repair. The chronic phase gives rise to long-term sequelae.
Abbreviations: BBB, blood brain barrier; ICP, intracranial pressure.

Challenges such as standardization in extraction, purification, and scalable production have yet to be addressed. Achieving efficient and cost-effective production of clinical-grade exosomes remains a major obstacle to their translational use.

Therefore, this review elaborates on the application of exosomes as biomarkers and neurorepair factors in TBI, and the application of exosomes as biomedical materials in TBI. In addition, we explore existing challenges and future directions to promote the transformation of exosome research into clinical practice.

This review employed a systematic approach to identify studies related to the application of exosomes in TBI. A comprehensive search was conducted across several databases, including Web of Science, PubMed, Embase, Cochrane Library, CNKI, and Wanfang Data, covering the period from January 2010 to November 2025. The search strategy combined controlled vocabulary and free-text terms, with the core query structured as: ("exosomes" OR "extracellular vesicles") AND ("traumatic brain injury" OR "TBI"). This was further supplemented by specific source keywords such as "biomarker", "diagnosis", "therapy", "tissue engineering", and "drug delivery", encompassing both Chinese and English publications on the biology of exosomes, tissue engineering strategies, and their foundational, preclinical, and clinical model research in TBI diagnosis and treatment.

Biogenesis and Transport Mechanisms of Exosomes

Exosomes originate from early endosomes, which are formed through the inward invagination of the plasma membrane and serve to initially enrich specific bioactive molecules such as proteins and nucleic acids. Subsequently, the membrane of these early sorting endosomes undergoes inward budding. Regulated by protein complexes such as Endosomal Sorting Complex Required for Transport, this process leads to intraluminal vesiculation, maturing the structure into multivesicular bodies (MVBs). The intraluminal vesicles within MVBs are exosome precursors, which are ultimately released into the extracellular matrix upon fusion of the MVBs with the plasma membrane.[25] (Figure 2) Owing to their endosomal origin, exosomes possess favorable properties such as low immunogenicity. In contrast, apoptotic bodies which are rich in DNA and nuclear fragments—are more prone to induce immune activation or

https://doi.org/10.2147/IJN.S580440

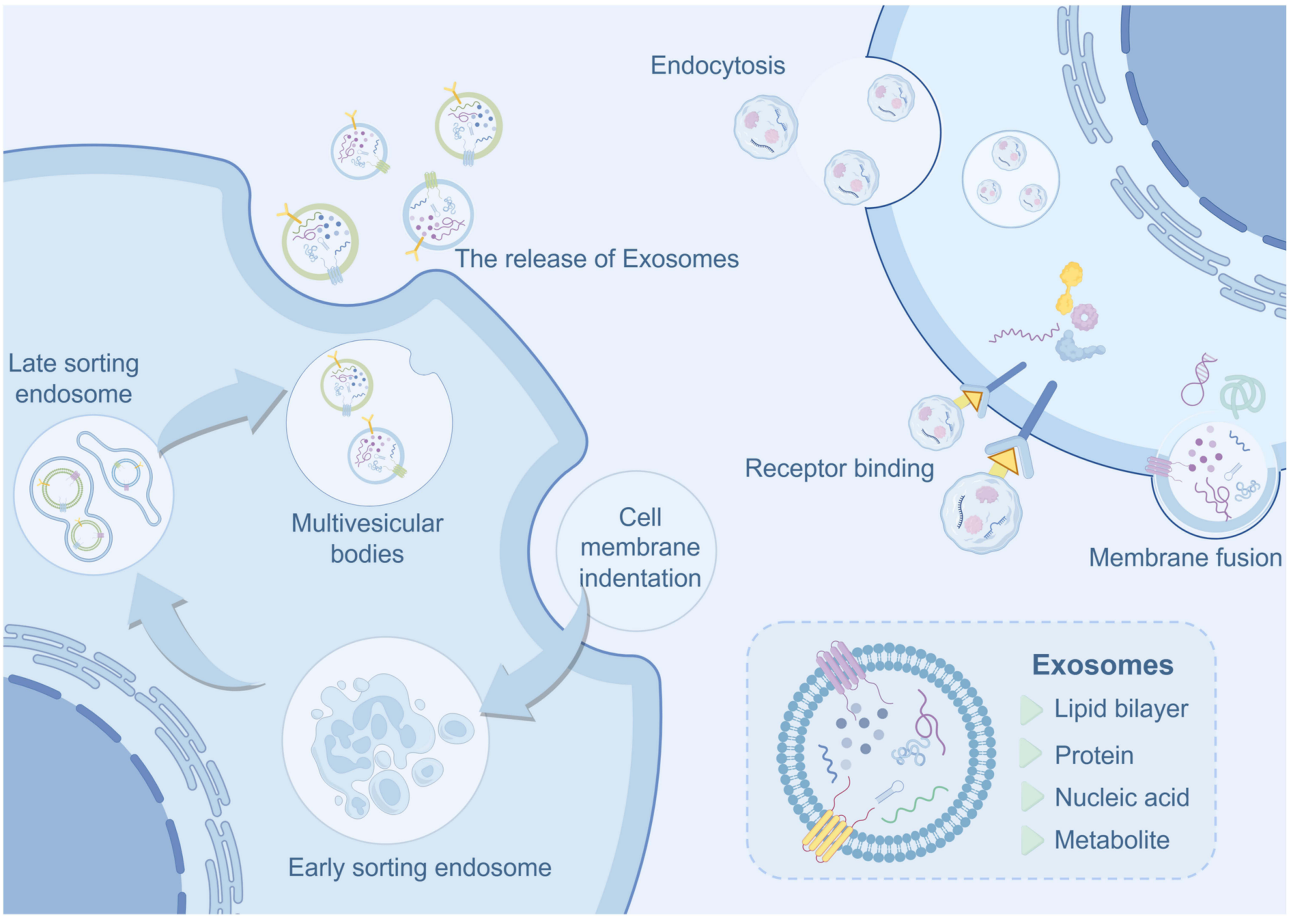


Figure 2 Schematic diagram of exosome biogenesis and information transmission. Originating from early endosomes, exosomes mature within multivesicular bodies and are secreted via membrane fusion. Subsequently, they mediate intercellular communication by entering target cells through endocytosis, membrane fusion, or receptor-ligand interactions.

inflammatory responses. Microvesicles (100–1000 nm), generated by direct outward budding of the plasma membrane, typically encapsulate cargo in a more stochastic and heterogeneous manner and exhibit considerable size variability, which limits their capacity to elicit consistent biological effects. Exosomes not only demonstrate superior membrane stability but also carry well-defined surface markers, thereby providing a robust foundation for targeted delivery and engineered modifications.

As an important communication tool between cells, exosomes mainly rely on three ways to mediate information exchange: (i) internalization by recipient cells followed by release of their cargo into the cytoplasm; (ii) binding to receptors on the target cell membrane, thereby activating intracellular signaling pathways; (iii) direct membrane fusion with the target cell, allowing non-selective delivery of their bioactive contents into the cytosol.[13] (Figure 2) In the nervous system, the surface ligand proteins of exosomes (such as integrin αvβ3, transferrin, etc.) can specifically recognize and bind to the receptors on the surface of brain microvascular endothelial cells (BMECs), inducing the endocytosis of exosomes, and then released into the interstitium of brain tissue through the basal side of endothelial cells, achieving precise delivery across the BBB. After TBI, the permeability of the BBB significantly increases, but the adverse leakage response provides a shortcut for exosome delivery. Disruption of tight junction proteins enhances exosome diffusion, and the inflammatory microenvironment upregulates receptors such as Intercellular Adhesion Molecule-1 on endothelial cells, increasing exosomal transcytosis. These properties make exosomes a promising candidate for TBI therapy. Nevertheless, their pharmacokinetic profile remains incompletely elucidated. Although exosomes possess inherent BBB-crossing capability, their short circulation time and high clearance rate in vivo result in limited accumulation at the site of brain injury, posing a challenge for therapeutic efficacy.

Regulatory Effects of Exosomes After Brain Injury

Brain tissue is delicate and complex, with substances interacting and influencing each other, generating numerous cascades of reactions. Consequently, the regulatory effects of exosomes on brain function following TBI are multi-dimensional rather than a single targeted mechanism. Multiple signaling pathways interconnect and intertwine to form an integrated multidimensional regulatory system. Current research on the therapeutic effects of exosomes in TBI focuses on inflammatory responses, neuronal repair, angiogenesis and cognitive function, and we have conducted a summary of the corresponding mechanism pathway. (Figure 3).

Exosomes Regulate Inflammation

After TBI, the BBB is disrupted, the pathways such as nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) pathway and NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasomes are activated, releasing proinflammatory cytokines, leading to imbalanced astrocyte recruitment and microglial polarization, which in turn damages the BBB and creates a vicious cycle.[26,27] Microglia is a double-edged sword in the TBI cascade, they can differentiate into either pro-inflammatory microglia 1 phenotype (M1) or anti-inflammatory microglia 2 phenotype (M2).[28,29] The M1 phenotype mainly activates the NF-κB pathway and the NLRP3 inflammasome pathway, exacerbating neuronal death and BBB destruction; the M2 phenotype activates the Transforming Growth Factor-beta / Sma- and Mad-related protein pathway and the Interleukin-10 (IL-10) / Signal Transducer and Activator of Transcription 3 pathway, which are anti-inflammatory factors that contribute to tissue repair.[30,31]

Many studies have shown that exosomes can carry bioactive molecules such as proteins, RNAs, and lipids, which target the signaling pathways and interrupt the inflammatory cascade, thereby mitigating neuroinflammation after

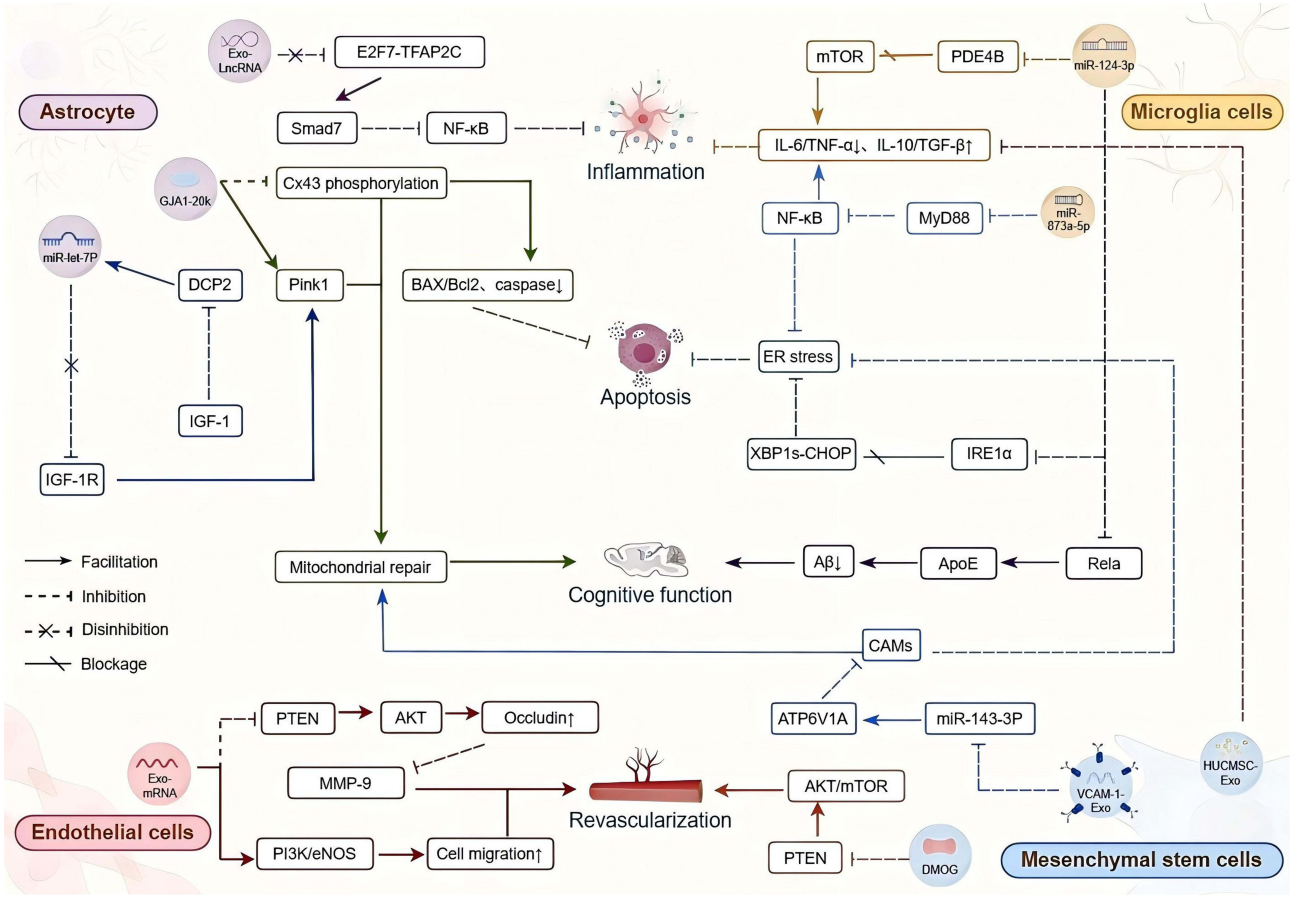


Figure 3 Exosome-mediated regulation of signaling pathways relevant to TBI. Exosomes from different cell sources exert synergistic effects of neuroprotection, anti-inflammation and promoting repair by delivering specific miRNA, lncRNA and proteins after TBI.

https://doi.org/10.2147/IJN.S580440

TBI.[26,32,33] MiR-124-3p is a chronic regulator of gene expression after TBI, and the decreased expression in the injured cortex is related to neuroinflammation.[34–36] Studies have shown that the level of miR-124-3p in microglial exosomes are significantly elevated during both acute and chronic phases of TBI. It can not only inhibit Toll-like receptor 4 signaling pathway to promote M2 polarization, but also target phosphodiesterase 4B to inhibit the mechanistic target of rapamycin (mTOR) signaling pathway, promote axonal regeneration of damaged neurons, and improve the neurological function of rTBI mice.[36,37] He et al discovered that astrocyte-derived exosomal lncRNA regulatory axis can inhibit the NF-κB signaling pathway. It reduced the pro-inflammatory phenotype of microglia and enhanced their phagocytic activity, offering a novel target for interventions aimed at modulating glial cell interactions.[38] Similarly, another miR-873a-5p derived from astrocyte exosomes (AS-Exos) was taken up by adjacent microglia after TBI, which block NF-κB phosphorylation, upregulating the anti-inflammatory factor Arg-1 while downregulating the pro-inflammatory factor inducible nitric oxide synthase.[39] Additionally, Exosomes from human umbilical cord mesenchymal stem cells also exhibited anti-inflammatory effects via NF-κB pathway modulation.[40] These studies suggest that the NF-κB pathway may be a common regulatory target of cross-cell origin. However, due to differences such as the source of exosomes and the administration window, more cross-model validation data are still needed for support.

Exosomes Promote Neuronal Regeneration

Neuronal injury is a major feature of secondary damage following TBI. In the lesion area, inflammatory and oxidative stress responses interact, leading to neuronal apoptosis and synaptic dysfunction, which impede brain tissue repair and regeneration.[41–43] Antioxidants can reduce free radicals accumulation and mitigate oxidative stress. However, traditional antioxidant drugs have a single target and obvious side effects. For instance, long-term use of vitamin E increases the risk of bleeding;[44] N-acetylcysteine has poor BBB penetration and low bioavailability.[45,46] Additionally, impairment of the gut-brain axis in TBI patients leads to gastrointestinal side effects with oral medications and considerable variability in treatment response.[47] Neurotrophic factors can suppress apoptotic protein expression and promote axonal regeneration.[48] However, endogenous levels are often insufficient, and exogenous supplementation is hindered by poor BBB penetration.

