Journal of Nanobiotechnology

REVIEW Open Access

Extracellular particles: emerging insights into central nervous system diseases

Shenyuan Chen[1,2†], Qinghua Bao[1†], Wenrong Xu[1,2*] and Xiao Zhai[3*]

Abstract

Extracellular particles (EPs), including extracellular vesicles (EVs) and non-vesicular extracellular particles (NVEPs), are multimolecular biomaterials released by cells that play a crucial role in intercellular communication. Recently, new subtypes of EPs associated with central nervous system (CNS), such as exophers and supermeres have been identified. These EPs provide new perspectives for understanding the pathological progression of CNS disorders and confer potential diagnostic value for liquid biopsies in neurodegenerative diseases (NDs). Moreover, EPs have emerged as promising drug delivery vehicles and targeted platforms for CNS-specific therapies. In this review, we delineate the landscape of EP subtypes and their roles in the pathophysiology of CNS diseases. We also review the recent advances of EP-based diagnosis in NDs and highlight the importance of analytical platforms with single-particle resolution in the exploitation of potential biomarkers. Furthermore, we summarize the application of engineered EVs in the treatment of CNS diseases and outline the underexplored potential of NVEPs as novel therapeutic agents.

Introduction

Under the dual drivers of neuroscience breakthroughs and global population aging, central nervous system (CNS) diseases have become a major healthcare crisis in the 21st century. According to The Lancet Neurology [1], neurological disorders affected approximately 3.4 billion people worldwide in 2021, which is 43.1% of the global population. These conditions resulted in 443 million disability-adjusted life years lost, making them the leading cause of global disease burden, surpassing cardiovascular diseases. Notably, neurodegenerative diseases (NDs) such as Alzheimer's and Parkinson's are growing exponentially, especially in aging Asia-Pacific populations [2, 3]. This public health issue leads to over $2.5 trillion in annual economic losses and highlights significant limitations in current treatments [4–7]. 97% of neurotherapeutics fail clinical trials due to the blood-brain barrier (BBB) impermeability [4, 8, 9].

This therapeutic challenge is driving the fourth revolution in drug delivery technologies. From the Nobel Prize-winning vesicular transport mechanism (2013), and the minimum information for studies of extracellular vesicles (MISEV) guidelines [10], to the first extracellular vesicles (EVs) therapy entering Phase III trials (2022) (NCT05354141), EVs have transcended their initial mischaracterization as "cellular debris" to become interdisciplinary bridges connecting nanotechnology, synthetic biology, and precision medicine [11, 12]. Additionally, cells also release a wide range of non-vesicular extracellular particles (NVEPs), comprising complex multimolecular structures that play roles in regulating various biological processes and facilitating cell-cell

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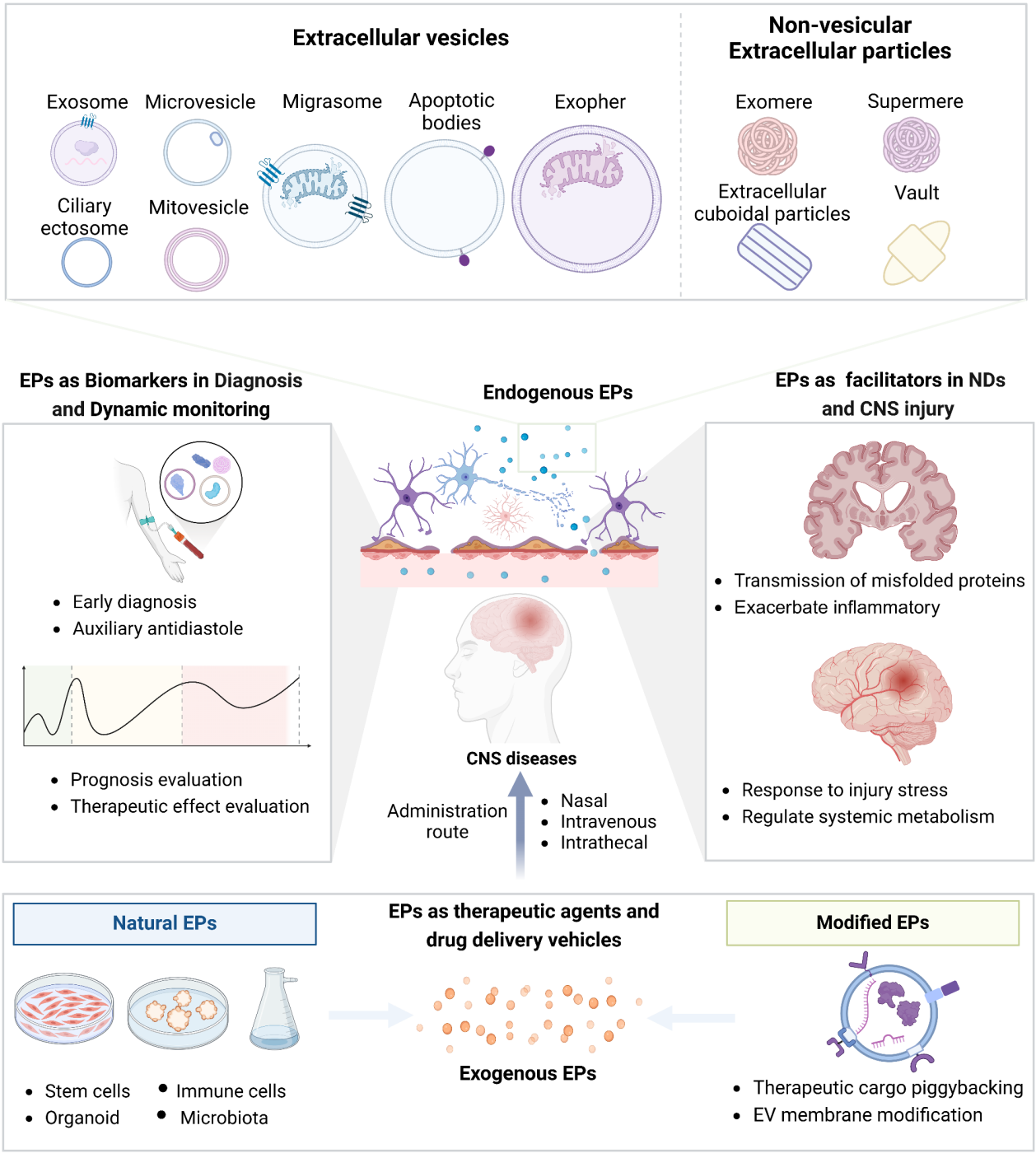
Graphical abstract



Keywords Extracellular particles, Central nervous system diseases, Heterogeneity, Pathological progression, Biomarkers, Therapeutic exploitation

communication [13–15]. For instance, supermeres, a subtype of NVEPs, are also enriched for NDs disease-associated molecules such as amyloid precursor protein (APP), suggesting that NVEPs may contribute to the pathological progression of NDs [14, 16]. According to the MISEV guidelines, both EVs and NVEPs are collectively termed

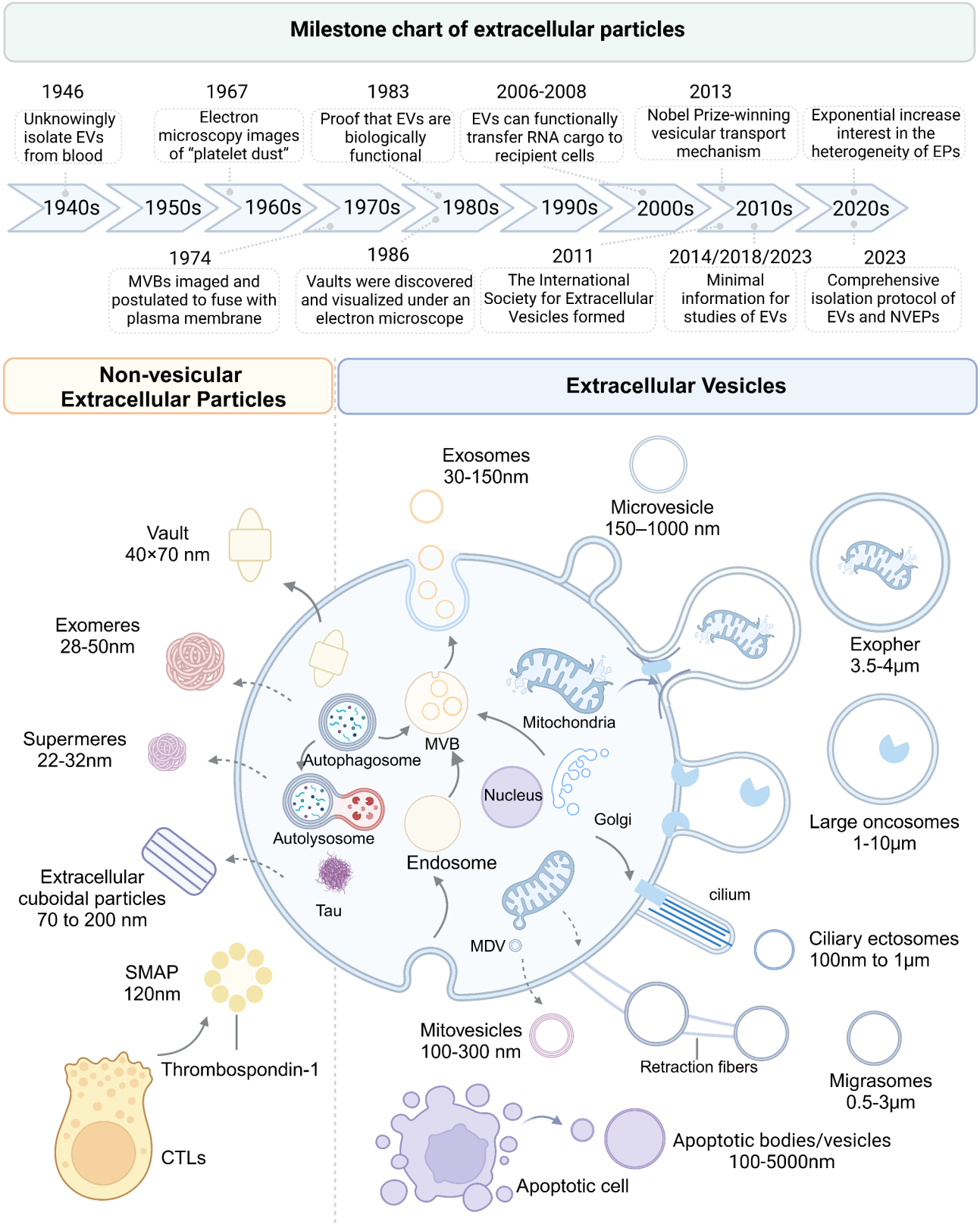


Fig. 1 (See legend on next page.)

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Fig. 1 Milestone chart of EPs and the biogenesis of the EP subtypes. Extracellular vesicles (EVs) coconstitute a heterogeneous group of lipid bilayer-enclosed particles released from cells into the extracellular space, including exosome, microvesicle, exopher, large oncosome, ciliary ectosome, migrasome, mitocesicle, apoptotic bodies, and apoptotic vesicle. Non-membrane extracellular particles (NVEP) are extracellular multimolecular aggregates that are released from cells and function without being enclosed by bilayer lipid membranes, including T cell-derived supramolecular attack particles (SMAP), vault, exomere, and supermere. MVB: multivesicular bodies; MDV: mitochondria-derived vesicles; CTL: cytotoxic T lymphocyte (Figure created with BioRender)

extracellular particles (EPs), a nomenclature adopted in this review.

The exploration of EPs in pathological microenvironments has been significantly advanced by cutting-edge single-particle omics technologies. For example, a recent study employed asymmetric flow field-flow fractionation (AF4) to identify distinct subpopulations of EPs and found distinct biological functions and potential roles in disease processes [17]. Similarly, the use of single-particle interferometric reflectance imaging sensing (SP-IRIS) and other orthogonal technologies has allowed for detailed phenotyping and sizing of EVs, revealing the complexity of their biomarker profiles and enhancing our understanding of their mechanistic roles in pathological conditions [18].These technologies have unveiled the spatiotemporal heterogeneity of EP subpopulations, providing insights into their diverse roles and interactions within CNS disease contexts [14, 16, 19].

