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NEUROPROTECTION

REVIEW

Mesenchymal stem cell-derived exosomes: Shaping the next era of stroke treatment

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Abstract

Exosome-based treatments are gaining traction as a viable approach to addressing the various issues faced by an ischemic stroke. These extracellular vesicles, mainly produced by mesenchymal stem cells, exhibit many properties with substantial therapeutic potential. Exosomes are particularly appealing for stroke therapy because of their low immunogenicity, effective cargo transport, and ability to cross the blood–brain barrier. Their diverse effects include neuroprotection, angiogenesis stimulation, inflammatory response modulation, and cell death pathway attenuation, synergistically promoting neuronal survival, tissue regeneration, and functional recovery. Exosomes also show potential as diagnostic indicators for early stroke identification and customized treatment options. Despite these promising qualities, current exosome-based therapeutics have some limitations. The heterogeneity of exosome release among cell types, difficulty in standardization and isolation techniques, and complications linked to dosage and targeted administration necessitates extensive investigation. It is critical to thoroughly understand exosomal processes and their complicated interactions within the cellular milieu. To improve the practicality and efficacy of exosome-based medicines, research efforts must focus on improving production processes, developing robust evaluation criteria, and developing large-scale isolation techniques. Altogether, exosomes' multifunctional properties offer a new route for transforming stroke treatment and significantly improving patient outcomes.

KEYWORDS

exosomes, inflammatory stress, ischemic stroke, mesenchymal stem cells, middle cerebral artery occlusion, oxidative stress

Highlights

- Mesenchymal stem cells (MSCs), with their unique abilities, present a prospective treatment option for ischemic stroke, overcoming the obstacle of the blood–brain barrier using MSC-Exosomes derived from stem cells.
- Exosomes, tiny extracellular vesicles, show promising outcomes in mitigating the complex pathophysiological pathways linked to ischemic stroke.
- MSC-Exosomes offer therapeutic benefits, modifying pathways, promoting angiogenesis, neurogenesis, neuroprotection, and triggering antioxidative and anti-inflammatory responses, while also providing diagnostic capabilities.

Arshi Waseem and Saudamini contributed equally to this study.

- The cumulative effects of MSC-Exosomes contribute to enhanced tissue repair, neuronal survival, and functional recovery post-ischemic stroke.

1 | INTRODUCTION

Stroke is the second leading cause of death worldwide, with an approximate mortality rate of 5.5 million, and 50% of survivors experience chronic disabilities. Ischemic stroke accounts for 87% of all stroke patients.[1] Therefore, developing a novel treatment strategy for ischemic stroke is crucial. Currently, the only treatments for a severe acute ischemic stroke are rapid recanalization (thrombectomy) and thrombolytic therapy (injection of tissue plasminogen activator).

Stem cell-based therapy has surfaced as a promising method for treating ischemic stroke due to stem cells' unique regenerative properties. Studies indicate that stem cells can differentiate into numerous cell lineages, including neurons, astrocytes, and endothelial cells.[2–4] Stem cells have multiple effects when transplanted into an ischemic brain, including the promotion of neurogenesis, angiogenesis, and the modulation of the immune system.[5–7] Collectively, these mechanisms contribute to tissue repair and functional recovery. Improved neurological outcomes and decreased infarct size have been observed in preclinical models following stem cell transplantation.[8] Additionally, clinical trials have demonstrated the safety and viability of stem cell therapy in stroke patients, although additional large-scale clinical trials are necessary to establish its efficacy and optimize treatment.[1] Furthermore, stem cell-based therapy faces obstacles such as ethical considerations with embryonic stem cells, tumorigenic potential of pluripotent stem cells, optimization of cell delivery methods, standardization of protocols, and immune response against allogeneic stem cells. In this context, the utilization of exosome therapy derived from stem cells emerges as a compelling alternative. This approach offers targeted delivery, holding the potential to overcome existing barriers by transporting bioactive molecules such as proteins and microRNAs (miRNAs). Through this method, therapeutic effects can be mediated without the necessity for direct transplantation, thereby fostering tissue repair.[9,10]

Exosomes, small extracellular vesicles secreted by various cell types, have attracted increasing interest as potential therapeutic agents in the treatment of ischemic stroke. In animal models, exosome treatment has been shown to improve neurological outcomes by promoting neuronal survival, reducing inflammation and apoptosis, enhancing angiogenesis, and facilitating tissue repair and limiting secondary brain damage.[11,12] In addition, exosomes have been demonstrated to stimulate angiogenesis and improve synaptic plasticity, thereby facilitating the restoration of neural circuits.[13] Exosome-based therapy for ischemic stroke is still in the early phases of research. However, encouraging preclinical data have fueled optimism about their potential as a novel and noninvasive treatment for stroke patients. The present review focuses on the impact of exosomes derived from MSCs in the context of ischemic stroke.

2 | EXOSOMES

Exosomes are small extracellular vesicles with a size range of 30–150 nm and a density between 1.13 and 1.19 g/mL that play a crucial role in intercellular communication by transferring bioactive molecules such as proteins, lipids, and nucleic acids between cells.[14] Exosomes contain a wide variety of complex nucleic acids, including DNA, messenger RNA (mRNA), and numerous noncoding RNA species, in addition to a vast array of proteins, lipids, and miRNAs. Similarly, there are several forms of extracellular vesicles, which are mentioned in Table 1. These exosomes may also serve as biomarkers for the diagnosis and prognosis of stroke.[20]

The production of exosomes begins with a double invagination of the plasma membrane, which results in the formation of intracellular multivesicular bodies (MVBs) containing intraluminal vesicles (ILVs).[21] During the initial invagination phase, cell-surface and soluble proteins from the extracellular environment are internalized, forming early-sorting endosomes (ESEs), which may coalesce with previously formed ESEs. The trans-Golgi network and endoplasmic reticulum influence the composition and formation of ESEs.[22] Later, ESEs transform into late-sorting endosomes or MVBs, distinguished by the inward swelling of the endosomal limiting membrane and the plasma membrane, resulting in the formation of multiple ILVs within MVBs. ILVs eventually develop into exosomes. MVBs can be degraded by fusing with lysosomes or autophagosomes, or they can connect with the plasma membrane to release ILVs and exosomes.[23]

Exosomes are a subset of extracellular vesicles formed as ILVs within MVBs via the endocytic pathway. Exosomes are distinguished by their distinctive lipid bilayer membrane, which contains specific lipid compositions, including sphingomyelin, cholesterol, and ceramides.[24] In addition, they express tetraspanins, including cluster of differentiation (CD9, CD63, and CD81), MVB-related endosomal sorting complexes required for transport proteins (Alix, TSG101), and heat shock proteins (HSPs) (HSP60, HSP70, HSPA5, CCT2, and HSPs) (HSP60, HSP70, HSPA5, and HSP80) that can be used as exosomal markers.[25]

Exosomes can mediate a range of cellular activities by cargo transfer, including gene expression regulation, cell proliferation, apoptosis, differentiation, and immune system modulation.[26] Exosomes are efficient transporters of biologically active molecules due to their stability and ability to secure their cargo from

TABLE 1 Types of extracellular vesicles: A comprehensive overview.

