

# Exosomes: Mediators of Neurodegeneration, Neuroprotection and Therapeutics

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**Abstract** Exosomes have emerged as prominent mediators of neurodegenerative diseases where they have been shown to carry disease particles such as beta amyloid and prions from their cells of origin to other cells. Their simple structure and ability to cross the blood–brain barrier allow great opportunity to design a “makeup” with drugs and genetic elements, such as siRNA or miRNA, and use them as delivery vehicles for neurotherapeutics. Their role in neuroprotection is evident by the fact that they are involved in the regeneration of peripheral nerves and repair of neuronal injuries. This review is focused on the role of exosomes in mediating neurodegeneration and neuroprotection.

**Keywords** Blood–brain barrier · Exosomes · Neurotherapeutics · Neurodegeneration

## Introduction

There have been many implications regarding various physiologically mechanistic phenomena in neurodegenerative diseases. Nominally, exosomes have been implicated in neurodegenerative diseases where they are seen to carry information on biological alteration in their cells of origin. As such, emerging evidences have suggested the therapeutic and neuroprotective roles of exosomes. However, very little is known about the intricacies of their involvement in neuronal diseases, disease pathologies, and use to rescue against neuronal pathologies and alterations. Before establishing detailed significance of exosomal implications in neurodegeneration

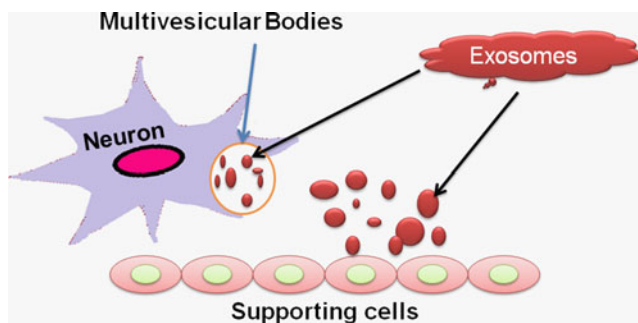
and neuroprotection, it is crucial to understand the essence of an exosome. Exosomes are the smallest membranous vesicles (40–100 nm) and depict homogenous shape (cup-shaped after fixation under electron microscope) with a buoyant density of 1.13–1.19 g/cm<sup>2</sup> [1–3]. These nanovesicles are secreted by diverse cell types (e.g., neurons, tumor cells, and kidney cells) and are found in various body fluids such as urine, amniotic fluid, malignant ascites, bronchoalveolar lavage fluid, synovial fluid, breast milk, saliva, blood, and cerebrospinal fluid. Intracellularly, exosomes are generated via the inward budding of endosomes to form multivesicular bodies (MVBs) that fuse with the plasma membranes to release exosomes into the surrounding environment (Fig. 1). Exosomes, depending on their parental origin, contain a variety of proteins, lipids, non-coding RNAs, mRNA, and miRNA, collectively termed as “cargo” contents, and are delivered to the surrounding cells or carried to the distal cells. Due to their cargo ability, exosomes represent a novel form of intercellular communication among cells without cell-to-cell direct contact [4, 5]. Exosomes are selectively taken up by the surrounding or distal cells and can reprogram the recipient cells due to their active cargo content [6].

Exosomes contain surface membrane proteins which act as markers for exosome identity and selection, and along with molecular cargo, these surface proteins provide a rich source of biomarkers for various pathological conditions [7]. Exosomes also aid in antigen presentation by immune cells, involved in cell signaling, and exhibit anti-inflammatory or pro-inflammatory properties [8, 9].

Furthermore, tumor-derived exosomes may deliver mRNAs, miRNAs, or proteins to the surrounding cells, which may aid in angiogenesis, cell proliferation, and cell survival. Interestingly, many cells of the nervous system have been shown to release exosomes in the form of extracellular membrane vesicles, which indicates their active role in function, development, and pathologies of this system [10]. Recent

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**Fig. 1** Exosome shedding from neuronal cells

findings insist the involvement of exosomes in the transfer of pathogens between cells, for example, the prion, the infectious particle responsible for the transmissible neurodegenerative diseases such as Creutzfeldt–Jakob disease (CJD) in humans or bovine spongiform encephalopathy (BSE) in cattle [11]. Apart from that, exosomes are also found to be associated with other proteins such as superoxide dismutase I and alpha-synuclein which are involved in amyotrophic lateral sclerosis and Parkinson's disease. Similarly, amyloid precursor protein (APP) associated with Alzheimer's disease has been reported to be processed through exosomes [12].

