Targeting capabilities of engineered extracellular vesicles for the treatment of neurological diseases

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From the Contents

Abstract

Recent advances in research on extracellular vesicles have significantly enhanced their potential as therapeutic agents for neurological diseases. Owing to their therapeutic properties and ability to cross the blood–brain barrier, extracellular vesicles are recognized as promising drug delivery vehicles for various neurological conditions, including ischemic stroke, traumatic brain injury, neurodegenerative diseases, glioma, and psychosis. However, the clinical application of natural extracellular vesicles is hindered by their limited targeting ability and short clearance from the body. To address these limitations, multiple engineering strategies have been developed to enhance the targeting capabilities of extracellular vesicles, thereby enabling the delivery of therapeutic contents to specific tissues or cells. Therefore, this review aims to highlight the latest advancements in natural and targeting-engineered extracellular vesicles, exploring their applications in treating traumatic brain injury, ischemic stroke, Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, glioma, and psychosis. Additionally, we summarized recent clinical trials involving extracellular vesicles and discussed the challenges and future prospects of using targeting-engineered extracellular vesicles for drug delivery in treating neurological diseases. This review offers new insights for developing highly targeted therapies in this field.

Key Words: Alzheimer's disease; amyotrophic lateral sclerosis; engineered extracellular vesicles; glioma; ischemic stroke; neurological diseases; Parkinson's disease; psychosis; targeted drug delivery; traumatic brain injury

Introduction

Neurological diseases are increasingly significant due to their high prevalence and substantial economic burden on healthcare systems. These diseases affect the brain, spinal cord, and nerves and could result in cognitive impairment, movement disorders, sensory disturbances, and disruptions in autonomic functions. According to the Global Burden of Diseases, Injuries, and Risk Factors Study, neurological diseases are among the leading causes of disability globally, contributing significantly to the overall disease burden (GBD 2016 Disease and Injury Incidence and Prevalence Collaborators, 2017). Neurological diseases are broadly categorized into cerebrovascular, neurodegenerative, and immunological diseases such as Guillain-Barre syndrome, tumors, central nervous system (CNS) infections, peripheral neuropathies, and movement disorders. Among these, traumatic brain injury (TBI), ischemic stroke (IS), Parkinson's disease (PD), Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), glioma, and psychosis are particularly prominent. These conditions are characterized by their high incidence, severe symptoms, poor prognosis, and lack of effective treatments.

Extracellular vesicles (EVs) are bioactive molecule-encapsulated nanoparticles secreted by various cell types and found in different body fluids. These lipid-bilayer vesicles facilitate intercellular communication by protecting molecules from degradation and delivering bioactive compounds to adjacent or distal areas (van Niel et al., 2022). Together with their low immunogenicity, high modifiability, and high permeability while crossing the blood–brain barrier (BBB) (Meng et al., 2020; Izquierdo-Altarejos et al., 2024), EVs are ideal for drug delivery, particularly for efficient intracerebral administration. Studies show that natural and drug-loaded EVs are beneficial in treating neurological diseases. Studies also indicate that endothelial cell-derived EVs provide neuroprotective and anti-inflammatory effects during IS (Yue et al., 2019; Gao et al., 2023). However, applications of EVs in neurological diseases are still limited by insufficient targeting abilities, raising safety concerns. To address this, multiple strategies—physical, chemical, and genetic—have been established to enhance EVs with high neural cell–targeting ability. These engineering strategies greatly boost the use of EVs in neurological treatments. Therefore, this review aims to

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comprehensively introduce current treatment strategies, recent research progress, and engineering techniques for developing neural cell-targeting EVs in neurological diseases while also summarizing related clinical trials. This review provides insights into the current status and future prospects of EV treatments in neurological diseases.

Search Strategy

In this narrative review, we sourced relevant literature published between July 1983 and May 2024 from the PubMed database using a combination of keywords including "extracellular vesicles," "exosome," "microvesicle," "ectosome," "engineered," "targeted drug delivery," "neurological diseases," "brain Injuries," "traumatic brain injury," "ischemic stroke," "Parkinson's disease," "Alzheimer's disease," "glioma," "amyotrophic lateral sclerosis," and "psychosis." The research and review articles were thoroughly examined.

Current Treatment Strategies of Neurological Diseases

Traumatic brain injury

TBI, commonly resulting from conditions such as traffic accidents, falling from heights, or physical assaults, presents a critical medical emergency from prehospital care to the emergency department. Severe cases often require transfer to a neurocritical care unit. TBI management generally currently involves two key approaches: prehospital care and monitoring with goal-directed therapy in the neurocritical care unit. Prehospital care, including airway protection, oxygen administration, and perfusion management, is crucial, as insufficient oxygenation and perfusion can lead to irreversible secondary brain injuries (Khellaf et al., 2019; Basit et al., 2023). For patients with TBI, regular monitoring of brain-specific parameters is essential for guiding subsequent goal-directed supportive treatment. In general, the key monitored parameters include intracranial pressure, brain tissue oxygenation, and cerebral microdialysis (Hawryluk et al., 2019; Lazaridis and Foreman, 2023). These monitoring measures enable clinicians to monitor the condition of patients and optimize clinical management in a timely manner. Additionally, treatments primarily focus on managing complications such as seizures. However, effective medications to directly mitigate brain damage and restore neurological function during TBI remain few. Direct and effective methods for tackling perfusion and metabolite issues in TBI remain limited. Given that TBI could affect the cortical area and potentially lead to seizures, preparing antiepileptic drugs is essential. Hypothermia treatment, which aims to reduce intracranial pressure and inhibit secondary brain injury (van Veelen and Brodmann Maeder, 2021), shows mixed results. Some clinical trials indicate possible adverse outcomes (Andrews et al., 2018; Rösli et al., 2020). In addition, in some cases, decompressive craniectomy is also considered (Al-Jehani et al., 2021). An article published in the *Lancet* reports that the antifibrinolytic agent tranexamic acid effectively reduces TBI-related mortality with a death rate of 12.5% in the tranexamic acid group compared to 14% in the placebo group (485 *vs*. 525 events; risk ratio 0.89 [95% confidence interval 0.80–1.00]) (CRASH-3 trial collaborators, 2019). However, two other clinical studies show that it did not significantly improve long-term clinical outcomes (Cone et al., 2020; Nelson Yap et al., 2020). In conclusion, TBI is a critical condition that poses challenges for long-term treatment. EVs, which can be administered repeatedly over extended periods, may potentially target lesion areas over time.

Ischemic stroke

Stroke is a severe cerebrovascular accident characterized by high incidence, recurrence, disability, and mortality rates, resulting in approximately 5.5 million deaths annually and accounting for approximately 34% of global healthcare costs (Yu and Chen, 2019; Saini et al., 2021; DeLong et al., 2022). Stroke consists of IS and hemorrhagic stroke, with IS being the more prevalent type. Early-stage IS treatment is effectively managed using revascularization methods, including thrombolysis via intravenous injection (fibrinolytic drugs, anticoagulants, and antiplatelet drugs) and endovascular mechanical thrombectomy (Herpich and Rincon, 2020; Rabinstein, 2020; Wang et al., 2024a). However, the therapeutic time windows for thrombolysis and thrombectomy are too short to meet the clinical needs. Although studies show some progress in extending these time windows, a large number of patients still miss the optimal period for treatment. In the middle and late stages of treatments, clinicians primarily focus on preventing complications through blood pressure control, brain edema amelioration, vertigo alleviation, and nutritional support. However, medications reducing brain damage in the early stage and enhancing neurological recovery are still lacking. Recent studies show multiple targets with the potential to inhibit neural cell death, reduce inflammation, and promote nerve regeneration. Ge et al. (2022) reported that exogenous recombinant C–X3–C motif chemokine ligand 1 could significantly reduce gasdermin D and NLRP3 inflammasome-mediated pyroptosis, decrease infarct volume and improve neurological deficits with a higher laterality index. Another study reports that recombinant human fibroblast growth factor 21 could reduce infarct volume and ameliorate neurological deficits and sensory function by inhibiting inflammatory cytokines, maintaining high levels of anti-inflammatory cytokines, and suppressing M1 microglia polarization. Additionally, it could inactivate the proinflammatory nuclear factor kappa B and activate the peroxisome proliferator-activated receptor gamma (Wang et al., 2020). Xue et al. (2023) reported that miR-181b promotes angiogenesis and neurological recovery in the penumbra area by inhibiting phosphatase and tensin homolog (PTEN) generation and activating the Akt pathway. Ji et al. (2023) introduced a new molecule called ISO-1, which inhibits the activity of proinflammatory macrophage migration inhibitory factor and alleviates apoptosis in the penumbra area. Despite the promising findings regarding these therapeutic molecules and their targets, practical application remains challenging. One major issue is identifying the most effective route to deliver these molecules to the brain with minimal toxicity. Additionally, further exploration of more targets is needed.

Parkinson's disease

PD is a neurodegenerative disease characterized by the accumulation of Lewy bodies and loss of neurons in the substantia nigra. It is identified by bradykinesia (slowness of movement), rigidity (increased muscle tone), rest tremor, and alterations in gait and postural reflexes (Kalia and Lang, 2015; Ye et al., 2023; Dong et al., 2024). PD also presents with nonmotor features, including olfactory function decline, cognitive impairment, psychiatric dysfunction, sleep disorder, autonomic dysfunction, fatigue, pain, and rapid eye movement sleep behavior disorder. As the disease progresses, choking and dementia often emerge after 12–14 years after the onset of motor symptoms (Kalia and Lang, 2015). Gastrointestinal syndromes, such as dental problems, drooling, and swallowing difficulties, are also common (Fasano et al., 2015; Bloem et al., 2021). With the increasing number of disabilities and deaths among patients with PD, medications to alleviate symptoms and control the progression are urgently needed (The Lancet Neurology, 2022). Current treatment approaches, such as deep brain stimulation and the administration of levodopa or

carbidopa (Gao et al., 2020), are adopted to ameliorate motor symptoms. Additionally, dopamine agonists (Schuepbach et al., 2013), monoamine oxidase B inhibitors (PD Med Collaborative Group et al., 2014), methylphenidate (Seppi et al., 2011), and istradefylline (Ren and Chen, 2020) supplemented with neurotrophic agents, are also recommended. In the exploration of medications for PD, several agents show potential in alleviating inflammation associated with PD progression. These include α-synuclein, leucine-rich repeat kinase 2, β-glucocerebrosidase, CNS-penetrant dihydropyridine channel blocker (Isradipine), immunosuppressant drug (Azathioprine) (Vijiaratnam et al., 2021), iron chelation with deferiprone (Elkouzi et al., 2019), nonsteroidal anti-inflammatory drugs (Rees et al., 2011), human recombinant granulocyte-macrophage colony-stimulating factor (Sargramostim) (Abdelmoaty et al., 2022), the selective and irreversible myeloperoxidase inhibitor (AZD3241) (Jucaite et al., 2015), the microglial NLR family pyrin domain containing 3 inhibitor called Parkin (Yan et al., 2023), statins (Lewis et al., 2022), coenzyme Q10 (Yan et al., 2023), ursodeoxycholic acid (Bell et al., 2018), that supports mitochondrial function, and glycerophosphodiester phosphodiesterase-1, that reduces inflammation and α-synuclein accumulation (Yun et al., 2018). Although these medications represent significant advances in PD treatment, challenges remain due to side effects, medication tolerance, symptom progression, and irreversibility of the disease, highlighting the need for continued exploration of safer and more innovative therapies.