Another recent area of focus in EPs research involves their utilization as therapeutic tools and targeted delivery vehicles for therapeutic molecules in the treatment of CNS disorders [20]. EPs derived from tissue-engineered cells like mensenchymal stem cells (MSCs) and neural stem cells (NSCs), have been extensively demonstrated to enhance neuronal axon regeneration, modulate glial cell phenotypes, and alleviate NDs and CNS injuries [21–23]. Additionally, engineered EPs interact with cells through surface epitopes, such as specific receptor-binding proteins and glycoproteins, enabling cargo delivery preferentially to CNS cells over peripheral tissuestissues [24, 25]. Moreover, breakthrough engineering strategies like biomimetic membrane camouflage [19] and light/megnetic-controlled release systems [26, 27] are overcoming natural EP limitations, redefining neural repair paradigms. Owing to the inherent biocompatibility, stability, and targeting precision, engineered EPs are emerging as promising nanocarriers for treating a spectrum of neurological disorders [25, 28].

This review pioneers systematic deconstruction of EP heterogeneity in pathology and treatment of CNS disorders. Initially, we explore the current knowledge regarding the heterogeneity of EVs and NVEPs. Subsequently, We delve into the roles played by various EVs and NVEP subtypes in NDs and CNS injuries. Lastly, we highlight recent advances in utilizing engineered EPs as bioinspired approaches to promote CNS regeneration and propose a strategic roadmap for revolutionizing neurological disease treatment.

Heterogeneity of EVs and NVEPs

In the past decade, advancements in technology and methodologies, along with an improved understanding of the complexity and heterogeneity of EPs, have led to the discovery of numerous new EPs subtypes [13, 29, 30]. The heterogeneity and complexity of EPs arise from the diversity of cell types and functional states, as well as the combined effects of multiple secretion pathways [14, 31, 32]. Research on the formation of different EPs subtypes has been significantly intensified. However, the precise mechanisms governing their origin, transport, and release still only partially understood [15, 33–35]. Here, we will provide the milestone chart of EPs and a briefly outline of the biogenesis of EP subtypes (Fig. 1).

EVs subtypes

Three main subtypes of EVs have been identified based on their origin and formation process:: apoptotic bodies or vesicles, ectosomes, and exosomes [11, 36]. These vesicle populations are differentiated by their unique biogenesis pathways. Recent studies have suggested that mitovesicles, which have a double-membrane structure, is a new subtype of EVs as they have a different biogenesis from the others [37].

Exosomes

The biogenesis of exosomes begins with the formation of early sorting endosomes (ESEs), which are membrane-bound vesicles created through endocytosis to encapsulate extracellular components and membrane proteins. These ESEs can mature into multivesicular bodies (MVBs) either by fusing with other ESEs or by exchanging materials with other organelles [38]. During this maturation process, multiple small intraluminal vesicles (ILVs) are formed within MVBs. Ultimately, MVBs fuse with the cell membrane, releasing ILVs into the extracellular space as exosomes [39]. Various molecules are involved in the biogenesis of exosomes, including the endosomal sorting complexes required for transport (ESCRT) protein complex [40], the Rab protein family [41], dynamin [42], and neutral sphingomyelinase [43]. Each stage of exosome biogenesis is governed by multiple mechanisms that involve competition or coordination, resulting in distinct molecular cargoes into specific

exosome subpopulations and contributing to significant heterogeneity within exosomes themselves [13, 38]. Consequently, researchers have shown a growing interest in using single-vesicle technologies (such as super-resolution microscopy and nanoscale flow cytometry) to decipher the heterogeneity of exosomes [44–46].

Apoptotic bodlies or vesicles

Apoptotic bodies are vesicles ranging from approximately 1000 to 5000 nm in diameter that are formed during the cellular apoptosis process [47]. The initial formation of apoptotic bodies typically involves a series of apoptotic molecules and signaling pathways, including the initiation of apoptotic signal transduction, the activation of caspases and their downstream cascade reactions, and the fragmentation of the cell nucleus. In the later stages of apoptosis, phosphatidylserine (PS) is redistributed from the inner to the outer leaflet of the cell membrane, ultimately leading to the formation of vesicular structures [47–49]. Notably, cells also release smaller apoptotic vesicles (100–1000 nm) during apoptosis [50]. These apoptotic vesicles not only carry specific apoptotic markers such as PS and Fas but also contain exosome markers such as CD63, suggesting a potential involvement of MVBs in their biogenesis [14].

Ectosomes

Ectosomes are produced through plasma membrane budding and blebbing [51]. There are multiple subtypes of ectosomes, including microvesicles [52], arrestin domain-containing protein 1-mediated microvesicles (ARMMs) [53], ciliary ectosomes [54], exophers [55], large oncosomes [56], and migrasomes [57].

Microvesicles are considered "standard" ectosomes, characterized by the expression of Annexin A1 and A2, and ranging in diameter from 150 to 1000 nm [52]. ARMMs, on the other hand, distinguish themselves from microvesicles as their budding process is guided by ARRDC1, a protein primarily localized on the cytoplasmic side of the plasma membrane. ARRDC1 initiates the budding of ARMMs towards the outer membrane by recruiting the ESCRT-I complex protein TSG101 to the cell surface through a tetrapeptide motif [53, 58]. Ciliary ectosomes, a distinct type of ectosome, are released from the plasma membrane of cilia [54]. Similar to Ciliary ectosomes, Filopodia-derived ectosomes originate from the finger-like membrane projections of cells, called filopodia, which are formed under oxidative stress. These EVs propagate oxidative stress and contribute to cell death, particularly targeting mitochondria. A novel mechanism involving two enzymes (neutral sphingomyelinase 2 and acid sphingomyelinase) that regulate ceramide production, linking filopodia-derived ectosomes secretion to neurodegenerative processes [59]. Migrasomes are recently identified vesicles that develop on the contraction fibers of migrating cells and may stem from migratory cytokinesis [57, 60]. Exophers, secreted from C. elegans neurons, are giant membrane vesicles (~4 μm in diameter) produced under neurotoxic stress. These vesicles act as a detoxification mechanism, expelling aggregated proteins (such as, polyQ aggregates) and damaged organelles, such as dysfunctional mitochondria, to protect neuronal health. When C. elegans neurons are exposed to proteotoxic stress or mitochondrial toxins, they shed exophers to prevent the accumulation of toxic components, thereby delaying neurodegeneration. This process highlights a conserved cellular strategy for managing stress in aging or disease contexts [61, 62].

These distinct ectosomes are closely associated with specific cell types and various physiological and pathological conditions. It is also becoming increasingly apparent that the biogenesis of EVs can intersect with cell migration, secretory autophagy, tumor cell-related secretion, and the stress response [14, 56, 60, 62].

Mitovesicles

Mitovesicles are a recently identified subtype of extracellular vesicles (EVs) of mitochondrial origin, characterized by a unique double-membraned structure. They were first discovered through high-resolution density gradient separation of EVs isolated from murine and human brains, particularly in the context of Down syndrome (DS) and diploid controls [37]. Unlike other EVs, such as exosomes or microvesicles, mitovesicles are directly derived from mitochondrial components. Their biogenesis involves the release of mitochondrial material into the extracellular space, potentially through processes linked to mitochondrial dysfunction and endolysosomal abnormalities in aging and neurodegenerative conditions. Notably, mitovesicles are distinct from other mitochondrial-derived vesicles (MDVs) due to their extracellular localization and specific membrane architecture [37, 63, 64].

Mitovesicles are implicated in the pathophysiology of neurodegenerative disorders, including AD and DS, which are characterized by mitochondrial dysfunction and metabolic deficits. In these conditions, mitovesicles exhibit altered abundance and cargo composition, reflecting underlying mitochondrial damage [63, 65]. For example, in DS brains, mitovesicles show dysregulated levels of mitochondrial proteins and enzymes, such as monoamine oxidases (MAO-A/B), which contribute to synaptic dysfunction. Experimental studies demonstrated that mitovesicles isolated from DS model mice impair long-term potentiation (LTP), a key mechanism of synaptic plasticity, and this effect is reversible with MAO inhibitors [37]. These findings suggest that mitovesicles

act as vehicles for propagating mitochondrial-derived toxic factors, exacerbating neuronal dysfunction.

NVEPs subtypes

While most research on cell-secreted EPs has focused on EVs, it has long been acknowledged that numerous NVEPs, consisting of proteins, RNA, lipids, and DNA, are not encapsulated by bilayer membrane structures and be released from cells [11, 13, 66, 67]. Examples of these include exomeres [68], supermeres [16], extracellular cuboidal particles [69], vaults, nucleosomes [13], lipoprotein particles (LPPs) [67], and T cell-derived supramolecular attack particles (SMAPs) [70].

Exomeres

Exomeres were first identified in 2018 as a subtype of NVEPs using AF4 [17]. In 2019, Zhang et al. refined their isolation from EV-depleted cell supernatants using sequential ultracentrifugation, confirming their size (~ 35 nm, < 50 nm) and absence of an external membrane structure under transmission electron microscopy [71]. Proteomic and lipidomic analyses further revealed that exomeres lack typical membrane-associated proteins (e.g., CD9, CD81, CD63) and lipids (e.g., phospholipids, sphingomyelin), distinguishing them from EVs [17, 71].

In cancer biology, tumor-derived exomeres act as mediators of systemic metabolic reprogramming. They deliver cargo, such as palmitic acid, which induces pro-inflammatory responses in hepatic Kupffer cells, leading to the development of fatty liver, suppressed fatty acid metabolism, and reduced drug metabolism [72]. Mechanistic studies indicate that exomeres are carriers of functional proteins like β-galactoside α2,6-sialyltransferase 1 (ST6Gal-I) and the epidermal growth factor receptor (EGFR) ligand amphiregulin (AREG). The transfer of these proteins to recipient cells results in significant cellular reprogramming. For instance, ST6Gal-I in exomeres hypersialylates cell-surface proteins such as β1-integrin, while AREG-containing exomeres prolong EGFR signaling, alter EGFR trafficking, and enhance the growth of tumor organoids [71]. These findings highlight the role of exomeres as active contributors to the modulation of cellular signaling and behavior, with important implications for both normal and pathological cellular processes.

Emerging evidence highlights the biological significance of exomeres in various physiological and pathological contexts. In the case of chronic methamphetamine use disorder (MUD), exomeres are shown to harbor EV-associated miR-29a, a microRNA critical in promoting inflammation and synaptodendritic injury. This miRNA cargo, enriched in the exomere pool, contributes to neuroinflammation and synaptic damage, underscoring the roles of exomeres in modulating neurodegenerative processes [73].

Overall, exomeres emerge as critical mediators in intercellular communication, influencing inflammation, metabolic reprogramming, and cellular signaling. Their unique cargo and biological effects establish their importance in both health and disease, offering potential therapeutic and diagnostic avenues for conditions ranging from neurodegeneration to cancer. However, the mechanisms underlying the biogenesis and cellular uptake of exomeres remain ambiguous at present [71, 74].

Supermeres

Recently, in exomere-depleted cell supernatants, Zhang et al. identified 25–35 nm NVEPs termed supermeres using ultra-high-speed centrifugation [16]. Supermeres exhibit distinct morphological and structural characteristics compared to exomeres in terms of size, structure, and density [32, 71]. DiFi-derived supermeres demonstrate a unique proteome profile with elevated levels of TGFBI and a range of disease-related proteins, including argonaute protein, APP, and its key cleavage enzyme, β-site APP cleaving enzyme 1 (BACE-1) [16]. Interestingly, supermeres can also incorporate extracellular RNAs (exRNA), such as miRNAs and snRNAs. These exRNAs exhibited different distribution patterns in DiFi-sourced exomeres and supermeres, with miRNAs being the predominant component in both types. Notably, supermeres exhibit higher RNA abundance, with the most abundant miR-1246 expressed at a level 1024-fold higher than in cells [14, 16, 75]. The discovery further highlights their role in extracellular signaling and stability. These RNAs are shielded from degradation by ribonucleases through protein complexes such as Argonaute 2 (AGO2) and ribosomal components, underscoring the molecular adaptability of supermeres in maintaining RNA functionality [16, 76]. Zhang et al. suggested the biogenesis of supermeres may associated with autophagy or phase separation processes. They are enriched with proteins involved in chaperone-mediated autophagy (such as, HSPA8, HSP90) and contain cargo with KFERQ motifs, which are targeted by autophagic mechanisms. Additionally, supermeres include phase separation-associated RNA-binding proteins and metabolic enzymes, suggesting phase separation plays a role in their biogenesis [14].