Although exosomes have been implicated in neurodegenerative diseases where they carry information on the biological alteration in their cells of origin, however, little is known about their role in neurodegenerative diseases. Exosomes not only act as vehicles for the transfer of biomolecules and pathogens associated with neurodegenerative diseases, but also help in neuroprotection by encapsidating biomolecules and this needs to be explored. Apart from diagnosis and pathogenesis, exosomes have also been implicated in therapeutics where they are used to deliver drugs or genetic elements like siRNA or miRNA to target tissues, e.g., in the treatment of neuroinflammatory diseases and gene therapy. Therefore, this review aims to highlight the role of exosomes in neurodegeneration and how they can be utilized in therapeutics for neuroprotection.

### Biogenesis and Contents of Exosomes

The exosomal content varies from diverse proteins to lipids and nucleic acids. Exploring the biogenesis and trafficking of exosomes may be helpful in understanding how cells utilize exosomes for cell-to-cell communication and environment modification. The generation of exosomes is initiated by inward invaginations of clathrin-coated microdomains on the plasma membrane [13]. After invagination, the endosomal sorting complex required for transport (ESCRT) facilitates the development of invaginated vacuoles into early endosomes (EE) that carry ubiquitinated cargos. This is followed by secondary invagination into the EEs to form intraluminal vesicles (ILVs) which accumulate and mature

inside the endosomes that are now referred to as large multivesicular bodies [13]. The multivesicular bodies have two fates: either they can be processed to lysosomes for degradation (degradative MVBs) or fused with plasma membrane (exocytic MVBs) for the release of ILVs into the extracellular space where they are referred to be as exosomes [14]. Trajkovic et al. demonstrated that in oligodendrocytes, the release of ILVs is ESCRT-dependent, and the distribution of sphingolipid ceramide in MVBs guides the extracellular release of ILVs as exosomes [15]. Some studies demonstrate that the release of exosomes depends upon Rab27 and Rab35 and can be blocked with an inhibitor of neutral sphingomyelinase [15–17]. In another study, the release of exosomes is shown to be induced by  $\text{Ca}^{2+}$  and ionophore A23187 treatment [18].

Since exosomes originate from endosomes, they contain membrane transport and fusion proteins (GTPases, annexins, flotillin), tetraspanins (CD9, CD63, CD81, and CD82), heat shock proteins (heat shock cognate (Hsc70), heat shock protein (Hsp 90)), proteins involved in MVB biogenesis (Alix and TSG101), and lipid-related proteins and phospholipases [19]. These proteins are used as positive 'markers', although there are wide variations in proteins among exosomes derived from different origins. The most widely used markers include TSG101, Alix, flotillin, and Rab5b which are detected by antibody-based techniques such as western blot and ELISA to confirm the presence of exosomes. Apart from the membrane-associated proteins, over 4,400 different proteins have been identified usually by mass spectrometry and were found to be associated with exosomes and serving as cargo for cell-to-cell communication [20].

Exosomes are rich in lipids, and different types of exosomes have different lipid composition, depending upon their cells of origin. A variety of lipid compounds have been identified to form exosomes, which include phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine (PS), lysobisphosphatidic acid, ceramide, cholesterol, and sphingomyelin [21]. The lipid composition contributes different biophysical properties to exosomes and determines their rigidity and delivery efficiency, e.g., sphingomyelin and *N*-acetylneuraminy-galactosylglucosylceramide (GM3) [22]. Phosphatidylserine is expressed on the exosome membrane through floppase, flippase, and scramblase activities, and it is involved in signaling and fusion to plasma membrane by docking the outer proteins [23]. Hence, the difference in the levels of PS on exosomal membrane may affect the communication functions of exosomes. Exosomes have also been reported to contain saccharide groups on their surface membranes. Batista et al. have reported mannose, poly lactosamine,  $\alpha$ -2,6 sialic acid, and complex N-linked glycans in exosomes [24].