As an emerging intercellular communication medium, exosomes show considerable potential in promoting neuronal survival and restoring synaptic plasticity. Specifically, miR-17-5p in AS-Exos can downregulate the expression of adenovirus E1B 19 kDa interacting protein 2, a protein closely associated with mitochondrial homeostasis and cell death pathways.[49] Through in vitro and in vivo experiments, Du et al demonstrated that this mechanism reduces neuronal apoptosis in hypoxic-ischemic brain injury.[50] Given the heterogeneity of the injury model, the repeatability of this mechanism in the TBI model still needs to be further confirmed. The protective role of nuclear factor erythroid 2-related factor 2 (Nrf2) in mitigating oxidative stress and neuronal damage has been well established.[51] Zhang et al further showed that AS-Exos upregulate Nrf2 expression, thereby suppressing oxidative stress and reducing neuronal damage after TBI. The effect abolished in Nrf2-knockout mice, confirming the earlier findings.[52] Zhuang et al provided different ideas: Bone marrow mesenchymal stem cell-derived exosomes (BMSC-Exos) can effectively inhibit glutamate-mediated excitotoxicity after TBI via the Mitogen-Activated Protein Kinase (MAPK) signaling pathway and reduce apoptosis.[53]

Exosomes Facilitate Cerebrovascular Regeneration

The neurovascular unit serves as a prerequisite for neural repair following injury.[54] Blood supply to the injured area not only delivers oxygen and nutrients that support neuronal survival and synaptic remodeling, but also modulates inflammation through paracrine signaling, thereby improving the local microenvironment.[55] More importantly, neovascularization creates favorable conditions for skull repair and provides essential support during bone remodeling and maturation.[56–59]

The role of exosomes in promoting vascular development and growth in neurological diseases has been confirmed. These studies provide a relevant theoretical basis for repairing vascular damage after TBI.[60–62] A study that used exosomes derived from human peripheral blood endothelial progenitor cells found that exosomes are taken up by endothelial cells and translated into proteins and by activating the phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) pathway and endothelial nitric oxide synthase, thereby enhancing endothelial proliferation and angiogenesis.[63] Similarly, Gao et al observed that BMECs efficiently took up exosomes formed by fluorescently

labeled endothelial cell colonies. In vitro experiments further revealed that these exosomes suppressed phosphatase and tensin homolog (PTEN) pathway, and activated the AKT signaling pathway, upregulating tight junction proteins such as Ceroid-lipofuscinosis neuronal protein 5 and occluding,[64] consistent with previous findings. Exosomes derived from mesenchymal stem cells (MSCs) are rich in angiogenic signaling proteins and have angiogenic capabilities.[65] Zhang et al reported that intravenous injection of MSC-derived exosomes (MSC-Exos) increased the number of newly formed endothelial cells and neurons in the peri-injury region and the hippocampal dentate gyrus after TBI.[66] Johnathon et al proposed that exosomes at low dose (1 μg/mL) and medium dose (10 μg/mL) were shown to promote angiogenesis by modulating the NF-κB signaling pathway.[67] Interestingly, not all exosomal effects are beneficial. For instance, AS-Exos can exacerbate BBB disruption after TBI by delivering miR-143-3p, which targets BMECs and inhibits autophagic degradation of cellular adhesion molecule, thereby worsening secondary injury.[22] Based on this founding, Wu et al developed a vascular cell adhesion molecule-1-targeted exosome delivery system to specifically deliver a miR-143-3p inhibitor, significantly improving neurological functional prognosis in TBI mice models.[22] Inhibiting the expression of miR-491-5p in mesenchymal stem cells can target the melatonin receptor 2-mediated HIF-1α/VEGF signaling pathway, increase the microvessel density and local cerebral blood flow at the site of brain injury.[68] Therefore, the rational utilization of the negative regulatory mechanism of exosomes can also be transformed into the pro-repair pathway of TBI.

Patients with TBI who have suffered mechanical injuries often have skull fractures or defects. Loss of this natural protective barrier, the brain tissue is more prone to difficult complications.[69–71] Researchers have gradually focused on the role of exosomes in the synergistic regulation of bone regeneration and vascular repair. Notably, MSC-Exos pre-treated with low-dose dimethyloxalylglycine further enhanced osteogenesis and angiogenesis via activation of the AKT/mTOR signaling pathway, demonstrating superior efficacy compared to untreated MSC-Exos.[72]

Exosomes Improve Cognitive Function

The neuroinflammatory cascade following TBI is complex and prolonged, driving neurodegenerative changes that persist for months to years after injury. These changes manifest as cognitive impairment, reducing motor coordination and emotional disturbances.[73] A long-term follow-up study reported rates of cognitive decline at 21% in mTBI and 26% in sTBI cases, with the risk of deterioration increasing over time.[74] Imaging studies showed that brain atrophy after TBI mainly involving areas the hippocampus and frontal cortex, which highly overlapped with regions of Amyloid-β (Aβ) protein deposition.[75]

Cognitive impairment following TBI is positively correlated with pituitary dysfunction.[76] And it has been found that mitochondrial autophagy function is impaired post-injury.[77,78] Insulin-like growth factor-1 (IGF-1) is one of the downstream factors of growth hormone. A study revealed that astrocyte-derived IGF-1 promoted the maturation of miR-let-7e via the 7-methylguanosine mRNA-decapping enzyme 2, which in turn suppressed IGF-1 receptor expression and restored mitophagic function.[79] This provides a new target for improving cognitive function after TBI. Xian et al found that MSC-Exos ameliorated mitochondrial dysfunction in hippocampal astrocytes of mice, exerted anti-inflammatory effects while improving cognitive function.[24] Zhuang et al reported that BMSCs-Exos reduced cortical lesion volume, further supporting the role of exosomes in improving cognitive recovery after TBI.[53] A recent study identified the PTEN-induced kinase 1 pathway as another mechanism modulating mitophagy and inflammasome NLRP3 activation. And the gap junction protein alpha-1 20 kDa isoform protein in AS-EXOs can inhibit pyroptosis, alleviating cognitive impairment and anxiety-like behaviors after TBI.[80]

Additionally, abnormal Aβ accumulation is a key feature of neurodegenerative changes following TBI, particularly in cases of repetitive mTBI.[81] Although neuron-derived exosomes have been implicated in driving the formation of Aβ fibrils, growing evidence suggests that exosomes predominantly contribute to the clearance of Aβ.[82–84] Ge et al demonstrated that miR-124-3p is altered after TBI and promoted Apolipoprotein E expression by targeting its inhibitory transcription factor, thereby suppressing Aβ pathology and attenuating neurodegenerative processes.[85]

Exosomes as Biomarkers for TBI

In the clinical diagnosis of TBI, commonly used assessment and detection methods include clinical scales, neuroimaging and biomarker analysis. GCS is widely employed to evaluate consciousness in TBI patients. However, it is relatively subjective, susceptible to medication interference, and lacks sensitivity in diagnosing mTBI.[86] CT and MRI serve as essential imaging modalities for TBI diagnosis, offering high sensitivity and specificity in detecting brain tissue damage. Nevertheless, CT carries the risk of ionizing radiation, while MRI is time-consuming and costly. Importantly, neither technique can reliably predict functional recovery in TBI patients. Given the long-term impact of TBI on neurological function, continuous monitoring of functional status is crucial. The emergence of fluid biomarkers, particularly the application of exosomes, has brought new opportunities in this regard.

The Traditional Fluid Markers

Cerebrospinal fluid is the primary source for detecting TBI-related biomarkers, but its invasive collection procedure and limited sample availability considerably reduce feasibility. Blood-derived biomarkers have also garnered considerable attention, such as neurofilament light chain (NfL), tau protein, S100 calcium-binding protein β, and glial fibrillary acidic protein (GFAP),[87–89] whose altered expression levels are typically associated with the severity of brain injury and functional prognosis.[21] Peltz et al found that combined measurement of phosphorylated tau protein, NfL and the inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-α can effectively assess cognitive function in TBI patients.[20] Guedes et al further validated this conclusion through blood analysis of 195 veterans.[90] However, the sources of the research samples have distinct population characteristics. Potential exposure histories and military-specific injury mechanisms may have shaped the kinetics of p-tau, NfL and inflammatory factors and their association with cognitive outcomes. TBI has been firmly associated with cognitive impairment.[91] After brain injury, the increased tau acetylation mediated by S-nitrosylated glyceraldehyde-3-phosphate dehydrogenase leads to neurodegeneration and behavioral disorder.[92] Patients with moderate to sTBI have a 2–3 times higher risk of developing dementia in later life.[93,94] Furthermore, repetitive brain injuries induce hyperphosphorylation of tau and structural changes resembling, yet distinct from Alzheimer's disease(AD), potentially resulting in chronic traumatic encephalopathy (CTE).[95] However, the accuracy of tau in predicting CTE remains suboptimal, with a negative predictive value of only 53%, limiting its utility in ruling out CTE.[96]

Large clinical cohorts have shown that plasma GFAP and Ubiquitin C-terminal Hydrolase L1 (UCH-L1) both change within 7 days of mTBI, and their combined use outperforms either marker alone.[97] This work provided the evidence base for their clinical adoption. In 2018, the Food and Drug Administration (FDA) first cleared a blood-based assay that quantifies GFAP and UCH-L1 to evaluate mTBI. A subsequent European multicentre prospective study confirmed that the test predicts intracranial haemorrhage with high sensitivity.[98] The Abbott i-STAT TBI Whole Blood Test, approved by the FDA in 2024, further simplifies testing, expands the settings in which it can be used, and reduces unnecessary CT scans.[99] Due to performance drops with increasing injury severity, these methods mainly support intracranial hemorrhage detection and offer limited utility in sTBI. The inherent instability of circulating proteins and their resulting narrow detection window constitute significant challenges for biomarker development.

Exosomes as Novel Markers

Exosomes are an emerging biomarker that has shown diagnostic value in various diseases such as pancreatic cancer and acute myocardial infarction.[100,101] Exosomes carrying molecules from neurons, glial cells, and other cell types can reflect early changes after brain injury and systemic immune responses, offering comprehensive pathological insights. In the context of neurological disorders, isolating exosomes released by specific cell types provides new insights into the complex molecular change.[102,103] The summary is shown in Table 1. (Table 1) Compared with cerebrospinal fluid sampling, exosomes can be obtained from peripheral blood. There is no need for lumbar puncture, which reduces patient discomfort and risk through non-invasive sampling. Moreover, exosomes are encapsulated by a phospholipid bilayer that

protects their cargo from enzymatic degradation and immune attack, conferring high stability in circulation, thereby enabling repeated and frequent monitoring.[13]

RNA in Exosomes

RNA is one of the main cargoes of exosomes, can be further categorized into miRNA, lncRNA, circRNA, and tRNA, whose expression changes may be associated with the disease development after TBI. Rodent TBI models have shown differential expression of both lncRNAs and circRNAs.[19,112] Among them, circRNA-enriched calcium-signaling cascades can activate the MAPK pathway, thereby contributing to post-injury inflammation and apoptosis, plausibly by competing for miRNA binding and modulating target-gene levels.[113] Studies in rat models have analyzed alterations in plasma exosomal miRNA profiles after TBI and revealed significant associations with the MAPK signaling pathway, actin cytoskeleton regulation, supporting the potential of miRNAs as TBI biomarkers.[114]

Clinical studies have provided robust evidence for the use of exosomal RNA as a rapid diagnostic marker after TBI. Devoto et al measured plasma exosomes in 153 military personnel with TBI and found differential expression of hsa-miR-139-5p and hsa-miR-18a-5p in exosomes between the mTBI and rTBI groups.[106] Further analysis revealed a negative correlation between miRNA levels in the rTBI group and Neurobehavioral Symptom Inventory scores, suggesting a potential link to post-TBI neurobehavioral symptoms.[106] Additionally, compared to TBI patients with normal consciousness and the healthy group, the plasma-exosomal small RNAs hsa-miR-1-3p, hsa-miR-155-5p and nine others changed expression as the GCS score varied in TBI patients with altered consciousness.[115] Similarly, serum-derived exosomes also exhibited differential RNA expression profiles. For instance, lncRNA VLDLR-AS1 was significantly downregulated in military personnel with chronic TBI.[104] This study highlights the potential of exosomal signaling as a blood-based biomarker for identifying chronic TBI patients. However, since lncRNA VLDLR-AS1 is also implicated in neuropsychiatric disorders, there is a risk of false positives. Cross-population and longitudinal validation are needed to establish its generalizability. In another study, serum exosomes isolated from severe and mTBI patients showed that miR-206 and miR-549a-3p were significantly elevated in patients with poor prognosis compared to those with favorable outcomes, indicating their predictive value.[18] A retrospective analysis revealed that the miR-338-3p in neural-derived exosomes significantly increased 48 hours after TBI, positively correlated with the New Injury Severity Score.[107] Notably, its expression exhibited TBI-specific and time-dependent patterns, potentially reflecting oxidative stress modulation or initiation of neuronal repair, supporting its utility for dynamic monitoring of TBI progression. To better understand the dynamic changes of exosomal RNA in human brain tissue after TBI, His et al built a 3D in-vitro brain model, using qPCR and NanoString, profiled multiple miRNAs (miR-124-3p, miR-137-3p, miR-204-5p and miR-320c, etc.) during the acute phase of TBI. The observed changes matched clinical data. The main limitation is the absence of a BBB, which reduces translational relevance. Incorporating a BBB layer while preserving the model's reproducibility would allow more accurate, longer-term miRNA tracking and open new avenues for longitudinal prediction of TBI progression.[116]

Protein in Exosomes

Proteins also represent crucial biological information carried by exosomes, which can be taken up by recipient cells and undergo changes during the onset of diseases, making them popular candidates for TBI biomarkers. Goetzl et al observed distinct expression patterns of exosomal proteins between acute and chronic TBI patients, Aquaporin-4 and synaptogyrin-3 are proteins carried by exosomes that are closely related to neurological functions, and their increase was significantly greater in acute TBI compared to chronic TBI.[108] While IL-6 and cellular prion protein remained elevated in chronic TBI, which is associated with the deposition of neurotoxic proteins following concussion.[117] Additionally, potassium-chloride cotransporter 2 (KCC2), a neuron-specific potassium-chloride cotransporter that regulates intracellular chloride homeostasis, was significantly reduced in blood exosomes from TBI patients. The ability of KCC2 to predict cognitive dysfunction after TBI has been validated, and its expression is significantly reduced in blood exosomes of TBI patients, which is also closely associated with depressive-like behavior.[111] Furthermore, AS-Exos carry complement proteins, such as C4b, factor D, C3b, which remain elevated in patients with mTBI.[118] These alterations may lead to long-term neural damage and dysfunction.