In vitro studies on cellular uptake kinetics revealed a notable slower uptake of supermeres compared to small EVs (sEVs). However, upon in vivo administration, supermeres showed higher in vivo uptake efficiency than both exomeres and sEVs. Moreover, supermeres were found to be capable of crossing the BBB, while exomeres displayed limited BBB penetration and brain absorption capabilitiens [16, 71]. Additionally, supermeres are highly enriched in circulating biomarkers, positioning them as potential diagnostic tools for CNS diseases. Their abundance in biofluids along with their disease-specific cargo,

provides a non-invasive pathway for the early detection and monitoring of conditions such as AD and other neurodegenerative disorders [16, 32, 75]. In summary, supermeres represent a unique class of extracellular nanoparticles with significant implications for CNS diseases. By serving as carriers of disease-relevant biomarkers and modulators of cellular processes, they offer new insights into intercellular communication in the CNS. Moreover, they hold promise as both diagnostic tools and therapeutic targets for neurodegenerative and other CNS-related diseases (Fig. 2).

Other NVEPs

Vaults are cargo delivery nanodevices (41 nm×72.5 nm) that self-assemble from peptides, showing extensive cellular distribution and high evolutionary conservation among eukaryotes [77]. Recent research has demonstrated that vaults can be actively released through amphiphilic pathways, in addition to their passive release from deceased cells [13, 78]. Nucleosomes, complexes composed of histones and DNA, are present in both eukaryotes and archaea. A recent study identified nucleosomes within the NVEPs fraction of cell supernatants [13]. Š Bálint et al. described a specific type of NVEPs (120 nm) termed SMAPs, released by cytotoxic T-lymphocytes, which are equipped with cytotoxic substances like perforins and granzymes. These particles, released upon immune activation, contain potent cytotoxic proteins that can directly bind to and eliminate target cells when discharged into the extracellular environments [70, 79].

In summary, as detection and isolation techniques advance, the heterogeneity of NVEPs is being increasingly acknowledged. Based on proteomic analysis of the cellular secretome, it is speculated that each cell could potentially secrete over 20 subtypes of EPs, suggesting that the complexity and heterogeneity of the EPs landscape may surpass the currently identified subtypes [13, 31, 80]. Importantly, this intricate EPs landscape may offer a detailed molecular "snapshot" of the body's cells, tissues, and systems, including the CNS. Additionally, the presence of unique EP subtypes in the CNS, such as mitovesicles and exophers, could provide insights into underlying pathologies by transporting specific molecular signals outside the CNS [31, 65, 81].

EPs as mediators of pathologic molecules in NDs

Pathological deposition of misfolded protein is a prevalent pathogenic mechanism in many NDs, including Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS). Importantly, these misfolded and aggregated proteins have the ability to propagate from one or more focal points to nearby neuroanatomical regions [82, 83]. EPs play pivotal roles in the transmission of misfolded proteins and the pathological progression of NDs [69, 84, 85]. Here, we will present several examples to elucidate the impact of EPs on the pathogenesis of NDs.

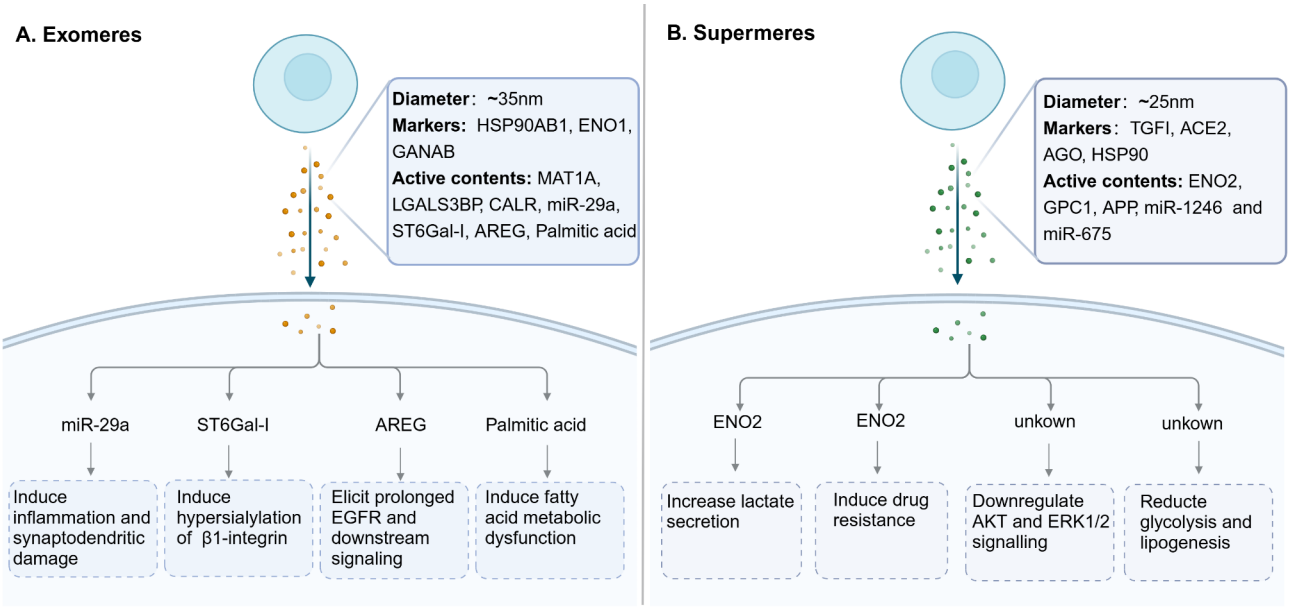

Fig. 2 Characteristics and biological functions of exomeres and supermeres. (**A**) Neuron-derived exomere induces neuroinflammatory responses by delivering miRNA-29a; Colorectal cancer (CRC) cell line DiFi-derived exomere promotes organoid growth by delivering β-galactoside α2,6-glycosyltransferase 1 (ST6Gal-I) and the epidermal growth factor receptor ligand amphoteric trypsin (AREG); Melanoma derived-exomere fatty acid metabolism in hepatocytes by delivering palmitic acid. (**B**) DiFi-derived supermere promotes secretion of lactate and drug resistance in tumor cells by delivering ENO1; DiFi-derived supermere affect the levels of liver lipids and glycogen via AKT and ERK1/2 pathways (Figure created with BioRender)

Alzheimer's disease

AD is a debilitating neurodegenerative disorder characterized by continuous and worsening deterioration. Pathologically, it is defined by the buildup of β-amyloid (Aβ) plaques in the cerebral cortex and subcortical grey matter, as well as development of neurofibrillary tangles resulting from excessive hyperphosphorylation of tau proteins. The accumulation of Aβ and presence of neurofibrillary tangles contribute to substantial synapses and neurons loss [86, 87].

Aβ peptides are generated through the proteolytic cleavage of APP, and the intracellular trafficking and processing of APP are closely associated with the endosomal vesicle cycle [88]. APP undergoes partial cleavage within endosomes, leading to the subsequent release of Aβ peptides from cells via exosomes [88, 89]. Similarly, recent findings demonstrate that tau filaments, primarily composed of truncated tau, are selectively packaged within EVs in the AD brain. These EVs, enriched in endo-lysosomal proteins, tether tau filaments to their limiting membranes through specific molecular interactions, facilitating their prion-like propagation [90, 91]. Meanwhile, EVs derived from AD brains, enriched in tau oligomers, preferentially target specific neuronal populations, such as interneurons, to propagate tau pathology and impair synaptic function [92]. Notably, the inhibition of exosome synthesis has been shown to effectively prevent this propagation [93]. Additionally, EVs facilitate the prion-like spread of Aβ among various brain cells, including astrocytes and microglia [94, 95]. Söllvander et al. discovered that microvesicles containing N-terminally truncated Aβ, derived from astrocytes, trigger apoptosis in cortical neurons [95]. These subtype-specific differences underscore the complexity of EV-mediated pathology in AD and present potential therapeutic targets to halt disease progression.

Significantly, NVEPs are enriched in unique protein and RNA cargo that distinguish them apart from EVs, making them a compelling focus for understanding their role in CNS pathophysiology. Supermeres demonstrate a high capacity for in vivo uptake and carry cargo associated with neurodegenerative diseases. They are notably enriched in APP, a central molecule implicated in AD pathology, suggesting their potential involvement in propagating and modulating disease processes in AD [16]. Additionally, recent observations in AD patients have identified extracellular cuboidal particles (a new NVEPs subtype) ranging from 70 to 200 nm within β-amyloid plaques, which are absent in tissues from non-demented individuals. These particles exhibit unique internal characteristics, including regularly spaced high-density striations with intervals of 2.5–2.8 nm. Researchers suggest that these particles may be associated with AD pathology or Aβ formation, and potentially representing a cellular response to amyloid proteins [69]. The discovery of supermeres and extracellular cuboidal particles introduces novel perspectives for comprehending NDs pathology and the process of Aβ formation (Fig. 3).

Parkinson's disease

Pathologically, PD is characterized by the degeneration of nigrostriatal dopaminergic neurons, a significant reduction in striatal dopamine levels, and the formation of abnormal α-synuclein (α-syn) protein aggregates known as Lewy bodies within neurons [96, 97]. The neuropathological changes involving oligomeric α-syn with neurotoxic properties typically progress throughout the brain in a specific and predictable manner, suggesting that the progression of PD is associated with the intercellular propagation of α-syn [97, 98].

The transmission of α-syn through EVs across multiple brain regions accelerates the progression of PD pathology. Both cerebrospinal fluid (CSF)-derived and blood-derived EVs from PD patients have been identified to carry significant amounts of α-syn [99, 100]. The reduction in intracellular degradation of α-syn due to lysosomal dysfunction has been proposed as a potential mechanism for the cellular release of α-syn through EVs [101]. Additionally, abnormal autophagy in neuron induced by PD-associated stress is likely to also involve the release of α-syn. For example, a study identified an enrichment of α-syn-containing autophagosomes in CSF-derived EVs from PD patients [102]. Moreover, α-syn was also released in a calcium-dependent manner via externalization from EVs, suggesting a nonclassical secretory pathway for α-syn [103]. Microglia-derived EVs have been demonstrated to induce neuronal α-syn aggregation and facilitate the progression of AD pathology [104]. Furthermore, pathological EPs influence neuroinflammation and immune responses. For example, Blood-EVs from PD patients exacerbate the intense inflammatory activation of monocytes and resting microglia induced by pathological α-syn [105, 106] (Fig. 4).

