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REVIEW

Plant-Derived Exosome-Like Nanoparticles: A Promising Therapeutic for Neurological Disorders and Drug Delivery

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Abstract: Neurological disorders, including ischemic stroke, Alzheimer's disease, and Parkinson's disease, exhibit high incidence rates and pose significant health challenges. Current pharmacological treatments often fail to adequately address clinical needs due to obstacles such as limited penetration of the blood-brain barrier and suboptimal efficacy. Plant-derived exosome-like nanoparticles (PELNs) have emerged as promising therapeutic agents due to their superior biocompatibility, low toxicity, ability to traverse the blood-brain barrier, and abundance of lipids, microRNAs, and other bioactive compounds. This review provides a comprehensive overview of recent advancements in PELNs preparation technologies, elucidates the mechanisms of action of their principal bioactive components, and explores their therapeutic applications across various neurological disorders, thereby offering a theoretical foundation for the development of related treatment strategies. Nonetheless, researches on PELNs continue to encounter significant challenges. At the production level, there is an absence of standardized isolation protocols, and the yields remain inadequate to satisfy clinical requirements. Clinically, the efficacy in humans has yet to be established, and the available safety data are insufficient. Technically, the lack of standardized storage conditions and the susceptibility of biological stability to external factors further complicate the field. This review delineates these challenges to offer insights for advancing both fundamental research and the clinical translation of PELNs.

Keywords: plant-derived exosome-like nanoparticles, neurological disorders, blood-brain barrier, neuroprotection, anti-inflammation, oxidative stress

Introduction

Neurological disorders comprise a spectrum of diseases characterized by structural damage, functional impairment, and degenerative changes in the central or peripheral nervous system. Major subtypes include Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic lateral sclerosis (ALS), Multiple sclerosis (MS), Huntington's disease (HD), and ischemic stroke (IS). According to 2021 statistics, approximately 3.4 billion people worldwide (43.1% of the global population) suffer from these neurological disorders.[1] As the second leading cause of mortality after cardiovascular diseases, neurological disorders account for a significant number of deaths annually, with stroke (4.88 million deaths), AD (0.98 million), and PD (1.9863 million) ranking as the top three lethal neurological conditions.[2] Accordingly, neurological disorders have emerged as a significant global health challenge, and their pathogenesis is now understood to involve a complex interaction of various factors and structures, including the blood–brain barrier (BBB), which contributes to the lack of availability of effective treatment options.

Current therapeutic strategies for neurological disorders primarily focus on symptom alleviation and disease progression delay. For instance, cholinesterase inhibitors used for treating AD merely provide symptomatic relief for cognitive

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impairment and behavioral/psychological symptoms of dementia.[3] While FDA-approved anti-Aβ monoclonal antibodies can slow down disease progression of AD patients, few adverse reactions have been observed in clinical trials.[4] A fundamental obstacle in treating neurological disorders is the BBB, which severely restricts drug delivery to the CNS. This biological barrier has consequently become a critical bottleneck in developing effective neurological therapies. Given these challenges, the identification of safe, effective BBB-penetrating therapeutic strategies has emerged as a paramount priority in neurology research.

In recent years, the potential of plant-derived exosome-like nanoparticles (PELNs) in the treatment of neurological diseases has attracted significant interest. Research has demonstrated that exosomes derived from various medicinal plants, such as *Panax notoginseng*,[5] *Pueraria lobata*,[6] and *Gardenia*,[7] exhibit preventive and therapeutic effects in neurodegenerative diseases and ischemic nerve injury. PELNs are phospholipid bilayer membrane-enclosed nanovesicles (30–300 nm) secreted by plant cells, which contain various bioactive molecules including proteins, lipids, nucleic acids (eg, miRNAs, mRNAs), and secondary metabolites.[8] PELNs exhibit pharmacological actions similar to the plant from which they are derived. Moreover, PELNs exhibit excellent biocompatibility, low immunogenicity and the ability to traverse biological barriers compared to traditional therapeutic drugs.[9–11] These advantages collectively underscore PELNs' potential as promising therapeutic drugs for neurological disorders.