Alzheimer's disease

AD, the leading cause of dementia worldwide, is becoming one of the most expensive, lethal, and burdensome diseases of the 21st century. According to a cohort study by the European Memory Clinic, the median survival time after a dementia diagnosis is only 6 years (AD dementia: 6.2 (6.0–6.5) years) (Scheltens et al., 2021). Additionally, with the growing age population, the number of patients with AD continues to rise. Generally, AD progression involves two principal pathological features: senile plaques and neurofibrillary tangles (Soares Martins et al., 2021; Yin et al., 2023). Senile plaques are extracellular deposits of amyloid-beta (Aβ) peptides, which form through two pathways related to amyloid precursor protein: the non-amyloidogenic and amyloidogenic pathways. Moreover, neurofibrillary tangles consist of hyperphosphorylated and misfolded Tau proteins. Current treatments include galantamine (Koola, 2020), rivastigmine (Birks and Grimley Evans, 2015), donepezil (Birks and Harvey, 2018), and memantine (Birks and Harvey, 2018) are routinely used. Molecular therapy for AD began with the Aβ immunotherapeutic drug AN1792, which entered clinical trials in 1999 but was discontinued due to the occurrence of aseptic meningoencephalitis (Jucker and Walker, 2023). Recently, novel drugs have emerged, such as sodium oligomannate targeting the brain–gut axis (Syed, 2020) and aduhelm targeting Aβ aggregates (Han et al., 2021). Monoclonal antibodies such as aducanumab (Aduhelm), lecanemab (Leqembi), and donanemab were developed to help remove abnormal Aβ from the brain and slow early AD progression (Söderberg et al., 2023). Other therapies, including stem cell treatment, remain limited due to the imprecise targeting of its effects (Wang et al., 2022d). Although these medications significantly improve AD therapy, they are insufficient for complete remission. Developing new targets and drugs capable of halting or reversing the process of AD is urgently needed.

Amyotrophic lateral sclerosis

ALS is a neurodegenerative disease characterized by the degeneration of upper or lower motor neurons, leading to progressive paralysis, respiratory failure, and death within 2–5 years. Early symptoms typically present as bulbar-onset, marked by weakness in the neck and facial muscles, or as spinal-onset, manifested by limb and other body part weakness (Goutman et al., 2022; Wang et al., 2024b). Cognitive and behavioral impairments also affect a significant proportion of proportions and may indicate a worse prognosis (Bersano et al., 2020). The lifetime risk of developing ALS is 1 in 400 to 800 (Feldman et al., 2022). Given that ALS is currently incurable, treatment focuses on prolonging survival time and improving quality of life. A retrospective cohort study recommends multidisciplinary therapy as an effective approach (Klavžar et al., 2020). Briefly, the anti-glutamate agent riluzole and antioxidant edaravone, which show efficacy in prolonging survival and slowing disease progression, are in clinical use in several countries (Jaiswal, 2019). In the United States, a combination of dextromethorphan and quinidine is approved to manage the pseudobulbar effect (Fralick et al., 2019). Regular respiratory monitoring and noninvasive ventilation are also crucial for improving survival and quality of life (Cooksey and Sergew, 2020). Additionally, percutaneous endoscopic gastrostomy (PEG) is recommended to alleviate dysphagia-related malnutrition and demonstrates a significant survival advantage (PEG: 23 (15–35) months; No PEG: 11 (4.75–8.5) months) (López-Gómez et al., 2021). Recent studies show the popularity of genetic therapy for ALS, with the most commonly targeted genes being superoxide dismutase 1 (SOD1), TAR DNA-binding protein (TARDBP/TDP43), FUS RNA-binding protein (FUS), and C9orf72-SMCR8 complex subunit (C9orf72). Antisense oligonucleotide drugs, such as ION363 (jacifusen) (Korobeynikov et al., 2022) and BIIB067 (tofersen) (Mullard, 2021) have been developed to inhibit the expression of these target genes and show efficacy in clinical trials (Akçimen et al., 2023). Monoclonal antibodies targeting mutant C9orf72 and TDP43 are in preclinical development. In immunotherapy, a phase 2a clinical trial (ClinicalTrials.gov, NCT02059759) demonstrates that low-dose interleukin-2 could effectively reduce inflammatory cytokines and promote the M2 shift of monocytes, although it may also activate macroglia and microglia through receptors on these cells (Camu et al., 2020). Moreover, stem cells derived from various sources could offer neurotrophic support to neural cells, though they have not shown long-term efficacy. More efficient targets and intervention strategies are needed to better manage ALS.

Glioma

Glioma is the most common and malignant primary brain tumor, originating from glial cells, and accounts for approximately 40%–50% of all brain tumors. It is generally categorized into astrocytoma, glioblastoma (GBM), and oligodendroglioma based on the cell types involved. Of these, GBM is the most malignant type, with a median survival time of < 2 years (Yang et al., 2022); it could be further classified into isocitrate dehydrogenase (IDH) wild-type and IDH mutation groups that exhibit distinct molecular profiles and responses to medications. Common clinical symptoms of glioma, particularly in its early stages, include headache (30%), which is more prevalent in high-grade tumors (38%) than in low-grade tumors (22%), indicating increased intracranial pressure; seizure (35%), which is generally more common in low-grade glioma patients (58%) than in high-grade glioma (24%); cognitive decline (36%); and neurological deficits such as aphasia (20%) and motor deficits (20%) (van den Bent et al., 2023). Prompt recognition of these symptoms is crucial for developing appropriate treatment plans based on the different conditions of the disease. For glioma treatment, especially given the rapid growth and malignancy, surgical resection combined with chemotherapy and radiotherapy is the primary approach.

In GBM, surgical removal followed by temozolomide and short-term radiation improves overall survival (9.3 months *vs*. 7.6 months; HR, 0.67 [95% confidence interval, 0.56–0.80]; $P < 0.001$) (Perry et al., 2017). For low-grade glioma treatment, patients treated using maximal safe surgical resection combined with procarbazine, lomustine, and vincristine, in addition to radiotherapy, demonstrate better overall survival (Darlix et al., 2019). In recent years, immunotherapy, which leverages the immune system to attack tumors, has been advancing rapidly (Xu et al., 2020). Multiple strategies show promise in treating glioma (Morrison et al., 2018; Borgers et al., 2021; Li et al., 2021b). Immune checkpoint blockade therapies, including inhibitors of cytotoxic T-lymphocyte-associated protein 4 and prephenate dehydratase 1, show limited effectiveness due to the heterogeneity of glioma but still benefit a sub-group of patients (Aslan et al., 2020). Additional checkpoints, such as CD47 and CD73, require further investigation. Chimeric antigen receptor T-cell therapy for glioma faces challenges owing to antigenic heterogenicity (Thomas et al., 2023). However, modified approaches, such as bispecific T-cell engagers that produce chimeric antigen receptor T cells, offer potential solutions to overcome antigen escape and show promise in anti-glioma therapy (Choi et al., 2019). Other promising and novel therapies, including oncolytic viruses and glioma-targeting vaccines, are under development and could provide future alternatives for patients. In addition to the therapies above, studies show genes influencing different processes (including proliferation, migration, invasion, stemness, and cell death) in glioma a. Non-coding RNAs, such as microRNAs (miRNAs) (Wang et al., 2021), long non-coding RNAs (Liang et al., 2022a), and circular RNAs (Lou et al., 2020), interact with genes involved in glioma progression. Despite these advancements, glioma remains incurable. Patients frequently experience recurrence, drug and radiation resistance, short survival times, and complications. Continued development is needed for new target genes to intervene in tumor progression, strategies to improve drug and radiation susceptibility, and advanced delivery systems for anti-tumor agents.

Psychosis

Psychosis is a syndrome characterized by disconnection from reality, resulting in disturbances in thoughts, emotions, and perceptions. Sundquist (2019) introduced the non-affective psychoses comprising schizophrenia, schizoaffective disorders, and schizophreniform disorders. Schizophrenia, the most prevalent psychosis, affects approximately 1% of individuals and typically emerges in early adulthood, generally after age 16 (Westhoff et al., 2021). This disorder is linked to a growing number of environmental and genetic risks and often imposes significant societal burdens (McCutcheon et al., 2020). Additionally, it is associated with a reduction in life expectancy by approximately 15 years and a 5%–10% lifetime risk of suicide. Furthermore, patients with schizophrenia experience a lifelong decline in intelligence quotient, averaging 7–8 points (Jauhar et al., 2022). Fusar-Poli et al. (2022) presented a "third psychosis" besides schizophrenia and bipolar disorder, termed brief psychotic episodes, which are characterized by their transient nature with a mean duration of 10.2 days. Additionally, postpartum psychosis—marked by the sudden onset of affective and psychotic symptoms within 2 weeks of childbirth—is a type of psychosis that could be easily overlooked (The Lancet Psychiatry, 2021) but is increasingly recognized by psychiatrists and social psychologists. For the treatment of schizophrenia, current options primarily include medications that inhibit D2/3 receptors of dopamine (Howes et al., 2017), and 5-hydroxytryptamine receptor 2A antagonists could be prescribed if the D2 receptor antagonist worsen symptoms (Peng et al., 2024). Besides medications, various treatments such as electroconvulsive therapy, transcranial magnetic stimulation, transcranial direct-current stimulation, and deep-brain stimulation, can be utilized, often alongside social skills training administered after the acute phase (Lieberman and First, 2018). Owing to the lack of pathogenic data, curing psychosis remains challenging, though these treatments can help mitigate symptoms. Although these conventional treatments lay the foundation for current strategies, they are often accompanied by challenges that perplex clinicians, and their efficacy and success rates remain difficult to predict. Further development of molecular mechanisms, therapeutic targets, and agents is necessary.

In conclusion, while strategies have been developed to target etiology, ameliorate symptoms, and slow the progression of neurological diseases, current treatments are still limited by delayed onset, incomplete intervention at complex pathological targets, drug resistance, inefficient brain-targeted delivery, and unclear pathogenesis. Advancing the development of new therapeutic targets and agents, as well as creating a delivery system with efficient brain-targeting abilities, low toxicity, extended circulation time, and capacity to carry drugs or bioactive materials to multi-targets, could accelerate the research progress of drug development.