Apart from proteins and lipids, exosomes contain nucleic acids in the form of miRNA, mRNA, and other non-coding RNAs [19, 25]. Many studies report that the RNA cargo of

exosomes is different from that of the parent cell [19, 26, 27]. Others have shown that exosomes originating from cancer cells contain the same miRNA content as their parent cells which can be used as biomarkers [19, 28, 29]. miRNA may provide a better tool for the confirmation of exosomal presence and disease biomarkers since verification of exosomes through EM is costly and time consuming. The mRNAs carried by exosomes can be translated in the recipient cells, while the miRNA and ncRNAs may regulate the gene expression. Exosomes also contain DNA, but their function has yet to be validated [30, 31]. Due to large increase in finding the exosomal contents, there is a need to accommodate these molecules in web-based database, and in this direction, ExoCarta has been introduced to record exosomal proteins, RNA, and lipids (<http://www.exocarta.org/>) [20]. Table 1 shows the important content of neuronal exosomes and their roles.

Overall, exosomes are secreted by a vast range of cells and contain constituents such as proteins, lipids, and nucleic acids from their parent cells. Thus, exosomes can be viewed as transporters, bridging the communicatory gap between cells, shuttling material from their cells of origin to the other cells. By observing the contents and considering candidates for potential biomarkers, experimental investigation can give credence to the neuroprotective role of exosome in neurodegenerative disease. Further explication of the proposed functionality of exosomes in cell-to-cell communication will tender this credence.

### Neuronal Communication Via Exosome

Exosomes function as a means of cell-to-cell communication and interact with the neighboring cells to facilitate the delivery of active molecules (Fig. 2). Studies by Skog et al. have shown the transfer of proteins and micro-RNAs from glial cells to

axons [32]. In response to glutamatergic synaptic activity, the hippocampal neurons and cultured cortical cells release exosomes into the extracellular environment that carry GluR2/3 subunits which are receptor molecules [33]. The characteristic myelin lipids—galactocerebrosides, sulfatides, and cholesterol—are released into the exosomes via oligodendrocytes which are characteristics of myelin sheath and nerve conduction [34].

In pathological condition, microglial cells, which are macrophages of the CNS, become activated and serve as antigen-presenting cells via secreting exosomes [35]. The exosomes released from the plasma membranes of the microglial cells and astrocytes in response to ATP stimulation and sphingomyelinase activation contain the pro-inflammatory cytokine IL- $\beta$  [36]. Cultural astrocytes release exosomes in response to oxidative and heat stress that contains heat shock protein 70 and synapsin-1 [37]. Guescini et al. have reported that exosomes released from astrocytes contain mitochondrial DNA [38]. Brain tumor cells which originate from astrocytes and glioblastomas release exosomes that carry immunosuppressive and oncogenic factors [39]. Hence, exosomes serve as vehicles for cell-to-cell communication via transferring molecules of diverse origin.

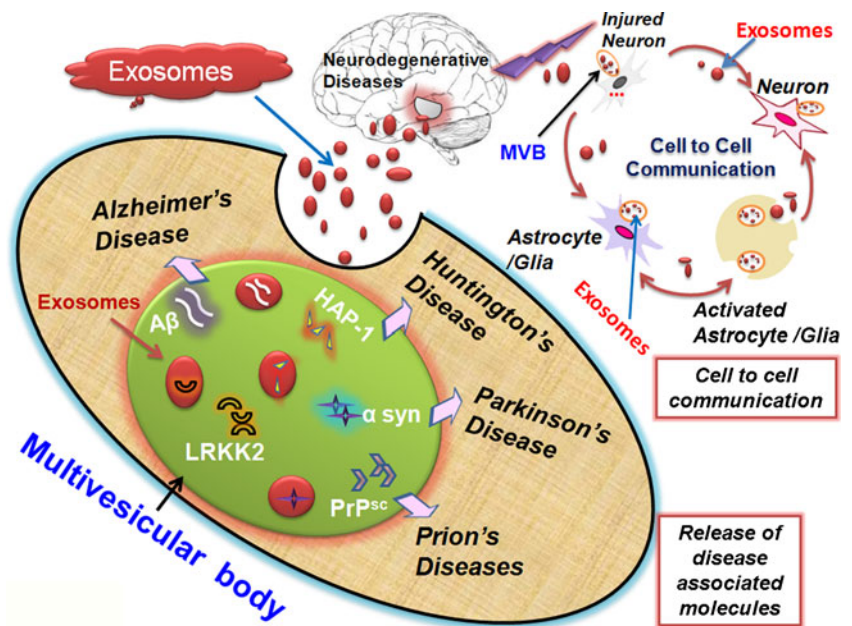
### Exosome in Neurodegeneration

One of the major causes of neurodegeneration is neuronal cell death which is a hallmark of neurodegenerative disorders such as Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD), Niemann–Pick disease, frontotemporal dementia, and amyotrophic lateral sclerosis. All these diseases have been realized to have a common molecular and cellular mechanism which involves protein