EPs as bimarkers in NDs

Bulk particles analysis

In the early stages of neurodegenerative diseases, subtypes of EPs carrying pathologic molecules may play a significant role in facilitating communication between lesion and healthy cells. Additionally, several specific subpopulations of EPs, such as supermere, appear to readily cross the BBB [16]. In the context of neurodegenerative diseases such as AD and PD, this heterogeneity and availability provides a valuable opportunity for screening and diagnosis of the disease [107]. Proteomics, RNAomics, and metabolomics of bulk EPs have greatly enhanced our understanding of the alterations of EPs during NDs and

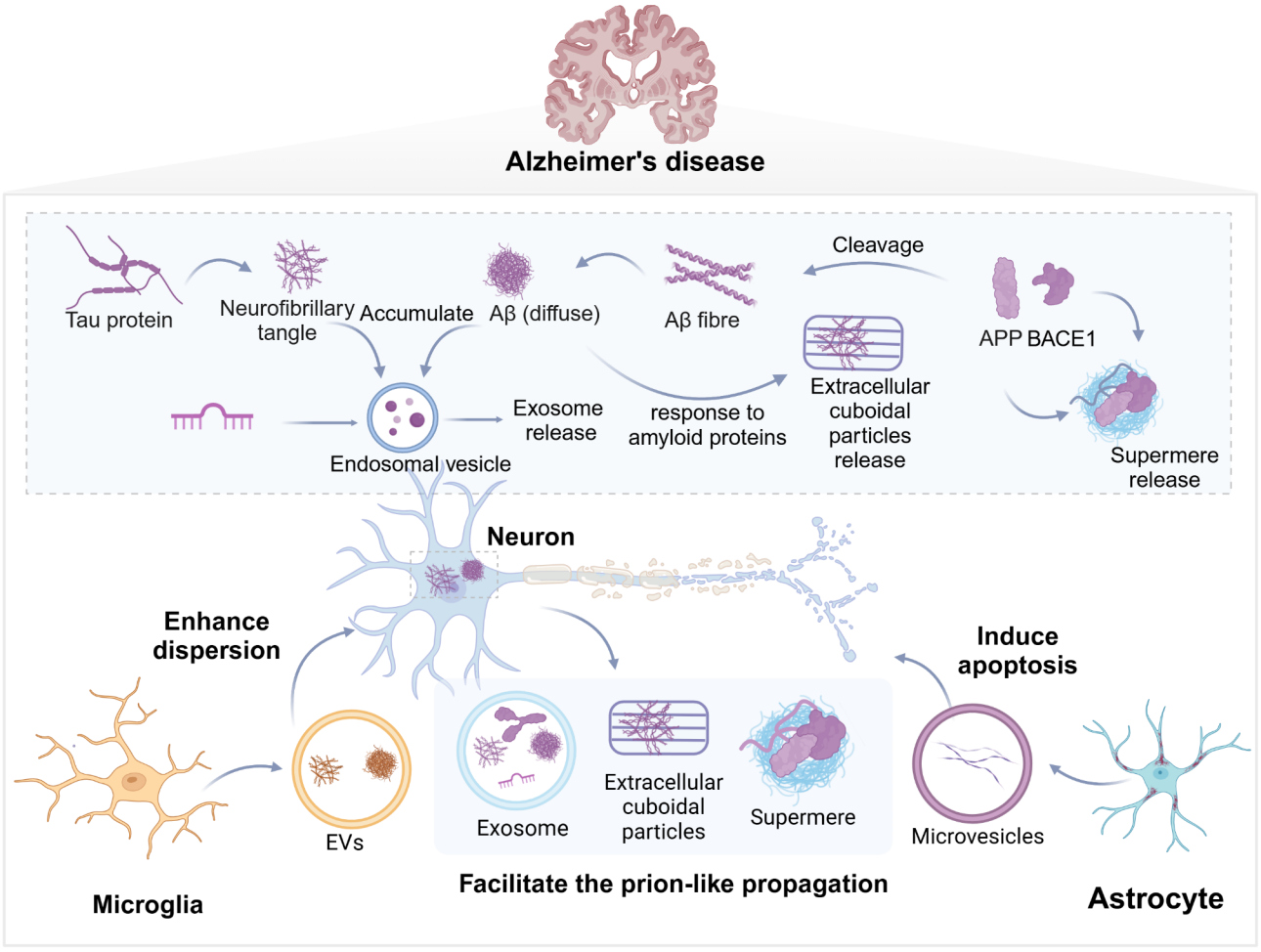

Fig. 3 EPs as novel actors for intercellular communication and potential diagnostic tool in Alzheimer's disease(AD). In the pathological conditions of AD, neuronal, astrocyte and microglia-derived EVs and NVEPs can accelerate the spread and aggregation of pathological proteins such as Aβ and Tau. Additionally, the portion of EPs that crosses the blood-brain barrier can be used as a potential biomarker for AD diagnosis. APP: Amyloid precursor protein; BACE1: β-Site APP-cleaving enzyme; APLP2: Amyloid Beta Precursor Like Protein 2 (Figure created with BioRender)

have advanced the development of EPs as potential diagnostic markers [108–110].

For instance, brain-derived EVs show upregulated the levels of disease-associated miRNAs, correlating with peripheral EV profiles, suggesting their potential as "liquid biopsies" for the early AD detection [111]. Additionally, longitudinal analyses of plasma neuronal-enrichedenriched EVs (elevated levels of p-tau, Aβ42, and pIRS-1) have demonstrated their predictive value for preclinical AD diagnosis, showcasing high accuracy and clinical relevance [112]. Proteomic studies also identified novel EV-associated proteins, such as ANXA5 and VGF, expanding our understanding of EV-mediated mechanisms in AD [113]. Moreover, astrocyte-derived EVs carrying α-synuclein significantly increase in PD patients, with their levels correlating with lysosomal dysfunction. These α-synuclein-containing EVs have also shown strong diagnostic capabilities in differentiating PD from other α-synucleinopathies [114, 115].

Single-particle analysis

As previously mentioned, the heterogeneity of EPs and the complexity of the humoral components present significant challenges for EPs-based diagnosis [116]. Recent advancements in single-particle technology have greatly enhanced our ability to analyze EPs at a detailed level, particularly in the context of NDs. By overcoming the limitations of bulk EV analysis, such as the loss of subtle molecular differences among vesicle populations, single-vesicle techniques can accurately identify specific EV subpopulations carrying disease-relevant biomarkers [44, 117]. This enhanced granularity aligns with the multifactorial and highly individualized nature of neurodegenerative diseases, offering a more nuanced approach to diagnostics and personalized medicine.

Nanoscale flow cytometry and enhanced plasmonic assays have significantly improved the sensitivity and specificity of EV biomarker detection, allowing for differentiation among AD, mild cognitive impairment (MCI),

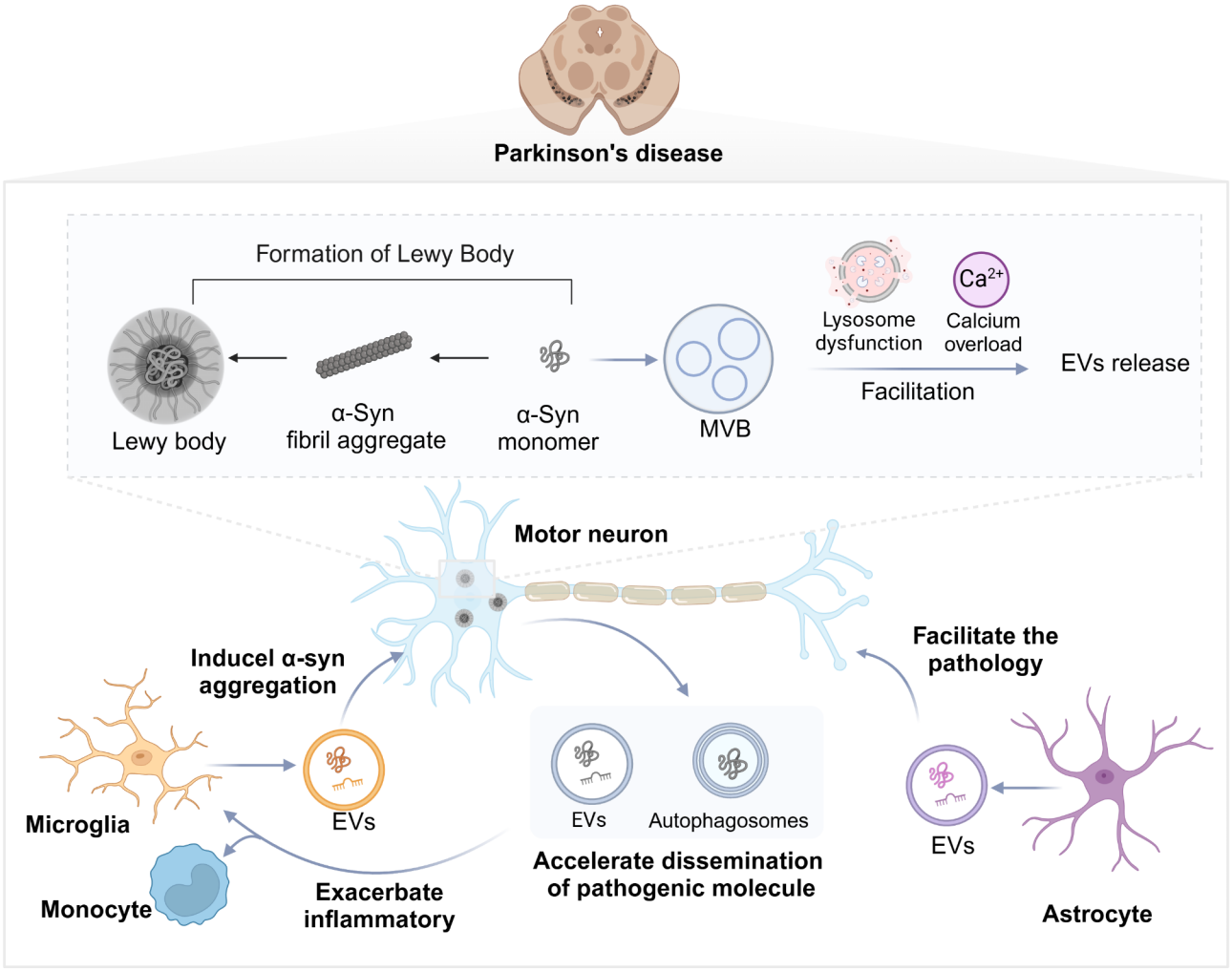

Fig. 4 EPs as novel actors for intercellular communication and potential diagnostic tool in Parkinson's disease (PD). In the pathological conditions of PD, damaged motor neurons accelerate microglial and macrophage inflammation through EPs, and microglial or astroglia-derived EPs induce aggregation of α-syn, accelerating disease progression. Additionally, the portion of EPs that crosses the blood-brain barrier can be used as a potential biomarker for PD diagnosis. (Figure created with BioRender)

and healthy control. AD plasma EVs show elevated levels of phosphorylated tau proteins (p-TS235, p-TS396, p-TS404) and Aβ42 compared to MCI. These biomarkers, particularly when combined (such as, p-tauS235 with Aβ42), enable high diagnostic accuracy (AUC 0.989) for distinguishing AD from healthy controls using nanoscale flow cytometry (nFC) without requiring EV isolation [118]. Additionally, the proximity barcoding assay (PBA) provides a transformative approach to AD diagnosis by enabling multiplexed profiling of single-EV surface proteins from noninvasive biofluids like urine. This technique has identified 183 proteins and highlighted specific urinary EV subpopulations, such as those marked by PLAU, ITGAX, and ANXA1, achieving an impressive 88% diagnostic accuracy. PBA integrates seamlessly with machine learning, enhancing precision and scalability while uncovering disease-specific EV signatures crucial for early detection. Its noninvasive nature, high reproducibility, and potential for clinical application highlight its an importance in advancing biomarker-based diagnostics for AD and other neurodegenerative diseases [110]. Moreover, the development of the EV identification and detection biochip (EVID-biochip) enables efficient isolation and quantification of L1CAM-positive neuronal EVs from serum, providing a rapid and minimally invasive platform with high diagnostic accuracy (AUC = 0.973) for differentiating PD from healthy controls [119]. Complementary to these findings, new vesicle analysis tool and novel imaging technologies, including deep-learning algorithms and super-resolution microscope (SRM), are contributing to a more comprehensive understanding of the heterogeneous progression of PD, paving the way for targeted interventions and precise patient stratification [45, 120](Fig. 5).