Classification of Extracellular Vesicles

Various cell types secrete EVs and exist in all bodily fluids, including blood, urine, bronchoalveolar lavage fluid, breast milk, amniotic fluid, synovial fluid, pleural effusions, and ascites (Simpson et al., 2008). The lipid bilayer of EVs encapsulates diverse contents such as miRNAs, mRNAs, proteins, metabolites, chromosome fragments, and organelles, which vary across different EVs and they are considered key mediators of intercellular communication. In addition, the membrane of EVs contains common proteins related to their assembly (e.g., lysosomal-associated membrane protein 2 (Lamp2b), CD9, CD63, CD81) and specific proteins that reflect their cell type (e.g., transferrin receptor, prephenate dehydratase 1). Furthermore, EVs are characterized by low immunogenicity (Ma et al., 2023b) and a relatively long half-life in blood circulation (Murphy et al., 2019). These features enable EVs to transport bioactive molecules to distant parts of the body, playing crucial roles in physiological and pathological processes, making them ideal vehicles for drug delivery. While EVs share structural and biological characteristics, they can mainly be categorized into exosomes, ectosomes (microvesicles), and apoptotic bodies based on their size and biological origin. Recently, novel EVs such as mitochondrial EVs (mitoEVs) were identified, which originate from distinct sources compared to those of other EVs (Picca et al., 2023). The following sections provide an overview of representative EVs and discuss methods for their isolation and administration.

Exosome

EVs were first identified as exosomes in 1983 when two studies described that reticulocytes could secrete exosomes enriched with transferrin receptors (Harding et al., 1983; Pan and Johnstone, 1983). In detail, exosomes are nano-sized single membrane-enclosed vesicles (30–150 nm in diameter) (Yang et al., 2020), originating from endosomal membrane invagination, multivesicular bodies (MVBs) formation, fusion of MVBs with the plasma membrane, and release of intraluminal vesicles containing proteins, miRNAs, mRNAs, DNAs, lipids, metabolites, and cytosolic molecules (Pegtel and Gould, 2019). The formation process begins with the invagination of the plasma membrane,

creating a cup-shaped structure that incorporates materials from the membrane and extracellular space. These structures then develop into early-sorting endosomes, with the assistance of the Golgi network and endoplasmic reticulum, through direct contact or by fusing with pre-existing early-sorting endosomes. These early-sorting endosomes developed into late-sorting endosomes, which then form MVBs containing numerous intraluminal vesicles. The MVBs can be degraded by lysosomes or fused with the plasma membrane to release the intraluminal vesicles as exosomes (Kalluri and LeBleu, 2020). The heterogeneity of EVs is due to the wide variety of specific molecules they carry, including distinct surface proteins that serve as markers, which depend on the cell type from which the EVs originate (Kanninen et al., 2016). Among the various types of EVs, exosomes are the most extensively studied, particularly in terms of their assembly mechanisms and potential applications.

Ectosome

Ectosomes are vesicles of varying sizes (100–1000 nm in diameter) that bud directly from the plasma membrane into the extracellular space (Zhu et al., 2021). Their formation begins with the assembly of membrane proteins and receptors in a particular region of the plasma membrane, creating a specialized domain. Cargoes such as proteins and nucleic acids accumulate within these domains, facilitated by anchoring proteins. The budding process, including the pinching and detachment of ectosomes, could be influenced by phospholipid redistribution in the plasma membrane, Rho-kinase-mediated phosphorylation of myosin light chains, and contractile machinery (Rädler et al., 2023).

Apoptotic body

Apoptotic bodies are plasma membrane blebs formed during apoptosis, with sizes ranging from 500–2000 nm in diameter (Tang et al., 2022). Compared to exosomes and ectosomes, which serve as vehicles for physiological intercellular communication, apoptotic bodies appear when apoptotic cells break down into subcellular fragments (Santavanond et al., 2021). The cargo of apoptotic bodies includes not only proteins, nucleic acids, and metabolites but also organelles (Kang et al., 2021). These components were once considered waste products destined for degradation by neighbor cells. However, a previous study reported that apoptotic bodies can also act as intercellular messengers, playing a role in regulating pathophysiological processes (Gregory and Rimmer, 2023).

Mitochondrial extracellular vesicles

In recent studies, a subpopulation of nonexosomal EVs originating from mitochondria, known as mitoEVs, was identified. The formation of mitoEVs may be mediated by PTEN-induced kinase 1/Parkin-dependent pathway, supported by the translocase of the outer mitochondrial membrane 20 (Howard et al., 2022) and microtubule-associated protein 1 light chain 3 (Yamano et al., 2018). Alternatively, it may occur through the dynamin-related protein 1-dependent pathway, supported by mitochondrial fission factor, mitochondrial elongation factor 2, and mitochondrial elongation factor 1 (König et al., 2021). The fusion of mitoEVs with MVBs and their release as EVs may be regulated by CD38/cyclic ADP ribose signaling (Suh et al., 2023), sorting nexin 9 signaling (Zecchini et al., 2023), OPA1 mitochondrial dynamin-like GTPase (Todkar et al., 2021), and inhibited by Parkin (Matheoud et al., 2016). MitoEVs contain mitochondrial fragments, full-length mitochondrial DNA, mitochondrial proteins, or intact mitochondria (Al Amir Dache et al., 2020). These mitochondrial components transferred by mitoEVs hold the potential for disease diagnosis and treatment.

Basic Techniques in Extracellular Vesicle Study

Isolation and characterization of extracellular vesicles

For isolating EVs, various methods were employed depending on the specific requirements, including differential ultracentrifugation (Deng et al., 2022), iodixanol/sucrose density gradient centrifugation (Yang et al., 2020), size exclusion chromatography (You et al., 2022), ultrafiltration (Dong et al., 2020), affinity selection (Theel and Schwaminger, 2022), and chemical precipitation (Shtam et al., 2020). Briefly, differential ultracentrifugation was used for isolating microvesicles and exosomes, involving two rounds of centrifugation at 120,000 × *g* for 70 minutes at 4°C. Iodixanol/sucrose density gradient centrifugation was employed to achieve high-purity small EVs and exclude non-vesicular components (Onódi et al., 2018). Size exclusion chromatography columns such as IZON qEV (IZON, Medford, MA, USA) were used to separate particles larger than 70 nm without selectively targeting vesicles or non-EV materials (Suthar et al., 2020). Ultrafiltration used membranes with a 500 kDa molecular weight cut-off to retain proteins and isolate small EVs (0.1 μm). However, this method could damage EVs due to clogging and shear stress (Jalaludin et al., 2023). Affinity selections involved using antibodies that target surface proteins on EVs (e.g., CD9, CD63, CD81) or cell-specific proteins (e.g., neural cell adhesion molecule 1 for neuronal EVs, solute carrier family 1 member 3 for astroglial EVs, CD11b for microglial EVs). These antibodies were immobilized on microplates or microbeads to selectively bind EVs with high specificity and purity (Chawla et al., 2019; Kumar et al., 2021; Liu et al., 2022). For chemical precipitation, reagents such as Exoquick (SBI, Palo Alto, CA, USA) (Tangwattanachuleeporn et al., 2022) and PEG6000/NaCl mixtures (Yang et al., 2016) were employed to separate EVs at lower centrifugation speeds. Although this method is economical and straightforward, it is limited by high levels of impurities. To isolate various types of EVs, different conditions (centrifugation speed, antibodies for affinity separation, and pore size of filtration membranes) were utilized. Statistical analysis reveals the frequency of different isolation methods: differential ultracentrifugation (31%), size exclusion chromatography (29%), polyethylene glycol-based chemical precipitation (4%), and other methods, including tangential flow filtration, density gradient, ultrafiltration, and microfluidics (10%). Recent studies indicate a trend toward combining methods (26%) to achieve higher purity EVs at a relatively low cost. Combining chemical precipitation with differential ultracentrifugation/ultrafiltration/size exclusion chromatography enhances isolation accuracy (Williams et al., 2023).

For EV characterization, a newly developed particle size analyzer (NanoFCM, Xiamen, Fujian, China) was used to assess the size distribution and surface protein expression of EVs (Wang et al., 2023b). Traditional methods, including transmission electron microscopy, nanoparticle tracking analysis, and Western blotting, remain gold standards for identifying morphologies, sizes, and biological features of EVs. In detail, transmission electron microscopy reveals the cap-shaped morphology of EVs (Deng et al., 2022; You et al., 2022; Wang et al., 2023a, b; Xie et al., 2023). Nanoparticle tracking analysis provides information on the size distribution and concentration of EVs (Cho et al., 2021). Western blotting was utilized to detect the specific, cell-specific, and negative proteins of Eva, revealing their biological features, cell origins, and purity (Fan et al., 2022; Yao et al., 2022). Together, these separation and characterization methods form the essential foundation for the application of EVs in disease treatment and diagnosis.

Routes of administration

Routes for administering EVs can be broadly classified into local and systemic administration. Various methods include skin patches (Xu et al., 2024), subcutaneous injections (Shin et al., 2020), eye drops (Ma et al., 2023a), intravenous injections (Pu et al., 2023), nebulization (Shi et al., 2021), intranasal (spray) administration (Zhou et al., 2023), intracochlear application (Warnecke et al., 2021), and intravitreal injections (Reddy et al., 2023), can be used with or without the assistance of ultrasound and hydrogel. In the intracranial administration of EVs, intranasal and intravenous injections are the most commonly used approaches (Akbari et al., 2020). Although intravenous injections could distribute EVs to the brain with delayed clearance, they result in a substantial amount of EVs internalized by unintended organs, which can limit efficacy and raise safety concerns. To enhance the efficiency of intracranial administration, various routes were tested. Intranasal administration is a noninvasive approach that allows EVs to reach the brain through the trigeminal and olfactory pathways from the nasal cavity (Erdő et al., 2018). This method is promising due to its convenience, noninvasiveness, reduced plasmatic fluctuation of drug levels, moderate compliance, and cost-effectiveness (Xu et al., 2021). However, distribution imbalances could restrict its application. Moreover, in a previous study, stereotactic injection was employed for delivering EVs to the mouse brain (Gu et al., 2023). To expand usage scenarios of EVs, hydrogels were adopted to enable slow-release or conditional response properties (e.g., photoresponse, thermal response, pH response, etc.) during the administration of EVs (Ju et al., 2023). The choice of administration route significantly influences the availability and effectiveness of EVs, which, to a great extent, affect subsequent treatment outcomes.

Therapeutic Applications of Natural Extracellular Vesicles in Neurological Diseases

The bioactive components of EVs could induce biological effects upon internalization by recipient cells, making their therapeutic potential a prominent focus in neurological disease research. Studies report the numerous therapeutic mechanisms of natural EVs derived from various cell types or even plants in treating neurological diseases (Cheng and Hill, 2022; Lian et al., 2022; Raghav et al., 2022). In this regard, we present studies (**Table 1**) showcasing the potential of natural EVs (**Figure 1**).