**Table 1** Exosome constituents and their roles

Exosomes/biomolecules	Examples	Role	Reference
Membrane transport and fusion proteins	GTPases, annexins, flotillin	Helps in membrane transport and fusion	[14, 17]
Tetraspannins	CD9, CD63, CD81, CD82	Morphogenesis, fission, and fusion processes	[14, 19, 20]
Heat-shock proteins	Hsc70, Hsp 90	Inherent characteristic of exosomes	[14, 19, 20]
Multivesicular body biogenesis proteins	Alix, TSG101	Proteins involved in multivesicular body biogenesis and potentially used as markers for the identification of exosomes	[14, 19, 20]
Lipid-related proteins and phospholipases	Phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, lysobisphosphatidic acid, ceramide, cholesterol, sphingomyelin, <i>N</i> -acetylneuraminyl-galactosylglucosylceramide, mannose,	Determines exosome rigidity and delivery efficiency, helps in signaling and fusion to plasma, helps in cell to cell communication	[20, 21]
Saccharides	Polylactosamine, $\alpha$ -2,6 sialic acid, and complex N-linked glycans	Inherent characteristic of exosomes	[24]
Genetic material	miRNA, mRNA, and other non-coding RNAs	Regulation of gene expression, transfer of information	[19, 25, 30]

**Fig. 2** Cell-to-cell communication through exosomes and processing of neurologic toxin proteins via exosomes. Cell-to-cell communication showing the exosome release from injured neuronal cell and processing to healthy neuronal cells. The release of neuronal toxins or disease-associated molecules is done via exosomes that are processed through multivesicular bodies and released to the extracellular spaces



aggregation and the formation of inclusion bodies in selected areas of the nervous system. Sorting of proteins correctly inside the cells and the degradation of cellular proteins is important for the health of neurons [40] (Fig. 2). Exosomes are involved in the spread of ‘toxic’ proteins in neurodegenerative disorders which are mutated or ‘misfolded’ proteins and serve as template for the formation of oligomers [34, 41, 42]. Neurons try to get rid of these accumulated proteins by processing them through endosomal pathway which either leads to degradation into lysosomes or incorporation into MVBs and release into extracellular space as exosomes. In this context, studies have described the incorporation of normal prion protein (PrP) and misfolded pathogenic prion

protein (PrP<sup>sc</sup>) into exosome [43, 44]. Vella et al. have shown that PrP<sup>sc</sup> which is associated with exosome is transferred to normal cells containing PrP. This mechanism by which proteins tend to seed their own aggregation with ‘infectious’ delivery via exosomes is involved in a number of neurodegenerative diseases [45]. Table 2 summarized some of the neurodegenerative diseases and disease-associated molecules. In such diseases, there is a spatiotemporal propagation of pathology, suggesting cell-to-cell spread, and for non-secreted proteins, it is mediated by exosomes or nanotubes [46, 47]. The involvements of exosomes in different neurodegenerative diseases have been studied, and some of the studies are summarized below.

**Table 2** Neurodegenerative diseases and associated molecules of exosomes

Neurodegenerative disease	Associated molecules in exosomes	Role	Reference
Alzheimer’s disease	Aβ42	Accumulated in MVBs and are released as exosomes, spread to other tissues through exosomes	[49, 50]
Huntington’s disease	Huntingtin protein	Mutated huntingtin protein was found to be accumulated in MVBs and speculated that the pathology of HD is closely determined through exosomes	[53, 54]
Parkinson’s disease	α-syn, LRRK2, VPS35	Toxic form of α-syn is deposited and transferred from one to other cells by exosomes, LRRK2 plays an important role in exosome secretion and fusion of MVBs with plasma membrane; a mutation in the LRRK2 gene R1441C induces the formation of skein-like abnormal MVBs that induce large number of exosomes that contain toxic form of α-syn; essential component of retromer complex and dysfunction of retromer causes increased exosomal secretion of APP	[2, 62–64, 70, 74]
Prion diseases	PrP <sup>c</sup> and PrP <sup>sc</sup>	Associated with exosomes and infectious in both animal and cell bioassays	[44, 76]