No.	Type	Size	Biogenesis	Composition	References
1.	Exosomes	50–100 nm	Multivesicular body exocytosis	Heat shock proteins, actin, tubulin, MHC molecules, tetraspannins (CD63, CD81, CD82, CD9), miRNA, mRNA	[15]
2.	Microvesicles	100–1000 nm	Plasma membrane budding	Actin, tubulin, β1 integrin, VAMP3, miRNA	[16]
3.	Apoptotic bodies	100–5000 nm	Plasma membrane budding in apoptosis	Annexin V, C3b, thrombospondin, any cellular components	[16]
4.	Retrovirus-like particles	90–100 nm	Direct plasma membrane budding	Retroviral proteins such as Gag, cytoskeletal proteins, plasma membrane components	[17]
5.	Ectosomes	100–500 nm	Blabbing of plasma membrane towards outside	β1 integrins, selectins, CD40, MMP, lineage markers, erzin	[18]
6.	Nanovesicle	30–150 nm	Plasma membrane cleavage and direct budding are facilitated through calcium influx and cortical cytoskeleton remodeling	Lipid membrane, proteins, nucleic acids, tetraspanins	[19]

Abbreviations: CD, cluster of differentiation; MHC, major histocompatibility complex; miRNA, microRNA; MMP, matrix metalloproteinase; mRNA, messenger RNA; VAMP, vesicle-associated membrane protein.

extracellular degradation. Their small size and lipid membrane allow them to cross biological barriers, including the blood–brain barrier (BBB),[27] facilitating their access to diverse body fluids, including blood, cerebrospinal fluid, urine, and saliva, thereby making them potential biomarkers for a variety of diseases. Due to their nonimmunogenic nature, exosomes derived from adult stem cells can be utilized for therapeutic applications, thereby avoiding ethical concerns associated with immune rejection and embryonic stem cell use.

3 | MSCS AS THERAPEUTIC CHOICE FOR ISCHEMIC STROKE

MSCs have emerged as a promising therapy for treating ischemic stroke due to their ability to proliferate, differentiate, and modulate the immune system.[28] MSCs, derived from bone marrow, adipose tissue, and the placenta, share essential traits such as cell migratory patterns and immunomodulatory properties.[29] Because of their ease of separation from various sources, they provide a readily available cell source for therapeutic purposes. The ability of MSCs to develop into neurons, astrocytes, and endothelial cells facilitates tissue repair and regeneration in the post-stroke brain.[30] MSCs also release trophic factors and cytokines that improve neuroprotection, reduce inflammation, and stimulate angiogenesis, all of which contribute to their therapeutic actions in ischemic stroke.[31] MSCs have minimal immunogenicity and immunomodulatory characteristics, allowing them to avoid immune responses and promote tissue regeneration without generating unfavorable effects. MSCs are good candidates for cell-based therapeutics due to their unique combination of features. MSCs have been proven in animal models of ischemic stroke to migrate to injured brain locations, supporting tissue repair, angiogenesis, and neurogenesis. These regenerative and anti-inflammatory characteristics aid in stroke patients' neurological function and recovery. Clinical trials evaluating the safety and efficacy of MSC therapy in stroke have yielded promising results, paving the path for novel cell-based therapeutics for this debilitating condition.[32]

Given the above qualities, MSCs are naturally the ideal choice for cellular therapy in treating ischemic stroke. MSCs execute an essential role in the production of exosomes.[33] MSCs find widespread application in stroke cell therapy, primarily owing to their abundant source, ease of cultivation, controlled proliferation, and notable survival rate in the brain posttransplantation.

4 | ADVANTAGES OF MSC-EXOSOMES (MSC-EXOS) OVER MSCS

There are advantages and disadvantages to using MSCs and MSC-Exos to treat ischemic stroke, as shown in Table 2. However, MSC-Exos are gaining

TABLE 2 Advantages and disadvantages of MSCs and MSC-Exos in neurodegenerative disorders.

Stem cell/ exosome/origin	Isolation of MSCs	Exosomes isolation methodology	Animal models/human	Advantage	Disadvantage	References
MSCs						
Bone marrow	Posterior iliac crest of patients	–	– Human ischemic stroke patients	– 12 stroke patients received BM-MSCs treatment without experiencing any negative side effects.	–	[34]
Bone marrow	Posterior iliac crest of healthy adult volunteers	–	– MCAO in male Wistar rats, 250–300 g	– BDNF- or GDNF-transfected MSCs increased function and decreased ischemia damage.	–	[35]
Bone marrow	Femurs of C57BL/6J mice	–	– Photothrombotic model in male C57BL/6J mice, 8 weeks old, 20–23 g	– Enhanced stroke recovery through hydrogel-mediated. – Delivery of BDNF-overexpressing MSCs, prolonged survival, and improved ischemic stroke recovery.	–	[36]
Human umbilical cord	Clinical-grade hUC-MSCs	–	– tMCAO in male C57BL/6 mice, 6-8 weeks old, 22–25g	– A rapidly proliferating, low tumorigenicity, highly therapeutic source of MSCs.	–	[37]
Human umbilical cord	Umbilical cord tissues	–	– MCAO in CB17 male and female mouse pups	–	– Long-term stroke healing dose is difficult to determine.	[38]
Adipose tissue	Adipose tissues	–	– MCAO in male adult SD rats, 200–300 g	–	– The study failed to examine ADMSCs' direct effect on brain cells, limiting AD-MSC-Exos.	[39]
MSC-Exos						
Bone marrow	–	Precipitation by ExoQuick	– MCAO in SD rats, 2 months old, 250–280 g	– MSC-Exos improved function and MCAO model regulation.	–	[40]
Bone marrow	–	Ultracentrifugation	– TBI in Male Wistar rats, 2-3 months old 325 ± 11 g	– MSC cell-free exosomes improved cognitive and sensorimotor functional recovery – Resulted in an increased population of newborn neuroblasts and mature neurons in the DG (dentate gyrus).	–	[41]
Bone marrow	–	Ultracentrifugation	– TBI in female Yorkshire swine 35–45 kg	– MSC-derived exosomes minimize neuronal injury. – Demonstrated the ability to promote neurocognitive recovery.	–	[42]
Bone marrow	–	Ultracentrifugation	– SCI in male Wistar rats, 200–250 g	–	– Inhibition of complement activation.	[43]

TABLE 2 (Continued)

Stem cell/ exosome/origin	Isolation of MSCs	Exosomes isolation methodology	Animal models/human	Advantage	Disadvantage	References
Bone marrow	–	Exosome-depleted FBS media	– SCI in male SD rats, 200–250 g	–	– Pericyte migration control by extracellular vesicles is unknown. – Pericytes only started migrating 6 h after OGD exposure. – BMSC-EV's effect cannot be determined in such a short time.	[44]
Bone marrow	–	Chromatography	– TBI in male C57BL/6J mice, 7–8 weeks old	–	– EV quality depends on producer cell type and physiological status. – Isolation procedures are hard to scale up – Efficacy testing is hard to design.	[45]