Traumatic brain injury

Our previous study demonstrates that endothelial cell–derived exosomal miR-155-5p could exert protective effects by inhibiting astrocyte-induced inflammation through the regulation of the cellular Fos/Jun proto-oncogene pathway (Gao et al., 2023). Long et al. (2020) conducted *in vivo* TBI models and *in vitro* cultured microglia experiments, showing that astrocyte-derived exosomal miR-873a-5p could promote microglial M2 polarization and inhibit nuclear factor kappa B-induced inflammation. This could lead to a decrease in brain lesion size, cerebral edema, and neurological deficits. Similarly, Zhang et al. (2023) reported that exosomes derived from the human umbilical cord mesenchymal stem cell (MSC) could significantly improve motor function in a TBI mice model and ameliorate brain edema. Additionally, these exosomes decrease cortical lesion volume, the number of apoptotic cells, and pyroptosis induced by TBI. Another study shows that TBI-induced EVs enriched with miR-21-5p enhance the proliferation of human bone marrow MSC and boost bone volume, tissue volume, bone volume fraction, and bone mineral density in patients with TBI, highlighting the active role of TBI in bone fracture healing (Lin et al., 2023).

Moreover, He et al. (2023) reported that astrocyte-derived exosomal long non-coding RNA 4933431K23Rik acts as a sponge for miR-10a-5p, inhibiting its function and thereby enhancing the expression of transcription factor AP-2 gamma and SMAD family member 7. This mechanism could increase neurovascular unit formation in the peri-lesion cortex and ameliorate microglia-associated neuroinflammation. However, previous studies have shown that brain-derived EVs could induce rapid cerebral vasoconstriction, which failed to be ameliorated by epinephrine, and they could contribute to secondary TBI-induced coagulopathy and inflammation (Wang et al., 2022a; Li et al., 2024). Thus, EVs could function as a double-edged sword in TBI.

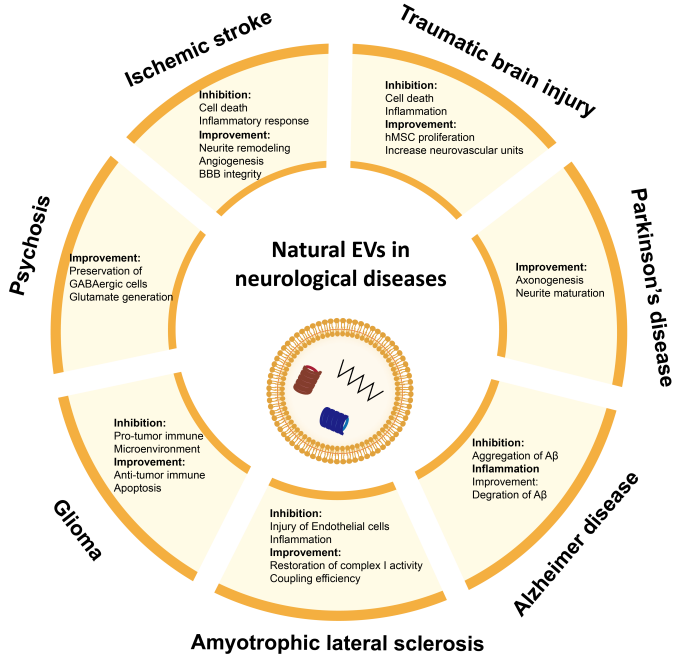

Figure 1 | Therapeutic functions of natural EVs in neurological diseases.
Natural EVs can transmit various bioactive contents to the lesions, potentially inhibiting neuronal cell death pathways, reducing inflammation and anti-peroxidation damage, improving revascularization, and accelerating the degradation of pathological substances in neurological diseases. These therapeutic effects make EVs promising agents for advancing the development of medications. Although natural EVs possess intrinsic homing capabilities, this capacity is not yet fully optimized, especially in therapeutic use. Engineering techniques to enhance the targeting capacity of EVs could facilitate their clinical translation. Future research should focus on elucidating more therapeutic mechanisms of EVs and developing engineered EVs with greater therapeutic payloads and improved targeting abilities. Created using Adobe Illustrator. Aβ: Amyloid-beta; BBB: blood–brain barrier; circRNA: circular RNA; EVs: extracellular vesicles; hMSC: human mesenchymal stem cell; lncRNA: long noncoding RNA; miRNA: microRNA.

Table 1 | **Natural EV-based treatment for representative neurological diseases**

Disease	Source	Content	Model	Effect	Methods of isolation	Reference
AD	MSCs	miR-29a	Mouse	Downregulate the level of Aβ and HDAC4	Ultracentrifugation	Chen et al., 2021
		Cytokine	Mouse	Neuroprotection and anti-inflammation	Ultracentrifugation	Losurdo et al., 2020
	hucMSCs	Proteins	Mouse	Reduce Aβ plaques, boost M2 transition of microglia, and inhibit inflammatory reaction	ExoQuick-TC	Ding et al., 2018
PD	hUCB-MNCs	miR-124-3p	Mouse	Enhance axonogenesis and neurite maturation	Density gradient centrifugation	Esteves et al., 2022
Glioma	HEK293Tcells	miR-124	Human	Upregulate M1 polarization and downregulate M2 polarization	Ultracentrifugation	Hong et al., 2021
	Ginsengs	phospholipids	Rat and mouse	Inhibit glioma growth, facilitate glioma cell apoptosis, and inhibit M2 macrophage polarization	Density gradient centrifugation	Kim et al., 2023
	MSCs	miR-7 and TRAIL	Human	Increase apoptosis and suppress tumor growth of glioma	Sequential ultracentrifugation	Zhang et al., 2017
IS	ADSCs	miR-30d-5p	Mouse	Inhibit neuronal apoptosis and expression of inflammatory factors	Sequential ultracentrifugation	Jiang et al., 2018
	MSCs	miR-138-5p	Mouse	Inhibit apoptosis of astrocytes and inflammatory response	Ultracentrifugation	Deng et al., 2019
		miR-138-5p	Mouse	Inhibit apoptosis of astrocytes	Ultracentrifugation	Deng et al., 2019
		miR-133b	Rat	Increase axonal density and neurite remodeling	Ultracentrifugation	Xin et al., 2013
		miR-134	Rat	Inhibit apoptosis of astrocytes	Ultracentrifugation	Xiao et al., 2019
		miR-22-3p	Rat	Inhibit apoptosis of astrocytes	Ultracentrifugation	Zhang et al., 2021
	Plasma	miR-451a	Human	Inhibit inflammation, apoptosis, and oxidative stress	Ultracentrifugation	Li et al., 2021a
	BMECs	miR-29b-3p	Rat	Inhibit apoptosis of neurons and reverse repression in angiogenesis	Ultracentrifugation	Hou et al., 2020
	Astrocytes	Nampt	Mouse	Reduce infarct volumes and neuronal loss	Differential centrifugation	Deng et al., 2022
TBI	Astrocytes	lncRNA 4933431K23Rik	Mouse	Increase neurovascular units and ameliorate inflammation	Exoquick kit	He et al., 2023
		miR-873a-5p	Mouse	Inhibit inflammation, decrease brain defect area, cerebral edema content, and ameliorate neurological deficit	Ultracentrifugation	Long et al., 2020
	Serum	miR-21-5p	Mouse	Improve hMSC proliferation, BV, TV, BV/TV, and BMD in CT analysis	Ultracentrifugation	Lin et al., 2023
	hucMSCs	Not mentioned	Mouse	Improve motor performance, decrease brain edema, cortical lesion volume, the number of apoptotic-positive cells, and pyroptosis	Ultracentrifugation	Zhang et al., 2023
ALS	ADSCs	Proteins	Mouse	Restore complex I activity, coupling efficiency, and mitochondrial membrane potential	PureExo exosome isolation kit (density separation)	Calabria et al., 2019
				Improve motor ability	PureExo exosome isolation kit	Bonafede et al., 2020
Schizophrenia	MSCs	Not mentioned	Mouse	Preserve GABAergic cells and boost glutamate concentration	Ultracentrifugation	Tsivion-Visbord et al., 2020

AD: Alzheimer's disease; ADSC: adipose-derived stem cell; ALS: amyotrophic lateral sclerosis; Aβ: amyloid-beta peptide; BMD: bone mineral density; BMEC: brain microvascular endothelial cell; BV/TV: bone volume fraction; BV: bone volume; CT: computerized tomography; EV: extracellular vesicle; GABAergic: gamma-aminobutyric acidergic; GBM: glioblastoma; HDAC4: histone-modifying enzyme histone deacetylase 4; HEK293T: human embryonic kidney 293T; hMSC: human bone marrow mesenchymal stem cell; hUCB-MNC: human umbilical cord blood-derived mononuclear cell; hucMSC: human umbilical cord mesenchymal stem cell; IS: ischemic stroke; lncRNA: long noncoding RNA; MDD: major depressive disorder; MSC: mesenchymal stem cell; PD: Parkinson's disease; TBI: traumatic brain injury; TRAIL: TNF-related apoptosis-inducing ligand; TV: tissue volume.

Ischemic stroke

Our previous study shows that endothelial cell-derived exosomal miR-1290 exerts a neuroprotective effect by downregulating neuronal apoptosis *in vitro* and *in vivo* (Yue et al., 2019). Additionally, a study reports that adipose-derived stem cell (ADSC)-derived exosomes overexpressing miR-30d-5p decrease infarct volume induced by middle cerebral artery occlusion (MCAO) and inhibit ischemia-induced neuronal apoptosis by targeting the 3′ untranslated region site of autophagy-related proteins Beclin1 and autophagy-related 5. These exosomes also suppress the oxygen-glucose deprivation (OGD)-induced expression of the inflammatory factors, including tumor necrosis factor-alpha, interleukin 6 (IL-6), and inducible nitric oxide synthase (Jiang et al., 2018). Similarly, Deng et al. (2019) reported that bone marrow MSC-derived exosomal miR-138-5p inhibits apoptosis and inflammatory responses in OGD-injured astrocytes while promoting astrocyte proliferation by targeting the 3′ untranslated region of lipocalin 2 sites. This leads to upregulation of β-cell lymphoma-2 (Bcl-2), cyclin-dependent kinase 4, Cyclin D1, and Cyclin E expression, and downregulation of IL-6, interleukin 1 beta (IL-1β), tumor necrosis factor alpha, cleaved caspase 3, and Bcl2-associated X-protein. MSC-derived exosomes carrying miR-133b, administered via the tail vein in rats, significantly increase axonal density and neurite remodeling in the ischemic boundary zone and boost the synaptophysin immunoreactive area in this zone (Xin et al., 2013). Zhang et al. (2020) reported that miRNAs of exosomes isolated from cerebral endothelial cells of ischemic rats and nonischemic rats improve axonal growth and growth cone extension by decreasing the axonal inhibitory proteins Pten, ras homolog family member A, and semaphorin 6A in axons and somata, which was linked to the communication between distal axons and their parent somata.