Aβ42 amyloid beta 42, α-syn α-synuclein, LRRK2 leucine-rich receptor kinase 2, VPS35 vacuolar-sorting protein 35, PrP<sup>c</sup> and PrP<sup>sc</sup> prion proteins, APP amyloid-β precursor protein

### Alzheimer's Disease

The involvement of MVBs was first suggested in 1970 in Alzheimer's disease patients when it was found that MVBs were enlarged more in number in the cortical neurons of the forebrain [48]. Amyloid  $\beta$ 42 ( $A\beta$ 42), a peptide fragment which is found to be accumulated in the MVBs, is the main constituent of plaque characteristic of Alzheimer's disease [49]. The oligomeric fibrils of the  $A\beta$  peptide initiate the accumulation by serving as a seeding center for AD pathology in naïve mice and become neurotoxic [50]. The amyloid precursor protein is proteolytically processed to generate peptides at the plasma membrane which are taken up into endosomes, further processed in the MVBs, and are released as exosomes from the cell [51]. The role of exosomes in Alzheimer's disease is attributed to the inappropriate sorting and accumulation of amyloid-beta and spread to other tissues through exosomes. Oral administration of amyloid A1 (amyloidosis) among cheetahs suggests their transmission with exosomes present in saliva and fecal matter [52]. Exosomes play a role in both the degradation of toxic  $A\beta$  and the accumulation of toxic peptides when the clearance pathway is overwhelmed [12]. However, it is unclear whether the protein aggregates caused the impaired clearing or the impaired clearing caused the amyloid-beta aggregates and transmission.

### Huntington's Disease

Huntington's disease is a progressive neurodegenerative disease, and the role of MVBs in this disease was first discovered in 1997 when the huntingtin protein that is mutated in the disease was found to be accumulated in MVBs [53, 54]. Apart from MVBs, huntingtin has been also found in other membrane structures such as endoplasmic reticulum, lipid rafts, and late endosomes [55–57]. Huntington's associated protein (HAP-1) interacts with ESCRT-O, and its overexpression is associated with impaired trafficking of the EGF receptor through the MVBs and lysosomes [58]. It is speculated that the pathology of HD is closely related to the recycling and sorting of cellular proteins through MVBs and maintaining efficient endosomal–lysosomal trafficking [56, 59].

### Parkinson's Disease

Parkinson's disease is the second most common neurodegenerative disorder after Alzheimer's disease and is characterized by selective degeneration of dopaminergic neurons in the substantia nigra of the Lewy bodies that are composed of fibrillar  $\alpha$ -synuclein ( $\alpha$ -syn) and ubiquitinated proteins in the surviving neurons [60, 61]. Ninety percent of PD are sporadic, but familial cases have also been associated to different genes such as  $\alpha$ -syn, leucine-rich receptor kinase 2

(LRRK2). However, the exact mechanism for the disease onset and progression are unclear. Exosomes play a role in PD by transferring the toxic form of  $\alpha$ -syn to other cells, and  $\alpha$ -syn deposits are released by exosomes [2, 62–64].  $\alpha$ -syn which is transferred from one neuron to another is able to form aggregates in the recipient cells [64, 65]. The  $\alpha$ -syn deposits which are released by neurons are cleared by astrocytes and microglia by endocytosis [66–68]. However, excessive uptake of  $\alpha$ -syn can produce glial inclusions and trigger inflammatory response [68, 69]. Understanding the cell-to-cell transmissions of the toxic forms of  $\alpha$ -syn and inflammatory mechanisms in the brain cells may provide an insight into the disease onset and progression of PD and help in identifying novel strategies for PD therapeutics.

LRRK2 plays an important role in exosome secretion and fusion of MVBs with plasma membrane as it has been found to co-localize with MVBs [70]. Shin et al. (2008) have demonstrated that LRRK2 interacts with Rab5b which is a regulator of endocytic vesicle trafficking [71]. A mutation in the LRRK2 gene R1441C induces the formation of skein-like abnormal MVBs. These abnormally large MVBs due to the pathological LRRK2 activity release large number of exosomes which may contain the toxic form of  $\alpha$ -syn and thus lead to the spread of the disease [70]. Tau protein which is involved in the pathogenesis of Alzheimer's disease can accelerate exosome-mediated release of  $\alpha$ -syn toxin from injured neurons as it can interact with  $\alpha$ -syn, promoting oligomerization and toxicity of these proteins [72]. Vacuolar sorting protein 35 (VPS35) which is involved in PD is an essential component of the retromer complex (which performs retrograde transport from an endosome to the golgi apparatus). Sullivan et al. have reported that cells with defective retromer activity have increased exosomal secretion of APP [73]. In conclusion, exploring the LRRK2, tau, and VPS35 in context with exosomes may provide important clues about the spreading of  $\alpha$ -syn and progression of PD.