Abbreviations: BDNF, brain-derived neurotrophic factor; BMSC, bone marrow-derived MSC; BMSC-EV, BMSC extracellular vesicle; DG, dentate gyrus; FBS, fetal bovine serum; GDNF, glial cell line-derived neurotrophic factor; hUC-MSCs, human umbilical cord–MSC; MCAO, middle cerebral artery occlusion; MSC, mesenchymal stem cell; SCI, spinal cord injury; SD, Sprague–Dawley; SVZ, subventricular zone; TBI, traumatic brain injury.

attraction as a therapy for ischemic stroke due to the more straightforward, cheaper, and quicker production of MSC-Exos compared with MSCs.[46] Exosomes are approximately one-millionth the size of MSCs, which are simpler to produce and store.[33] MSC-Exos have less stringent storage requirements and retain their activity even when stored at −80°C for protracted periods. Therapies based on MSCs face obstacles such as cell survival, regenerative capacity, immune rejection, and tumorigenic differentiation. The use of exosomes as a cell-free therapy can circumvent these issues. Exosomes contain a negligible quantity of membrane-bound proteins, resulting in a low probability of immunological rejection following allogeneic injection. The exosomes facilitate effective cell-to-cell communication by transporting active substances to recipient cells. Their membrane contains proteins with a high affinity for target cell membranes or extracellular matrix ligands, enabling targeted delivery to particular organs or microenvironments. In addition, the exosomal membrane can be altered to allow specific substances to reach their intended cells and tissues.[47]

MSC-Exos is beneficial in the treatment of ischemic stroke. For instance, in an experiment conducted by Doeppner et al., mice intravenously injected with exosomes following an ischemic stroke exhibited long-term neuroprotection associated with enhanced angioneurogenesis, whereas, in contrast to MSCs, they failed to decrease neuroinflammation.[48] In comparison, intra-arterial delivery of MSC-Exos overcomes this limitation and attenuates neuroinflammation.[49]

In addition, studies have shown that curcumin encapsulated in exosomes has improved stability, solubility, blood concentration, and anti-inflammatory effects.[50] Moon et al. reported that rats in the exosomes group had substantially lower mortality rates and faster recoveries than those in the bone-marrow-derived MSCs (BMSCs) and other study groups.[51] Using MSC-extracellular vesicles (MSC-EVs) instead of MSCs to treat preterm infants with hypoxic-ischemic brain injury can help avoid the risks associated with systemic delivery of living cells.[52] Based on the aforementioned evidence and facts, it is a reasonable deduction to assert that MSC-Exos therapy is more effective in treating ischemic stroke compared to MSCs.

5 | EXOSOMES IN ISCHEMIC STROKE THERAPY: HARNESSING THERAPEUTIC POTENTIAL

Following a stroke, brain cells produce and release exosomes that can cross the BBB and reach cerebrospinal fluid or peripheral blood. Exosomes can cross the BBB when administered systemically or directly into the brain and convey their cargo to the affected areas. As a cell-based therapy for ischemic stroke, MSC-Exos hold great promise due to their favorable properties, which include minimal immunogenicity, tumorigenicity, efficient cargo transport, stability, and paracrine effects.[53] MSC-Exos offer a compelling treatment option for ischemic stroke (see Table 3). They can

TABLE 3 MSC-Exos: Origins and roles in stroke.

No.	Source of MSCs	Exosomes isolation methodology	Model		Approach to achieve ischemic pathology	Dose and mode of administration	Results	References
			Cell	Rodent				
1.	Bone marrow of C57BL/6 mice	Ultracentrifuged	–	C57BL/6 mice	Photo-thrombotic model	100 µg MSC-exos injected intravenously	– MSC-Exos reduced infarct volume, improved neurological function. – MSC-Exos increased angiogenesis, neurogenesis white matter regeneration after stroke.	[54]
2.	MSCs isolated from bone marrow of adult male Wistar rats	Multistep centrifugation	–	Adult male Wistar rats, 270–300 g	MCAO	3×10^6 MSCs in 1 mL PBS injected via tail vein	– MSC-Exos with *miR-133b* affect gene expression, neurite remodeling, and brain cell function. – Cellular interactions promote neuronal connection and function.	[55]
3.	MSCs isolated from femur and tibia marrow of Wistar rats	Multistep centrifugation	–	Adult male Wistar rats, 270–300 g	MCAO	3×10^6 MSCs injected via tail vein	– MSCs-Exos transmitted *miR-133b* to post-MCAO brain extract neurons, increasing neurite branch number and length.	[56]
4.	BM-MSC isolated from femora of adult male mice	Ultracentrifugation	–	Male C56BL/6 mice, 8–10 weeks old, 22–25 g	Photothrombotic model	At 1 dpi, DiI-Exos injected intravenously	– MSC-Exos with *miR-124* induced cortical neurogenesis and protected against ischemic injury.	[57]
5.	Mini-pig abdominal adipose tissue-isolated ADMSCs	Exosomes isolated from the culture medium	–	Male SD rats, 350–375 g	MCAO	100 µg of MSC-Exos injected intravenously	– AD-MSC-Exos reduced brain-infarct zone and improved neurological performance.	[58]
6.	SD-rat bone marrow MSCs	Ultracentrifugation	–	Male SD rats, 260–280 g	MCAO	200 µL/rat MSC-Exos injected intravenously through tail vein	– MSC-Exos overexpressing *miR-223-3p* reduced brain infarct volume and neurological deficits. – Boosted learning and memory.	[59]
7.	Adult male Wistar rat bone marrow MSCs	Multistep centrifugation	–	Adult male Wistar rats, 270–300 g	MCAO	100 µg protein of MSC-exos injected through tail vein	– MSC-exos promoted neurite remodeling, neurogenesis, and angiogenesis.	[60]
8.	Healthy donor bone marrow MSCs	EVs isolated by ultracentrifugation	–	Male SD rats	Permanent distal MCAO	2×10^6 MSC-EVs administered intravenously	– MSC-EV remodel persistent distal MCAO rats peri-infarct brains.	[61]

TABLE 3 (Continued)