Table 1 The Source and Function of Exosomes as Biomarkers of TBI

Source		Expression	Function	Refs
Serum	miR-206 miR-549a-3p	↑	The prognosis of patients with sTBI deteriorates	[18]
Serum	VLDLR-AS1	↓	Chronic mTBI related to combat is positively correlated with depressive symptom	[104]
Plasma	miR-203b-5p miR-203a-3p miR-185-5p	\	The brain-derived map composed of 8 RNAs effectively classifies the injury status of TBI	[105]
Plasma	hsa-mir-139-5p	↑	The prognosis of patients with mTBI deteriorates	[106]
Tissue	miR-338-3p	↑	It is positively correlated with the severity of injury, reflecting the activation of neuronal repair or stress regulation mechanisms	[107]
Blood	p-tau, TNF-α, IL-6	↑	Cognitive function impairment occurs in the chronic stage of TBI	[20]
Blood	NfL	↑	It is associated with rTBI as well as Post-Traumatic Stress Disorder and depressive symptoms	[91]
Plasma	CD81	↓	A significant decrease in its content indicates acute mTBI	[108]
Plasma	tau	↑	The worse the performance in the fields of memory and psychomotor speed	[96]
Plasma	CD63, Iba1	↑	It indicates that it is in the acute stage	[109]
Plasma	CD11b, ACSA-2	↑	It indicates that it is in the post-acute stage	[109]
Serum	UCH-L1	↑	Its significant increase predicts early death in patients with TBI	[110]
Serum	KCC2	↓	Depression-like behaviors increase after TBI	[111]

Notes: ↑: up-regulated expression;↓: down-regulated expression; \: non-unidirectional changes.
Abbreviations: sTBI, severe TBI; mTBI, mild TBI; rTBI, repetitive TBI.

A study by Moyron et al identified that plasma exosomes collected within 24h of injury carry proteins whose expression patterns differ by TBI severity. Sixteen proteins (Ig kappa V–I Gal, tetranectin and adiponectin etc) were unique to mild–moderate TBI, whereas fourteen (Ig kappa V–I AG, Ig lambda V–II NIG-84 and mannose-binding protein C etc) appeared only in severe caseses.[119] However, nine proteins were shared by uninjured controls and mild–moderate patients, underscoring an overlap zone that may complicate single-subject classification. The concentration of GFAP isolated from plasma exosomes was significantly higher in patients with altered consciousness, and approximately ten times greater than in those without.[115] In a separate study, Mondello et al collected serum daily for five days from 21 patients with moderate-to-severe TBI. Exosome-derived UCH-L1 identified early mortality with 100% sensitivity and specificity, while Exosome-derived GFAP and NfL were significantly higher in diffuse injury than in focal lesions. GFAP remained four-fold elevated at 48h, indicating a prolonged sampling window.[110] Relative to the FDA-cleared assays for free GFAP and UCH-L1, exosome-associated biomarkers may offer broader temporal coverage and better lesion-phenotype discrimination. Small cohorts and undefined cut-offs have so far limited clinical translation. Validation in large and prospective studies using standardized protocols and blinded clinical end-points is required to establish incremental value.

Lipid in Exosomes

In contrast to RNAs and proteins, the potential of lipids as biomarkers should not be ignored either. After TBI, lipid metabolism in brain tissue is disrupted, compromising membrane integrity. The resulting phospholipid metabolites and oxidation products increase BBB permeability, allowing exosomes, which are bilayer lipid vesicles, to cross the barrier more readily and become selectively enriched or depleted.[120] A multivariate serum lipid panel distinguished TBI within 24 h in rats with 90% sensitivity and accuracy.[121] In a multicentre European cohort of 716 patients, metabolomic profiling revealed that choline-phospholipid concentrations declined in proportion to acute injury severity.[122] Lipid markers of blood origin are the attracting wide attention, yet exosome-derived lipids remain under-studied in TBI. Pioneering work in other neurological diseases shows the diagnostic value of exosomal lipids. Pergande et al reported early elevation of sulfatides in mouse brain-derived exosomes that rose progressively with metachromatic

leukodystrophy severity,[123] while exosomes from AD brain tissue displayed reduced glycerophospholipids and poly-unsaturated fatty acids, with DHA depletion correlating closely with cognitive decline.[124] These neuron-enriched lipid cargoes sensitively mirror cerebral metabolism, providing a solid rationale for exploring exosomal lipids as TBI biomarkers.

Collectively, exosomes hold promise for the diagnosis and longitudinal tracking of TBI. Although findings from clinical samples are promising, it is important to acknowledge that current studies on exosomal biomarkers remain limited by small sample sizes and a scarcity of large-scale cohort investigations. Furthermore, there is considerable heterogeneity in the biomarkers identified across different studies, compounded by variability in sample sources and injury severity. The generalizability of these results is also constrained by differences in injury mechanisms—such as blast-induced versus impact-induced trauma—and demographic or clinical confounders including age, sex, and comorbidities, all of which may significantly influence exosomal cargo profiles. Additionally, variations in sampling timepoints and inter-individual differences continue to pose challenges for achieving reliable longitudinal monitoring. The difference of sampling time between nodes and individuals is still the key parameter to realize accurate longitudinal monitoring.

Exosome Tissue Engineering Strategies for Treating TBI

We already know that exosomal low immunogenicity, high biocompatibility, and ability to penetrate the BBB make them ideal delivery vehicles for treating brain injury. However, natural exosomes are limited by insufficient targeting specificity, low drug-loading efficiency, and uncontrolled in vivo distribution. In recent years, advances in sophisticated technologies have enabled the functional enhancement of exosomes through tissue engineering strategies. Innovations in drug loading, material integration, and delivery approaches have significantly improved the therapeutic potential. Example of research on organizational engineering is shown in the Figure 4.

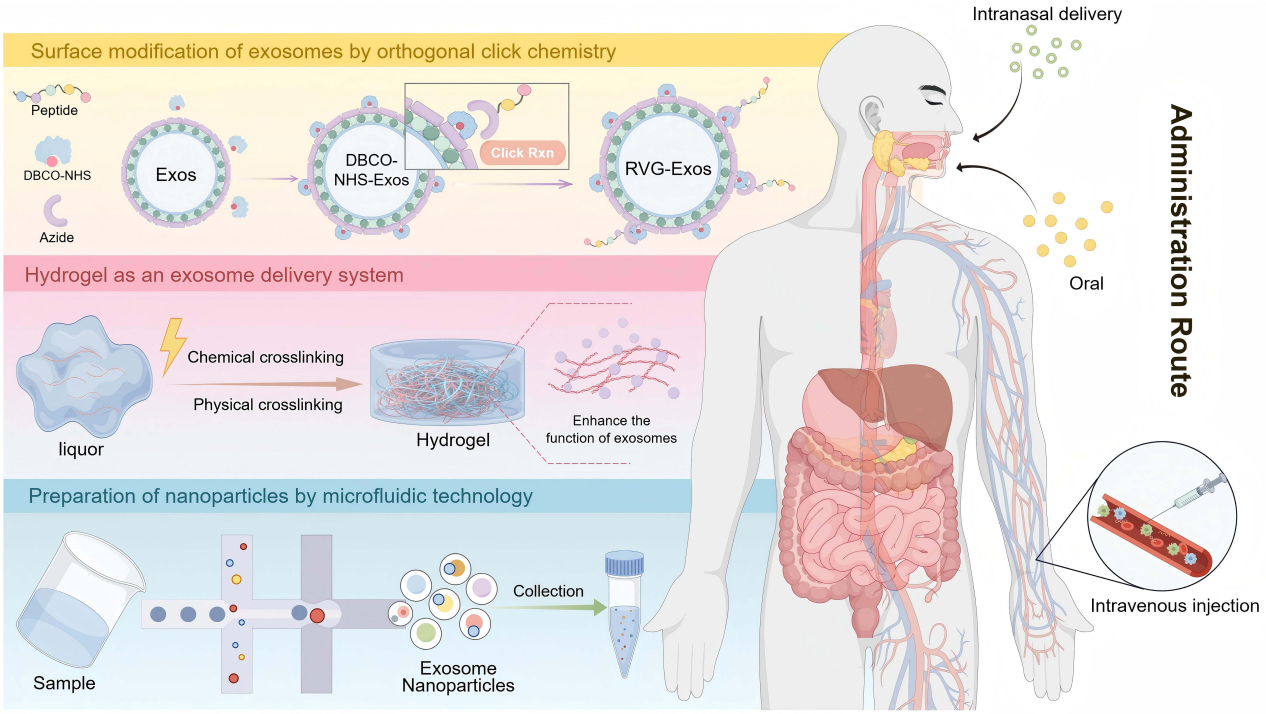


Figure 4 Exosome-based tissue engineering strategies mainly encompass surface modification via orthogonal click chemistry, the construction of hydrogel delivery systems through chemical or physical cross-linking, and nanoparticle preparation using microfluidic technology, with intravenous, oral, and intranasal administration serving as key delivery routes.

Abbreviations: Exos, exosomes; DBCO, dibenzocyclooctyne; NHS: N-hydrox-ysuccinimidyl ester; RVG, Rabies Virus Glycoprotein.

https://doi.org/10.2147/IJN.S580440

Functional Loading and Surface Modification

Through genetic engineering and other means to load therapeutic molecules, combined with techniques such as orthogonal biochemical surface modification, the targeting capability and stability of exosomes can be enhanced.[125] Haroon et al used orthogonal click chemistry to modify the surface of exosomes secreted by microglia with rabies virus glycoprotein-derived 29-aa peptide, achieving brain-targeted and efficient delivery of the neuroprotective NH2-terminal tandem PDZ-binding motif of the N-methyl-D-aspartate receptor 2B subunit. Evaluation data from the oxygen-glucose deprivation model showed that the modified exosomes significantly increased the viability of Neuro-2a cells, reduced oxidative stress levels and decreased the extent of brain injury.[126] Studies have shown that under inflammatory conditions, macrophage-derived exosomes can readily cross the BBB, greatly improving the uptake rate of brain-derived neurotrophic factor (BDNF) encapsulated within them.[127] Extensive research indicates that exosomes act as reactive oxygen species (ROS) scavengers by regulating ROS-related signaling pathways while also delivering antioxidant proteins or enhancing ion reduction capacity mainly of iron ions, thereby effectively alleviating oxidative stress responses.[125,128–130] In addition, the modified macrophage exosomes can deliver pioglitazone in a targeted manner, reduce the intracellular reactive oxygen species content after TBI, and repair the mitochondrial membrane potential to improve its respiratory metabolic function. Specifically, the researchers successfully connected the complement C3-derived peptide to the exosome membrane via fusion protein plasmid transfection, and then hybridized the modified exosome membrane with the platelet membrane to construct ROS-responsive multi-fusion nanosystem.[131]

Preparation of Biological Scaffolds by Combining with Nanomaterials

With the progression of research, diverse engineering strategies have emerged to incorporate exosomes into biomaterial scaffolds, enabling investigators to tailor delivery systems that increasingly unlock the therapeutic potential of exosomes for TBI. 3D printing technology has become a widely adopted method for fabricating biological scaffolds in materials synthesis. Chen et al used this method to prepare a collagen/chitosan bioscaffold loaded with exosomes derived from NSCs pretreated with interferon γ.[132] This scaffold was designed to mimic the extracellular matrix, providing a supportive microenvironment for neural tissue repair. It enhanced endogenous neurogenesis and angiogenesis while mitigating neuroinflammation. Similarly, 3D-printed biological scaffolds delivering exosomes from BDNF-preconditioned cells promoted axonal and myelination regeneration, and behavioral assessment indicates recovery of motor and cognitive functions.[133] 3D ultrafoam scaffolds made of type I collagen can also simulate the extracellular matrix. Zhang et al seeded multipotent mesenchymal stem cells into 3D collagen scaffolds, and the amount of exosomes secreted by the cells was twice that of culture dishes. Subsequent experiments demonstrated that exosomes isolated from 3D-cultured cells significantly increased the number of newborn endothelial cells in the dentate gyrus and improved cognitive function in mice after TBI.[134] In another study, exosomes derived from human adipose-derived stem cells were immobilized on a PLGA scaffold via a dopamine coating. Both in vitro and in vivo experiments confirmed that this method significantly enhanced the homing ability of MSCs in skull tissue and effectively induced bone regeneration of skull defects after TBI.[135] Electrospinning represents another viable strategy for fabricating nanofibrous scaffolds. By modifying electrospun polycaprolactone nanofibers with polydopamine, researchers successfully incorporated exosomes from two types of stem cells—MSCs and NSCs. This innovative exosome delivery system not only improved exosome loading efficiency but also exhibited combined anti-inflammatory and neurorestorative effects.[136]

Hydrogels are polymeric materials characterized by a three-dimensional network structure, high water content and excellent plasticity, which can carry various therapeutic substances such as drugs and biological factors. As such, they serve as outstanding biological scaffolds in the treatment of TBI.[137,138] Researchers have leveraged polydopamine's mussel-mimetic adhesion to reinforce hydrogel adhesiveness, and this strategy has been widely adopted to fine-tune hydrogel synthesis and design,[139,140] after decorating polydopamine nanomotors with a brain-targeting peptide, the trans-BBB flux was significantly boosted.[141] Li et al prepared a photo-crosslinkable hyaluronic acid derivative by chemical modification. After mixing it with collagen, a hydrogel that simulated the extracellular matrix of brain tissue was constructed to achieve sustained release of exosomes.[142] Exosomes isolated from human deciduous dental stem cells were encapsulated in a poly citrate-gallic acid(PLGA) composite hydrogel, which exhibited good antioxidant activity,

effectively scavenging free radicals and reducing ROS production in microglia. It also had the ability to promote the production of anti-inflammatory cytokines and inhibit neuroinflammation.[143]

Short peptide chains can self-assemble into hydrogels or peptide-decorated gels through non-covalent interactions, closely mimicking the architecture and function of the extracellular matrix. Their injectability allows in-situ gelation within the injured brain, conforming precisely to the lesion cavity.[144] RADA-16, a self-assembling peptide composed of 16 amino acids with repeating Arg-Ala-Asp-Ala sequences, has been previously demonstrated to serve as an ideal backbone for nanoscaffolds. When combined with bioactive factors and stem cell transplantation, it can mitigate inflammatory responses and apoptosis after TBI.[145–147] Based on this, Mehrdad et al used stromal cell-derived factor-1 α combined with RADA-16 peptide to create a new type of nanoscaffold for delivering exosomes derived from human embryonic NSCs. Their findings showed that the regeneration of neurons in the subventricular ventricle of the treatment group was significantly increased, and the activation of glial cells in the injury site was reduced.[148] Compared with polymer hydrogels, peptide gels assembled from natural amino acids offer a markedly better safety profile and rarely trigger allergic or inflammatory responses.[137] Their soft consistency reduces compression of brain tissue, however, the limited mechanical strength hampers neovascular adhesion to the matrix, and potential scaffold collapse can lead to uneven exosome release —— an instability that must be weighed in design.

Skull defects are often concomitant symptoms of TBI, leading researchers to focus increasingly on the repair of cranial injuries after TBI to provide more comprehensive treatment for affected patients. Using microfluidic technology, PLGA- Polyethylene Glycol -PLGA triblock copolymer microspheres were prepared to encapsulate exosomes derived from human dental pulp stem cells, which enhance the mineralization capacity of osteoblasts and promote cranial bone regeneration.[149] The ionic composition of β-tricalcium phosphate (β-TCP) is similar to that of bone tissue. Zhang et al combined it with exosomes derived from MSCs and revealed the mechanism by which exosomes enhance bone regeneration by activating the PI3K/Akt signaling pathway.[150] These studies used bone volume or density as the end point; they did not record the residual amounts of microspheres or β-TCP and provide no degradation kinetics. Because calvarial repair is slow, scaffolds are normally engineered for prolonged degradation, yet their long-term persistence at the brain–bone interface may exert chronic physical pressure or sustained inflammatory stimuli on the underlying neural tissue.