Therefore, exosomes from ischemic rats show a better therapeutic effect than those from nonischemic rats by further increasing pre-miRs in axons and their somata. MSC-derived exosomes carrying cargoes such as miR-134 (Xiao et al., 2019), miR-138-5p, miR-30d-5p (Jiang et al., 2022), and miR-22-3p (Zhang et al., 2021), ameliorate brain injury by downregulating neuronal apoptosis. Li et al. (2021a) reported that exosomal miR-451a, derived from cerebral ischemic preconditioning plasma, plays a neuroprotective role in MCAO or OGD/reperfusion-induced injury by targeting Rac family small GTPase 1 and protecting N2a cells. Deng et al. (2022) showed that astrocyte-derived exosomes stimulated by OGD/reperfusion, containing nicotinamide phosphoribosyltransferase (Nampt), decrease autophagy plaques and autophagosomes, thereby promoting cell viability. Additionally, these exosomes further reduce infarct volumes and neuronal loss *in vivo* by upregulating phosphorylated adenosine monophosphate-activated protein kinase and downregulating phosphorylated mechanistic target of rapamycin kinase levels. D'Souza et al. (2021) demonstrated that EVs derived from brain endothelial cells injured by OGD/reperfusion transfer mitochondria improve endothelial cell survival. Moreover, in recent years, neural stem cell-derived EVs have been developed into the drug AB126 and entered clinical trials to inhibit inflammation during IS (Spellicy et al., 2024). Overall, EVs show great potential in treating IS, with treatments primarily focused on anti-inflammatory actions, apoptosis suppression, and neuronal protection during and after revascularization. These EVs carry a variety of cargoes, including non-coding RNAs, mitochondrial fragments, and proteins, which are protected from degradation and transmitted to the lesion site to exert therapeutic effects.

Parkinson's disease

A previous study explored the diagnostic use of EVs, highlighting their role in the propagation of pathological contents such as α-synuclein. This process resulted in the aggregation of α-synuclein in neurons, which further exacerbates the progression of PD (Guo et al., 2020). In a study by Blommer et al. (2023), plasma samples from 273 participants were analyzed, including 103 cognitively normal and 121 cognitively impaired individuals with PD. Next, they tested various molecules, such as tyrosine-phosphorylated insulin receptor substrate-1, serine-phosphorylated insulin receptor substrate-1, α-synuclein, and phosphorylated Tau181, all enriched in neuronal origin EVs, to distinguish between PD subtypes. Neuronal origin-enriched EVs are more effective in separating these groups (area under the curve for neuronal origin-enriched EV α-synuclein = 0.772 (0.658–0.886) *vs.* area under the curve for plasma α-synuclein = 0.696 (0.573–0.820)), indicating their enhanced diagnostic value.

Alzheimer's disease

MSC-EVs were used to target hippocampal CA1 pyramidal neuron microglia, significantly decreasing the density of ionized calcium-binding adapter molecule 1-positive cells. The average cell body size and CD68 expression are downregulated compared to that of the control. The intranasal administration route of EVs was first described (Losurdo et al., 2020). Another study indicates that exosomal miR-29a in MSC-derived exosomes could benefit AD by upregulating memory/synaptic plasticity-related genes through the targeting of the histone-modifying enzyme histone deacetylase 4. Reductions in Aβ and histone deacetylase 4 levels are observed in AD transgenic mice (Chen et al., 2021). Molecules such as annexin A5, pT181, pS396 tau, and Aβ1-42 in AD brain-derived EVs are identified as potential biomarkers to distinguish AD from other neurodegenerative diseases (Muraoka et al., 2020). Ding et al. (2018) reported that human umbilical cord MSC-exosomal CD63 and CD9 could increase the expression of two key Aβ-degrading enzymes, insulin-degrading enzyme and neprilysin, thereby reducing Aβ plaque aggregation. These exosomes also inhibit the production of inflammatory cytokines and promote the M2-to-M1 transition of microglia. Moreover, a study shows that miR-124-3p in small EVs derived from human umbilical cord blood-derived mononuclear cells could alleviate symptoms of PD by enhancing axonogenesis and neurite maturation and improve motor function deficits by modulating cytoskeletal proteins and ras homology growth-related pathway (Esteves et al., 2022).

Amyotrophic lateral sclerosis

A previous study reported that ADSC-derived EVs can inhibit motor neuronal degeneration and delay ALS progression (Wang et al., 2022b). Calabria et al. (2019) prepared a murine NSC-34 cell line transfected with the human mutant SOD1 (G93A) gene, mimicking the motor neuron phenotype of patients with ALS, and administered ADSC-derived exosomes to these cells. These EVs ultimately restore the mitochondrial membrane potential in the G93ADOXY-positive cells (cells expressing the G93A mutant gene doxycycline). Similarly, Bonafede et al. (2020) conducted an *in vivo* study using mice with SOD1 (G93A) mutation, and they observed that the ADSC-derived exosomes significantly improved the motor function of these transgenic mice. Furthermore, a prospective study shows that human bone marrow-derived endothelial progenitor cell-derived EVs significantly ameliorate the damage of vascular endothelial cells induced by plasma with SOD1 (G93A) mutation (Garbuzova-Davis and Borlongan, 2021).

Glioma

The signal transducer and activator of transcription 3, which play a critical role in promoting tumorigenesis, is downregulated by 0.75-fold ($P < 0.05$) following treatment with miR-124 carried in HEK293T-derived EVs. Additionally, M2 polarization of microglia, which indicates an action of tumorigenesis, is suppressed. This was evidenced by the upregulation of IL-6, representing the M1 subtype (1.86-fold, $P < 0.01$), and the downregulation of transforming growth factor beta and down-regulation of IL-10, markers of M2 subtype (0.62 and 0.41 folds, respectively) (Hong et al., 2021). Kim et al. (2023) reported that ginseng-derived exosomes carrying phospholipids increase the expression of BAX family genes, decrease BCL-2 levels, and facilitate apoptosis in glioma cells in a concentration-dependent manner. Furthermore, these exosomes downregulate the production of growth factors and inhibit M2 macrophage polarization. Zhang et al. (2017) showed that miR-7 and TRAIL in MSC-exosomes could increase apoptosis and suppress tumor growth in glioma. Studies increasingly utilize EVs as vehicles to deliver artificial drugs rather than relying solely on natural EVs to treat glioma.

Psychosis

Tsivion-Visbord et al. (2020) reported that MSC-derived EVs could improve the behavioral symptoms of schizophrenia by preserving parvalbumin-positive interneurons, a type of gamma-aminobutyric acidergic cells in the prefrontal cortex, and increasing glutamate levels in phencyclidine-treated mice. Honorato-Mauer et al. (2023) conducted cross-sectional and longitudinal analyses, confirming the relationship between major depressive, anxiety, and attention-deficit/hyperactivity disorders with various miRNAs from serum-derived EVs, such as miR-409-3p and miR-432-5p. Research on psychosis and EVs remains limited due to the complex and poorly understood pathogenesis of the disorder.

Therapeutic Functions of Engineered Extracellular Vesicles in Neurological Diseases

Targeted engineering of extracellular vesicles

As mentioned, the therapeutic application of EVs is limited by inadequate therapeutic contents and nonspecific uptake in unintended organs. The targeting issue is exacerbated during systemic administration of EVs to the brain, as organs with a high phagocytic ability, such as the liver, spleen, and lungs, would sequester most of the EVs. Here, we primarily discuss current strategies for the targeted EV delivery. Strategies are generally classified into passive and active targeting. Passive targeting utilizes the natural properties of EVs to accumulate at specific body sites. Direct administration of EVs to the target site minimizes their loss due to non-specific internalization. In intracerebral administration, a common approach involves delivering EVs as a spay or droplets via the intracranial pathway. These EVs then travel along the olfactory or trigeminal nerve pathway to reach the brain. However, because EVs would initially reach areas associated with the olfactory and trigeminal nerve, uptake is uneven. Moreover, once EVs reach the brain, they can be internalized by various cell types, such as neurons, microglia, astrocytes, and endothelial cells, amongst others. Passive targeting does not meet the need for neuronal cell-specific delivery. To further improve targeting efficiency and specificity, active targeting is employed, utilizing the surface proteins and lipid membrane of EVs. This approach is known as targeted engineering.

Engineering approaches for modifying EV surfaces include genetic engineering (Khan et al., 2016), chemical engineering (Zhang et al., 2019; Guo et al., 2021; Tian et al., 2021), and physical engineering, such as metabolic labeling, affinity binding, and enzymatic ligation (Gopalan et al., 2020; Liu et al., 2023a). Genetic engineering modifies EVs surface proteins by constructing fusion proteins that combine targeting peptides (arginine-glycine-aspartic acid (RGD), rabies virus glycoprotein (RVG), antibody-Fc), and surface proteins (Lamp2b, TfR, CD9, CD63, and CD81) or overexpressing existing surface proteins (TfR) (Alvarez-Erviti et al., 2011; Wang et al., 2017). This genetic modification can be achieved through techniques such as viral transduction (Wang et al., 2019), plasmid transfection (Du et al., 2021), or CRISPR/Cas9 gene editing (Hazrati et al., 2022). While genetic engineering provides numerous benefits for EV modification, limitations also exist. Firstly, the process is complex and time-consuming, with potential risks such as off-target effects and immunogenicity. Additionally, modifications to surface proteins are limited to proteins and peptides, reducing flexibility. Moreover, this modification can be unstable due to changes in the gene-edited parental cells.

Chemical engineering is a widely used method for targeted modification, offering both flexibility and efficiency. Crosslinkers mediate the conjugation of biomolecules to the EV membrane including 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide (NHS) (Rayamajhi and Aryal, 2020), maleimides (Di et al., 2019), and succinimides (Veneziano et al., 2017), PEG (Wang et al., 2023c), polyethyleneimine (Lu et al., 2023) and dibenzocyclooctyne (Tu et al., 2020). These crosslinkers operate through various conjugation principles (amide bond formation, thiol-maleimide coupling, click chemistry). Combining these crosslinkers allows for efficient modification of EVs with desired biomolecules such as antibodies, peptides, proteins, single-strand DNAs, and aptamers, resulting in an extended circulation half-life. Lee et al. (2016) engineered EVs by modifying their parental cells with membrane fusogenic liposomes containing azide lipids, enabling customization with agents via copper-free click chemistry. Our data also confirms that Maleimides-PEG6-NHS ester could incorporate peptides and single-strand DNAs into the EV membrane, enhancing their targeting capability. Nevertheless, chemical engineering could affect the physical properties and functions of EVs, potentially leading to loss of functionality or toxicity. Moreover, some modifiers are environmentally sensitive and may be unstable during storage.

In recent years, physical engineering has encompassed a range of innovative methods for EV modification, including noncovalent ligation, liposome fusion, magnetic modification, and ultrasound modification. In noncovalent ligation, the photosensitive, thermal-responsive, or pH-responsive hydrogels are used to carry and controllably release EVs to targeted regions (Tsintou et al., 2021). In liposome fusion, EVs are fused with artificial liposomes to acquire pre-modified targeting ligands on their membranes. In magnetic guidance, magnetic nanoparticles are incorporated into the EV surface through covalent conjugation or lipid-based interactions. This allows the EVs to be directed by magnetic forces and specifically accumulated in targeted regions. Moreover, a recent study utilized ultrasound to facilitate the incorporation of targeting molecules into EVs, enabling the construction of targeted EVs (Xu et al., 2021). Despite the flexibility and controllability, physical engineering is limited by its complexity and potential safety issues.