No.	Source of MSCs	Exosomes isolation methodology	Model		Approach to achieve ischemic pathology	Dose and mode of administration	Results	References
			Cell	Rodent				
							– MSC-EV promoted neurogenesis in SVZ region and angiogenesis in peri-infarct cortex of young and aged rats.	
9.	MSCs isolated from healthy human bone marrow	Ultracentrifugation	–	Male and female C57Bl6/j mice, 8–10 weeks or 15–24 months old	Transient MCAO	2×10^6 MSCs administered intravenously	– MSC-EVs reduced neurological deficits, infarct volume, brain edema, and neuronal injury in young and aged mice of both sexes.	[62]
10.	Albino male wistar rats' subcutaneous adipose tissue yielded ADSCs	Exosomes isolation kit	BMECs	–	– OGD/R		– ADSCs-Exos stimulated angiogenesis of BMEC after OGD insult in vitro via *miRNA-181b/TRPM7* axis.	[63]
11.	ADMSC from healthy human or rat adipose tissue	Supernatant double centrifuged and exosome protein isolated by Exosomal Protein Extraction kit	SH-SY5Y	Male SD rats, 230–280 g	– OGD/R – MCAO	100 μg/kg/day exosomes was injected via lateral cerebral ventricle daily for 3 days before MCAO surgery	– PEDF-modified ADSC exos protect SH-SY5Y cells from OGD-induced apoptosis and autophagy. – PEDF-exosomes reduced MCAO-induced brain damage.	[64]
12.	MSCs isolated from bone marrow of SD rats	Sequential centrifugation	–	Male SD rats	MCAO	200 μL/rat MSC-exos intravenously through the tail vein	– MSC-Exos significantly reduced neurological severity score, improved spatial learning and memory ability.	[65]
13.	hUMSC isolated from human umbilical cord	Multistep centrifugation	– hUMSCs	C57BL/6 mice, 8 weeks old, 20–30 g	– OGD – MCAO	50 μg hUMSC-Exos was injected into tail vein, 4 h postreperfusion	– hUMSC-Exos miR-146a-5p attenuated microglial pro-inflammatory activity in vitro through suppression of the IRAK1/TRAF6 signaling pathway. – Microglia-mediated neuroinflammation and neural deficits resulting from ischemic stroke are attenuated by hUMSC-Exosomal *miR-146a-5p* in mice.	[66]

(Continues)

TABLE 3 (Continued)

No.	Source of MSCs	Exosomes isolation methodology	Model		Approach to achieve ischemic pathology	Dose and mode of administration	Results	References
			Cell	Rodent				
14.	BM-MSCs isolated from femur of C57BL/6 mice	– Exosomes were isolated by ExoQuick-TC™ kit	– Astrocyte	– C57BL/6 mice, 8–10 weeks old	– OGD – MCAO	–	– MSC-Exos overexpressing *miR-138-5p* prevented OGD-injured astrocyte death. – BMSCs-derived exosomal *miR-138-5p* decreased stroke-induced neuron damage in vivo.	[67]
15.	Mouse bone marrow tibias and femur MSCs	Multistep centrifugation	–	Male C57BL/6 mice, 8–10 weeks old	– MCAO	100 μg RGD-Exos was administered via tail vein	– RGD-exo:*miR-210* intravenously increased lesion VEGF expression and angiogenesis.	[68]
16.	MSC isolated from adipose tissue of SD rats	– EVs were extracted by miRCURYTM Exosome Isolation Kit, EXIQON	–	Male SD rats, 8–9 weeks old, 200–250 g	Subcortical infarct by endothelin-1	100 μg MSC-Exos protein through intravenous administration	– MSC-EV in improving functional outcome by mediating axonal sprouting and growth, oligodendrocyte formation, tract connectivity, and remyelination.	[69]
17.	BM-MSC isolated from femora and tibias of SD rats	Exosomes Isolation Kit (EXIQON)	–	– Male SD rats, 8 weeks old, 270–300 g	tMCAO	30 μg of MSC-Exos protein injected intravenously	– MSC-EVs promoted neurogenesis and angiogenesis.	[51]
18.	Mouse abdominal adipose tissue-derived AD-MSC	ExoQuick kit	–	Male C57BL/6J mice, 3–4 months old	Bilateral CCAO	200 μg EVs injected intracerebroventricularly	– MSC-EVs restored basal synaptic transmission, plasticity, and spatial memory.	[70]
19.	MSCs isolated from umbilical cord of	hWJ-MSC-derived EVs isolated by serial centrifugations	N2a cell	–	OGD/R	0.1 or 1 μg/mL EVs	– hWJ-MSC-derived EVs reduced OGD/R-induced N2a cell apoptosis.	[71]
20.	MSC-EV	EV isolation kit	–	C57BL/6J mouse	– Ischemia was induced in C57BL/6J mouse by placing them in hypoxia chamber	100 μg of EVs injected intracardially	– Reduced neuroinflammation and apoptosis.	[72]

Abbreviations: ADMSCs, adipose-derived mesenchymal stem cells; BMSC, bone marrow-derived MSC; CCAO, common carotid arteries occlusion; EVs, extracellular vesicles; EXO-FBS, exosome-depleted fetal bovine serum; hUMSC, human umbilical cord-derived MSCs; MCAO, middle cerebral artery occlusion; MCAO/R, middle cerebral artery occlusion/reperfusion; MI, myocardial ischemia; miRNA, microRNA; MSC, mesenchymal stem cell; MSC-EV, MSC extracellular vesicle; MSC-Exos, MSC-Exosomes; N2a, Neuro-2a; OGD, oxygen-glucose deprivation; PBS, phosphate-buffered saline; PEDF, pigment epithelium-derived factor; SD, Sprague–Dawley; tMCAO, transient MCAO; TRPM7, transient receptor potential melastatin 7; VEGF, vascular endothelial growth factor.

promote brain plasticity, allowing internalization by cell bodies and axons to stimulate axonal growth.[60]

Exosomes derived from BM-MSCs promote angiogenesis[73] by inducing cerebral endothelial cell proliferation.[73] By inhibiting the caspase-8 apoptotic pathway,[74] these exosomes also prevent oligodendrocyte mortality. In addition, adipose-derived stem cell exosomes enriched with *miRNA-181b-5p* promote angiogenesis by inhibiting transient receptor potential melastatin 7 poststroke.[63] In addition, exosomes containing *miR-30D-5p*, reducing inflammation and ameliorating neurological impairment in rodents with ischemic stroke.[75] In addition, Valadi et al. demonstrated that intravenous MSC-Exo administration in a rat model of transient middle cerebral artery occlusion (MCAO) improved functional recovery by promoting neurogenesis, neurite remodeling, and angiogenesis.[76] Notably, MSC-Exos treated with oxygen-glucose deprivation (OGD) or brain extract demonstrated enhanced therapeutic benefits, potentially as a result of the enrichment of specific functional proteins.[77] In a primate stroke model, intravenous administration of MSC-Exo improved recovery of fine hand motor function.[78] Mechanistic studies demonstrated that MSC-Exos inhibits injury-induced hyperexcitability, restores excitatory–inhibitory equilibrium in primates, reduces neuroinflammation, and directs microglia to perform restorative functions.[79] Overall, these findings highlight MSC-derived exosomes as a versatile therapeutic strategy, influencing various cellular pathways to enhance stroke recovery and facilitate repair.