Biological scaffolds further expand the utility of exosomes. 3D printing allows the geometry and dimensions of a scaffold to be tailored to the defect, yet the shear forces, temperature shifts, or photopolymerization inherent in the process can disrupt exosomal membranes or inactivate their cargo. Optimizing cross-linking chemistry and printing parameters is therefore essential. Although polymer-based scaffolds offer high mechanical strength and stable structural support, their stiffness may compress or injure brain tissue, so material design must explicitly match or buffer the modulus mismatch with native neural tissue. Clearance rates of scaffolds vary with the time-course of repair, and long-term safety data are still lacking. Moreover, incorporating robust behavioral readouts when evaluating biomaterial-based therapies will accelerate the clinical translation of exosome-laden tissue-engineered constructs.

Clinical Translation and Challenges

Progress in Preclinical Research

Large animal models, characterized by cerebral structures and injury mechanisms that more closely resemble those of humans, serve as a critical bridge for advancing exosome research toward clinical therapy. Primates are one of the more commonly used large animal models in TBI research. Zhou et al observed a significant reduction in glutamate receptor 2/3 in the cerebral cortex of aged rhesus monkeys following injury, along with enhanced coupling signals between microglial complement protein C1q and synapses in primary motor cortex.[151] Canine and pig models also share certain similarities in human physiological structure and can be used for related research after TBI. Liu et al encapsulated hypoxia-induced umbilical cord MSC exosomes in 3D-printed collagen silk scaffolds and implanted them into the TBI area of beagle dogs. The results showed that the composite biomaterial can significantly promote nerve fiber regeneration and axon extension, and reconstruct the neurovascular unit while inhibiting inflammation. This study offers a novel technical strategy for the clinical translation of exosome-based therapies.[152] Williams et al established a dual-hit model of

TBI combined with hemorrhagic shock in female Yorkshire pigs and treated them with exosomes derived from human BMSCs (hBMSCs), which markedly shortened recovery time. Notably, this study incorporated a "delayed treatment" paradigm into the experimental design, effectively replicating the positive effects of delayed administration in a porcine model, which is more consistent with the actual situation of injury.[153] Furthermore, they also found that early single-dose hBMSCs-derived exosome treatment increased the level of brain-derived neurotrophic factor, upregulated genes involved in synaptic transmission and neuronal development and differentiation while downregulated inflammatory genes, and protected the integrity of the BBB.[154–156] Bollard et al have made efforts in the standardization research of cross-species exosomes. Following the MISEV2018 guidelines, they isolated and characterized EVs from human and canine plasma, excluded non-EV contaminants, and showed that canonical markers such as CD63 and HSP70 are consistently expressed in both species, thereby providing standardized evidence for cross-species exosome comparisons.[157] In short, large animal models bridge the gap between rodent studies and non-human primate trials in exosome research, thereby laying a critical translational foundation for future clinical applications.(Figure 5).

Encouragingly, clinical validation of exosomes has entered the experimental stage. The Michigan Center for Regenerative Medicine reported a clinical case of hBMSCs treating sTBI. The patient was a 33-year-old male who had sustained sTBI in a traffic accident more than two years prior and had reached a rehabilitation plateau. The patient received intravenous infusion of the commercially available exosome product ExoFlo™ (Manufactured by Direct Biologics of the United States in accordance with current Good Manufacturing Practice (GMP) standards. The concentration of exosomes is 60–80billion/mL) during the first week of each month, for a total treatment period of six

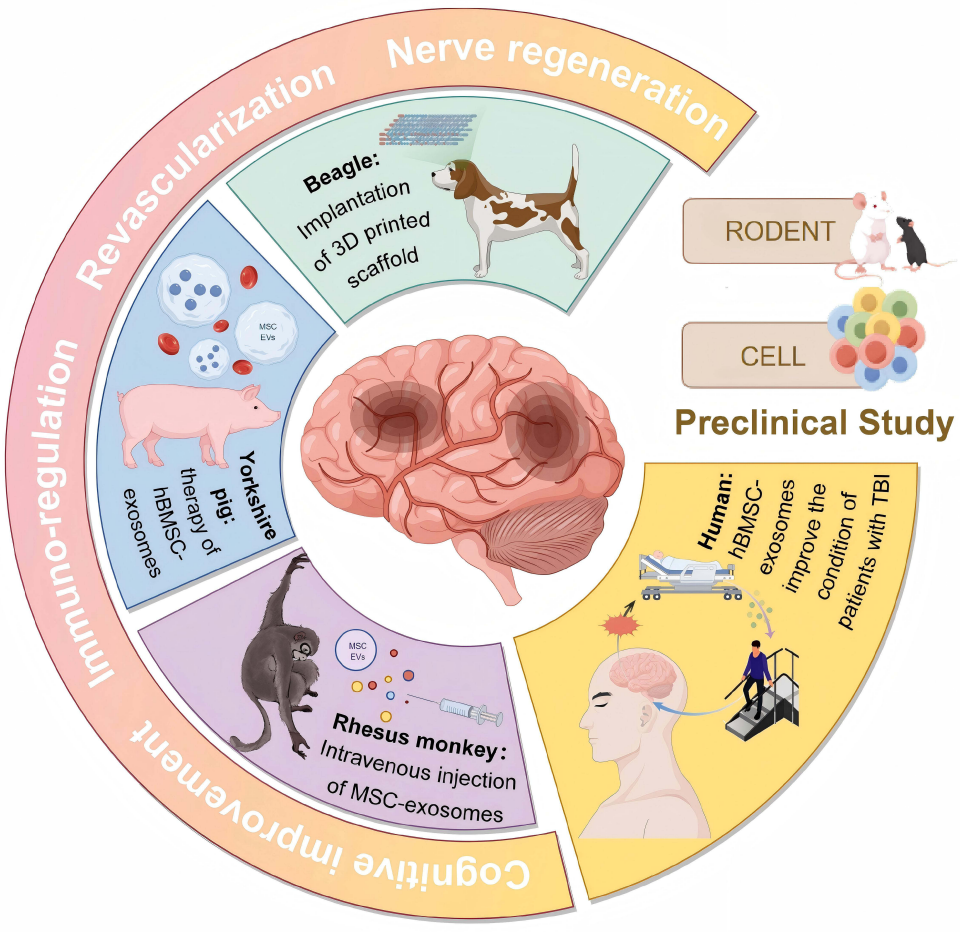


Figure 5 The translational research approach of exosome therapy for TBI. The research evolved from validation at the cellular level and in rodent models, progressed to evaluating preclinical efficacy and safety in large animal models, and ultimately advanced to validation in clinical applications.
Abbreviations: hBMSC, human bone marrow mesenchymal stem cells; MSC, mesenchymal stem cells; TBI, traumatic brain injury.

months. After 36 weeks, the overall score of the Functional Independence Measure/Functional Assessment Measure increased by 48–55%.[158] In a separate Phase 1 trial, Wharton's jelly-derived MSC exosomes were administered through combined intrathecal and intramuscular injections to five chronic-phase moderate–severe TBI patients whose injuries were combat-related. Over the 18-week observation period, although gains in cognition and spasticity did not reach statistical significance, the FIM motor scores and global quality of life improved significantly.[159] Both studies had small sample sizes and lacked randomized controls, but they undeniably accelerated the clinical transformation of exosome treatment regimens. In addition, breakthroughs have also been made in exosome-assisted clinical diagnosis. Using plasma-derived neuronal exosomes from the Vietnam Era Twin Study of Aging —— a cohort in which 40% of participants had a history of mild-to-moderate TBI, Michaela et al quantified AD-relevant proteins (Aβ40, Aβ42, p-tau and NfL) with single-molecule arrays. All analytes were significantly altered in participants with prior TBI, and the magnitude of change tracked with injury frequency, age at first injury and severity. The findings suggest that neuron-derived exosome cargo proteins could serve as accessible biomarkers of TBI-related neurodegenerative risk.[160] Hu et al developed an immunomagnetic exosome polymerase chain reaction platform to distinguish AD patients from healthy people by detecting Aβ and phosphorylated tau protein in blood exosomes. This method is capable of detecting biomarker concentrations as low as 10 fg/mL with a specificity of 95%.[161] Meanwhile, Ko et al proposed a brain-derived extracellular vesicle–miRNA panel strategy to assess the severity and time window of TBI. They validated the strategy's diagnostic efficacy in 60 patients with Abbreviated Injury Scores of 2–5.[162] These developments indicate that exosomes are progressively advancing toward clinical application in the diagnosis and treatment of neurological disorders, establishing a scalable technological paradigm for precision neurology.

Optimization of Administration Routes

When exploring exosomes as a novel therapeutic modality, the route of administration and the dose are the two variables that most strongly govern efficacy. Current administration routes for exosomes include intravenous injection, intramuscular injection, intranasal delivery, and oral administration (Table 2). Owing to the physiological characteristics of exosomes, intravenously injected exosomes can cross the BBB after reaching the brain tissue through the blood circulation, targeting neurons, astrocytes and microglia, to achieve brain tissue damage repair.[40,85] But intravenous injection needs to overcome the obstacles of its short half-life in the blood circulation and the existence of "off-target effects". In a clinical case report, a patient with chronic TBI received repeated intravenous infusions of 15 mL ExoFlo™ diluted in 85 mL normal saline.[158] This dose was chosen on the basis of safety data from an Expanded Access Protocol (NCT05215288) and a Phase II trial.[163] For post-TBI spasticity, intramuscular injection is a reasonable option. One study administered 3×10^8 exosomes in 3 mL via intrathecal and intramuscular routes per treatment cycle; six cycles were completed, giving a cumulative dose of 3.6×10^{10} exosomes. The regimen was well tolerated, with only mild and transient adverse events.[159] Intranasal and oral administrations enhance patient compliance and are considered favorable drug delivery options. Oral administration is more limited due to the gastrointestinal barrier and is primarily used for delivering milk-derived exosomes in the treatment of gastrointestinal disease, and their efficiency after TBI remains to be verified.[164,165] In contrast to oral or intravenous routes, intranasal delivery allows exosomes to bypass the BBB and directly enter the central nervous system via the olfactory nerve pathway. While this approach has been validated, its efficacy is constrained by factors such as ciliary movement reducing exosomal retention, inefficient transport along the olfactory/trigeminal nerve pathways, and low brain enrichment rates.[166–168] The intranasal dose varies with the animal model, therapeutic window and exosome source. Rats received 200μg of BMSC-EVs, whereas mice were given 10μg of exosomes derived from human adipose-derived stem cells.[169,170] Cross-species studies show significant dose variations, which directly limit the reliability of clinical translation. Therefore, the pharmacodynamics and pharmacokinetics of exosomes should be thoroughly investigated to establish safe and effective starting doses.

Standardization Challenges in Exosome Isolation

Heterogeneity of Exosome Sources and TBI

The functional of exosomes is tightly coupled to their cellular origin and molecular cargo. The heterogeneity of sources and the pathological complexity of TBI constitute the primary obstacles to the clinical transformation of exosomes.

Table 2 Research Progress on Exosome Therapy for TBI

Research Object	Administration Route	Endpoint Criteria	Refs
Rats (msTBI)	A single intranasal administration of hypoxia-pretreated BMSC-Exos (200 μg/100 μL) at 30 min post-injury, with a follow-up period of 28 days.	Early functional improvement; Restoration of cerebral blood flow; Reduction of neuronal apoptosis	[170]
Mice (mTBI)	A single dose of hAD-MSC exosomes (10 μg, containing MALAT1) was administered intranasally at 48 hours post-injury, with a follow-up period of 6–7 weeks.	Improvement in motor function and cognitive function; Reduction in cortical lesion volume	[169]
Beagle dog (msTBI)	A scaffold loaded with hypoxia-induced MSC exosomes (200 μg) was implanted immediately after injury, with a follow-up period of 6 months.	Improved neurological function; Reduced impaired consciousness	[152]
Yorkshire pig (sTBI + HS)	Initial intravenous infusion of hMSC-Exos at 9 hours post-injury, with additional administrations on days 1, 5, 9, and 13, dose 1×10^{13} particles/4mL, follow-up for 30 days.	Accelerated neurological recovery within 1 day postoperatively; Long-term neurological improvement	[153]
	A single dose of hMSC-Exos (1×10^{12} particles/5 mL LR) was administered intravenously 1 hour post-injury, with a follow-up period of 6 hours.	Reduction in brain swelling; Reduction in brain lesion size; Decrease in intracranial pressure	[155]
	A single dose of hMSC-Exos (1×10^{12} particles/5 mL LR) was administered intravenously 1 hour post-injury, with a follow-up period of 7 days.	Brain lesion reduction; Inflammation mitigation; Early neurological improvement	[154]
Aging rhesus monkeys (Cortical injury of M1)	Intravenous infusion of MSC-Exos was administered at 24 hours and 14 days postoperatively, with a dose of 4×10^{11} particles each time, with a follow-up period of 14–16 weeks.	Recovery and enhancement of fine motor function in the hand; Reduced inflammatory response	[151]
Human (Chronic TBI)	Combined intrathecal (3 mL) and intramuscular (3 mL) injection of Wharton's jelly-derived MSC exosomes (30×10^{9} particles/3 mL) was administered at weeks 0, 2, 6, 10, 14, and 18 post-injury, with a follow-up period of 1 year.	Improvement in FIM motor score and KPS score	[159]
Human (sTBI)	Intravenous infusion of hBMSC-Exos (ExoFlo™) was administered at a dose of 15 mL per infusion, three times per week for 6 months, followed for 36 weeks.	Improvement in FIM motor score and FAM score	[158]

Abbreviations: msTBI, moderate-severe TBI; mTBI, mild TBI; sTBI, severe TBI; HS, haemorrhagic shock; M1, primary motor cortex; Exos, exosomes; BMSC, Bone marrow stromal cell; hAD-MSC, human adipose-derived mesenchymal stem cell; MSC, mesenchymal stem cell; hMSC, human mesenchymal stem cell; LR, lactated ringer; FIM, functional independence measure; KPS, karnofsky performance status; FAM, functional assessment measure.

Secretory state adds a second layer of variability —— the same cell type can release phenotypically distinct exosomes under different physiological or pathological conditions. MSCs exposed to neuroinflammatory cues, for example, package exosomes that simultaneously up-regulate both pro- and anti-inflammatory mediators, producing a dual immunomodulatory signature.[30] By contrast, hypoxia biases the cargo toward neuroprotective molecules that limit neuronal apoptosis.[171] In tissue-engineered therapies, scaffold chemistry and surface modification govern loading efficiency and release kinetics, indirectly shaping downstream bioactivity. As biomarkers, exosomes are typically harvested from peripheral blood, but brain-derived vesicles are interfered with by ubiquitous circulating exosomes, thereby reducing the specific enrichment of central nervous system signals.

TBI is highly heterogeneous. The type and severity of injury, the stage of disease course, and individual patient differences all lead the brain tissue to present distinct pathological states. Especially in the aspect of biomarker monitoring, injuries of different degrees induce the release of different exosomes as signalling molecules, and their expression levels change dynamically with injury time and disease progression, resulting in limited timeliness. This heterogeneity makes it difficult to personalize the therapeutic effect of exosomes. Therefore, identifying an appropriate therapeutic window according to the manifestations of acute and chronic injury is a central step for clinical application.