Recent innovative engineering methods for metabolic labeling involve supplying the culture medium of parental cells with azide-bearing amino acids (Qian et al., 2022) or azide-containing saccharides (Lim et al., 2021). By absorbing and reusing labeled metabolites, these chemical groups could be incorporated into the EV membrane. These azide-bearing EVs could further be modified with targeted molecules using click chemistry (Smyth et al., 2014; Armstrong et al., 2017; Deng et al., 2023). For affinity binding, specific peptides are utilized for their affinity to EV surface proteins. To achieve targeted modification, the peptide CP05, which binds to the second extracellular loop of CD63, has been previously conjugated with targeted molecules. EVs could bind CP05 and acquire targeted ability through simple mixing (Yu et al., 2024). In enzymatic ligation, protein ligases recognize specific amino acid sequences in two peptides and mediate their ligation (Pham et al., 2021). For targeted modification, peptides pre-linked with recognition amino acid sequences are mixed with EVs. Conjugation occurs between EV protein and the targeted peptides in the presence of ligases. This method forms a covalent bond between targeted peptides and EVs without using genetic or chemical approaches.

Overall, while targeted engineering is widely used in EV-based therapeutics, current strategies face limitations such as complexity, inflexibility, time and high costs, loss of original properties, and safety concerns. Methods require further improvements. Studies in neurological diseases have also shown enhanced therapeutic functions of targeted engineered EVs. Next, we will discuss the therapeutic applications of engineered EVs in neurological diseases (**Figure 2** and **Table 2**).

Engineered extracellular vesicles in traumatic brain injury

Wu et al. engineered EVs with vascular cell adhesion molecules-1-targeting peptides and simultaneously encapsulated miR-143-3p inhibitors. Subsequently, they administered these engineered EVs into a murine model of TBI and observed a significant reduction in cell adhesion molecules, which are known to promote neutrophil infiltration in the brain. This reduction in cell adhesion molecules

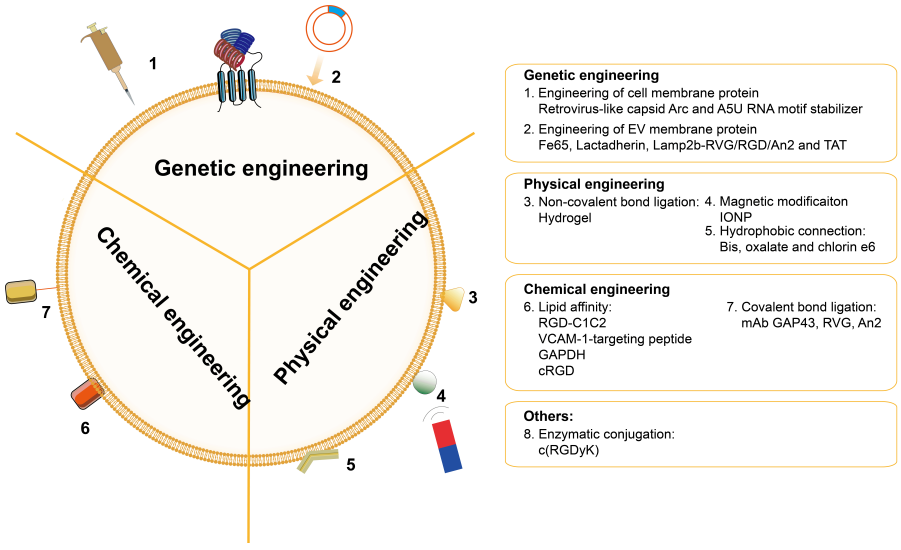

Figure 2 | Strategies for brain-targeted engineering of EVs.
Created using Adobe Illustrator. A5U: Arc 5′-untranslated-region; An2: angiopep-2; Arc: capsid-forming activity-regulated cytoskeleton-associated protein capsid; Bis: 2,4,5-trichloro-6-carbopentoxyphenyl; c(RGDyK): cyclo(arginine-glycine-aspartic acid-tyrosine-lysine); cRGD: cyclo-arginine-glycine-aspartic acid; EVs: extracellular vesicles; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; IONP: iron oxide nanoparticle; Lamp2b: lysosome-associated membrane glycoprotein 2b; mAb GAP43: monoclonal antibody against growth-associated protein-43; RGD: arginine-glycine-aspartic acid; RGD-C1C2: arginine-glycine-aspartic acid-C1C2 region of lactadherin; RVG: rabies virus glycoprotein; TAT: trans-activator of the transcription; VCAM: vascular cell adhesion molecules.

resulted in decreased neuronal apoptosis and improved motor ability in mice (Wu et al., 2024). Haroon et al. (2024) reported using a neuroprotective macromolecule (NR2B9C), which targets the N-methyl-D-aspartate receptor and neuronal nitric oxide synthase death signaling complex. The macromolecules were loaded into exosomes derived from BV2 cells engineered with the rabies virus glycoprotein (RVG29), which specifically targets Ach receptors on the neuronal membrane. The dibenzocyclooctyne-PEG4-NHS and Azido-RVG29 were used in the study. Consequently, administering the engineered exosomes improved motor function and reduced neuronal apoptosis in a rodent model of TBI.

Engineered extracellular vesicles in ischemic stroke

A study innovatively introduced plasmids encoding the retrovirus-like mRNA-packaging capsid-forming activity-regulated cytoskeleton-associated (Arc) protein and capsid-stabilizing Arc 5′-untranslated-region RNA motif stabilizer into leukocytes. This approach produced engineered retrotransposon Arc EVs modified with retrovirus-like capsid protein Arc and Arc 5′-untranslated-region stabilizer protein incorporated into the membrane. By utilizing Arc, engineered retrotransposon Arc EVs enhance mRNA encapsulation and significantly improve EV uptake by neurons (Gu et al., 2024). Khan et al. (2021) reported on various engineered exosomes for therapeutic use. These include exosomes expressing rabies RVG peptide on their membranes and carrying circular RNA derived from scm polycomb group protein homolog 1, exosomes transporting neurotrophic factor mRNA and protein to target cells to combat brain inflammation, and interferon gamma-preconditioning exosomes, which not only do not affect exosome secretion but also promote neurogenesis, angiogenesis, apoptosis as well as the inhibition of neuroinflammation. Yang et al. (2017) reported that exosomes genetically engineered with the fused protein Lamp2b-RVG effectively targeted the ischemic brain area. They also demonstrated that exosomal miR-124 functionally promotes neurogenesis in progenitor cells. Moreover, quercetin-loaded monoclonal antibody growth-associated protein-43 conjugated exosomes could direct to the ischemic penumbra. This targeting stimulates the nuclear factor erythroid 2-related factor 2/heme oxygenase 1 pathway, reducing both apoptotic nuclei and ROS levels in OGD-treated SH-SY5Y cells. In MCAO mice, this approach reduced infarction volume compared to those in the control group (Guo et al., 2021). In another study, exosomes derived from the neural progenitor cell line ReN were modified with RGD-C1C2, which binds to integrin receptors and specifically targets the ischemic brain. After being administered to an MCAO mouse model, RGD-C1C2-modified EVs exhibited anti-inflammatory effects by suppressing mitogen-activated protein kinase signaling through their cargo of seven miRNAs (Tian et al., 2021). In addition, Zhang et al. (2019) developed bone marrow MSC-derived exosomes conjugated with cyclo(Arg-Gly-AspD-Tyr-Lys) peptides and loaded with cholesterol-modified miR-210. As a result, exosomes modified with cyclo(arginine-glycine-aspartic acid-tyrosine-lysine) (c(RGDyk)) enhanced angiogenesis by upregulating integrin β3, vascular endothelial growth factor, and CD34. These modified exosomes demonstrated greater positioning efficiency than natural exosomes, which was confirmed by increased near-infrared fluorescence intensity in imaging studies. Similarly, Tian et al. (2018) conjugated the c(RGDyk) peptide to exosomes using a two-step reaction involving dibenzocyclooctyne-sulfo-NHS and packaged curcumin into these exosomes. Through the interaction with integrin αvβ3, the c(RGDyk) peptide enables engineered exosomes to deliver curcumin to the lesion site, inhibiting pro-inflammatory microglia activation and reducing apoptosis in the damaged tissue. Furthermore, a recent study employed magnetic nanoparticles to improve targeting efficiency. In the study, magnetically engineered MSC-derived EVs were more effectively attracted to ischemic regions and delivered therapeutic effects through the use of a magnetic helmet (Kim et al., 2020).

Engineered extracellular vesicles in neurodegenerative diseases

The binding of glyceraldehyde 3-phosphate dehydrogenase to EVs through a phosphatidylserine-binding G58 domain could induce EV clustering and increase particle size, enhancing the delivery and gene silencing efficacy of therapeutic small interfering RNAs (siRNAs) in Huntington's disease (Dar et al., 2021). This introduces a novel approach to increasing cargo delivery to targeted regions by aggregating EVs rather than relying on overloading modifications. Cui et al. (2019) suggested that MSC-exosomes conjugated with a CNS-specific RVG peptide via a dioleoylphosphatidylethanolamine-NHS linker effectively reduced plaque deposition, Aβ accumulation, pro-inflammatory cytokines release, while also lowering glial fibrillary acidic protein levels. These improvements contributed to enhanced learning and memory function. Another study reports that exosomes engineered with Fe65 to encapsulate corynoxine-B effectively targeted amyloid precursor protein and improved the autophagy-