Despite considerable advancements in the application of PELNs for disease treatment, several challenges persist, including a lack of standardized isolation protocols, insufficient characterization of their cargo and biological functions, difficulties in large-scale production, and inadequate safety evaluation for clinical translation. In the future, technological innovations in exosome separation (such as microfluidic technology) and the application of artificial intelligence are expected to enhance production efficiency and quality. It is believed that exosomes will become a powerful weapon in the treatment of neurological diseases.

This review summarizes recent advancements in the preparation of PELNs from various sources and illustrates their main characteristics. In addition, the review expounds on the therapeutic potential and applications of PELNs. Indeed, a more comprehensive understanding of the mechanisms and clinical translation potential of PELNs may provide novel strategies for developing safe, effective, and blood-brain barrier-penetrating therapies for neurological diseases.

Overview of PELNs

PELNs are phospholipid bilayer membrane-enclosed nanovesicles (30–300 nm) secreted by plant cells, which contain various bioactive molecules including proteins, lipids, nucleic acids (eg, miRNAs, mRNAs), and secondary metabolites.[8] PELNs exhibit remarkable source diversity and production scalability, as they can be isolated from a wide range of edible and medicinal plants, including fruits, vegetables, cereals, and herbs. PELNs are typically obtained from various plant tissues such as leaves, fruits, and seeds, offering a sustainable and cost-effective alternative to conventional synthetic nanoparticles.

Current evidence suggests that PELNs demonstrate high biocompatibility and a favorable safety profile. Derived from commonly consumed plants (eg, ginger, grapes, broccoli, and tomatoes),[12–15] PELNs exhibit significantly different compositions from mammalian cells, resulting in low immunogenicity and high clinical safety profiles. Furthermore, studies have demonstrated that PELNs do not exhibit long-term accumulation in any major organs, exhibiting low systemic toxicity while maintaining optimal cellular uptake efficiency.[16] For instance, Chen et al demonstrated the biosafety of cucumber-derived exosomes through hemolysis assays (<5%), cytokine profiling (no significant differences vs PBS), and histopathological evaluation (no organ damage), thereby validating their biocompatibility in vivo.[17] Compared to synthetic lipid nanoparticles, nanovectors derived from grapefruit lipids demonstrate superior safety profiles, with studies showing no placental barrier penetration following intravenous administration in pregnant mice, further supporting their potential clinical applicability.[18]

In addition, PELNs have been demonstrated to cross various physiological barriers, including the BBB, primarily through receptor-mediated cellular transport and membrane fusion. The core characteristics enabling PELNs to cross the BBB can be summarized as a triple advantage: "natural targeting, multi-pathway penetration, and cross-species regulation". First, specific ligands (eg, glycoproteins, sphingolipids) present on the surface of the PELN lipid bilayer bind with high affinity to receptors on brain microvascular endothelial cells, triggering receptor-mediated transcytosis or, under

specific conditions, facilitating direct fusion with the plasma membrane to transiently open transmembrane channels. This mechanism constitutes an "unmodified, high-efficiency" natural delivery system. Second, PELNs possess multiple parallel internalization pathways: they can be internalized by brain endothelial cells via clathrin-/caveolae-mediated endocytosis, macropinocytosis, or phagocytosis, while also harnessing lipid raft structures to achieve rapid membrane fusion.[19]

In summary, PELNs, leveraging their inherent biocompatibility, multi-modal crossing mechanisms, and cross-species gene regulatory capabilities, have emerged as optimal natural carriers for BBB penetration, treatment of neurological disorders, and promoting neural regeneration.