### Prion Diseases

Prion diseases are neurodegenerative disorders that are transmissible and often fatal, e.g., CJD, Gerstmann–Straüssler–Scheinker syndrome in humans, BSE in cattle, and scrapie in sheep. Prion disease is caused by the abnormal form of the prion protein  $PrP^{Sc}$  which is a toxic scrapie confirmation of the normal prion protein  $PrP^c$  and eventually results in neuronal death causing large spongiform vacuoles in the brain tissue [74]. Both forms of prion protein  $PrP^c$  and  $PrP^{Sc}$  have been found to be associated with exosomes, and exosome-containing  $PrP^{Sc}$  was infectious in both animal and cell bioassays [44, 75]. Apart from cell cultures used to isolate the exosomal vesicles, primary cultured neurons and CSF have also been used as a source of exosomes for detecting prion particles [76, 77]. It has been suggested that the conversion of

PrP<sup>c</sup> to PrP<sup>sc</sup> occurs in the lipid raft region as PrP<sup>c</sup> is attached to the plasma membrane by a glycosylphosphatidylinositol anchor [78]. Baron et al. [79] suggested that the generation of new PrP<sup>sc</sup> during infection requires the insertion of PrP<sup>sc</sup> into lipid rafts, showing that the presence of lipid rafts in exosomes aids in their ability to transmit PrP<sup>sc</sup>. The observation that MVBs are abnormal and increased in prion disease state [80, 81] provides a strong evidence of the role of exosomes in the progression of prion pathology.

### Aging

Aging is associated with neurodegenerative changes, and there is an increase in the exosome number in degenerating neurons such as neuromuscular junctions of aging mouse, exon terminals of sympathetic ganglia, and dorsal column nuclei [82]. In rats also, the increase in the accumulation of exosomes has been found along with other neurodegenerative changes, e.g., in neurons and oligodendrocytes [83]. An increase in exosomes was also noted in monkeys and in humans as a response to aging and degeneration [84, 85]. In aged brains, ultrastructural changes, for example, increase in exosome number, are most likely to be related to neurodegenerative changes that occur with aging.

Besides chronic neurodegenerative diseases, exosomes have also been reported in acute degenerative diseases for example stroke. A recent study by Xin et al. describes the role of exosome in neurological recovery after stroke [86]. They genetically engineered mesenchymal stem cells (MSC) to release exosomes laden with microRNAs (particularly miR133b) and injected these MSCs in the blood stream of rats after 24 h of stroke. These MSCs enter the brain and release exosomes enriched with miR133b. The rats injected with miR133b-enriched MSCs showed more neurological recovery and axonal plasticity as compared to the rats injected with MSCs deprived of miR133b.

### Exosomes in Neuroprotection

Many studies have shown the role of exosomes in neuronal protection, nerve regeneration, neuronal development, and synaptic plasticity, indicating the release of exosomes by neurons [76], microglia [87], astrocytes [88], oligodendrocytes [89], and neural stem cells [90]. During early neurogenesis in developing mouse brain, exosomes are released into the ventricular fluid in the neural tube of small (50–80 nm) and large (600 nm) vesicles [90]. The transfer of mRNAs that encode pluripotent transcription factors may be mediated by exosomes which have the capacity to reprogram the recipient cells [6]. Spatial and temporal gradients, which are critical in neuronal development, are thought to be mediated by exosomes [91]. Coufal et al. have demonstrated

that exosomes may participate in the genomic plasticity (genomic changes) of embryonic cells by mediating retroposon sequences and allowing cell-to-cell transfer of genomic plasticity and changes in gene expression [92].

The role of exosomes in different developmental processes has been shown in *Drosophila*. Exosome-like bodies in *Drosophila* are termed as argosomes, and during wing development, they transport morphogenic *Wnt* signaling proteins along the spatial and temporal gradients [93]. Argosomes may also carry Hedgehog, Notch, decapentaplegic, and wingless signaling proteins for the developmental gradients in other tissues [94, 95]. Hence, exosomes are involved in the systemic and local interneuronal transfer of information which is superior to direct cell-to-cell contact [96].