6 | NEURONAL PLASTICITY INDUCED BY MSC-EXOS

Within the ischemic brain, MSC-Exos are internalized by neurons, oligodendrocytes, and microglia.[69] For instance, in cultured primary cortical neurons, MSC-Exos exhibits internalization by both neuronal cell bodies and axons, resulting in heightened axonal extension.[80] Notably, the content of exosomal miRs emerges as a key mediator of neural plasticity, as evidenced by the suppression of the argonaute 2 protein—an essential component of the miR machinery in MSC-Exos—which prevented their effects on axonal outgrowth.[80] Moreover, engineered MSC-Exos overexpressing the *miR-17-92* cluster demonstrated an enhanced capacity to stimulate the phosphatase and tensin homolog/mammalian target of the rapamycin signaling pathway, leading to increased axonal development in recipient neurons.[80] Consequently, MSC-Exos possess the capability to deliver their miR cargo directly to recipient neurons, thereby influencing neuronal remodeling poststroke. Furthermore, a notable augmentation in therapeutic effects was observed in stroke-prone rats when exosomes produced by MSCs overexpressing the *miR-133b* gene were employed.[81] Intriguingly, the heightened neuronal plasticity induced by *miR-133b* MSC-Exos was attributed to the accelerated secondary release of exosomes by astrocytes, promoting neurite growth.[81]

7 | ASTROCYTE-MEDIATED BRAIN PLASTICITY

Astrocytic EVs potentially play a role in fostering brain plasticity post-stroke. In vitro studies suggest that the secondary release of EVs from astrocytes may contribute to some of the neurodegenerative effects observed with MSC-EVs.[81] A study reported that exosomes derived from astrocytes can suppress autophagy, diminish the production of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β), and mitigate neuronal damage.[82] Another study identified lipocalin 2 as the target of *miR-138-5p*-enriched exosomes, enhancing neurological recovery in ischemic stroke mice by promoting astrocyte growth and reducing inflammation.[67] The transfer of *miR-133b* to both neurons and astrocytes via MSC-derived exosomes resulted in the downregulation of connective tissue growth factor, leading to the subsequent release of exosomes from astrocytes that facilitated neurite outgrowth.[56,83]

8 | MICROGLIA-MEDIATED BRAIN PLASTICITY

In aged *Macaca mulatta*, MSC-Exos not only mitigated neuroinflammation but also shifted the role of microglia towards a reparative function.[84] The intranasal delivery of MSC exosomes before ischemia, possibly involving the Toll-like receptor 4/CD14/NF-κB signaling pathway, demonstrated a capacity to minimize neuronal mortality and suppress microglia-mediated neuroinflammation.[85] Adipose-derived MSC (ADMSC)-Exosomes enriched with *miR-30d-5p*, by promoting M2 microglia polarization, offering enhanced protection against brain damage compared with control and *miR-30d-5p* knockdown groups. Mechanistic investigations unveiled that *miR-30d-5p* targeted autophagy-related genes, specifically *beclin-1* and *autophagy-related gene 5*, resulting in a reduction in autophagy-mediated M1 microglia polarization.[75] Systemically administered M2 microglial exosomes exhibited a reduction in infarct size, contributed to neurological function restoration, and suppressed neuron apoptosis, potentially through the transfer of *miR-124* and modulation of ubiquitin-specific protease 14 in neurons.[86]

9 | EXOSOME-MEDIATED ANTIOXIDATIVE EFFECTS OF MSC IN ISCHEMIC STROKE

The role of oxidative stress in the development of poststroke pathophysiology has been well established.[87] This oxidative stress environment poses challenges for the survival and colonization of transplanted neural stem cells. Treatment of senescent $CD4^+$ T cells with exosomes derived from human placenta-derived mesenchymal stem cells resulted in notable reductions in oxidative stress-induced damage, including reactive oxygen species (ROS) and 8-Hydroxy-2'-deoxyguanosine, as well as diminished numbers of cells expressing Senescence-Associated

β-galactosidase. Moreover, expression of aging-related proteins such as p53 and γ-H2A histone family member X, along with senescence-associated secretory phenotype components like IL-6 and Osteopontin, were significantly decreased.[88] Exosomes released by AdMSCs carry miR-25, which effectively suppresses the autophagic response. This mechanism promotes cell survival both in vitro under hypoxic conditions and in vivo during cerebral ischemia, thus facilitating recovery of post-stroke neurological function. Co-culturing with exosomes containing *circular RNA Akap7* (exo-*circAkap7*) demonstrated a decrease in cellular injury induced by OGD-reoxygenation (OGD-R). This effect was attributed to the absorption of *miR-155-5p*, which in turn promoted *ATG12*-mediated autophagy while reducing *NRF2*-mediated oxidative stress.[54] Exosomes act as mediators of intercellular communication in the brain, influencing various cell types such as neurons, microglia, oligodendrocytes, astrocytes, endothelial cells, and pericytes. Their cargo plays a pivotal role in regulating diverse brain functions, including responses to oxidative stress, maintenance of BBB integrity, and synaptic activity.[89] Through improvements in neurological functioning, reduction of pathological and structural damage to neurons, attenuation of oxidative stress, mitigation of neuronal apoptosis, and enhancement of normal neuron count, Exosomes derived from BMSCs (BMSCs-Exo) exhibit a protective effect against hypoxic-ischemic brain injury.[90] Exosomes derived from stem cells, post-stem cell implantation, have been shown to enhance post-stroke recovery in mice subjected to MCAO/reperfusion (MCAO/R).[91]

10 | ANTI-INFLAMMATORY EFFECTS OF MSC EXOSOMES IN ISCHEMIC STROKE

Inflammation serves a crucial role in the pathogenesis of brain damage resulting from cerebral ischemia, a key pathogenetic pathway. MSCs-Exos have the potential to reduce the severity of acute ischemic or ischemia–reperfusion injury by modulating the inflammatory responses. For instance, when hMSC-Exos is given to mice with a brain injury, the inflammation in the brain goes down in a dose-dependent way.[45] This helps rats with traumatic brain injuries recover their abilities and reduces inflammation-induced neuronal degeneration, microgliosis, and reactive astrogliosis.[92] On similar counts, when MSC-IL-1-Exos is added to astrocytes, the amount of inflammatory factors (*p-P65* and *P-65*) and antioxidant factors (*Nrf2*, *Keap1*, and *HO-1*) goes down.[93] Further, exosomes derived from BMMSCs have been shown to decrease inflammatory factors, promote neuronal survival, and ameliorate degenerative changes and neurological function in rodents with MCAO.[94] Furthermore, MSC-Exos protects against ischemic stroke by directing immune cells to take on a protective character.[95] Additionally, MSC-Exos also enhances neurogenesis to treat ischemic stroke. Three hours after an ischemic stroke, rats treated with ADMSC-Exos exhibited enhanced neurological function, decreased lesion volume, increased angiogenesis, and anti-inflammatory and immunomodulatory effects.[58] MSC-EVs can augment the secretion of *TGF-β* and IL-10 by $CD11c^+$ dendritic cells, thereby inhibiting lymphocyte proliferation.[96] The AMP-activated protein kinase and *JAK2/STAT3/NF-κB* signaling pathways may also be regulated by MSC-Exos to protect rodents with MCAO injury.[97] Therefore MSC-Exos can be utilized in neuroprotection, neurogenesis, immunomodulation, and angiogenesis (Figure 1).