Table 2 | **Targeting-engineered EVs in treating neurological diseases**

Source	Engineered molecule (targeted region/cell)	Methods of isolation	Methods of engineering	Content	Model	Disease	Reference
Leucocytes	Arc protein capsid and the A5U RNA motif stabilizer (neurons)	Ultrafiltration and density gradient centrifugation	Genetic engineering (Cell membrane protein engineering)	mRNA	Rat	Neural inflammation	Gu et al., 2024
MSCs	Lamp2b-RVG (neurons)	Sequential ultracentrifugation	Genetic engineering (EV membrane protein engineering)	miR-124	Mouse	IS	Yang et al., 2017
WB	mAb GAP43 (neurons)	Gradient centrifugation	Chemical modification (covalent bond ligation)	Que	Rat	IS	Guo et al., 2021
NPCs	RGD-C1C2 (neurons)	Ultracentrifugation	Chemical modification (lipid affinity)	7 miRNAs	Mouse	IS	Tian et al., 2021
MSCs	c(RGDyK) (vessels)	Ultracentrifugation	Biochemical modification (enzymatic conjugation)	miR-210	Mouse	IS	Zhang et al., 2019
MSCs	c(RGDyK) (neurons)	Ultracentrifugation	Biochemical modification (enzymatic conjugation)	Curcumin	Mouse	IS	Tian et al., 2018
M2-BV2	DA7R, SDF-1	Ultracentrifugation	Chemical modification (covalent bond ligation)	miRNAs	Mouse	IS	Ruan et al., 2023
MSCs	IONP (neurons)	Density gradient centrifugation	Physical modification (hydrophobic connection)	GF	Rat	IS	Kim et al., 2020
HEK293T	VCAM-1 targeting peptide (neurons)	Ultracentrifugation	Chemical modification (lipid affinity)	miR-143-3p inhibitor	Mouse	TBI	Wu et al., 2024
Microglia	RVG29 (neurons)	Ultracentrifugation	Chemical modification (covalent bond ligation)	NR2B9c	Mouse	TBI	Haroon et al., 2024
Primary astrocytes	Plasmids expressing Bcl-2 and Bax shRNA (neurons)	Ultracentrifugation	Genetic engineering (plasmid transportation)	Bcl-2 and Bax shRNA	Mouse	TBI	Wang and Han, 2019
MSCs, HEK293T, SKOVE-3,B16-F10,Hela cells	GAPDH (neurons)	Ultrafiltration	Chemical modification (lipid affinity)	siRNA	Mouse	HD	Dar et al., 2021
MSCs	RVG (neurons)	Gradient centrifugation	Chemical modification (covalent bond ligation)	Cytokine	Mouse	AD	Cui et al., 2019
Hippocampus neurons	Fe65 (neurons)	Total exosome isolation reagent	Genetic engineering (EV membrane protein engineering)	Corynoxine-B	Mouse	AD	Iyaswamy et al., 2023
ADSCs	Hydrogel	Differential centrifugation	Physical modification (noncovalent bond ligation)	Not mentioned	Mouse	AD	Huang et al., 2024
Murine BV-2 microglia	Lactadherin (phagocytes)	Differential Ultracentrifugation	Genetic engineering (EV membrane protein engineering)	IL-4	Mouse	MS	Casella et al., 2018
HEK293T	An2 and TAT (BBB and tumor tissue)	Gradient centrifugation	Genetic engineering (EV membrane protein engineering)	DOX	Mouse	Glioma	Zhu et al., 2022
HEK293FT	Lamp2b-RGD (tumor tissue)	Total exosome isolation reagent and centrifugation	Genetic modification (EV membrane protein engineering)	DOX and siRNA	GBM cells	Glioma	Gečys et al., 2022
RAW264.7 culture medium	cRGD (tumor tissue)	Ultracentrifugation	Chemical modification (lipid affinity)	Panobinostat and siRNA	Mouse	Glioma	Shan et al., 2022
THP-1	An2 (tumor tissue)	Ultrafiltration	Chemical modification (covalent bond ligation)	siRNA	U87MG cell	Glioma	Liang et al., 2022b
Brain tissue	An2 and TAT (tumor tissue)	Not mentioned	Genetic engineering (EV membrane protein engineering)	Cas9/c	Mouse	Glioma	Liu et al., 2023b
M1-like macrophage	Bis, oxalate, and chlorin e6 (tumor tissue)	Ultracentrifugation	Physical modification (hydrophobic connection)	Banoxantrone	Mouse	Glioma	Wang et al., 2022c
L929 cells	DWFKAFYDKVAEKFKEAF, RLTRKRGLKLA, and KLAKLAK-KLAKLAK (tumor tissue)	Gradient centrifugation	Physical modification (hydrophobic connection)	Methotrexate	Mouse	Glioma	Ye et al., 2018
DCs	Lamp2b-RVG (neurons)	Differential centrifugation	Physical modification (EV membrane protein engineering)	Dicer siRNA	Mouse	Psychosis	Chivero et al., 2020
MSC	LJM-3064 (myelin)	ExoQuick kit	Chemical modification (covalent bond ligation)	Natural contents	Mouse	MS	Hosseini Shamili et al., 2019

A5U: Arc 5′-untranslated-region; AD: Alzheimer's disease; ADSC: adipose-derived stem cell; An2: angiopep-2; Arc: capsid-forming activity-regulated cytoskeleton-associated protein capsid; Bax: Bcl-2-associated X-protein; Bcl-2: β-cell lymphoma-2; Bis: 2,4,5-trichloro-6-carbopentoxyphenyl; c(RGDyK): cyclo(arginine-glycine-aspartic acid-tyrosine-lysine); Cas9/sgGSS: sgRNA-glutathione single guide synthetase complex; DC: dendritic cell; DOX: doxorubicin; DWFKAFYDKVAEKFKEAF: c-I mimetic peptide 4F; EV: extracellular vesicle; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; GBM: glioblastoma; GF: growth factor; HEK293T: human embryonic kidney 293T; IL-4: interleukin-4; IONP: iron oxide nanoparticle; IS: ischemic stroke; KLAKLAK-KLAKLAK: therapeutic KLA; Lamp2b-RVG: lysosome-associated membrane glycoprotein 2b-rabies virus glycoprotein; mAb GAP43: monoclonal antibody against growth-associated protein-43; MS: multiple sclerosis; MSC: mesenchymal stem cell; NPC: neural progenitor cell; Que: Quercetin; RGD-C1C2: arginine-glycine-aspartic acid-C1C2 region of lactadherin; RLTRKRGLKLA: ApoB LDLR binding domain, shRNA: short hairpin RNA; siRNA: small interfering RNA; SKOVE-3: human ovarian cancer cell-3; TAT: trans-activator of the transcription; TBI: traumatic brain injury; THP1: human myeloid leukemia monocyte; VCAM: vascular cell adhesion molecules; WB: whole blood.

lysosomal degradation pathway in AD mice (Iyaswamy et al., 2023). Hydrogel can reduce EV degradation and promote their retention. Huang et al. (2024) introduced the proteases into MSC-EVs membranes, enabling hydrogel degradation and allowing for controlled and targeted release of the EVs. Intranasal administration enhances the therapeutic effects of neurogenesis in AD mice. Furthermore, Casella et al. (2018) engineered IL-4^{+} EVs from murine BV-2 microglia by incorporating Mfg-e8 (lactadherin), which improved their targeting ability to phagocytes such as monocytes and microglia. This approach enhances the ability of EVs to upregulate anti-inflammatory cytokines CD206, arginase-1, and chitinase 3-like 3 while downregulating proinflammatory cytokines IL-17 and inducible nitric oxide synthase in multiple sclerosis (MS). RVG peptide and lactadherin are commonly used in targeted engineering processes, demonstrating their effectiveness in directing EVs to specific areas in neurodegenerative diseases.

Engineered extracellular vesicles in glioma

Liang et al. (2022b) identified angiopep-2 (An2), which enables exosomes to target GBM cells and cross the BBB. They observed a 41-fold increase in exosomes-An2 uptake by U87MG cells compared to exosomes without An2, with significantly stronger fluorescence detected in the brain of the exomes-An2 group. When loaded with siRNA, engineered exosomes more effectively inhibited signal transducers and activators of the transcription 3 pathway, resulting in approximately 94.55% apoptosis of U87MG cells. A study constructed a dual peptide-modified small EV from HEK293T cells, incorporating An2 for high affinity to lipoprotein receptor-related protein 1 and trans-activator of the transcription (TAT) to enhance BBB penetration and improve glioma targeting. Engineered EVs loaded with doxorubicin (DOX) hydrochloride demonstrated enhanced anti-tumor effects both *in vitro* and *in vivo* (Zhu et al., 2022). Another study also combined An2 and TAT modifications to enhance the cell membrane penetration of EVs, resulting in dual-modified EVs containing a Cas9/sgGSS (single guide glutathione synthesis) complex. An2/TAT-sgGSS-EVs were shown to induce ferroptosis and improve radio sensitization by depleting glutathione synthesis (Liu et al., 2023b). Although dual-peptide modification could enhance targeting ability, considering the associated workload and potential cost burden is essential. Dovydas Gečys et al. (2022) engineered DOX, and epigenetic modulator siRNAs encapsulated EVs using a Lamp2b-RGD fusion protein. Quantitative analysis revealed that GBM cells internalized approximately 40% more RGD-EVs compared with natural EVs. Research also shows that siRNA-loaded RGD-EVs are more effective at glyceraldehyde 3-phosphate dehydrogenase knockdown. Moreover, a study developed macrophage-derived EVs modified with cyclo (arginine-glycine-aspartic acid (cRGD) and encapsulating panobinostat and siRNAs pre-linked to micelles, aiming to target GBM through BBB. Briefly, panobinostat was first encapsulated in positively charged micelles (DEP). PPM1D siRNA was then electrostatically adsorbed onto the surface of these DEP micelles, resulting in DEP-siRNA. Macrophage exosomes from RAW264.7 were conjugated with the tumor-targeting peptide-cRGD using click chemistry. Finally, the exosomal membrane was extracted and used to encapsulate DEP-siRNA via a coextrusion process, resulting in cEM@DEP-siRNA. The engineering strategy improved the brain-targeting delivery of Panobinostat, which has poor water solubility and low BBB penetration efficiency (Shan et al., 2022). To target the immune microenvironment, M1-like macrophage-derived EVs were demonstrated to modulate the GBM immune microenvironment by promoting M2-to-M1 translation and increasing reactive oxygen species (ROS) production. Wang et al. (2022c) first incubated M1-like macrophages with the inactivated chemotherapy agent banoxantrone (AQ4N, A) to produce EVs carrying AQ4N (A-M1EVs). Subsequently, A-M1EVs were functionalized into CCA-M1EVs by incorporating the hydrophobic bis (2,4,5-trichloro-6-carbopentoxyphenyl), oxalate (CPPO, C) and chlorin e6 (Ce6, C) into the EV membrane to increase the production of ROS further. As a result, CCA-E1EVs exhibited an improved anti-glioma effect with 30% penetration efficiency after 8 h. In another study, Ye et al. (2018) engineered functionalized methotrexate-loaded EVs incorporating ApoA-I mimetic peptide 4F (DWFKAFYDKVAEKFKEAF), ApoB LDLR binding domain (RLTRKRGLKLA), and therapeutic KLA (KLAKLAK-KLAKLAK) into the membrane. They demonstrated that U87 cells took up significantly more EVs-LDL and EVs-KLA-LDL than that of natural EVs.

Engineered extracellular vesicles in psychosis

A recent study on EV engineering in psychosis are limited. One such study reported that engineered dendritic cell (DC)-derived EVs carrying miR-124, which downregulates TLR4, MYD88, and signal transducers and activators of transcription 3, could ameliorate cocaine-mediated microglia activation (ionized calcium-binding adapter molecule 1) in cocaine use disorder. This effect was achieved by co-transfecting mouse DCs with Dicer siRNA and Lamp2b-RVG plasmid to deplete endogenous miRNAs separately and target the CNS (Chivero et al., 2020). Despite the complex pathogenesis limiting treatment development in psychosis, the therapeutic potential of engineered EVs remains promising and warrants further investigation, given their success in other neurological diseases.