### Role of Exosomes in Synaptic Activity

Faure et al. have shown that undifferentiated cortical neurons in culture release exosomes and are stimulated by depolarization [76]. The exosomes contain GluR2/3 subunits of subunits of AMPA receptors and a neuronal cell adhesion protein L1, which shows their role in the synaptic function. In agreement to this study, another study by Lachenal et al. showed the same phenomena in fully differentiated cortical neurons in culture. In this study, the exosome was stimulated by GABA<sub>A</sub> receptors which result in increased spontaneous neuronal activity [33]. In another experiment, exosomes were found to incorporate heavy chains of tetanus toxin in neurons; it was demonstrated that exosome contained GluR2 subunits, and there was an increased release with depolarization which could modulate the synaptic activity [97].

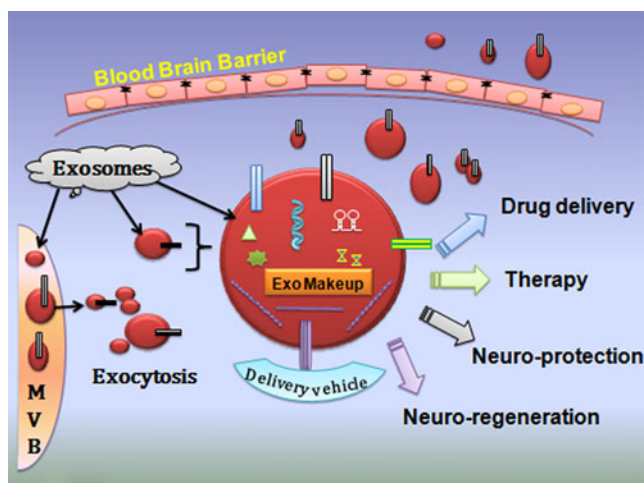
### Role of Exosomes in the Regeneration of Peripheral Nerves











Exosomes play a protective role in injury and regeneration. Brain injury accumulates toxic proteins, and their degradation is mediated by the expression of Ndfip1 which interacts with Nedd4 ubiquitin ligases [98]. These two proteins, Ndfip1 and Nedd4, are postulated to serve important roles in removing toxic proteins after injury and are found in exosomes released by neurons [99]. Bianco et al. have demonstrated that during the damage of the nervous tissue, there is an increase in extracellular ATP which leads to the release of exosomes from microglia and astrocytes via sphingomyelinase-dependent process [36]. These exosomes contain inflammatory cytokine IL-1 $\beta$  which induces inflammatory response. In stress conditions, exosomes released by astrocytes contain synapsin 1, which is a neuronal specific protein and is associated with synaptic vesicles. Exosomes are also released by oligodendrocytes which contain myelin and stress protective proteins [89]. Schwann cells which surround a damaged or degenerating peripheral nerve translocate vesicles containing polyribosomes into the axon, and the contents are released here [100,

[101]. Hence, exosomes serve as a medium for delivering mRNA and ribosomes to injured nerves and promote local protein synthesis which is needed for regeneration. In this context, Court et al. have shown that labeled ribosomes in the nerve are derived from the Schwann cells [102].

### Exosomes in Therapeutics—As Delivery Vehicles

The use of exosomes has several advantages over liposomes as delivery vehicles which were used as a nanodelivery system over the past decades. Ideal liposomes should evade immune detection system and should have a longer half-life in the circulation system for therapeutic cargo delivery, and all these properties are well presented by exosomes [103]. Due to low immunogenicity, remarkable delivering properties, and the ability to cross the BBB, exosomes have been used efficiently for therapeutic delivery systems [104–107]. The MVBs that produce exosomes can be genetically engineered to package mRNA, siRNA, proteins, and drugs into exosomes or into exosome lipid bilayers. Hence, exosomes are now efficiently being used in the RNAi therapy, immunotherapy, and drug delivery (Fig. 3).