Further, MSC-Exos also regulates pro-inflammatory factor secretion that reduces inflammation in ischemic stroke. Changes in inflammatory mediators such as cytokines and chemokines, as well as immune cell participation have been observed in the ischemic area following ischemia episodes. For instance, pro-inflammatory factors such as TNF-α, IL-1β, and IL-6 are upregulated following ischemia.[98] MSC-Exos containing factors such as *lncRNA ZFAS1*, *lncRNA H19*, *miR21-3p*, *miR-146a-5p*, *miR-138-5p*, and *miR182-5p* have been found to inhibit the release of *TNF-α*, *IL-1β*, and *IL-6*, resulting in reduced immunosuppression.[39,99,100] Additionally, exosomes derived from BM-MSCs enriched in *miR-138-5p* or *miR-1906* have been demonstrated to reduce inflammatory responses and suppress pro-inflammatory signaling cascades, thereby enhancing stroke recovery.[101] MSC-Exos can also inhibit the pro-inflammatory mediators *IFN-γ*, *iNOS*, and *IL-8*.[102] Furthermore, in the context of brain damage, some MSC-Exos upregulate anti-inflammatory factors such as *TGF-β*, *IL-4*, and *IL-10*, whereas downregulate pro-inflammatory factors.[75] Furthermore, MSC-Exos have been demonstrated to influence the levels of pro-inflammatory cytokines such as *TNF-α*, *IL-1β*, and *IL-6*, as well as anti-inflammatory molecules such as *IL-4* and *IL-10*, contributing to the reduction of inflammation in the infarcted brain.[75]

11 | INFLAMMATORY ROLE OF T-CELL INFILTRATION IN POSTSTROKE

T-cell recruitment and infiltration through the BBB after stroke play an important role in inflammation. Following the onset of ischemic stroke, innate immune cells are first activated, followed by T lymphocytes, which then infiltrate into brain tissue via BBB. Within 24 h of the stroke, T cells begin to invade the ischemic brain tissue, and 4 weeks later, they are still present.[103] T lymphocytes have been found to infiltrate ischemic brain tissue, according to numerous investigations. Depending on their functional properties, various T-cell subsets will affect ischemic brain tissue in different ways. It has been demonstrated that an excessive infiltration of inflammatory T cells might aggravate the inflammatory response and encourage tissue damage in the brain.[104] However, T cells may have immunosuppressive effects in the later stages of stroke that encourage the healing of damaged nerves, which may help to improve the prognosis for stroke.[105] T cells may also be advantageous in regulating

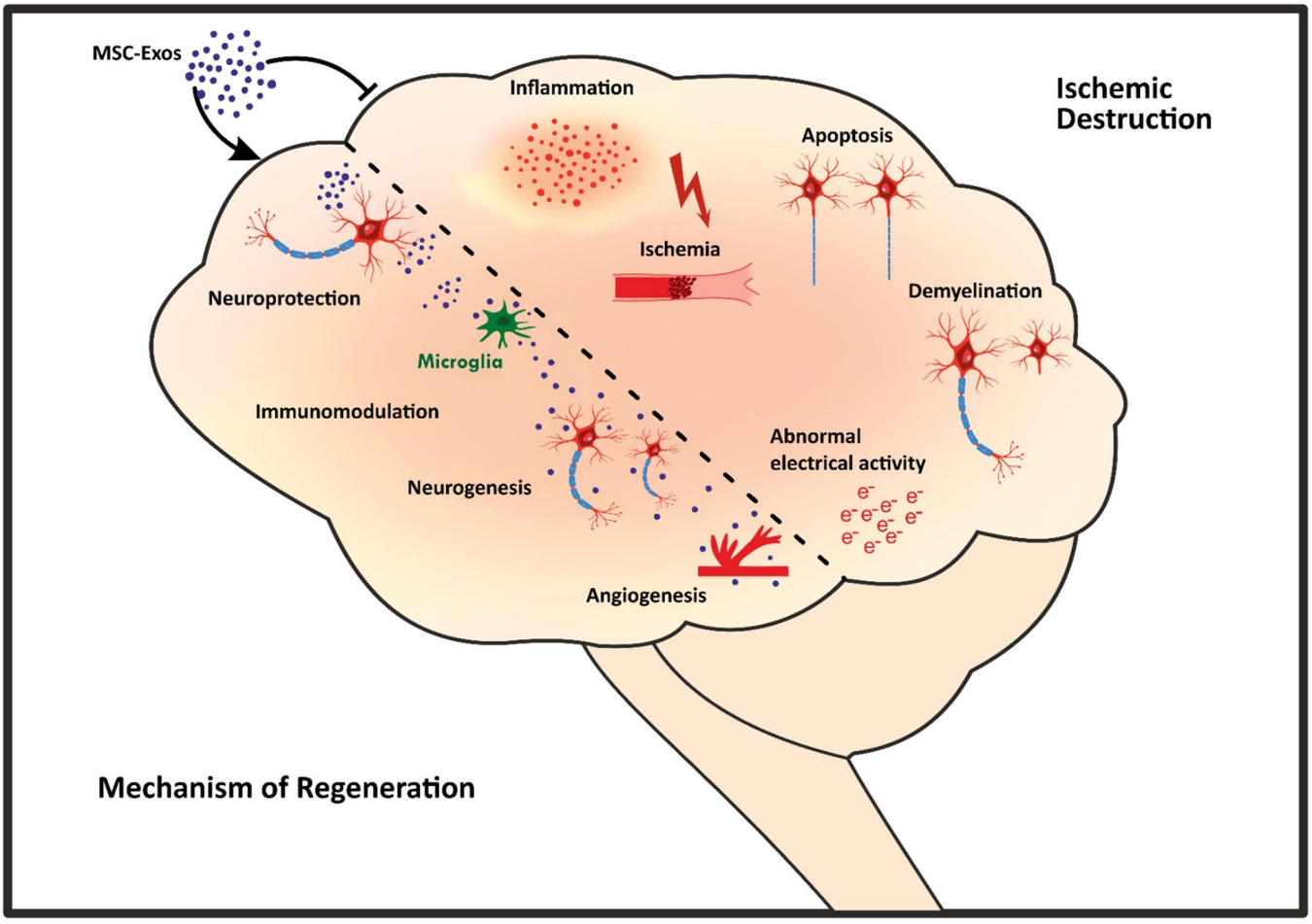

FIGURE 1 Ischemic stroke-induced damage triggers inflammation, apoptosis, ischemia, demyelination, and abnormal electrical activity. The administration of MSC-Exos to the ischemic brain facilitates restoration by enhancing neurogenesis, angiogenesis, neuroprotection, and the modulation of immune cell responses. MSC, mesenchymal stem cell.

inflammation, increasing poststroke repair, and encouraging clearance of wounded tissue debris throughout the chronic phases of stroke recovery, in addition to the detrimental effects in the acute period post-stroke.[106]

Extracellular vesicles generated by MSC-Exos have an active role in immunomodulation, resulting in a change in the microglial phenotype targeted at lowering stress in the context of ischemic stroke. Following OGD, treatment with MSC-EV enhances BV-2 cell survival and *miR-21a-5p* levels. In both in vitro and in vivo models of ischemic injury, decreased *miR-21a-5p* levels in MSC-Exos reduce the effects on microglial polarization and STAT3 phosphorylation.[107] Zhao et al.[59,65] showed that BM-MSCs' exosomes have anti-inflammatory properties by showing that they change the *miR-223-3p*-mediated *CysLT2R-ERK1/2* signaling pathway, which affects microglia M1 polarization. Further, it has been reported that exosomes derived from BMSCs inhibit inflammation-related signaling pathways, promote microglial polarization from M1 to M2, and reduce endothelial cell injury and stroke-related neurological impairment.[108] AD-MSCs promote the polarization of M2 macrophages by activating the transcription factors *Stat6* and *MafB* via exosomes.[109] Exosomes derived from AD-MSCs have been found to promote microglial polarization from M1 to M2 in response to pro-inflammatory microenvironment signals,[110] indicating their function in activating pro-inflammatory microenvironment signals. HU-MSC-derived exosomes can reach the site of ischemic injury and internalize by cells both in vivo and in vitro. Treatment with HUMSC-exosomes reduces OGD-induced microglial inflammation in vitro. After transient cerebral ischemia in vivo, therapy with HUMSC-exosomes results in decreased infarct volume improved behavioral impairments, and decreased microglial activation.[66]