Clinical Trials of Extracellular Vesicles in Neurological Diseases

Numerous clinical trials have explored the therapeutic use of EVs across various diseases (**Table 3**). While fewer trials focus on neurological diseases, promising results support the application of EVs in this area. Bang et al. (2022) initiated a prospective randomized controlled trial for patients with chronic major stroke. MSC-EVs were administered intravenously, resulting in a 5-fold increase in EV levels within 24 hours. The result showed that EV level was significantly correlated with motor function improvement (odds ratio, 5.718 for EV numberLog [95% confidence interval, 1.144-28.589]; $P = 0.034$) and the integrity of the ipsilesional corticospinal tract and intrahemispheric motor network ($P < 0.05$). Another preclinical trial demonstrated that neural stem cell-derived EVs, named AB126, could significantly reduce white matter lesions and improve motor activity and exploratory behavior. T2-weighted sequences revealed a significant decrease in edema-corrected lesion volume in MCAO pigs (6.0 ± 1.4 cm^3 *vs.* 10.7 ± 1.4 cm^3; $P < 0.05$). A reduction in swelling of the affected ipsilateral hemisphere (113.7% ± 2.6% *vs*. 126.8% ± 3.4%) was also observed (Webb et al., 2018). According to a summary from Aruna Bio, Inc. (Athens, GA, USA), AB126 significantly reduced the neurofilament light chain in serum and inflammatory cytokine levels in the spinal cord in the SOD1 ALS mouse model. The study of AB126 treatment for ALS is ongoing in SOD1 murine to determine the optimal dosing regimen and route of administration. In AD research, clinical trials investigated the relationship between exercise and AD mitigation by collecting plasma samples from patients in both exercise and control groups. This trial showed that neuron-derived EVs carrying neuroprotective brain-derived neurotrophic factor, pro-brain-derived neurotrophic factor, and humanin were significantly upregulated in the exercise group (Delgado-Peraza

Table 3 | **Clinical trials investigating EVs for neurological diseases**

Organization	EV	Disease	Stage/reference
Samsung Medical Center	MSC Serum-derived EVs	IS	Phase III (NCT01716481)/Bang et al., 2022
ArunA Biomedical	NSC-EV	IS	Preclinical/Webb et al., 2018
Rigshospitalet, Denmark	Neuron-derived EVs	AD	Phase III (NCT01681602)/Delgado-Peraza et al., 2023
University Hospital, Lille	Ectosome	AD	Recruiting (NCT03381482)
Fondazione Don Carlo Gnocchi Onlus	Saliva-derived EVs	PD	Recruiting (NCT05320250)
Michael J. Fox Foundation for Parkinson's Research University Health Network, Toronto Indiana University University of Rochester	CSF-derived EVs	PD	Completed (NCT03775447)

AD: Alzheimer's disease; CSF: cerebrospinal fluid; exoSTING: EVs loaded with cyclic dinucleotide agonists of the stimulator of interferon genes; EV: extracellular vesicle; IS: ischemic stroke; MCAO: middle brain artery occlusion; MSC: Mesenchymal stem cell; NSC EV: neural stem cell-derived EV; PD: Parkinson's disease.

et al., 2023). A trial recruited patients with AD to investigate whether tau proteins in ectosomes, precursors of neurofibrillary tangles, could be detected in plasma and cerebrospinal fluid before causing pathological changes (NCT03381482). While clinical trials investigating therapeutic EVs in glioma remain unknown, Jang et al. (2021) conducted both *in vivo* mice and human clinical trials. They proposed that engineered EVs loaded with cyclic dinucleotide agonists of the stimulator of interferon response cGAMP interactor (STING), termed exoSTING, could improve immune efficacy approximately 100-fold more than pure cyclic dinucleotide or cyclic dinucleotide co-administered with EVs in growth suppression tests for various solid tumors. The clinical potential of exoSTING appears promising. In PD, EVs are currently being evaluated in clinical trials for diagnostic purposes. Carlomagno et al. (2021) used saliva-derived EVs to assess the effect of rehabilitation therapy on patients with PD. Another trial used EVs to measure leucine-rich repeat kinase 2 levels in cerebrospinal fluid samples from patients with PD (NCT03775447). Further advancements are needed to enhance the therapeutic use of EVs in neurological diseases.

Limitation

This review highlights that most studies on engineered or natural EVs are conducted using animal models. The clinical trials evaluating EVs on human bodies are insufficient. Moreover, this review examines the advancements in the targeted engineering of EVs. Pathological functions, cargo-loading strategies, and diagnostic potential of EVs in these representative neurological diseases are not comprehensively discussed.

Conclusion

In conclusion, this review addresses the advanced development of targeted drug delivery using engineered EVs for neurological diseases, including TBI, IS, PD, AD, ALS, glioma, and psychosis. Compared with the traditional drug delivery system, EVs derived from parent cells are highly effective at protecting their contents from degradation and penetrating the BBB. Furthermore, while these EVs can partially target the location of their parental cells, their effectiveness is limited. A significant amount of EVs could still accumulate in certain organs, including the liver, lung, and spleen, due to their strong phagocytic capacity. Physical, chemical, and biogenetic methods have been developed to enhance the targeting ability of EVs (**Figure 3**). Diverse materials and molecules including Fe_3O_4, peptides, monoclonal antibodies, and DNA aptamers are used for targeted engineering (**Figure 4**; Jiao et al., 2017; Hosseini Shamili et al., 2019; Wu et al., 2022). Consequently, these strategies significantly enhanced neural cell-targeting ability. Recent studies increasingly combine multiple strategies and molecules for EV-targeted engineering to improve specificity, affinity, and biological stability. The development of novel targeting molecules could further optimize the use of CNS-targeting EVs. However, significant changes remain in translating engineered EVs into clinical practice due to safety concerns, low modification efficiency, high costs, and the complexity of modifications. Safety concerns, including immunogenicity and cytotoxicity from engineering and side effects from the accumulation of engineered EVs or interactions between engineered molecules and neural cells, should be thoroughly evaluated in cells, animals, and humans. Additional targeting strategies and modifiers should be investigated to further improve the specific recognition of neural cells in CNS. Nevertheless, targeting-engineered EVs clearly have the potential to influence the treatments of neurological diseases significantly in the future. Therefore, further studies are essential to advance the development of engineered EVs.

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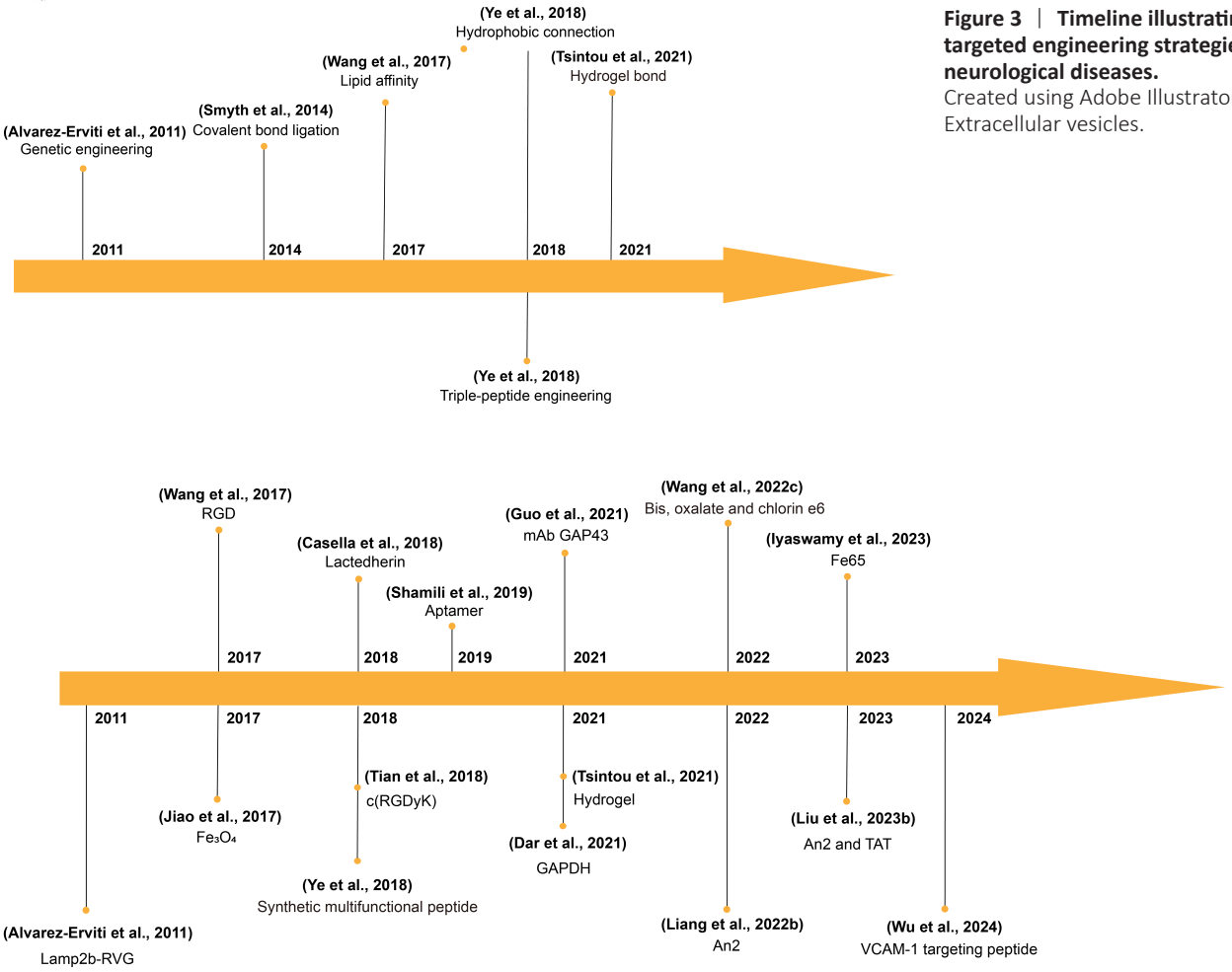

Figure 3 | Timeline illustrating targeted engineering strategies of EVs in neurological diseases.
Created using Adobe Illustrator. EVs: Extracellular vesicles.

Figure 4 | Timeline illustrating molecules or materials used in targeted engineering of EVs in neurological diseases.
Created using Adobe Illustrator. An2: Angiopep-2; Bis: 2,4,5-trichloro-6-carbopentoxyphenyl; c(RGDyK): cyclo(arginine-glycine-aspartic acid-tyrosine-lysine); EVs: extracellular vesicles; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; Lamp2b: lysosome-associated membrane glycoprotein 2b; mAb GAP43: monoclonal antibody against growth-associated protein-43; RGD: arginine-glycine-aspartic acid; RVG: rabies virus glycoprotein; TAT: trans-activator of the transcription; VCAM: vascular cell adhesion molecules.

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