Isolation and Extraction of Plant-Derived Extracellular Vesicles

The efficient extraction and purification of PELNs are critical prerequisites for studying their biological functions and potential applications. The rigid structure of plant cell walls (primarily composed of cellulose, hemicellulose, and pectin) necessitates an extraction process that balances efficient disruption of cell walls with the preservation of exosome integrity. For different plant tissues (such as fruits, seeds, leaves, and roots), differentiated pretreatment methods (eg, juicing, enzymatic digestion, or grinding) are required, combined with various separation techniques such as ultracentrifugation, density gradient centrifugation, and size-exclusion chromatography for isolation and purification (Figure 1). The following section will systematically elaborate on the extraction strategies for PELNs and the principles, advantages, disadvantages, and applicability of mainstream separation methods (Table 1).

Pretreatment

PELNs are typically obtained from various plant tissues, including seeds, roots, stems, fruits, and leaves. Prior to extraction, plant materials require thorough washing, decontamination, and homogenization to remove cellular debris, large tissue fragments, and other impurities. The rigid plant cell wall, primarily composed of cellulose, hemicellulose, and pectin, presents a key challenge in PELN isolation, necessitating efficient cell wall disruption while preserving vesicle integrity and bioactivity. For succulent tissues such as tomatoes, grapes, and lemons.[39,40] PELN-rich juice can be directly obtained through mechanical pressing or screw extrusion; however, potential contamination with polysaccharides and pigments must be considered. Seed-derived PELN extraction involves pretreatment through buffer immersion and seed coat removal (eg, *Raphanus sativus* seeds), followed by powderization under liquid nitrogen and subsequent dissolution in phosphate-buffered saline (PBS) at a 1:1 ratio.[41] For leaf-derived exosome isolation, initial enzymatic digestion with pectinase and cellulase is essential to facilitate efficient vesicle release from the cellular matrix while preserving structural integrity.[42] is essential to facilitate efficient vesicle release from the cellular matrix while preserving structural integrity. Subsequent centrifugation effectively removes protoplast contaminants, yielding a supernatant enriched with intact vesicles. Root tissue processing requires specialized handling due to its robust structure, beginning with precise sectioning of fresh specimens to maximize surface area and enhance subsequent enzymatic cell wall degradation. A critical PBS washing step precedes enzymatic treatment to eliminate intracellular contaminants released during tissue preparation, followed by a two-hour enzymatic hydrolysis in optimized buffer conditions prior to lysate collection.[43] Overall, tissue-specific pretreatment is essential for optimal vesicle recovery, with enzymatic digestion proving most effective for leaves and roots, while direct mechanical pressing is sufficient for fruits and fleshy stems. Importantly, seed-derived vesicles necessitate hydration and mechanical disruption prior to extraction. These tailored pretreatment methodologies significantly enhance both extraction efficiency and vesicle purity, establishing an essential foundation for subsequent purification processes.

Differential Ultrafiltration (DUC)

The ultracentrifugation method is now understood to facilitate the effective separation of plant-derived exosomes through a sequential centrifugation protocol. Initial low-speed centrifugation steps (2000×g followed by 10,000×g) progressively remove large particulate contaminants, including plant debris and cellular aggregates. Subsequent ultrahigh-speed centrifugation at 100,000×g facilitates the sedimentation and concentration of extracellular vesicles (EVs).[20,21] The entire isolation procedure is carefully maintained at 4 °C to preserve vesicle integrity and biological activity.[44] Widely