In primary hippocampal astrocytes stimulated with lipopolysaccharide, MSC-Exos has been shown to decrease the expression of markers associated with astrocyte activation, pro-inflammatory phenotype (C3), and cell proliferation (Ki67). These effects included less reactive astrogliosis and less *NF-κB* activation. In vivo and in vitro administration of MSC-Exos reversed inflammatory phenotypes, such as *NF-κB* activation and translocation, and hippocampal astrocyte oxidation, by upregulating and translocating *Nrf2*.[111] In conclusion, there could potentially be additional stroke therapies that utilize the modification of immune responses to mitigate inflammation through the utilization of MSC-Exos.

11.1 | MSC-Exos in cell death pathway

MSC-derived exosomes exhibit promise in modulating cell death pathways and promoting cell survival. Experimental evidence indicates their capability to inhibit apoptosis, leading to a reduction in cell mortality. MSC-Exos have demonstrated the ability to diminish proapoptotic proteins while increasing antiapoptotic factors, thereby enhancing cell survival in ischemic conditions and neurodegenerative diseases. Moreover, MSC-Exos protected ischemia/reperfusion (I/R) damage in mice by inhibiting microglial apoptosis, potentially through exosomal *miR-26a-5p*-mediated inhibition of *CDK675*.[112] Mahmoudi et al.[113] illustrated that MSC-Exos enhances phagocytosis and minimizes neutrophil apoptosis. AD-MSC-derived exosomes prevent apoptosis and inflammation by activating *MAT2B* and downregulating *miR-21-3p* in hypoxia or reoxygenation-exposed cells.[39]

Additionally, exosomes also impact autophagy, a vital physiological process influencing cell fate and survival. The proposed methods have been found to enhance autophagy in injured cells, facilitating the removal of toxic aggregates and damaged organelles, thereby promoting cell survival and tissue repair.[114] Along the preceding lines, excessive autophagy may also lead to cell death, however, MSC-derived exosomes can prevent autophagy-induced cell death. Adipose-derived MSC exosomes, transmitting *miR-25*, show promise in reducing autophagy and enhancing cell survival under hypoxia and cerebral ischemia, facilitating neurological function recovery after stroke.[115] Pigment epithelium-derived factor (PEDF)-modified ADSC-derived exosomes protect against in vivo cerebral I/R injury by increasing autophagy and preventing apoptosis.[64]

12 | IMPACT OF MSC-EXOS ON PYROPTOSIS

MSC-Exos exhibits the ability to inhibit the expression of the NLRP3 inflammasome and pyroptosis-associated proteins on neuronal surfaces. Through the modulation of microglial polarization and the suppression of NLRP3 inflammasome-mediated inflammation, BMSC-Exos effectively reduces cerebral I/R injury.[116] In a rat model of MCAO, Sarmah et al.[117] observed a parallel reduction in *NLRP-1* and *NLRP-3* inflammasomes, along with associated components such as IL-1β, caspase-1, and ASC, following intra-arterial MSC injections. The interplay between astrocyte and microglial polarization and the initiation of the post-stroke inflammatory cascade, linked to NLRP3 apoptotic bodies, is mitigated by BMSC-Exos, contributing to the dampening of the inflammatory response after stroke.[117] Furthermore, human mesenchymal stem cell-derived exosomes, through the *miR-138-5p*/*Sirt1* axis of *lncRNA KLF3-AS1*, demonstrate a reduction in cardiomyocyte pyroptosis and myocardial infarction.[118]

13 | IMPACT OF MSC-EXOS ON FERROPTOSIS

Exosomal circBBS2 derived from UC-MSCs enhances *SLC7A11* expression by sequestering *miR-494*, thereby inhibiting ferroptosis and mitigating ischemic stroke effects.[119] Moreover, ADSC-derived exosomes exhibit positive outcomes in a mouse neuroblastoma cell line N2a model of OGD. The study reveals that *miR-760-3p* in ADSC-Exo, through its interaction with *CHAC1* in neurons, plays a role in preventing ferroptosis.[120] Ferroptosis occurs during the late stages of cerebral ischemia when blood containing free iron and ferritin infiltrates the brain parenchyma through a compromised BBB.[121]

14 | CUPROPTOSIS IN NEUROLOGICAL DISORDERS

Recent research has revealed that cuproptosis, a unique and copper-dependent form of controlled cell death, is triggered by copper's direct binding to lipoylated tricarboxylic acid cycle components. It causes iron-sulfur cluster protein loss and lipoylated protein aggregation, which trigger proteotoxic stress and cell death.[122] According to experimental data, neurotoxicity and cerebral copper dyshomeostasis are also factors in the pathogenesis of Alzheimer's disease.[123] However, a recent study found that both male and female rats exposed to chronic copper have reduced learning and memory, as well as oxidative stress in the hippocampus.[124] Additionally, high Cu exposure impairs mice's learning and memory by causing Cu accumulation in their brain tissue. Cu excess causes oxidative damage and cell death, which result in the pathological injury of brain tissue. Through *CREB*/*BDNF* pathways, Cu reduces synaptic plasticity and activates cuproptosis, which aids in cell death, according to mechanism studies.[125] The intricate interplay between MSC-Exos and cuproptosis holds significant implications, offering new avenues for understanding and potentially intervening in copper-induced cell death mechanisms.[126]

15 | EXOSOMES AS DIAGNOSTIC BIOMARKERS FOR ISCHEMIC STROKE

Exhibiting unique attributes, exosomes show promise as potential therapeutic agents for ischemic stroke detection. These attributes encompass minimal immunogenicity, inherent stability, effective transmission, and the capability to traverse the BBB. Exosomes are secreted by all cell types and can be found in biological fluids, making them useful for liquid biopsies to monitor the progression of disease.[127] Therefore, exosomes have the potential to identify various stages of ischemic stroke and function as potential diagnostic biomarkers. Along these lines, investigating the presence of differentially regulated miRNAs in exosomes, particularly those originating from neural sources, would be of