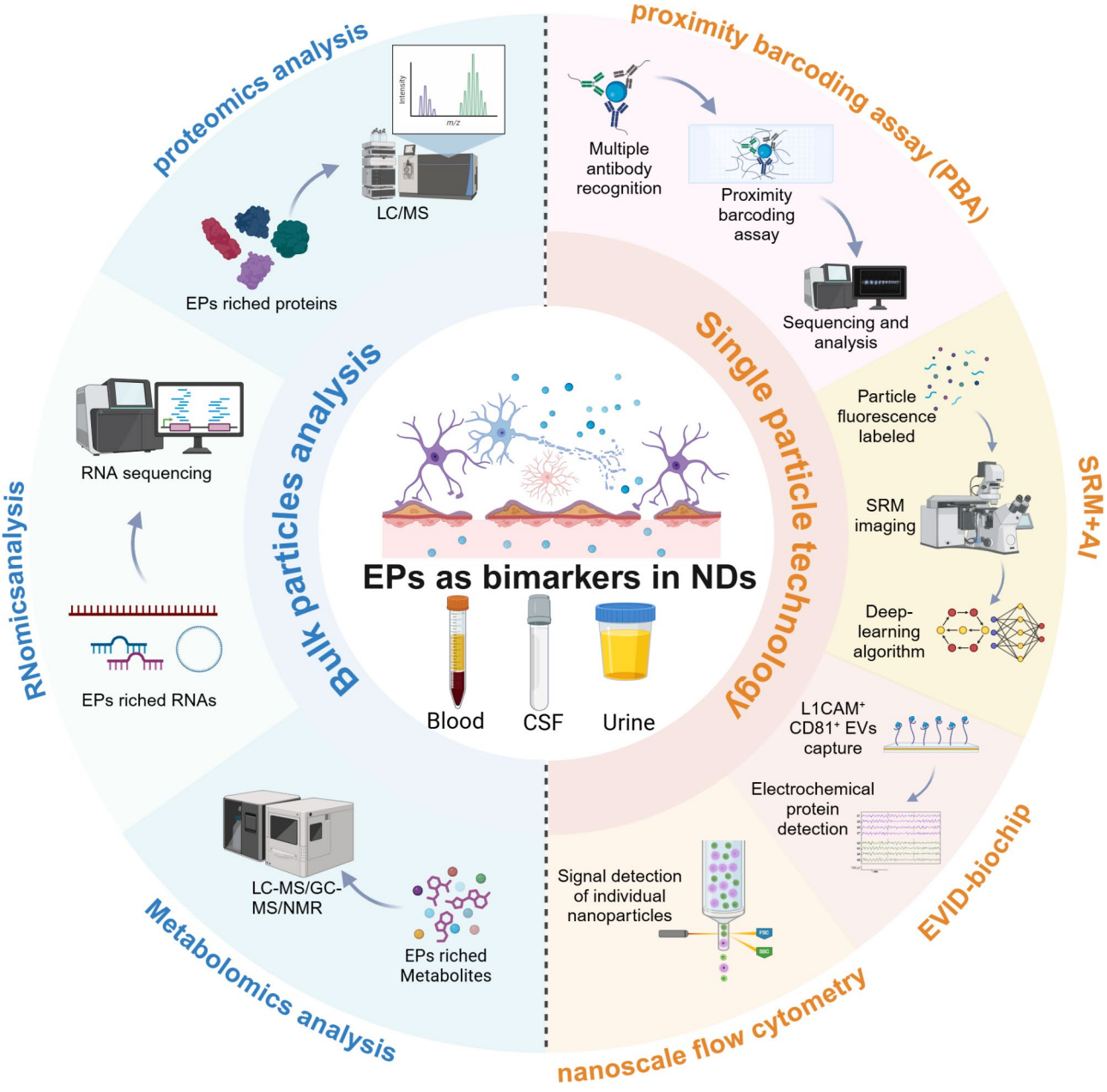

Fig. 5 Representative techniques for the identification of biomarkers associated with EPs in Central Nervous System diseases. Bulk particles analysis techniques include proteomics, RNomics, and Metabolomics analysis. Single particle technology include proximity barcoding assay (PBA), Super-resolution microscope (SRM) integrated with artificial intelligence (AI), EV identification and detection biochip (EVID-biochip) and nanoscale flow cytometry (Figure created with BioRender)

To date, over ten single-particle technologies have been developed, resulting in a significant increase in single vesicle technologies in recent years [44]. Innovative platforms such as HaloTag-based quantitative platforms system and CRISPR-assisted barcoding enable detailed analysis of subpopulation, thereby enhancing our understanding of EV heterogeneity and biogenesis [121, 122]. Advancements like catch and display for liquid biopsy (CAD-LB), charge-based fractionation methods for EPs, and photosensitive nanoprobes facilitate the rapid, high-purity isolation and characterization of EVs, effectively addressing the challenges related to scalability and clinical applicability [123–125]. However, these methods also face limitations, including high costs, technical complexity, and a lack of standardization across platforms, which hinder their widespread adoption. Additionally, the throughput of single-vesicle analysis remains limited compared to bulk approaches, posing challenges for large-scale clinical studies [44, 126]. Despite these obstacles, ongoing advancements in bioinformatics,

automation, and integration with complementary techniques promise to address these limitations, paving the way for single-vesicle technologies to transform the diagnosis and management of neurodegenerative diseases.

Double-edged sword role of EPs in CNS injury

Acute CNS injury, such as stroke, traumatic brain injury (TBI) and spinal cord injury (SCI), leads to extensive cell death, triggering inflammation and secondary injury. EPs are involved in various stages of the injury process, including propagating inflammation, mediating neuroprotection, and regulating systemic metabolism [127, 128]. This section comprehensively examines the evidence for EP involvement in the pathogenesis of CNS injury, specifically focusing on the different subtypes of EPs.

Traumatic brain injury

During the acute phase of TBI, direct brain tissue damage, such as crush or laceration, can rapidly impair brain function. Subsequently, a cascade of injury reactions further exacerbates the damage [129, 130]. Ischemia and edema may trigger various secondary injury mechanisms, including the release of excitatory neurotransmitters, intracellular calcium influx, production of free radicals, and cytokine release. These processes lead to additional cellular damage, increased edema, and elevated intracranial pressure [131, 132]. Additionally, both centrally resident and peripheral immune cells swiftly sterile immune responses rapidly in response to TBI [132].

After severe TBI, an increased quantity of EVs, including microvesicles and exosomes, are observed in human CSF. Secondary damage from TBI also influences the characteristics of microglia- and astrocyte-derived EVs [133, 134]. Cerebral hemorrhage and edema induce astrocyte-derived EVs rich in miR-143-3p, which are transported into brain microvascular endothelial cells (BMECs). miR-143-3p directly targets ATP6V1A, leading to impaired lysosomal hydrolysis, reduced autophagic degradation of cell adhesion molecules (CAMs), and inhibited vascular remodeling [127]. Additionally, activated microglia secrete EVs that inhibit neurite growth and synaptic recovery during the acute phase of TBI, a process mediated by EV-derived miR-5121 [135].

In addition to their presence in the CNS, EVs in the peripheral circulatory system and other biological fluids are altered after TBI and contribute to systemic complications. Evidence shows a rapid increase in serum concentrations of EVs in TBI patients [136]. For example, a study suggests that the expression of inflammatory factor in salivary exosomes serves as a potential diagnostic marker for TBI [137]. Another study reported a significant presence of EVs with high plasma levels of high mobility group box 1 (HMGB1) protein in TBI patients, which can induce endothelial dysfunction by activating endothelial cell pyroptosis [138]. Additionally, brain-derived sEVs and microvesicles have been shown to activate the coagulation cascade and inflammatory cells, such as platelets and leukocytes, leading to systemic coagulation dysfunction and inflammation [139]. Moreover, the circulating microvesicles from TBI patients promotes coagulation dysfunction and affect prognosis [138, 140]. Furthermore, the BMECs contribute to vascular remodeling by releasing microvesicles containing tight junction proteins and endothelial markers. Researchers have found that this shedding vesicle assay may provide a novel approach for real-time monitoring of cerebrovascular health, BBB status, and neuroinflammation following TBI events [140].

These findings suggest that brain-derived exosomes and microvesicles can cross the BBB and facilitate communication between the central system and other systemic bodily fluids in the context of TBI pathology [140, 141]. However, additional research is necessary determine the specific cellular origins of these brain-derived EV subtypes [142, 143]. Notably, extracellular mitochondria have been found in brain-derived microvesicles following TBI, indicating that EV subtypes containing mitochondria may play a role in the pathological progression of TBI [141, 144]. Further exploration of this hypothesis in animal models of CNS injury would be beneficial (Fig. 6).

Stroke

EVs have been recognized as crucial participants in the complex pathophysiology of stroke, playing roles in cell-to-cell communication, immune regulation, and tissue repair. These vesicles, including exosomes and microvesicles, transport a wide range of biomolecules such as proteins, RNAs, and lipids, enabling the transfer of functional signals between cells [128, 145]. In the context of stroke, which is characterized by ischemic conditions leading to neuronal death and tissue damage, EVs derived from different brain cells (such as neurons, astrocytes, microglia, and endothelial cells) exhibit distinct functions that collectively influence stroke outcomes [146–149].

One of the key roles of EVs is to facilitate intercellular communication and repair mechanisms following a stroke. For instance, astrocyte-derived EVs have been demonstrated to promote axonal repair and improve functional recovery, highlighting their therapeutic potential [148]. Microvesicles released from brain endothelial cells can transfer mitochondria to recipient cells, significantly enhancing mitochondrial function and ATP production, which are crucial for cell survival under ischemic conditions [147]. Additionally, microglial EVs contribute to modulating immune responses, preventing immune cell senescence, and promoting oligodendrogenesis, which aids in myelin repair and functional recovery

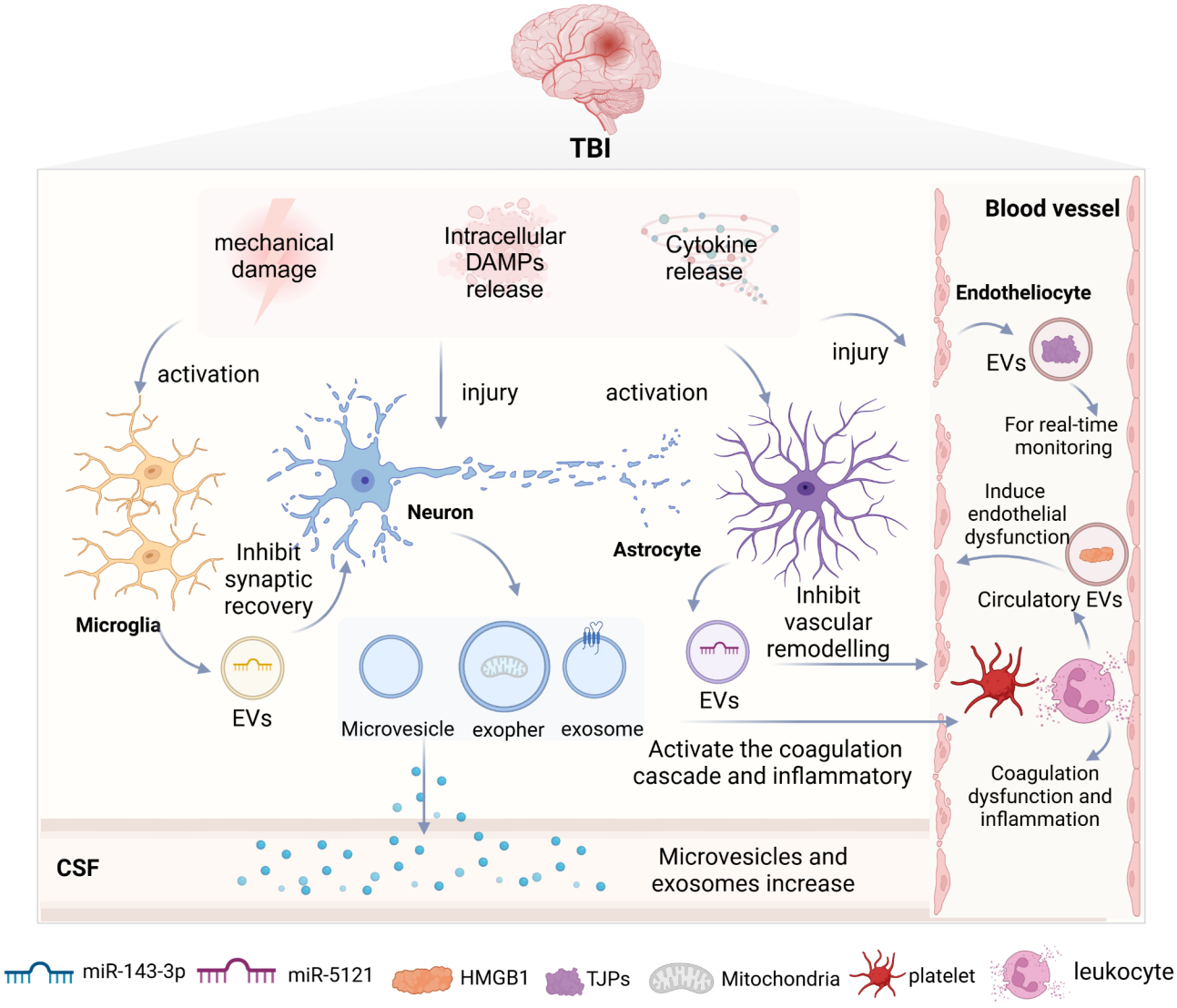

Fig. 6 EPs contribute to local and systemic crosstalk after traumatic brain injury (TBI). After TBI, the EPs released from activated microglia and astrocytes inhibit nerve function recovery and vascular remodeling. Brain tissue derived EPs at the site of injury can also activate systemic inflammation, while circulatory EPs induce dysfunction of vascular endothelial cells. HMGB1: High mobility group box-1 protein; TJPs: tight junction proteins; CSF: cerebrospinal fluid (Figure created with BioRender)

[150]. These findings emphasize the ability of EVs to impact cellular processes that are essential for recovery following stroke.