**Fig. 3** Exosome makeup and their use as delivery vehicles. The figure showing the exosome makeup with biological molecules, genetic element (such as miRNA and siRNA) or potential drugs for vascular-neuro-associated pathologies. The exosome could also be processed/trained in artificial multivesicular bodies (MVBs). Because of their nanometer size, they could cross the blood–brain barrier; therefore, desired exosome could be targeted to the brain in order to cure brain-associated pathologies.  Exosome transmembrane proteins,  desired drug embedded in exosome bilayer membrane,  artificially processed transmembrane proteins,  artificially processed exosome,  artificially unprocessed exosome,  genetic element,    natural or artificial biomolecules,  lipid molecule

### Exosomes in RNAi therapy

RNAi therapies have been explored for targeting human diseases like cancer, genetic disorders, and HIV, and may involve the use of ribozymes, aptamers, and siRNA [108]. siRNAs are short (~21–23 nt) single-stranded RNA molecules that bind to mRNAs with perfect or imperfect Watson–Crick base pairing and lead to post-transcriptional gene silencing [109, 110]. The siRNA is loaded onto the RISC complex for mRNA targeting, and after binding, it leads to the degradation of mRNA by endonuclease Argonaute 2. The siRNA is protected from the degradation by the RISC complex and can be used repeatedly to degrade other mRNAs [111, 112]. Hence, siRNA serves as an ideal candidate for RNAi therapy [108]. siRNAs can be immunogenic and are susceptible to degradation by endonucleases present in the serum, cells, and extracellular space [113]. They require efficient delivery vehicles like exosomes which can cross the BBB, preserve mRNAs and miRNAs, and deliver functional RNAs to the target cells [25, 27]. Alvarez-Erviti et al. demonstrated the delivery of siRNA into the mouse brain by systemic injection of targeted exosomes [3]. In this study, self-derived dendritic cells were genetically engineered to express Lamp2b, an exosomal membrane protein, fused with rabies glycoprotein for targeting brain cells. Purified exosomes expressing RGV+Lamp2b on the membrane were loaded with siRNA by electroporation and intravenously injected into mouse [3]. The exosomes specifically delivered siRNA (for GAPDH) to the neurons, microglia, and oligodendrocytes in the brain, resulting in specific gene knockdown [3]. Hence, exosomes can be used efficiently as delivery vehicles for targeting RNA molecules to specific cells, though more studies are required to address their use in clinical trials.

### Exosomes in Drug Delivery

The first experiment to demonstrate the potential of exosomes as drug delivery system was done by Sun et al. [106]. The study reported successful loading of curcumin into exosomes which provided higher bioavailability and solubility than curcumin alone. The exosomal curcumin significantly decreased LPS-induced inflammatory activity. The curcumin-loaded exosomes could be purified at 45–60 % sucrose density gradient instead of 30–45 % for empty exosomes. Similar to this study, Zhuang et al. reported successful delivery of exosomal curcumin and JS1124 (a signal transducer and activator of stat3 inhibitor) to the rodent brain via intranasal injection, crossing the BBB [105]. Exosome-mediated delivery of curcumin significantly decreased the LPS-mediated inflammation and myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis [105]. Adding to this, the glioma growth in the brain was suppressed by the intranasal administration of GL26. These studies

encourage the use of exosomes as delivery vehicles, though safety parameters like immunogenicity should be taken into account.

### Exosomes as Biomarkers in Neuronal Injury

Exosome are the tiny surrogates of their cells of origin and possess unique signature, or biomarkers, which describe the status of the cell. Skog et al. have shown that exosomes derived from the human glioblastoma cells contain proteins and micro-RNAs specific to the tumor cells and can be detected in the patients' serum [32]. The study suggests the potential role of exosomes to be used as non-invasive diagnostic and therapeutic tools in patients with brain tumors. The glioblastoma cells increase the number and type of exosomes released during transformation to malignant brain tumor cells and progression. This has been shown in vitro by Balaj et al. who compared the glioblastoma cells and normal cells in conditioned media [31]. The tumor cells use exosomes to modify the normal cells in their vicinity to promote tumor growth [31]. These modifications include (1) suppression of immune response to tumor, (2) facilitating tumor growth and invasion, and (3) stimulation of angiogenesis [114, 115]. The micro-RNAs specific to these tumor progressions can be detected in the serum and can be used as biomarkers [32].

### Conclusion

Exosomes represent a novel area to explore, stimulating and groundbreaking the field of neuroscience. Their involvement in neuronal diseases and their use in neurotherapeutics could be further checked and used as drug delivery vehicles.

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**Conflict of Interest** The authors confirm that there are no conflicts of interest.

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