Immunogenicity and Regulatory Risks

As a cell-free therapy, exosomes lack a nucleus and proliferative capacity, avoiding the potential tumorigenesis risks and intercellular fusion toxicity that may occur in traditional stem cell transplantation. Their surface carries only trace major histocompatibility complex molecules, so their immunogenicity is markedly lower than that of living cells, dampening both immune rejection and inflammatory responses. Nevertheless, exosomes are not risk-free: if the parental cells or body fluids harbour pathogens, cross-infection remains possible. Regulators such as the FDA, the European Medicines

Agency and National Medical Products Administration have not yet approved any exosome product. They classify exosomes as "biologics" or "advanced therapy medicinal products" and subject them to the same stringent evaluation pathway used for similar cell therapies.[172] Establishing a robust quality-control framework that meets Good Manufacturing Practice standards is an essential step toward clinical translation.

Functionalization strategies have improved the therapeutic potential of natural exosomes, yet their clinical translation is hindered by stringent regulatory hurdles and safety concerns. Genetic editing or chemical grafting shifts exosomes from simple biologics to engineered "bio-hybrids" that defy existing classification frameworks, creating a formidable regulatory barrier. Surface decoration with foreign proteins or non-natural motifs can remodel exosome surface antigens, disturb immune recognition and trigger inflammatory or allergic reactions. Although exosomes themselves lack nuclear DNA, the parental cells are often modified with integrating vectors such as adenovirus or lentivirus; the genomic stability and biosafety profile of these engineered cell lines must therefore be rigorously scrutinized.

Challenges in Separation and Purification

The MISEV2023 guidelines issued by the International Society for Extracellular Vesicles (ISEV) update the criteria for isolating and characterizing extracellular vesicles: After isolation, vesicles should be profiled against three positive markers (transmembrane/lipid-bound proteins such as CD9 and CD63, or cytosolic/endosomal proteins like Alix and TSG101) and one negative marker (GM130 or Calnexin). Particle concentration should be determined with nanoparticle tracking analysis or flow cytometry. Functional studies must state the exact vesicle source and provide a defined dose to allow replication.[173]

However, current isolation strategies still have limitations: (1) ultracentrifugation is a mature operation and remains the primary standard for exosome isolation, yet its non-selective sedimentation co-pellets abundant protein complexes and lipoproteins, and the high centrifugal force can compromise exosome structural integrity;[174,175] (2) density-gradient centrifugation effectively removes lipoproteins and yields high-purity fractions, but the protocol is labor-intensive, time-consuming, and gives low recovery;[176] (3) size-exclusion chromatography efficiently depletes free proteins, yet VLDL and IDL lipoproteins overlap closely with exosomes in size, leading to co-elution;[177] (4) asymmetric-flow field-flow fractionation avoids shear-induced damage and provides high-resolution particle analysis, but the procedure is complex, the instrumentation is costly, and scale-up is challenging.[178] In addition, immunoaffinity capture and polyethylene-glycol precipitation have been incorporated into commercial exosome kits, but their performance still requires validation.[179] Figure 6 schematically compares the yield and specificity of these isolation methods, and each has inherent limitations. (Figure 6) Consequently, the MISEV2023 guideline recommends sequential purification with complementary techniques to improve specificity. Researchers have begun corresponding explorations. Tayebi et al coupled microfluidics with acoustics and raised exosome purity to 95%.[180] The same acoustofluidic approach efficiently isolates plasma exosomes.[181] A tilted-angle standing surface acoustic wave (taSSAW) device has further expanded the microfluidic toolbox by adding precise control of acoustic pressure, flow rate and tilt angle.[182] Many studies still do not report all isolation parameters; future work should meet the transparency requirement so that the protocols can be more widely adopted.

Lack of Clinical Evidence

Research on exosomes in TBI remains largely preclinical. Although studies in rodent models have shown clear neuroprotection, the differences in brain architecture, immune responses and injury complexity between these animals and humans prevent direct translation. Most reports focus on histological end-points and short-term functional recovery, while the cognitive deficits and mood disturbances common in human TBI are rarely addressed. Preclinical data from large-animal models supply partial evidence, yet sample sizes are small and engineered exosomes have seldom been tested. Clinical trials have begun, but they are typically single-centre, include few participants, suffer from patient selection bias, lack standardized outcome measures and provide limited safety information. Progress from bench to bedside will require adequately powered randomized controlled trials and large cohort studies to establish both efficacy and long-term safety.

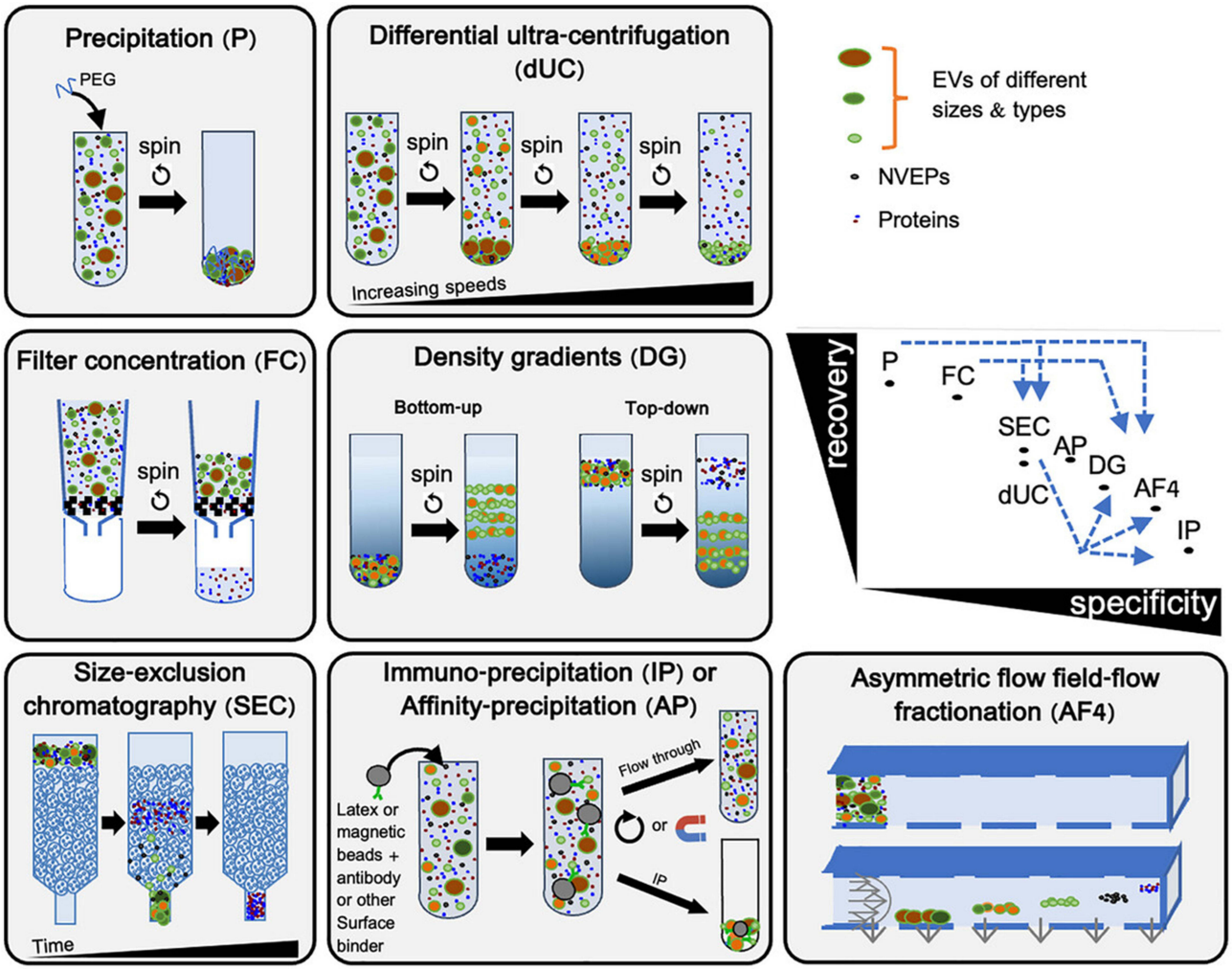


Figure 6 The yield and specificity of various exosome isolation methods. The dashed blue arrow indicates that multiple methods are combined to increase specificity. Sourced from Welsh, J. A.[173] via the Journal of Extracellular Vesicles under a CC BY 4.0 license. Canonical URL:https://creativecommons.org/licenses/by/4.0/.

Disputes Over Ethical Norms

The ethical debate surrounding exosomes stems mainly from their cellular origin and the associated biosafety concerns. Research involving exosomes derived from both animal and human sources must undergo strict ethical review. Stem-cell exosomes are a common research source, yet any recovery from human tissue must follow the principle of voluntary, non-remunerated donation and be approved by the ethics committee of the relevant medical institution. The clinical translation of exosomes remains at an early stage, and their potential risks are still unclear. Hazards such as pathogen transmission and cross-reactive immune responses, particularly those associated with exosomes derived from embryonic or gene edited cells, have not been ruled out. In addition, the regulatory classification of exosomes is ambiguous, some companies may skip adequate safety assessment, and the compliance of exosome-based applications is highly uncertain. Regulatory agencies worldwide need to establish standardized ethical review and oversight mechanisms and to formulate review principles.

Conclusion and Prospect

The Potential of Exosomes in the Application of TBI

Exosomes assemble a multi-target neuroprotective network by modulating inflammation, fostering neuronal regrowth, participating in vascular repair, and ameliorating cognitive deficits. Exosomes play a crucial role in regulating the NF-κB and PI3K/AKT pathways to alleviate inflammation and apoptosis, and their high repeatability verification provides

reliable targets for treatment. However, the inflammatory network triggered by TBI is intricate. During the acute phase, exosomes released by injured neurons or activated microglia are rich in miR-21 and can aggravate the inflammatory milieu.[183] Because the cell of origin shapes the therapeutic outcome, the molecular cargo reported by different groups overlaps only modestly, with miR-124-3p being the most consistently examined. These cargo molecules have shown efficacy in rodent models, and MSC-derived exosomes have already produced functional gains in early-stage clinical trials, indicating that exosome-based therapy is feasible. To enhance targeting, investigators have engineered the surface of exosomes with specific ligands or homing peptides.[184,185] Combining exosomes with biocompatible scaffolds prolongs their sustained release, while the scaffold itself mimics the extracellular matrix, furnishing structural support to the injured brain and providing a stable niche for the exosomes, thereby cooperatively promoting neuronal adhesion and microvascular formation.

In diagnostics, exosomes carry multi-omics information, and their phospholipid bilayer shields the cargo, enabling a wider and more stable detection window that supports repeated sampling and longitudinal tracking. Researchers have identified RNA and proteins that are differentially expressed in TBI-derived exosomes, which help to grade injury severity; moreover, the time-dependent profile of certain cargoes allows for dynamic disease assessment. Plasma GFAP and UCH-L1 are already FDA-approved biomarkers, and both are enriched in exosomes. Their combined readout has been shown to outperform either marker alone.

Future Research Direction

Exosomes, as a cell-free therapy, greatly reduce the risks of tumorigenicity, immune rejection, and cell-fusion toxicity associated with cell transplantation, offering a new paradigm for overcoming the limitations of traditional TBI diagnosis and treatment. However, the marked heterogeneity of TBI has created bottlenecks for clinical translation. Future work should therefore dissect the pathological differences among TBI subtypes and across injury phases to clarify the activation profiles of core regulatory pathways and the key molecular targets at each stage, thereby helping to define optimal treatment windows. To date, the therapeutic efficacy of exosomes for TBI has been validated mainly in animal models, and large-scale randomized controlled trials are still lacking. Studies should therefore expand clinical cohorts, recruit patients from diverse regions and populations, adhere strictly to randomized controlled designs, and analyze optimal routes of administration and dosing regimens while systematically assessing both short-term efficacy and long-term prognosis. For engineered exosomes, functional benefits and safety profiles need to be balanced by refining targeting modifications that enhance specificity without introducing additional hazards. Parallel efforts must address issues such as scaffold biodegradability to ensure long-term material safety. In addition, investigators can explore exosome-based combination therapies to construct multimodal treatment strategies.

In the biomarker field, sample sources must be expanded and multicenter prospective cohorts established so that confounding effects on marker expression can be systematically evaluated and phenotype-specific panels for distinct TBI subtypes can be defined. Active efforts are also needed to enrich brain-derived exosomes and to minimize contamination by peripheral exosomes, improving diagnostic accuracy. In addition, isolation and purification procedures should be optimized in accordance with MISEV guidelines. Current challenges, such as low yield and limited purity, should be addressed by combining complementary techniques or developing novel separation methods. A production process and quality-control system that meet GMP standards are also required to ensure batch-to-batch consistency and to provide a basis for reproducible and comparable clinical studies.

As clinical research advances, technology evolves and regulatory frameworks mature, exosomes may become a new tool for both the treatment and diagnosis of TBI.

Acknowledgments

The authors thank all the reviewers for valuable comments. Thanks to the Figdraw platform (https://www.figdraw.com). for its assistance in drawing the images in this article.

Funding

This research was sponsored by National Natural Science Foundation of China (82272255 to XC).

Disclosure

The authors declare no conflicts of interest in this work.