Moreover, changes in the heterogeneity of EVs and the composition dynamics of their cago also affect the fate of neurons during a stroke. Following ischemic events, there is a shift in the origin of EVs, for example, astrocytes emerge as the primary generators of EVs after a stroke, taking over from microglia, which are the main source under normal circumstances. This shift is accompanied by changes in EVs content, such as increased levels of prion protein, influencing their uptake by neurons and glial cells [146, 149]. Additonally, Hypoxia induces neurons to secrete EV carrying specific miRNA (such as, miR-21a-5p) that may serve as biomarkers of stroke severity and recovery progression [151]. Moreover, proteins like fused in sarcoma (FUS) protein facilitate the selective packaging of circular RNAs into neuron-derived-EVs under hypoxic conditions, ensuring the delivery of functional RNAs to target cells [152]. These insights into the mechanisms and heterogeneity of EVs underscore their potential as both therapeutic agents and biomarkers in stroke management, opening up avenues for innovative interventions to enhance recovery and minimize damage (Fig. 7).

EPs as delivery vehicles for CNS diseases

EPs, have inherent advantages over other emerging CNS drug delivery platforms such as liposomes, Viral Vectors, and Micelles, with biocompatibility and minimal

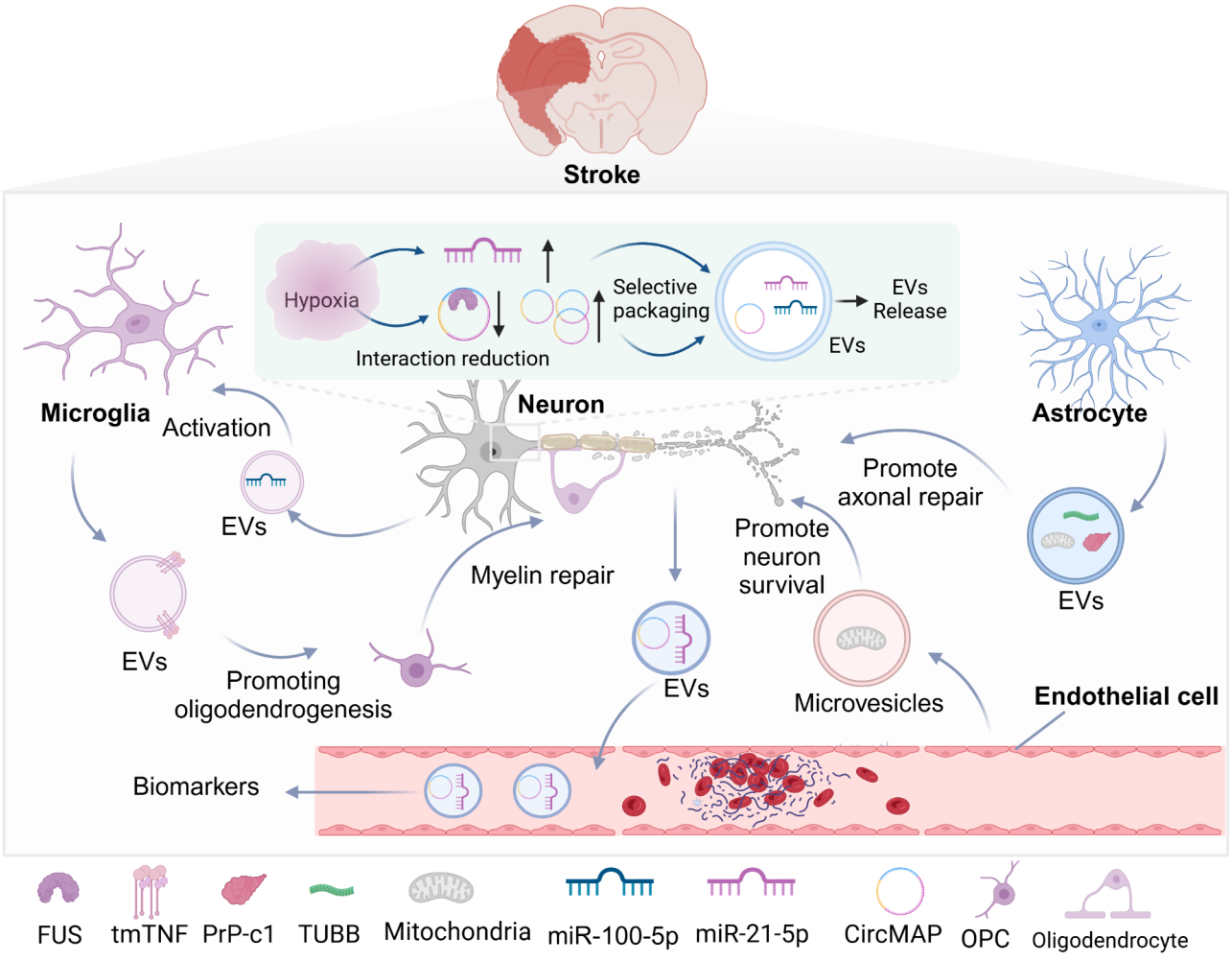

Fig. 7 EPs contribute to local and systemic crosstalk after Stroke. Neuron-derived EVs selectively enrich specific non-coding RNAs under hypoxia induction, and these EVs may serve as diagnostic biomarkers. Additionally, reactive microglia and astrocytes promote OPC differentiation and axon repair through EVs. Similarly, endothelium-derived microvesicles can also protect neurons from survival after stroke. MVB: multivesicular bodies; OPC: oligodendrocyte precursor cell; FUS: fused in sarcoma; tmTNF: transmembrane tumor necrosis factor; PrP-c1: prion protein C1 fragment; TUBB: β-tubulin (Figure created with BioRender)

immunogenicity (Table 1). These properties make EPs drug delivery vehicles and therapeutic agents for therapeutic drugs for neurological disorders, including AD, PD, TBI, and SCI [20, 153]. Based on their innate advantages, researchers are refining EP engineering strategies to optimize their therapeutic potential, thereby increasing the precision and efficacy [154, 155]. Currently, techniques for modifying EPs include therapeutic cargo piggybacking and surface modification [156, 157]. In this section, we will delve into emerging technologies for engineered EVs and vault particles and discuss their current applications in therapeutic delivery platforms for CNS disorders (Table 2).

Engineered EVs

EVs cargo loading

As previously discussed, EVs inherently transport various therapeutic cargoes derived from parental cells. However, the assortment of cargoes within natural EVs is diverse, yet the quantity of therapeutic cargoes is constrained [180]. Therefore, to further enhance the therapeutic potential of EVs for neurological disorders, genetic manipulation of parental cells and post-purification physical methods have been applied to encapsulate endogenous or exogenous molecules (such as nucleic acids, proteins, or small molecule drugs) into the interior of EVs [181–183].

To our knowledge, the first reported molecular delivery vehicles for receptor cell therapy were exogenous siRNA piggybacked EVs [184]. Zhang et al. loaded purified exosomes with exogenous BACE1-siRNA (a therapeutic

Table 1 Comparison of CNS drug delivery systems

Delivery System	BBB Penetration Mechanism	Targeting Precision	Key Advantages	Key Limitations	Ref.
Extracellular Particles (EPs)	●●●●○ (Native transcytosis)	Engineering-based (e.g., RVG peptide)	→ Optimal biocompatibility → Endogenous immune evasion → Native drug protection capabilities	→ Complex isolation protocols → High batch-to-batch heterogeneity	[116]
Liposomes	●●○○○ (Surfactant-enhanced)	Ligand-mediated (e.g., TfR antibody coating)	→ High drug-loading capacity (≥ 70%) → Customizable surface functionalization	→ Rapid clearance due to serum protein adsorption → Risk of oxidative degradation	[158]
Viral Vectors	●●○○○ (Serotype-dependent)	Transcriptional targeting (Tissue-specific promoters)	→ Long-term gene expression (months to years) → High transfection efficiency (> 90% in vivo)	→ Neutralizing antibodies limit redosing → Insertional mutagenesis risks	[159]
Polymeric NPs (PLGA)	●●●●○ (PEGylation-dependent)	Active targeting (e.g., lactoferrin conjugation)	→ Controlled release (1–4 weeks sustained release) → High physical stability (> 6 months storage)	→ Inflammatory response to degradation byproducts → Low ligand conjugation efficiency	[160]
Micelles	●●●●○ (Membrane translocation)	Charge-driven (Non-specific adsorption)	→ Rapid cytoplasmic delivery (minutes) → No cargo size limits (> 500 kDa)	→ Rapid plasma protease degradation → Non-specific accumulation in liver/kidneys	[161]

target for AD) using electroporation at 400 V and 125 μF, achieving a loading efficiency of approximately 50% for the siRNA [185]. In addition to nucleic acid cago, exogenous therapeutic proteins can also be directly loaded into the lumen of EVs. Catalase, an antioxidant protein, was loaded into EVs via sonication, demonstrating important neuroprotective effects in both in vitro and in vivo models of PD [182]. Moreover, specific exogenous small molecule drugs such as resveratrol can be encapsulated into EVs through sonication or incubation, allowing for targeted delivery to the brain and providing relief from neuroinflammation [177]. However, these physical methods may lead to exosome aggregations and disruption of exosome membrane structure, potentially necessitating excessive purification steps.

Another effective method for loading cargo into EVs involves genetic engineering of the parental cells. This approach enables the production of modified EVs without compromising their functionality or integrity. For example, macrophages transfected with GDNF overexpression plasmid secreted GDNF-enriched EVs, which enhanced neuronal survival and reduced neuroinflammation in the brains mouse models of PD [186]. Similarly, MSCs overexpressing tyrosine phosphatase-2 (SHP2) were capable of secreting SHP2-containing EVs that significantly induced mitochondrial autophagy in neuronal cells, thereby attenuating mitochondrial damage-mediated apoptosis and NLRP3 inflammasome activation in a rat model of AD [183]. In addition to protein loading, researchers have also focused on loading diverse RNA species into EVs. For example, Yang et al. engineered HEK293T cells to overexpress circRNA-SCMH1, a therapeutic molecule for stroke, using an overexpression plasmid. They then harvested EVs enriched for circRNA-SCMH1. These engineered CircSCMH1 EVs were able to promote functional recovery in ischaemic stroke models of rodent and non-human primates [173]. While the genetically engineered approach avoids the additional processing steps required to load the desired cargo into the EV after separation, it still has limitations in terms of loading efficiency and cost. Fortunately, several emerging technologies are being developed for cargo piggybacking of EVs, such as EXPLORs (a technology for prompting protein loading into exosomes through photoreversible protein interactions), IDEA (an intracellular protein delivery platform using EVs as carriers) and MAPLEX (a photoinducible cargo protein release system for engineered exosome) [187–189]. The development of these new technologies holds promise for enhancing the potential of EVs as therapeutic vectors in CNS diseases.