considerable interest. miRNAs, which are among the most abundant components of exosomes, can be used for the early detection of ischemic stroke. As per clinical findings, the expression of serum exosomal *miR-9* and *miR-124* was significantly correlated with infarct volume, serum IL-6 concentration, and National Institutes of Health Stroke Scale scores.[128] The above two exosomal miRNAs are crucial for assessing the severity of ischemic injury and diagnosing ischemic stroke. Notably, patients with acute ischemic stroke have a significant increase in *miR-223* expression in their serum exosomes. Patients who had a bad outcome from their stroke had a higher level of *miR-223* expression in their exosomes than those who had a good outcome.[129] In addition, plasma exosomal *miRs 422a* and *125b-2-3p* may serve as blood-based biomarkers for ischemic stroke diagnosis and monitoring in patients with acute and subacute strokes.[130] Further, a combination of plasma exosomal *miR-21-5p* and *miR-30a-5p* has also been proposed as an outstanding biomarker for ischemic stroke diagnosis and phase determination.[131] Patients with an acute ischemic stroke have more *miR-134* in their plasma exosomes than healthy people, and the size of the infarct is a strong predictor of a worse prognosis.[132] Likewise, the increased levels of *GADD34* in the plasma exosomes of rodents with cerebral ischemia suggest that exosomal *GADD34* may be useful as a diagnostic biomarker and therapeutic target in ischemic strokes.[133] Along the above lines, stroke onset significantly elevates E-selectin, P-selectin, and platelet-derived EVs, with platelet-derived EVs remaining elevated, reflecting protracted platelet activation during the healing phase, while E-selectin and P-selectin decreased significantly 3–6 months after stroke, platelet-derived EVs remained elevated.[134] Also, extracellular vesicles from ASC, an inflammatory protein of NLRP3 inflammatory cascade, could be used as possible inflammatory biomarkers for ischemic stroke, since patients with ischemic stroke have been found to have higher levels of ASC.[135] In addition, Annexin V-positive exosomes derived from patients with acute ischemic stroke had elevated levels of platelets ($CD61^+$), erythrocytes ($CD235ab^+$), leukocytes ($CD45^+$), neural progenitor cells ($CD34^+$ and $CD56^+$), and leukocytes ($CD45^+$) in their blood samples.[136] In addition, a recent study comparing 200 nm EV profiles in the mouse brain under physiological conditions and 24 h after acute MCAO revealed that microglial-derived EVs predominated at baseline, whereas astrocytic-derived EVs predominated after ischemia.[137] These findings highlight the potential of exosomes as diagnostic biomarkers and also as therapeutic targets for ischemic stroke management.

15.1 | Limitations and gaps in existing exosome therapy

Exosome therapy now available has numerous limitations and research gaps that are impeding its practical deployment. The differential regulation of exosome release by distinct cell types is a major concern; for example, oligodendrocytes release exosomes under NaCl regulation, whereas astrocytes secrete them under high KCl concentrations. Because of inherent variation in size, cargo, and identifying markers, standardizing exosomes is difficult, and large-scale synthesis and isolation are expensive and time-consuming operations.[9] The lack of existing criteria makes determining the therapeutic effects of individual exosomes challenging, and assessing their cargo or markers presents significant challenges.[138] Furthermore, unanticipated side effects of exosome therapy have been identified, such as hypothalamic stem cell-derived exosomes altering the aging rate of mice.[139]

Before clinical application, the appropriate injection dose, therapeutic window, and route of distribution for exosomes must be determined. Exosomes from MSCs, for example, have dose-dependent effects on functional recovery in infarcted areas.[51] Low doses have been shown to have neuroprotective characteristics, whereas high concentrations have been shown to have neurotoxic effects.[140] For instance, a dose-dependent study found that delivering exosomes at low dosages (50–100 g) was important for enhanced functional recovery after stroke.[141] Exosome-based treatments for stroke or brain injury have major challenges in efficiently targeting specific recipient cells inside the CNS.[142] Exosome composition and activity can be influenced by metabolic processes in both recipient and donor cells, complicating exosome therapy.[143] Pure exosome isolation advances are required for clinical application.[144] Overall, further research is needed to overcome these obstacles and improve the efficacy of current exosome approaches.

15.2 | Exosomes as delivery vehicle of therapeutics of interest

Utilizing exosomes as carriers for targeted therapeutic delivery is an attractive avenue within nanotechnological frameworks, offering enhanced solubility and precision in directing natural substances. Along these lines, to specifically address neuronal injury within the ischemic penumbra, a monoclonal antibody GAP43, a neuron-specific protein, was consistently modified onto the surface of drug-loaded exosomes.[145] In essence, the anti-inflammatory and neuroprotective agent curcumin (cur) was loaded into macrophages (Ex-cur) and exosomes derived from embryonic stem cells (MESC-exocur). This innovative approach demonstrated the capability to reduce inflammation production, downregulate excitatory amino acid receptors, minimize ROS accumulation, alleviate BBB damage in lesions, and enhance neurovascular recovery.[146,147] Furthermore, the delivery of nucleic acids and peptides, in addition to molecular medications, was achieved. For targeted delivery to the ischemic brain, MSC-exos were loaded with cholesterol-modified *miR-210* and conjugated to arginylglycylaspartic acid c(RGDyK) peptides. Notably, near-infrared fluorescence imaging illustrated significant improvements in angiogenesis and survival

in MCAO/R mice.[68] Facilitating nose-to-brain transport of the anti-miRNA oligonucleotide, a receptor for advanced glycation end-products (RAGE)-binding peptide linked to exosomes (RBP-Exo) was employed. RBP-Exo not only delivered AMO181a more efficiently than unmodified exosomes but also mitigated damage to the ischemic brain by downregulating RAGE.[148] Moreover, MNVs derived from iron oxide nanoparticle-harbored MSCs enhanced the magnetic navigation targeting of the ischemic brain region.[149] Through bioorthogonal chemistry, Tian et al. coupled exosomes with the c(RGDyK) peptide surface for the specific treatment of ischemic stroke, concentrating on the lesion region. These modified exosomes, containing curcumin, demonstrated a notable reduction in cellular apoptosis and the inflammatory response within the targeted (lesion) area, showcasing promising therapeutic efficacy and targeting precision in the in vivo results of the cRGD-exosome delivery system.[150]

16 | CONCLUSION

Exosome-based therapy emerges as a promising paradigm for ischemic stroke treatment, holding transformative potential in the therapeutic landscape. Originating from diverse sources, with a notable emphasis on MSCs, exosomes demonstrate effectiveness in alleviating pathophysiological mechanisms associated with stroke. Their distinctive features, including low immunogenicity and efficient cargo transport across the BBB, contribute to their therapeutic prowess. Demonstrating neuroprotection, angiogenesis, anti-inflammatory responses, and the regulation of cell death pathways, exosomes enhance neuronal survival, tissue healing, and functional recovery. Additionally, they show promise as diagnostic biomarkers, enabling early detection and tailored therapeutic interventions for ischemic stroke. Despite these advancements, practical application faces challenges and research gaps that warrant further exploration. Delving into the underlying mechanisms and cellular interactions is essential for translating exosome therapy from a promising concept to a revolutionary clinical reality, necessitating interdisciplinary collaborations across neurology, biotechnology, and clinical research.

AUTHOR CONTRIBUTIONS

Arshi Waseem: Manuscript writing, collection, and/or assembly of table data. **Saudamini:** Manuscript writing. **Rizwanul Haque** and **Miroslaw Janowski:** Editing. **Syed S. Raza:** Conception and design, editing, and final approval of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Not applicable.

ETHICS STATEMENT

Not applicable.

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