References

1. GBD 2016 Traumatic Brain Injury and Spinal Cord Injury Collaborators. Global, regional, and national burden of traumatic brain injury and spinal cord injury, 1990–2016: a systematic analysis for the global burden of disease study 2016. *Lancet Neurol*. 2019;18(1):56–87. doi:10.1016/S1474-4422(18)30415-0
2. Maas AIR, Menon DK, Manley GT, et al. Traumatic brain injury: progress and challenges in prevention, clinical care, and research. *Lancet Neurol*. 2022;21(11):1004–1060. doi:10.1016/S1474-4422(22)00309-X
3. Zhang Z, Hong Y, Gregory JS. Trauma care in China: a systematic review. *J Emerg Crit Care Med*. 2017;1(11):11. doi:10.21037/jeccm.2017.10.05
4. Marino AN, Mui M, Dobryakova E, Sandry J. Classification systems for moderate-severe traumatic brain injury: review and recommendations. *Curr Phys Med Rehabil Rep*. 2025;13(1):32. doi:10.1007/s40141-025-00504-7
5. Pearn ML, Niesman IR, Egawa J, et al. Pathophysiology associated with traumatic brain injury: current treatments and potential novel therapeutics. *Cell Mol Neurobiol*. 2017;37(4):571–585. doi:10.1007/s10571-016-0400-1
6. Beck J, Fung C, Strbian D, et al. Decompressive craniectomy plus best medical treatment versus best medical treatment alone for spontaneous severe deep supratentorial intracerebral haemorrhage: a randomised controlled clinical trial. *Lancet Lond Engl*. 2024;403(10442):2395–2404. doi:10.1016/S0140-6736(24)00702-5
7. Harding C, Heuser J, Stahl P. Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes. *J Cell Biol*. 1983;97(2):329–339. doi:10.1083/jcb.97.2.329
8. Johnstone RM, Adam M, Hammond JR, et al. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). *J Biol Chem*. 1987;262(19):9412–9420.
9. Mathieu M, Martin-Jaular L, Lavieu G, Théry C. Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. *Nat Cell Biol*. 2019;21(1):9–17. doi:10.1038/s41556-018-0250-9
10. Colombo M, Raposo G, Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu Rev Cell Dev Biol*. 2014;30:255–289. doi:10.1146/annurev-cellbio-101512-122326
11. Herrmann IK, Wood MJA, Fuhrmann G. Extracellular vesicles as a next-generation drug delivery platform. *Nat Nanotechnol*. 2021;16(7):748–759. doi:10.1038/s41565-021-00931-2
12. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science*. 2020;367(6478):eaau6977. doi:10.1126/science.aau6977
13. van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. *Nat Rev Mol Cell Biol*. 2018;19(4):213–228. doi:10.1038/nrm.2017.125
14. Guo M, Yin Z, Chen F, Lei P. Mesenchymal stem cell-derived exosome: a promising alternative in the therapy of Alzheimer's disease. *Alzheimers Res Ther*. 2020;12(1):109. doi:10.1186/s13195-020-00670-x
15. Li Q, Fu X, Kou Y, Han N. Engineering strategies and optimized delivery of exosomes for theranostic application in nerve tissue. *Theranostics*. 2023;13(12):4266–4286. doi:10.7150/thno.84971
16. Branscome H, Paul S, Yin D, et al. Use of stem cell extracellular vesicles as a "holistic" approach to CNS repair. *Front Cell Dev Biol*. 2020;8:455. doi:10.3389/fcell.2020.00455
17. Pascual M, Ibáñez F, Guerri C. Exosomes as mediators of neuron-glia communication in neuroinflammation. *Neural Regen Res*. 2020;15(5):796–801. doi:10.4103/1673-5374.268893
18. Yang Y, Wang Y, Li P, Bai F, Liu C, Huang X. Serum exosomes miR-206 and miR-549a-3p as potential biomarkers of traumatic brain injury. *Sci Rep*. 2024;14(1):10082. doi:10.1038/s41598-024-60827-8
19. Zhao RT, Zhou J, Dong XL, et al. Circular ribonucleic acid expression alteration in exosomes from the brain extracellular space after traumatic brain injury in mice. *J Neurotrauma*. 2018;35(17):2056–2066. doi:10.1089/neu.2017.5502
20. Peltz CB, Kenney K, Gill J, Diaz-Arrastia R, Gardner RC, Yaffe K. Blood biomarkers of traumatic brain injury and cognitive impairment in older veterans. *Neurology*. 2020;95(9):e1126–e1133. doi:10.1212/WNL.0000000000010087
21. Ghaith HS, Nawar AA, Gabra MD, et al. A literature review of traumatic brain injury biomarkers. *Mol Neurobiol*. 2022;59(7):4141–4158. doi:10.1007/s12035-022-02822-6
22. Wu X, Liu H, Hu Q, et al. Astrocyte-derived extracellular vesicular miR-143-3p dampens autophagic degradation of endothelial adhesion molecules and promotes neutrophil transendothelial migration after acute brain injury. *Adv Sci Weinh Baden-Wurtt Ger*. 2024;11(5):e2305339. doi:10.1002/advs.202305339
23. Kumar A, Kumar S. Inhibition of extracellular vesicle pathway using neutral sphingomyelinase inhibitors as a neuroprotective treatment for brain injury. *Neural Regen Res*. 2021;16(12):2349–2352. doi:10.4103/1673-5374.313014
24. Xian P, Hei Y, Wang R, et al. Mesenchymal stem cell-derived exosomes as a nanotherapeutic agent for amelioration of inflammation-induced astrocyte alterations in mice. *Theranostics*. 2019;9(20):5956–5975. doi:10.7150/thno.33872
25. KS V, Daorong F. The Machinery of Exosomes: biogenesis, Release, and Uptake. *Int J Mol Sci*. 2023;24(2):1337.
26. Chen X, Chen C, Fan S, et al. Omega-3 polyunsaturated fatty acid attenuates the inflammatory response by modulating microglia polarization through SIRT1-mediated deacetylation of the HMGB1/NF-κB pathway following experimental traumatic brain injury. *J Neuroinflammation*. 2018;15(1):116. doi:10.1186/s12974-018-1151-3
27. Cash A, Theus MH. Mechanisms of blood-brain barrier dysfunction in traumatic brain injury. *Int J Mol Sci*. 2020;21(9):3344. doi:10.3390/ijms21093344
28. Ghosh M, Pearse DD. The yin and yang of microglia-derived extracellular vesicles in CNS injury and diseases. *Cells*. 2024;13(22):1834. doi:10.3390/cells13221834

29. Hegdekar N, Sarkar C, Bustos S, et al. Inhibition of autophagy in microglia and macrophages exacerbates innate immune responses and worsens brain injury outcomes. *Autophagy*. 2023;19(7):2026–2044. doi:10.1080/15548627.2023.2167689
30. Loane DJ, Kumar A. Microglia in the TBI brain: the good, the bad, and the dysregulated. *Exp Neurol*. 2016;275 Pt 3(3):316–327. doi:10.1016/j.expneurol.2015.08.018
31. Liao J, Li Y, Fan L, et al. Bioactive ceria nanoenzymes target mitochondria in reperfusion injury to treat ischemic stroke. *ACS Nano*. 2024;18(7):5510–5529. doi:10.1021/acsnano.3c10982
32. Li C, Qin S, Wen Y, Zhao W, Huang Y, Liu J. Overcoming the blood-brain barrier: exosomes as theranostic nanocarriers for precision neuroimaging. *J Control Release*. 2022;349:902–916. doi:10.1016/j.jconrel.2022.08.002
33. Kim J, Zhu Y, Chen S, et al. Anti-glioma effect of ginseng-derived exosomes-like nanoparticles by active blood-brain-barrier penetration and tumor microenvironment modulation. *J Nanobiotechnology*. 2023;21(1):253. doi:10.1186/s12951-023-02006-x
34. Vuokila N, Lukasiuk K, Bot AM, et al. miR-124-3p is a chronic regulator of gene expression after brain injury. *Cell Mol Life Sci*. 2018;75(24):4557–4581. doi:10.1007/s00018-018-2911-z
35. Vuokila N, Aronica E, Korotkov A, et al. Chronic regulation of miR-124-3p in the perilesional cortex after experimental and human TBI. *Int J Mol Sci*. 2020;21(7):2418. doi:10.3390/ijms21072418
36. Yang Y, Ye Y, Fan K, Luo J, Yang Y, Ma Y. MiR-124 reduced neuroinflammation after traumatic brain injury by inhibiting TRAF6. *Neuroimmunomodulation*. 2023;30(1):55–68. doi:10.1159/000528502
37. Huang S, Ge X, Yu J, et al. Increased miR-124-3p in microglial exosomes following traumatic brain injury inhibits neuronal inflammation and contributes to neurite outgrowth *via* their transfer into neurons. *FASEB J*. 2018;32(1):512–528. doi:10.1096/fj.201700673R
38. He X, Huang Y, Liu Y, et al. Astrocyte-derived exosomal lncRNA 4933431K23Rik modulates microglial phenotype and improves post-traumatic recovery via SMAD7 regulation. *Mol Ther*. 2023;31(5):1313–1331. doi:10.1016/j.ymthe.2023.01.031
39. Long X, Yao X, Jiang Q, et al. Astrocyte-derived exosomes enriched with miR-873a-5p inhibit neuroinflammation via microglia phenotype modulation after traumatic brain injury. *J Neuroinflammation*. 2020;17(1):89. doi:10.1186/s12974-020-01761-0
40. Zhang ZW, Wei P, Zhang GJ, et al. Intravenous infusion of the exosomes derived from human umbilical cord mesenchymal stem cells enhance neurological recovery after traumatic brain injury via suppressing the NF-κB pathway. *Open Life Sci*. 2022;17(1):189–201. doi:10.1515/biol-2022-0022
41. Bhowmick S, D'Mello V, Caruso D, Abdul-Muneer PM. Traumatic brain injury-induced downregulation of Nrf2 activates inflammatory response and apoptotic cell death. *J Mol Med*. 2019;97(12):1627–1641. doi:10.1007/s00109-019-01851-4
42. Muneer PMA, Schuetz H, Wang F, et al. Induction of oxidative and nitrosative damage leads to cerebrovascular inflammation in an animal model of mild traumatic brain injury induced by primary blast. *Free Radic Biol Med*. 2013;60:282–291. doi:10.1016/j.freeradbiomed.2013.02.029
43. Abdul-Muneer PM, Chandra N, Haorah J. Interactions of oxidative stress and neurovascular inflammation in the pathogenesis of traumatic brain injury. *Mol Neurobiol*. 2015;51(3):966–979. doi:10.1007/s12035-014-8752-3
44. Pastori D, Carnevale R, Cangemi R, et al. Vitamin E serum levels and bleeding risk in patients receiving oral anticoagulant therapy: a retrospective cohort study. *J Am Heart Assoc*. 2013;2(6):e000364. doi:10.1161/JAHA.113.000364
45. Thomale UW, Griebenow M, Kroppenstedt SN, Unterberg AW, Stover JF. The effect of N-acetylcysteine on posttraumatic changes after controlled cortical impact in rats. *Intensive Care Med*. 2006;32(1):149–155. doi:10.1007/s00134-005-2845-4
46. Pandya JD, Readnower RD, Patel SP, et al. N-acetylcysteine amide confers neuroprotection, improves bioenergetics and behavioral outcome following TBI. *Exp Neurol*. 2014;257:106–113. doi:10.1016/j.expneurol.2014.04.020
47. Hawryluk GWJ, Aguilera S, Buki A, et al. A management algorithm for patients with intracranial pressure monitoring: the Seattle international severe traumatic brain injury consensus conference (SIBICC). *Intensive Care Med*. 2019;45(12):1783–1794. doi:10.1007/s00134-019-05805-9
48. Wurzelmann M, Romeika J, Sun D. Therapeutic potential of brain-derived neurotrophic factor (BDNF) and a small molecular mimics of BDNF for traumatic brain injury. *Neural Regen Res*. 2017;12(1):7–12. doi:10.4103/1673-5374.198964
49. Adams JM, Cory S. The bcl-2 protein family: arbiters of cell survival. *Science*. 1998;281(5381):1322–1326. doi:10.1126/science.281.5381.1322
50. Du L, Jiang Y, Sun Y. Astrocyte-derived exosomes carry microRNA-17-5p to protect neonatal rats from hypoxic-ischemic brain damage via inhibiting BNIP-2 expression. *NeuroToxicology*. 2021;83:28–39. doi:10.1016/j.neuro.2020.12.006
51. Hoang TT, Johnson DA, Raines RT, Johnson JA. Angiogenin activates the astrocytic Nrf2/antioxidant-response element pathway and thereby protects murine neurons from oxidative stress. *J Biol Chem*. 2019;294(41):15095–15103. doi:10.1074/jbc.RA119.008491
52. Zhang W, Hong J, Zhang H, Zheng W, Yang Y. Astrocyte-derived exosomes protect hippocampal neurons after traumatic brain injury by suppressing mitochondrial oxidative stress and apoptosis. *Aging*. 2021;13(17):21642–21658. doi:10.18632/aging.203508
53. Zhuang Z, Liu M, Luo J, et al. Exosomes derived from bone marrow mesenchymal stem cells attenuate neurological damage in traumatic brain injury by alleviating glutamate-mediated excitotoxicity. *Exp Neurol*. 2022;357:114182. doi:10.1016/j.expneurol.2022.114182
54. Gu C, Li Y, Liu J, et al. Neural stem cell-derived exosomes-loaded adhesive hydrogel controlled-release promotes cerebral angiogenesis and neurological function in ischemic stroke. *Exp Neurol*. 2023;370:114547. doi:10.1016/j.expneurol.2023.114547
55. Andreone BJ, Lacoste B, Gu C. Neuronal and vascular interactions. *Annu Rev Neurosci*. 2015;38:25–46. doi:10.1146/annurev-neuro-071714-033835
56. Zhai Y, Zhou Z, Xing X, Nuzzle M, Zhang X. Differential bone and vessel type formation at superior and dura periosteum during cranial bone defect repair. *Bone Res*. 2025;13(1):8. doi:10.1038/s41413-024-00379-9
57. Schilling K, Zhai Y, Zhou Z, Zhou B, Brown E, Zhang X. High-resolution imaging of the osteogenic and angiogenic interface at the site of murine cranial bone defect repair via multiphoton microscopy. *eLife*. 2022;11:e83146. doi:10.7554/eLife.83146
58. Grellier M, Bordenave L, Amédée J. Cell-to-cell communication between osteogenic and endothelial lineages: implications for tissue engineering. *Trends Biotechnol*. 2009;27(10):562–571. doi:10.1016/j.tibtech.2009.07.001
59. Yu H, VandeVord PJ, Mao L, Matthew HW, Wooley PH, Yang SY. Improved tissue-engineered bone regeneration by endothelial cell mediated vascularization. *Biomaterials*. 2009;30(4):508–517. doi:10.1016/j.biomaterials.2008.09.047
60. Van Balkom BWM, de Jong OG, Smits M, et al. Endothelial cells require miR-214 to secrete exosomes that suppress senescence and induce angiogenesis in human and mouse endothelial cells. *Blood*. 2013;121(19):3997–4006,S1–15. doi:10.1182/blood-2013-02-478925