EVs surface modification

Natural EVs and specific EPs have been identified to traverse the BBB following intravenous or nasal delivery. However, recent research indicates that only a limited quantity of EVs actually reach the site of CNS [190]. By incorporating targeted peptide modifications on the membrane surface of EVs, these engineered vesicles can significantly enhance their BBB-crossing efficiency, thereby improving their accumulation within the CNS system [20, 28].

Currently, the most commonly utilized molecule for modifying the membrane of EVs to target the CNS system is rabies virus glycoprotein (RVG), which binds to the nicotinic acetylcholine receptor (nAChR), thereby selectively targeting neuronal cells and brain microvascular endothelial cells [191]. Importantly, amino acid modification of the key region of the RVG protein that binds to

Table 2 EPs for drug delivery and therapy in CNS diseases

Disease	EVs source	Surface modification	Cargo	Therapeutic effect	Ref.
Alzheimer's disease	MSCs	/	Undefined	Inhibit microglia activation & increase synaptic density	[162]
Alzheimer's disease	Dendritic cells	RVG (rabies virus glycoprotein)	BACE1-siRNA	Knockdown BACE1 in neurons	[163]
Alzheimer's disease	HT22 neurons	Fe65	Corynoxine-B	Induce autophagy in APP neurons	[164]
Parkinson's disease	Dendritic cells	RVG	SNCA-siRNA and curcumin	Clear α-synuclein aggregates	[165]
Parkinson's disease	Adipose-derived stem cells	Dopamine	Undefined	Rescue dopaminergic neurons	[155]
Traumatic brain injury	Microglia	RVG	NR2B9c (neuroprotective peptide)	Improve behavior & reduce lesions	[166]
Traumatic brain injury	MSCs	/	MiRNA–654–3p et al.	Inhibit NLRP3-p38 signaling	[167]
Traumatic brain injury	MSCs	/	MiR–124–3p	Reduce glutamate excitotoxicity	[168]
Spinal cord injury	$CD163^+$ macrophage	RGD (arginine-glycine-aspartic acid)	TGF-β	Enhance vascular regeneration	[169]
Spinal cord injury	iNSCs	CAQK peptide	CCL2-siRNA	Limit inflammation damage	[170]
Spinal cord injury	$CD146^+CD271^+$ MSCs	RGD	MiR–501–5p	Preserve blood-spinal cord barrier	[171]
ALS	iPSCs-induced MSCs	/	Undefined	Alleviate motoneuron related pathological changes and neuroinflammation	[172]
Stroke	HEK293T cells	RVG	CircSCMH1	Enhance neuroplasticity & inhibite glial reactivity	[173]
Stroke	MSCs	MAP	α-mangostin	Enhance neuroprotective activity with the synergistic effect of apoptotic vesicle and α-mangostin	[174]
Stroke	Macrophage	/	Heptapeptide	Alleviate mitochondria-mediated neuronal damage	[175]
Stroke	M2 microglia	RVG-^{125}I/SPIO-PDA	MiR-221-3p & miR-423-3p	Reduce neuronal apoptosis & enable the dynamic visualization	[176]
Multiple sclerosis	Macrophage	Sialic acid analogues	Resveratrol	Inhibite inflammatory responses through targeting microglia	[177]
Multiple sclerosis	Microglia,	Mfg-e8	IL-4	Alleviate neuroinflammation	[178]
Machado-Joseph disease	Livers self-assembly	RVG	mATXN3-siRNA	Inhibite the expression of mATXN3 protein in neurons	[179]

nAChR produces the neuronal cell-specific, low-immunoreactive RVG-derived peptide, RVG29-9R [192]. For instance, Haroon et al. engineered RVG29 membrane-modified exosomes for delivering the neuroprotective peptide NR2B9c and for treating TBI using bioorthogonal clickchemistry [166]. In another study, octadecyl chain-modified RVG29aa was embedded in exosomal phospholipid bilayer membrane structures under ultrasonic vibration, forming a brain-targeted drug delivery platform [165]. Furthermore, the membrane molecules of exosomes can be genetically modified to generate membrane-modified exosomes without affecting their structure. For example, exosome surface lysosome-associated membrane glycoprotein 2b (Lamp2b) can be utilized for RVG fusion and subsequent surface functionalization [163].

Modifying EV surface with RGD peptide (binding to integrins) could also lead to targeted therapy for neovascular endothelial cells in CNS injury [169, 193]. Intravenous administration of RGD-Lamp2b-sEVs isolated from $CD163^+$ macrophage effectively transmit TGF-β to neovascular endothelial cells, promoting regeneration and stabilisation of blood vessels at the site of SCI injury and reducing side effects [169]. Other studies have also demonstrated that the expression of specific ligands binding to other receptors on exosome surface proteins, such as the IKVAV peptide [194], Matrix metalloproteinase-activatable cell-penetrating peptid (MAP), and CAQK [170], enhances their ability to target the CNS. Futhermore, dopamine-conjugated on EVs surface (Dopa-EVs) represent a cutting-edge advancement in the design of specific neuron-targeted therapies, particularly for PD. By leveraging the natural affinity of dopamine for dopaminergic receptors, Dopa-EVs achieve selective targeting of dopaminergic neurons, a critical cell population affected in PD. These vesicles have demonstrated the

ability to induce autophagy, reducing protein aggregation and oxidative stress while promoting neuroprotection and cellular repair [155]. These advancements mark a significant step toward developing neuron-specific therapeutics for NDs (Fig. 8).

Engineered NVEPs

Vault particles, the earliest discovered subtype of NVEPs, are recognized as versatile and innovative drug delivery platforms due to their robust structure, biocompatibility, and lack of immunogenicity. The inherent ability to encapsulate a variety of therapeutic agents, including proteins, nucleic acids, and small molecules, with their chemical modifications makes the development of engineered vault promising [195]. Recent innovations, such as INT-labeled cargo protein technology, have optimized production workflows, enabling efficient large-scale manufacturing [196]. Additionally, Ding et al. demonstrate that engineered vault particles with HIV-1 Gag fragments enhances their internal flexibility, which improves cargo loading and release efficiency [197]. Structural modifications of the vault for controlled disassembly and symmetric disintegration allow for precise spatiotemporal drug delivery, thereby greatly increasing its therapeutic precision [66, 197].

Advances in genetic engineering have expanded the functional versatility of vault particles. For instance, modifying the C-terminal domain of the Major Vault Protein (MVP) permits the attachment of targeting ligands like epidermal growth factor (EGF)-binding peptides, enabling receptor-specific delivery to epithelial cancer cells [198]. Additionally, chemical functionalization strategies for Vault, such as disulfide exchange and nucleophilic substitution, can facilitate the attachment of imaging probes and cell-penetrating peptides to lysine or cysteine residues on the vault surface. For example, Benner et al. improved the archtop modification strategy for targeted delivery and intracellular tracking by converting lysine residues to thiol-terminated side chains using Traut's reagents [199]. Moreover, surface modifications with neuronal receptor-targeting ligands also show potential for crossing BBB, offering novel avenues for treating gliomas and neurodegenerative disorders [200]. In addition to vault particles, other NVEP subpopulations derived from specialized cell types exhibit therapeutic potential for CNS disorders, as their capability

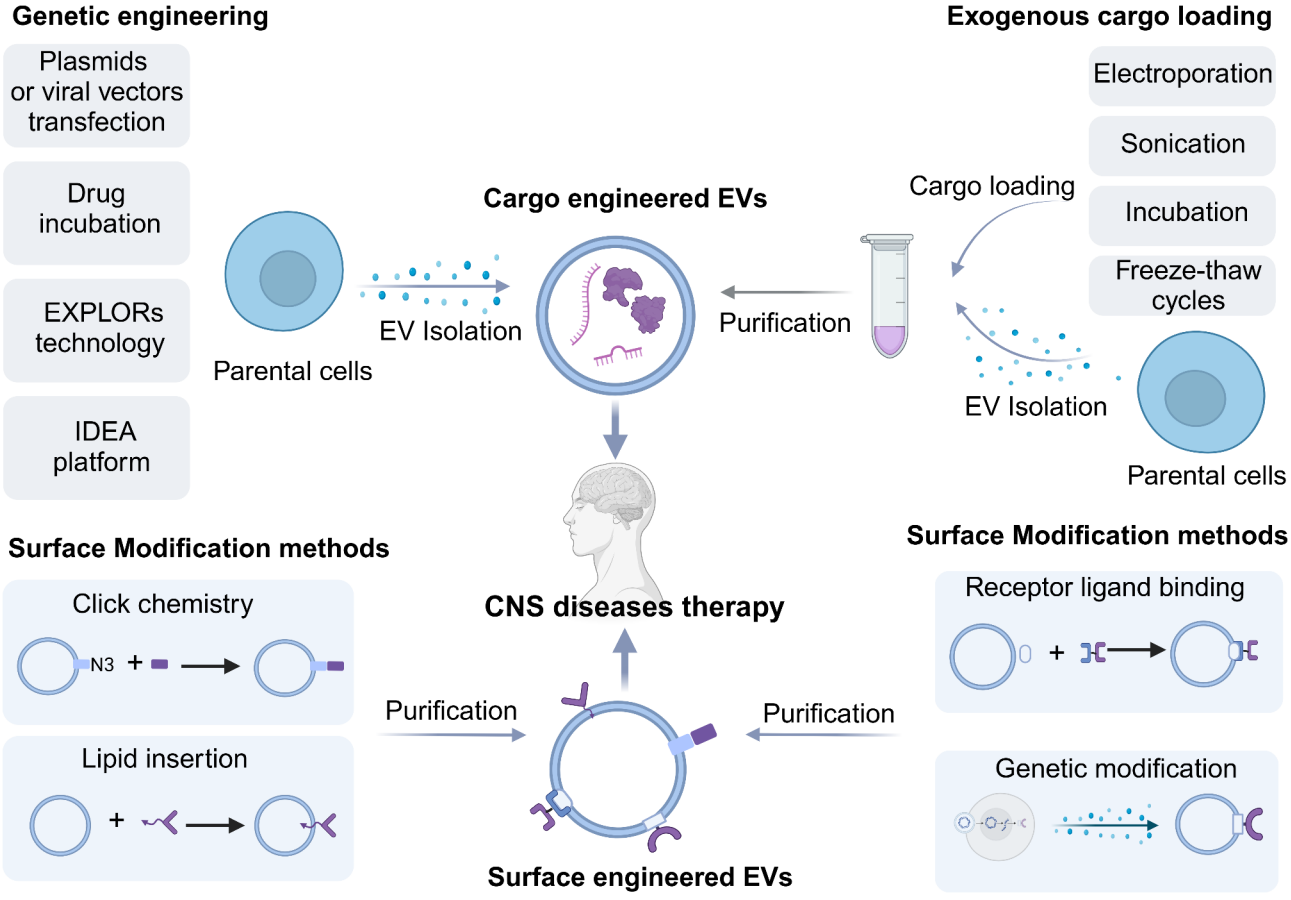

Fig. 8 Engineered EVs for the treatment of CNS disease. EVs cargo loading strategies include genetic engineering and exogenous cargo loading. Strategies for surface modification include genetic modification, click chemistry, and lipid insertion. (Figure created with BioRender)

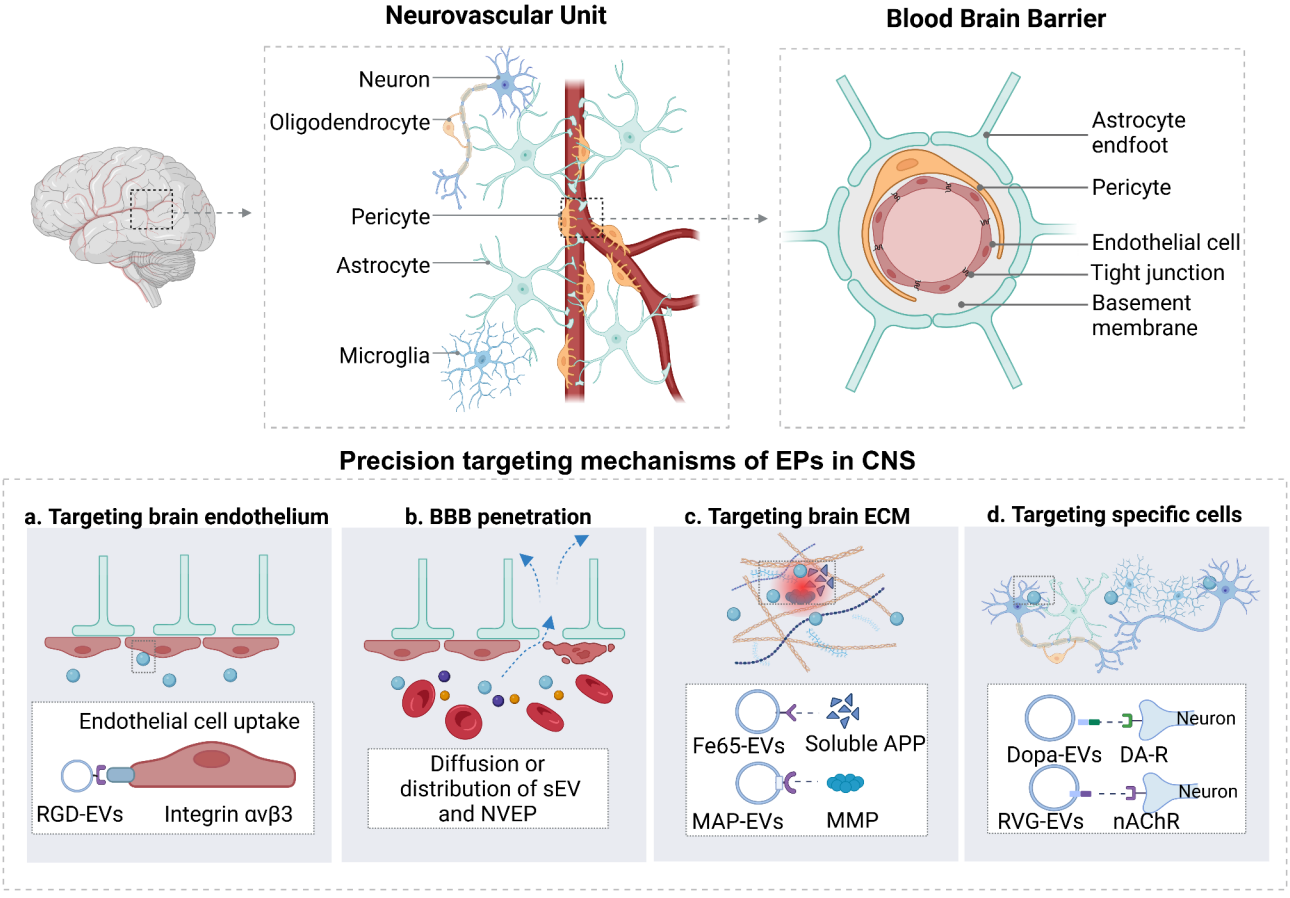

Fig. 9 Biological barriers and targeting mechanisms of EPs in CNS. The blood-brain barrier (BBB) consists of tightly-junctioned endothelial cells, astrocytes, and pericytes, which collectively form a selective protective interface to prevent large NPs from infiltrating the brain parenchyma. Precise targeting of EPs within the CNS requires a multifaceted strategy across four tiers: (**a**) Engineered EPs are designed to selectively bind endothelial cells. (**b**) small EPs exploit size-dependent mechanisms to traverse the BBB and access brain parenchyma. (**c**) Surface-modified EPs are designed to home to specific extracellular matrix components, such as soluble amyloid precursor protein (APP), matrix metalloproteinases (MMPs), within cerebral microenvironments. (**d**) Engineered EPs achieve cellular specificity through ligand-receptor interactions. RVG: Rabies virus glycoprotein; nAChR: nicotinic acetylcholine receptors; Dopa-EVs: dopamine-conjugated on EVs surface; DA-R: dopaminergic receptors. (Figure created with BioRender)

of delivering proteins or exRNA to target tissues. The smaller size of these particles also contributes to better tissue penetration and more effective targeting of specific cells or tissues within the CNS.

In summary, precision targeting of EPs in CNS employs a multi-tiered strategy: (1) Vascular targeting: Vascular targeting via functionalized EPs, such as RGD-modified EVs, enhances endothelial cell engagement; (2) BBB transmigration: Small EPs, such as sEVs and supermeres exploit size-dependent mechanisms to traverse the BBB; (3) Extracellular niche localization: Engineered EPs localize to extracellular niches, such as APP and MMPs, within cerebral microenvironments; and (4) Cell-specific delivery: Cell-specific delivery is achieved through ligand-receptor interactions, such as RVG-EVs targeting neuronal nAChRs or Dopa-EVs binding dopaminergic receptors, enabling precise therapeutic delivery to neurons [9, 28, 153]. (Fig. 9).

Challenges and perspectives

CNS diseases involve significant changes in cell-cell and cell-microenvironmental communication mechanisms. It has become increasingly clear that cells employ a diverse array of EPs to facilitate communication with both neighboring and distant cells [33]. However, the complexity and heterogeneity of EVs and NVEPs is hindering in-depth exploration of their detailed communication mechanisms [14, 15, 31]. Cellular activities, whether physiological or pathological, may leave different extracellular "footprints," such as those associated with migration, apoptosis, and ciliary movement [47, 60, 201]. Interestingly, the initial discovery of many novel EP subtypes appears to be closely related to CNS disorders, such as exophers, mitovesicles, supermeres, and extracellular cuboidal particles [16, 55, 63, 69]. These heterogeneous subtypes offer new insights into the pathological progression of CNS diseases. Additonally, specific subtypes of EPs also provide more efficient delivery platforms for

non-cell therapy of CNS disorders [16, 154, 180]. However, the EP heterogeneous present potential challenges for further research:

One of the challenges is the limited understanding of NVEPs and their biogenesis. While extensive studies have elucidated the roles of various EV subtypes in intercellular communication, the functions of NVEPs, especially in CNS, remain underexplored [28, 202]. Moreover, the biogenesis, cargo selection, packaging and release processes of NVEPs are poorly understood [13, 74].

Another challenge is the inadequate techniques for isolating and characterizing EPs. The exosomes isolated by most protocols are mixtures of exosomes, sEVs of non-endosomal origin and exomeres [32, 203]. Notably, minor differences in these protocols can lead to significant differences in results and conclusions [204]. Hence, the choice of isolation method is crucial for the isolation and collection of EVs and NVEPs [14, 75]. For example, For example, exosomes are best captured by immunoaffinity methods targeting markers such as CD63, while supermeres require ultrahigh-speed centrifugation combined with density gradients to be isolated from EVs. Similarly, mitovesicles require iodixanol gradients to capitalize on their unique buoyancy [37], while exophers require low-speed centrifugation to avoid structural damage [81]. Overlapping physical properties (such as, similar sizes of exomeres and sEVs) further complicate separation, requiring complementary techniques such as AF4 or charge-based separation to resolve ambiguities [68].

The heterogeneity of subpopulations of EPs also poses a significant challenge for EP-based therapies. Different subtypes of EPs from the same source can mediate different regenerative therapeutic effects, while some specific subtypes may produce undesired or contradictory effects [205]. For example, a recent study demonstrated that intravenous administration of high doses of MSC-LEVs (greater than 1 μg/g body weight) resulted in coagulation dysfunction and pulmonary thrombosis, potentially leading to pulmonary thromboembolism and acute death [206]. It is essential to identify which EP subpopulations are functionally active or carry specific therapeutic cargoes in order to effectively manipulate EP formulations, enhance their efficacy and improve reproducibility [28, 207].

Despite the challenges, EPs have shown remarkable progress in various biomedical fields in recent years. Future studies should prioritize the development of sound methodologies to accurately define EP subtypes and elucidate their functional roles in vivo and in vitro. As the current evidence comes mainly from cell culture models, it is imperative to validate the EV-mediated neural communication found in living mammalian systems [143]. Advanced tools such as gene genealogy tracking systems, optical EV reporters, and single-cell holography offer transformative potential to dissect the contribution of EVs and NVEPs in driving epigenetic remodeling and metabolic reprogramming within neurons and glial cells at single-cell resolution [208–210].

In EP-based therapies, strategies such as surface ligand coupling, cargo optimization, and biomimetic design have enhanced the potential efficacy of engineered EVs and Vault particles for the treatment of CNS disorders. These innovations not only promise to overcome BBB limitations, but also pave the way for precise, cell-specific interventions in regenerative medicine and immunomodulation [20, 28]. Future directions include refining their targeting capabilities and optimizing production methods to advance preclinical and clinical evaluations. Additionally, collaborative efforts to establish regulatory frameworks and share EP databases will improve reproducibility. By harmonizing protocols and leveraging scalable technologies, EP-based therapies can be robustly transferred from bench to bedside, unlocking their full potential for treating CNS disorders. Moreover, the long-term safety of EP-based therapies depends on the regulatory and manufacturing standards set for these therapies. Ensuring the consistency, purity and reproducibility of EPs used in clinical applications is essential to minimize risks [211, 212]. Futhermore, detailed preclinical studies, including toxicity assessments and biodistribution studies, must be conducted to identify potential safety concerns and optimize the therapeutic potential of Eps [205, 211].

Abbreviations

EPs	Extracellular particles
EVs	Extracellular vesicles
NVEPs	Non-vesicular extracellular particles
CNS	Central nervous system
NDs	Neurodengenerative diseases
BBB	Blood-brain barrier
APP	Amyloid precursor protein
MSCs	Mensenchymal stem cells
NSCs	Neural stem cells
ESEs	Early sorting endosomes
MVBs	Multivesicular bodies
ILVs	Intraluminal vesicles
ESCRT	Endosomal sorting complexes required for transport
PS	Phosphatidylserine
ARMMs	Arrestin domain-containing protein 1-mediated microvesicles
MDVs	Mitochondria-derived vesicles
LPPs	Lipoprotein particles
SMAPs	Supramolecular attack particles
AF4	Asymmetric flow field-flow fractionation
ST6Gal-I	β-galactoside α2,6-sialyltransferase 1
EGFR	Epidermal growth factor receptor
AREG	Epidermal growth factor receptor ligand amphiregulin
BACE-1	β-site app cleaving enzyme 1
sEVs	Small EVs
exRNA	Extracellular RNAs
AD	Alzheimer's disease
PD	Parkinson's disease
Aβ	β-amyloid
α-syn	A-synuclein
CSF	Cerebrospinal fluid
PBA	Proximity barcoding assay

TBI	Traumatic brain injury
SCI	Spinal cord injury
BMECs	Brain microvascular endothelial cells
CAMs	Cell adhesion molecules
HMGB1	High plasma levels of high mobility group box 1
FUS	Fused in sarcoma
GDNF	Glial cell line-derived neurotrophic factor
SHP2	Tyrosine phosphatase-2
RVG	Rabies virus capsid glycoprotein
nAChR	Nicotinic acetylcholine receptor
Lamp2b	Lysosome-associated membrane glycoprotein 2b
MAP	Matrix metalloproteinase-activatable cell-penetrating peptide

Acknowledgements
Not applicable.

Author contributions
X.Z. and WR.X. conceptualized this review. SY.C. and QH.B. drafted the manuscript and created the fgures. X.Z. and WR.X.revised the content of this manuscript. All authors read and approved the final manuscript.

Funding
This work was supported by the National Natural Science Foundation of China (Grant no. 82272179, 81971757); the Jiangsu Province's Major Project in Research and Development (BE2020680); the Priority Academic Program Development of Jiangsu Higher Education Institutions (Phase III) and Zhenjiang Key Laboratory of High Technology Research on Exosomes Foundation and Transformation Application (Grant no. SS2018003); Shanghai "Rising Stars of Medical Talent" Youth Development Program; the Excellent Young Talent Program of Shanghai Municipal Health Commission (2022YQ003); Changhong Talent Program of Changhai Hospital.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 13 January 2025 / Accepted: 24 March 2025
Published online: 01 April 2025

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