61. Todorova D, Simoncini S, Lacroix R, Sabatier F, Dignat-George F. Extracellular vesicles in angiogenesis. *Circ Res*. 2017;120(10):1658–1673. doi:10.1161/CIRCRESAHA.117.309681
62. Kalani A, Chaturvedi P, Kamat PK, et al. Curcumin-loaded embryonic stem cell exosomes restored neurovascular unit following ischemia-reperfusion injury. *Int J Biochem Cell Biol*. 2016;79:360–369. doi:10.1016/j.biocel.2016.09.002
63. Deregibus MC, Cantaluppi V, Calogero R, et al. Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA. *Blood*. 2007;110(7):2440–2448. doi:10.1182/blood-2007-03-078709
64. Gao W, Li F, Liu L, et al. Endothelial colony-forming cell-derived exosomes restore blood-brain barrier continuity in mice subjected to traumatic brain injury. *Exp Neurol*. 2018;307:99–108. doi:10.1016/j.expneurol.2018.06.001
65. Hu MS, Borrelli MR, Lorenz HP, Longaker MT, Wan DC. Mesenchymal stromal cells and cutaneous wound healing: a comprehensive review of the background, role, and therapeutic potential. *Stem Cells Int*. 2018;2018:6901983. doi:10.1155/2018/6901983
66. Zhang Y, Chopp M, Meng Y, et al. Effect of exosomes derived from multipluripotent mesenchymal stromal cells on functional recovery and neurovascular plasticity in rats after traumatic brain injury. *J Neurosurg*. 2015;122(4):856–867. doi:10.3171/2014.11.JNS14770
67. Anderson JD, Johansson HJ, Graham CS, et al. Comprehensive proteomic analysis of mesenchymal stem cell exosomes reveals modulation of angiogenesis via nuclear factor-KappaB signaling. *Stem Cells Dayt Ohio*. 2016;34(3):601–613. doi:10.1002/stem.2298
68. Tang W, Guo ZD, Chai WN, et al. Downregulation of miR-491-5p promotes neovascularization after traumatic brain injury. *Neural Regen Res*. 2022;17(3):577–586. doi:10.4103/1673-5374.314326
69. Zoerle T, Beqiri E, Åkerlund CAI, et al. Intracranial pressure monitoring in adult patients with traumatic brain injury: challenges and innovations. *Lancet Neurol*. 2024;23(9):938–950. doi:10.1016/S1474-4422(24)00235-7
70. Mastorakos P, Russo MV, Zhou T, Johnson K, McGavern DB. Antimicrobial immunity impedes CNS vascular repair following brain injury. *Nat Immunol*. 2021;22(10):1280–1293. doi:10.1038/s41590-021-01012-1
71. Sharma R, Shultz SR, Robinson MJ, et al. Infections after a traumatic brain injury: the complex interplay between the immune and neurological systems. *Brain Behav Immun*. 2019;79:63–74. doi:10.1016/j.bbi.2019.04.034
72. Liang B, Liang JM, Ding JN, Xu J, Xu JG, Chai YM. Dimethyloxaloylglycine-stimulated human bone marrow mesenchymal stem cell-derived exosomes enhance bone regeneration through angiogenesis by targeting the AKT/mTOR pathway. *Stem Cell Res Ther*. 2019;10(1):335. doi:10.1186/s13287-019-1410-y
73. Simon DW, McGeachy M, Bayır H, Clark RSB, Loane DJ, Kochanek PM. Neuroinflammation in the evolution of secondary injury, repair, and chronic neurodegeneration after traumatic brain injury. *Nat Rev Neurol*. 2017;13(3):171–191. doi:10.1038/nrneurol.2017.13
74. Brett BL, Temkin N, Barber JK, et al. Long-term multidomain patterns of change after traumatic brain injury: a TRACK-TBI LONG study. *Neurology*. 2023;101(7):e740–e753. doi:10.1212/WNL.0000000000207501
75. Buckner RL, Sepulcre J, Talukdar T, et al. Cortical Hubs Revealed by Intrinsic Functional Connectivity: mapping, Assessment of Stability, and Relation to Alzheimer's Disease. *J Neurosci*. 2009;29(6):1860–1873. doi:10.1523/JNEUROSCI.5062-08.2009
76. Bondanelli M, Ambrosio MR, Cavazzini L, et al. Anterior pituitary function may predict functional and cognitive outcome in patients with traumatic brain injury undergoing rehabilitation. *J Neurotrauma*. 2007;24(11):1687–1697. doi:10.1089/neu.2007.0343
77. Chen H, Chan YL, Nguyen LT, et al. Moderate traumatic brain injury is linked to acute behaviour deficits and long term mitochondrial alterations. *Clin Exp Pharmacol Physiol*. 2016;43(11):1107–1114. doi:10.1111/1440-1681.12650
78. Yin Y, Li E, Sun G, et al. Effects of DHA on hippocampal autophagy and lysosome function after traumatic brain injury. *Mol Neurobiol*. 2018;55(3):2454–2470. doi:10.1007/s12035-017-0504-8
79. Dabin R, Wei C, Liang S, Ke C, Zhihan W, Ping Z. Astrocytic IGF-1 and IGF-1R Orchestrate Mitophagy in Traumatic Brain Injury via Exosomal miR-let-7e. *Oxid Med Cell Longev*. 2022;2022:1–18. doi:10.1155/2022/3504279
80. Li Y, Chen W, Feng J. Astrocyte-derived exosomal GJA1-20 k targets Pink1-mediated mitophagy to attenuate traumatic brain injury. *Transl Stroke Res*. 2025. doi:10.1007/s12975-025-01374-w
81. Li G, Iliff J, Shofer J, et al. CSF β-amyloid and tau biomarker changes in veterans with mild traumatic brain injury. *Neurology*. 2024;102(7):e209197. doi:10.1212/WNL.0000000000209197
82. Yuyama K, Sun H, Mitsutake S, Igarashi Y. Sphingolipid-modulated exosome secretion promotes clearance of amyloid-β by microglia. *J Biol Chem*. 2012;287(14):10977–10989. doi:10.1074/jbc.M111.324616
83. Deng Z, Wang J, Xiao Y, et al. Ultrasound-mediated augmented exosome release from astrocytes alleviates amyloid-β-induced neurotoxicity. *Theranostics*. 2021;11(9):4351–4362. doi:10.7150/thno.52436
84. Huang S, Liao X, Wu J, et al. The Microglial membrane receptor TREM2 mediates exosome secretion to promote phagocytosis of amyloid-β by microglia. *FEBS Lett*. 2022;596(8):1059–1071. doi:10.1002/1873-3468.14336
85. Ge X, Guo M, Hu T, et al. Increased microglial exosomal miR-124-3p alleviates neurodegeneration and improves cognitive outcome after rmTBI. *Mol Ther*. 2020;28(2):503–522. doi:10.1016/j.ymthe.2019.11.017
86. Reith FCM, Van den Brande R, Synnot A, Gruen R, Maas AIR. The reliability of the Glasgow Coma Scale: a systematic review. *Intensive Care Med*. 2016;42(1):3–15. doi:10.1007/s00134-015-4124-3
87. Khalil M, Teunissen CE, Otto M, et al. Neurofilaments as biomarkers in neurological disorders. *Nat Rev Neurol*. 2018;14(10):577–589. doi:10.1038/s41582-018-0058-z
88. Wang KKW, Barton DJ, McQuillan LE, et al. Parallel Cerebrospinal Fluid and Serum Temporal Profile Assessment of Axonal Injury Biomarkers Neurofilament-Light Chain and Phosphorylated Neurofilament-Heavy Chain: associations With Patient Outcome in Moderate-Severe Traumatic Brain Injury. *J Neurotrauma*. 2024;41(13–14):1609–1627. doi:10.1089/neu.2023.0449
89. Amoo M, Henry J, O'Halloran PJ, et al. S100B, GFAP, UCH-L1 and NSE as predictors of abnormalities on CT imaging following mild traumatic brain injury: a systematic review and meta-analysis of diagnostic test accuracy. *Neurosurg Rev*. 2022;45(2):1171–1193. doi:10.1007/s10143-021-01678-z
90. Guedes VA, Kenney K, Shahim P, et al. Exosomal neurofilament light: a prognostic biomarker for remote symptoms after mild traumatic brain injury? *Neurology*. 2020;94(23):e2412–e2423. doi:10.1212/WNL.0000000000009577
91. Lai JQ, Shi YC, Lin S, Chen XR. Metabolic disorders on cognitive dysfunction after traumatic brain injury. *Trends Endocrinol Metab*. 2022;33(7):451–462. doi:10.1016/j.tem.2022.04.003

92. Shin MK, Vázquez-Rosa E, Koh Y, et al. Reducing acetylated tau is neuroprotective in brain injury. *Cell*. 2021;184(10):2715–2732.e23. doi:10.1016/j.cell.2021.03.032
93. Plassman BL, Havlik RJ, Steffens DC, et al. Documented head injury in early adulthood and risk of Alzheimer's disease and other dementias. *Neurology*. 2000;55(8):1158–1166. doi:10.1212/wnl.55.8.1158
94. Li Y, Li Y, Li X, et al. Head injury as a risk factor for dementia and Alzheimer's disease: a systematic review and meta-analysis of 32 observational studies. *PLoS One*. 2017;12(1):e0169650. doi:10.1371/journal.pone.0169650
95. Falcon B, Zivanov J, Zhang W, et al. Novel tau filament fold in chronic traumatic encephalopathy encloses hydrophobic molecules. *Nature*. 2019;568(7752):420–423. doi:10.1038/s41586-019-1026-5
96. Stern RA, Tripodis Y, Baugh CM, et al. Preliminary study of plasma exosomal tau as a potential biomarker for chronic traumatic encephalopathy. *J Alzheimers Dis*. 2016;51(4):1099–1109. doi:10.3233/JAD-151028
97. Papa L, Brophy GM, Welch RD, et al. Time course and diagnostic accuracy of glial and neuronal blood biomarkers GFAP and UCH-L1 in a large cohort of trauma patients with and without mild traumatic brain injury. *JAMA Neurol*. 2016;73(5):551. doi:10.1001/jamaneurol.2016.0039
98. Lagares A, de la Cruz J, Terrisse H, et al. An automated blood test for glial fibrillary acidic protein (GFAP) and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) to predict the absence of intracranial lesions on head CT in adult patients with mild traumatic brain injury: BRAINI, a multicentre observational study in Europe. *EBioMedicine*. 2024;110:105477. doi:10.1016/j.ebiom.2024.105477
99. Bazarian JJ, Welch RD, Caudle K, et al. Accuracy of a rapid glial fibrillary acidic protein/ubiquitin carboxyl-terminal hydrolase L1 test for the prediction of intracranial injuries on head computed tomography after mild traumatic brain injury. *Acad Emerg Med off J Soc Acad Emerg Med*. 2021;28(11):1308–1317. doi:10.1111/acem.14366
100. Wang JW, Yatim SMJM, Lim XC, et al. P2582Signature of plasma extracellular vesicles associated proteins in acute myocardial infarction patients. *Eur Heart J*. 2019;40(Supplement_1):ehz748.0908. doi:10.1093/eurheartj/ehz748.0908
101. Ariston Gabriel AN, Wang F, Jiao Q, et al. The involvement of exosomes in the diagnosis and treatment of pancreatic cancer. *Mol Cancer*. 2020;19(1):132. doi:10.1186/s12943-020-01245-y
102. Hill AF. Extracellular Vesicles and Neurodegenerative Diseases. *J Neurosci*. 2019;39(47):9269–9273. doi:10.1523/JNEUROSCI.0147-18.2019
103. Khan NA, Asim M, El-Menyar A, Biswas KH, Rizoli S, Al-Thani H. The evolving role of extracellular vesicles (exosomes) as biomarkers in traumatic brain injury: clinical perspectives and therapeutic implications. *Front Aging Neurosci*. 2022;14:933434. doi:10.3389/fnagi.2022.933434
104. Patel RS, Krause-Hauch M, Kenney K, Miles S, Nakase-Richardson R, Patel NA. Long noncoding RNA VLDLR-AS1 levels in serum correlate with combat-related chronic mild traumatic brain injury and depression symptoms in US veterans. *Int J Mol Sci*. 2024;25(3):1473. doi:10.3390/ijms25031473
105. Ko J, Hemphill M, Yang Z, et al. Multi-dimensional mapping of brain-derived extracellular vesicle MicroRNA biomarker for traumatic brain injury diagnostics. *J Neurotrauma*. 2020;37(22):2424–2434. doi:10.1089/neu.2018.6220
106. Devoto C, Lai C, Qu BX, et al. Exosomal MicroRNAs in military personnel with mild traumatic brain injury: preliminary results from the chronic effects of neurotrauma consortium biomarker discovery project. *J Neurotrauma*. 2020;37(23):2482–2492. doi:10.1089/neu.2019.6933
107. Hörauf JA, Leppik L, Weber B, et al. MiR338-3p expression in extracellular vesicles after severe trauma with or without traumatic brain injury. *Brain Commun*. 2025;7(4):fcaf242. doi:10.1093/braincomms/fcaf242
108. Goetzl EJ, Elahi FM, Mustapic M, et al. Altered levels of plasma neuron-derived exosomes and their cargo proteins characterize acute and chronic mild traumatic brain injury. *FASEB J*. 2019;33(4):5082–5088. doi:10.1096/fj.201802319R
109. Tang TZ, Zhao Y, Agarwal D, et al. Serum amyloid a and mitochondrial DNA in extracellular vesicles are novel markers for detecting traumatic brain injury in a mouse model. *iScience*. 2024;27(2):108932. doi:10.1016/j.isci.2024.108932
110. Mondello S, Guedes VA, Lai C, et al. Circulating brain injury exosomal proteins following moderate-to-severe traumatic brain injury: temporal profile, outcome prediction and therapy implications. *Cells*. 2020;9(4):977. doi:10.3390/cells9040977
111. Caccialupi Da Prato L, Rezzag Lebza A, Consumi A, et al. Ectopic expression of the cation-chloride cotransporter KCC2 in blood exosomes as a biomarker for functional rehabilitation. *Front Mol Neurosci*. 2025;18:1522571. doi:10.3389/fnmol.2025.1522571
112. Wang CF, Zhao CC, Weng WJ, et al. Alteration in Long Non-Coding RNA Expression after Traumatic Brain Injury in Rats. *J Neurotrauma*. 2017;34(13):2100–2108. doi:10.1089/neu.2016.4642
113. Xie BS, Wang YQ, Lin Y, et al. Circular RNA expression profiles alter significantly after traumatic brain injury in rats. *J Neurotrauma*. 2018;35(14):1659–1666. doi:10.1089/neu.2017.5468
114. Wang P, Ma H, Zhang Y, et al. Plasma exosome-derived MicroRNAs as novel biomarkers of traumatic brain injury in rats. *Int J Med Sci*. 2020;17(4):437–448. doi:10.7150/ijms.39667
115. Puffer RC, Garcia LMC, Himes BT, et al. Plasma extracellular vesicles as a source of biomarkers in traumatic brain injury. *J Neurosurg*. 2020;134(6):1921–1928. doi:10.3171/2020.4.JNS20305
116. Hsi P, Tandon V, Kaplan DL. Extracellular vesicles as biomarkers for traumatic brain injury using a 3D in vitro human brain tissue model. *Sci Rep*. 2025;15(1):26940. doi:10.1038/s41598-025-04835-2
117. Goetzl EJ, Ledreux A, Granholm AC, et al. Neuron-derived exosome proteins may contribute to progression from repetitive mild traumatic brain injuries to chronic traumatic encephalopathy. *Front Neurosci*. 2019;13:452. doi:10.3389/fnins.2019.00452
118. Goetzl EJ, Yaffe K, Peltz CB, et al. Traumatic brain injury increases plasma astrocyte-derived exosome levels of neurotoxic complement proteins. *FASEB J*. 2020;34(2):3359–3366. doi:10.1096/fj.201902842R
119. Moyron RB, Gonda A, Selleck MJ, et al. Differential protein expression in exosomal samples taken from trauma patients. *Proteomics Clin Appl*. 2017;11(9–10):1700061. doi:10.1002/prca.201700061
120. Hogan SR, Phan JH, Alvarado-Velez M, et al. Discovery of lipidome alterations following traumatic brain injury via high-resolution metabolomics. *J Proteome Res*. 2018;17(6):2131–2143. doi:10.1021/acs.jproteome.8b00068
121. Gier EC, Pulliam AN, Gaul DA, Moore SG, LaPlaca MC, Fernández FM. Lipidome alterations following mild traumatic brain injury in the rat. *Metabolites*. 2022;12(2):150. doi:10.3390/metabo12020150
122. Thomas I, Dickens AM, Posti JP, et al. Serum metabolome associated with severity of acute traumatic brain injury. *Nat Commun*. 2022;13:2545. doi:10.1038/s41467-022-30227-5

123. Pergande MR, Kang C, George D, et al. Lipidomic analysis identifies age-disease-related changes and potential new biomarkers in brain-derived extracellular vesicles from metachromatic leukodystrophy mice. *Lipids Health Dis*. 2022;21(1). doi:10.1186/s12944-022-01644-8
124. Su H, Rustam YH, Masters CL, et al. Characterization of brain-derived extracellular vesicle lipids in Alzheimer's disease. *J Extracell Vesicles*. 2021;10(7). doi:10.1002/jev2.12089
125. Dayem AA, Yan E, Do M, et al. Engineering extracellular vesicles for ROS scavenging and tissue regeneration. *Nano Converg*. 2024;11(1). doi:10.1186/s40580-024-00430-9
126. Haroon K, Zheng H, Wu S, et al. Engineered exosomes mediated targeted delivery of neuroprotective peptide NR2B9c for the treatment of traumatic brain injury. *Int J Pharm*. 2024;649:123656. doi:10.1016/j.ijpharm.2023.123656
127. Yuan D, Zhao Y, Banks WA, et al. Macrophage exosomes as natural nanocarriers for protein delivery to inflamed brain. *Biomaterials*. 2017;142:1–12. doi:10.1016/j.biomaterials.2017.07.011
128. Shen K, Jia Y, Wang X, et al. Exosomes from adipose-derived stem cells alleviate the inflammation and oxidative stress via regulating Nrf2/HO-1 axis in macrophages. *Free Radic Biol Med*. 2021;165:54–66. doi:10.1016/j.freeradbiomed.2021.01.023
129. Luo Q, Xian P, Wang T, et al. Antioxidant activity of mesenchymal stem cell-derived extracellular vesicles restores hippocampal neurons following seizure damage. *Theranostics*. 2021;11(12):5986–6005. doi:10.7150/thno.58632
130. Pan Q, Kuang X, Cai S, et al. miR-132-3p priming enhances the effects of mesenchymal stromal cell-derived exosomes on ameliorating brain ischemic injury. *Stem Cell Res Ther*. 2020;11(1):260. doi:10.1186/s13287-020-01761-0
131. Li Y, Xin X, Zhou X, et al. ROS-responsive biomimetic nanosystem camouflaged by hybrid membranes of platelet-exosomes engineered with neuronal targeting peptide for TBI therapy. *J Control Release*. 2024;372:531–550. doi:10.1016/j.jconrel.2024.06.018
132. Chen C, Chang ZH, Yao B, et al. 3D printing of interferon γ-preconditioned NSC-derived exosomes/collagen/chitosan biological scaffolds for neurological recovery after TBI. *Bioact Mater*. 2024;39:375–391. doi:10.1016/j.bioactmat.2024.05.026
133. Liu X, Zhang J, Cheng X, et al. Integrated printed BDNF-stimulated HUCMSCs-derived exosomes/collagen/chitosan biological scaffolds with 3D printing technology promoted the remodelling of neural networks after traumatic brain injury. *Regen Biomater*. 2023;10:rbac085. doi:10.1093/rb/rbac085
134. Zhang Y, Chopp M, Zhang ZG, et al. Systemic administration of cell-free exosomes generated by human bone marrow derived mesenchymal stem cells cultured under 2D and 3D conditions improves functional recovery in rats after traumatic brain injury. *Neurochem Int*. 2017;111:69–81. doi:10.1016/j.neuint.2016.08.003
135. Li W, Liu Y, Zhang P, et al. Tissue-engineered bone immobilized with human adipose stem cells-derived exosomes promotes bone regeneration. *ACS Appl Mater Interfaces*. 2018;10(6):5240–5254. doi:10.1021/acsami.7b17620
136. Li J, Li X, Li X, et al. Local delivery of dual stem cell-derived exosomes using an electrospun nanofibrous platform for the treatment of traumatic brain injury. *ACS Appl Mater Interfaces*. 2024;16(29):37497–37512. doi:10.1021/acsami.4c05004
137. Chen Y, Lin J, Yan W. A prosperous application of hydrogels with extracellular vesicles release for traumatic brain injury. *Front Neurol*. 2022;13:908468. doi:10.3389/fneur.2022.908468
138. Ma S, Zhou J, Huang T, et al. Sodium alginate/collagen/stromal cell-derived factor-1 neural scaffold loaded with BMSCs promotes neurological function recovery after traumatic brain injury. *Acta Biomater*. 2021;131:185–197. doi:10.1016/j.actbio.2021.06.038
139. Liu H, Zhao Y, Zou Y, et al. Heparin-poloxamer hydrogel-encapsulated rhFGF21 enhances wound healing in diabetic mice. *FASEB J*. 2019;33(9):9858–9870. doi:10.1096/fj.201802600RR
140. Wu J, Zhu J, He C, et al. Comparative study of heparin-poloxamer hydrogel modified bFGF and aFGF for in vivo wound healing efficiency. *ACS Appl Mater Interfaces*. 2016;8(29):18710–18721. doi:10.1021/acsami.6b06047
141. Xiao Z, Li Y, Chen T, et al. A novel nitric oxide-driven nanomotor for synergistic treatment of ischaemic stroke: enhanced deep brain penetration and therapeutic efficacy. *Chem Eng J*. 2024;496:154205. doi:10.1016/j.cej.2024.154205
142. Liu X, Wu C, Zhang Y, et al. Hyaluronan-based hydrogel integrating exosomes for traumatic brain injury repair by promoting angiogenesis and neurogenesis. *Carbohydr Polym*. 2023;306:120578. doi:10.1016/j.carbpol.2023.120578
143. Li Y, Wang M, Sun M, et al. Engineering antioxidant poly (citrate-gallic acid)-exosome hybrid hydrogel with microglia immunoregulation for traumatic brain injury-post neuro-restoration. *Compos Part B Eng*. 2022;242:110034. doi:10.1016/j.compositesb.2022.110034
144. Qin SY, Feng JQ, Cheng YJ, et al. A comprehensive review on peptide-bearing biomaterials: from ex situ to in situ self-assembly. *Coord Chem Rev*. 2024;502:215600. doi:10.1016/j.ccr.2023.215600
145. Shi W, Huang CJ, Xu XD, et al. Transplantation of RADA16-BDNF peptide scaffold with human umbilical cord mesenchymal stem cells forced with CXCR4 and activated astrocytes for repair of traumatic brain injury. *Acta Biomater*. 2016;45:247–261. doi:10.1016/j.actbio.2016.09.001
146. Gelain F, Luo Z, Zhang S. Self-assembling peptide EAK16 and RADA16 nanofiber scaffold hydrogel. *Chem Rev*. 2020;120(24):13434–13460. doi:10.1021/acs.chemrev.0c00690
147. Ghandy N, Ebrahimzadeh-Bideskan A, Gorji A, Negah SS. Co-transplantation of novel nano-SDF scaffold with human neural stem cells attenuates inflammatory responses and apoptosis in traumatic brain injury. *Int Immunopharmacol*. 2023;115:109709. doi:10.1016/j.intimp.2023.109709
148. Hajinejad M, Ebrahimzadeh MH, Ebrahimzadeh-Bideskan A, Rajabian A, Gorji A, Sahab Negah S. Exosomes and nano-SDF scaffold as a cell-free-based treatment strategy improve traumatic brain injury mechanisms by decreasing oxidative stress, neuroinflammation, and increasing neurogenesis. *Stem Cell Rev Rep*. 2023;19(4):1001–1018. doi:10.1007/s12015-022-10483-0
149. Swanson WB, Zhang Z, Xiu K, et al. Scaffolds with controlled release of pro-mineralization exosomes to promote craniofacial bone healing without cell transplantation. *Acta Biomater*. 2020;118:215–232. doi:10.1016/j.actbio.2020.09.052
150. Zhang J, Liu X, Li H, et al. Exosomes/tricalcium phosphate combination scaffolds can enhance bone regeneration by activating the PI3K/akt signaling pathway. *Stem Cell Res Ther*. 2016;7(1):136. doi:10.1186/s13287-016-0391-3
151. Zhou Y, Bhatt H, Mojica CA, et al. Mesenchymal-derived extracellular vesicles enhance microglia-mediated synapse remodeling after cortical injury in aging Rhesus monkeys. *J Neuroinflammation*. 2023;20(1):201. doi:10.1186/s12974-023-02880-0

152. Liu X, Wang J, Wang P, et al. Hypoxia-pretreated mesenchymal stem cell-derived exosomes-loaded low-temperature extrusion 3D-printed implants for neural regeneration after traumatic brain injury in canines. *Front Bioeng Biotechnol*. 2022;10:1025138. doi:10.3389/fbioe.2022.1025138
153. Williams AM, Dennahy IS, Bhatti UF, et al. Mesenchymal stem cell-derived exosomes provide neuroprotection and improve long-term neurologic outcomes in a swine model of traumatic brain injury and hemorrhagic shock. *J Neurotrauma*. 2019;36(1):54–60. doi:10.1089/neu.2018.5711
154. Williams AM, Wu Z, Bhatti UF, et al. Early single-dose exosome treatment improves neurologic outcomes in a 7-day swine model of traumatic brain injury and hemorrhagic shock. *J Trauma Acute Care Surg*. 2020;89(2):388–396. doi:10.1097/TA.0000000000002698
155. Williams AM, Bhatti UF, Brown JF, et al. Early single-dose treatment with exosomes provides neuroprotection and improves blood-brain barrier integrity in swine model of traumatic brain injury and hemorrhagic shock. *J Trauma Acute Care Surg*. 2020;88(2):207–218. doi:10.1097/TA.0000000000002563
156. Bambakidis T, Dekker SE, Williams AM, et al. Early treatment with a single dose of mesenchymal stem cell derived extracellular vesicles modulates the brain transcriptome to create neuroprotective changes in a porcine model of traumatic brain injury and hemorrhagic shock. *Shock Augusta Ga*. 2022;57(2):281–290. doi:10.1097/SHK.0000000000001889
157. Bollard SM, Howard J, Casalou C, et al. Comparative characterisation of extracellular vesicles from canine and human plasma: a necessary step in biomarker discovery. *Vet Res Commun*. 2024;48(4):2775–2782. doi:10.1007/s11259-024-10405-0
158. Nabity TS, Ransom JT. Treatment of severe traumatic brain injury with human bone marrow mesenchymal stem cell extracellular vesicles: a case report. *Brain Inj*. 2025;39(4):330–335. doi:10.1080/02699052.2024.2432967
159. Kabatas S, Civelek E, Savrunlu EC, et al. Efficacy and safety of exosomes from Wharton's Jelly-derived mesenchymal stem cells in traumatic brain injury. *World J Crit Care Med*. 2025;14(4):103782. doi:10.5492/wjccm.v14.i4.103782
160. Cullum-Doyle M, Panizzon MS, Risbrough V, et al. Impact of TBI on AD-associated proteins in neuron-derived small extracellular vesicles from the Vietnam era twin study of aging. *Alzheimers Dement*. 2024;20(S2):e091711. doi:10.1002/alz.091711
161. Hu S, Zhang L, Su Y, et al. Sensitive detection of multiple blood biomarkers via immunomagnetic exosomal PCR for the diagnosis of Alzheimer's disease. *Sci Adv*. 2024;10(13):eabm3088. doi:10.1126/sciadv.abm3088
162. Ko J, Hemphill M, Yang Z, et al. Diagnosis of traumatic brain injury using miRNA signatures in nanomagnetically isolated brain-derived extracellular vesicles. *Lab Chip*. 2018;18(23):3617–3630. doi:10.1039/c8lc00672e
163. Lightner AL, Sengupta V, Qian S, et al. Bone marrow mesenchymal stem cell-derived extracellular vesicle infusion for the treatment of respiratory failure from COVID-19: a randomized, placebo-controlled dosing clinical trial. *Chest*. 2023;164(6):1444–1453. doi:10.1016/j.chest.2023.06.024
164. Wu L, Wang L, Liu X, et al. Milk-derived exosomes exhibit versatile effects for improved oral drug delivery. *Acta Pharm Sin B*. 2022;12(4):2029–2042. doi:10.1016/j.apsb.2021.12.015
165. Mondal J, Pillarisetti S, Junnuthula V, et al. Extracellular vesicles and exosome-like nanovesicles as pioneering oral drug delivery systems. *Front Bioeng Biotechnol*. 2024:11. doi:10.3389/fbioe.2023.1307878
166. Ikeda T, Kawabori M, Zheng Y, et al. Intranasal administration of mesenchymal stem cell-derived exosome alleviates hypoxic-ischemic brain injury. *Pharmaceutics*. 2024;16(4):446. doi:10.3390/pharmaceutics16040446
167. Li P, Sun S, Zhu X, et al. Intranasal delivery of engineered extracellular vesicles promotes neurofunctional recovery in traumatic brain injury. *J Nanobiotechnology*. 2025;23(1):229. doi:10.1186/s12951-025-03181-9
168. Zhao J, Yang J, Jiao J, Wang X, Zhao Y, Zhang L. Biomedical applications of artificial exosomes for intranasal drug delivery. *Front Bioeng Biotechnol*. 2023;11. doi:10.3389/fbioe.2023.1271489
169. Moss LD, Sode D, Patel R, et al. Intranasal delivery of exosomes from human adipose derived stem cells at forty-eight hours post injury reduces motor and cognitive impairments following traumatic brain injury. *Neurochem Int*. 2021;150:105173. doi:10.1016/j.neuint.2021.105173
170. Chen L, Yang Y, Chen J, et al. Intranasal delivery of hypoxia-preconditioned extracellular vesicles derived from BMSCs alleviates neuroinflammation and brain dysfunction in TBI. *Stem Cell Res Ther*. 2025;16(1):544. doi:10.1186/s13287-025-04572-3
171. Zhuo H, Chen Y, Zhao G. Advances in application of hypoxia-preconditioned mesenchymal stem cell-derived exosomes. *Front Cell Dev Biol*. 2024;12. doi:10.3389/fcell.2024.1446050
172. Li Q, Li Y, Shao J, et al. Exploring regulatory frameworks for exosome therapy: insights and perspectives. *Health Care Sci*. 2025;4(4):299–309. doi:10.1002/hcs2.70028
173. Welsh JA, Goberdhan DCI, O'Driscoll L, et al. Minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches. *J Extracell Vesicles*. 2024;13(2). doi:10.1002/jev2.12404
174. Sidhom K, Obi PO, Saleem A. A review of exosomal isolation methods: is size exclusion chromatography the best option? *Int J Mol Sci*. 2020;21(18):6466. doi:10.3390/ijms21186466
175. Li P, Kaslan M, Lee SH, Yao J, Gao Z. Progress in exosome isolation techniques. *Theranostics*. 2017;7(3):789–804. doi:10.7150/thno.18133
176. Jeppesen DK, Fenix AM, Franklin JL, et al. Reassessment of exosome composition. *Cell*. 2019;177(2):428–445.e18. doi:10.1016/j.cell.2019.02.029
177. Karimi N, Cvjetkovic A, Jang SC, et al. Detailed analysis of the plasma extracellular vesicle proteome after separation from lipoproteins. *Cell Mol Life Sci CMLS*. 2018;75(15):2873–2886. doi:10.1007/s00018-018-2773-4
178. Zhang H, Freitas D, Kim HS, et al. Identification of distinct nanoparticles and subsets of extracellular vesicles by asymmetric flow field-flow fractionation. *Nat Cell Biol*. 2018;20(3):332–343. doi:10.1038/s41556-018-0040-4
179. Chu Z, Song Y, Wu M, et al. Programmable framework nucleic acid-modified nanomagnetic beads for efficient isolation of exosomes and exosomal proteomics analysis. *Anal Chem*. 2024;96(35):14099–14107. doi:10.1021/acs.analchem.4c01193
180. Tayebi M, Yang D, Collins DJ, Ai Y. Deterministic sorting of submicrometer particles and extracellular vesicles using a combined electric and acoustic field. *Nano Lett*. 2021;21(16):6835–6842. doi:10.1021/acs.nanolett.1c01827
181. Wang Z, Wang H, Becker R, et al. Acoustofluidic separation enables early diagnosis of traumatic brain injury based on circulating exosomes. *Microsyst Nanoeng*. 2021;7:20. doi:10.1038/s41378-021-00244-3
182. Peng T, Lin X, Li L, Huang L, Jiang B, Jia Y. Investigation on submicron particle separation and deflection using tilted-angle standing surface acoustic wave microfluidics. *Heliyon*. 2024;10(3):e25042. doi:10.1016/j.heliyon.2024.e25042

183. Song Y, Dong Y, Gao L. Research progress in exosomes for diagnosis and treatment of traumatic brain injury. *Chin J Trauma*. 2020;2020:664–669.
184. Mondal J, Pillarisetti S, Junnuthula V, et al. Hybrid exosomes, exosome-like nanovesicles and engineered exosomes for therapeutic applications. *J Control Release off J Control Release Soc*. 2023;353:1127–1149. doi:10.1016/j.jconrel.2022.12.027
185. Long F, Pan Y, Li J, et al. Orange-derived extracellular vesicles nanodrugs for efficient treatment of ovarian cancer assisted by transcytosis effect. *Acta Pharm Sin B*. 2023;13(12):5121–5134. doi:10.1016/j.apsb.2023.04.006