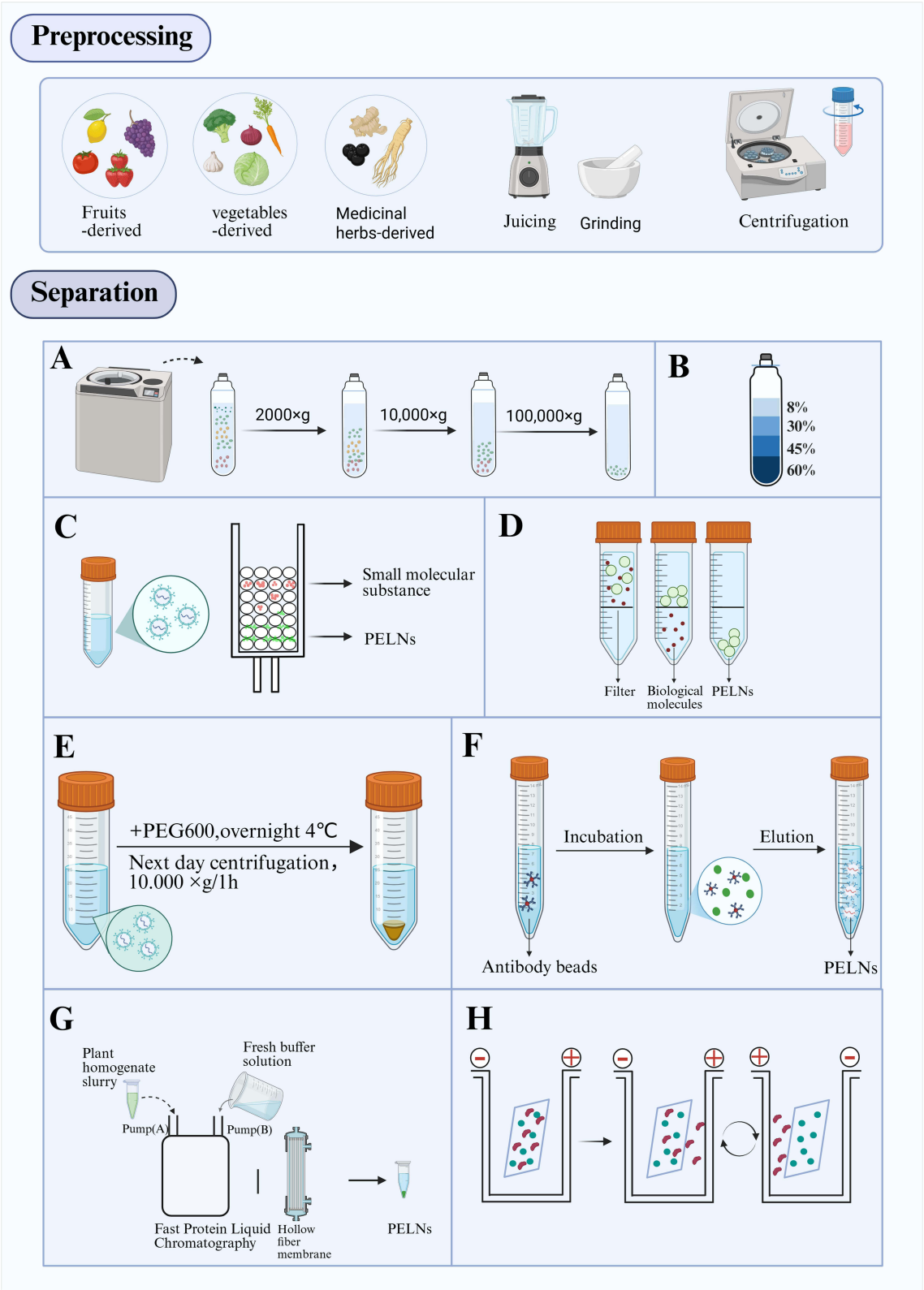

Figure 1 Isolation and Extraction methods for PELNs. (**A**) Differential Ultrafiltration; (**B**) Gradient ultracentrifugation; (**C**) Size-exclusion chromatography; (**D**) Ultrafiltration; (**E**) Polymer-based precipitation; (**F**) Immunoaffinity Capture; (**G**) Continuous tangential flow filtration; (**H**) Electrophoresis with dialysis.

Table 1 The Summary of Each Isolation and Extraction Method of PELNs

Method	Principle	Advantages	Limitations	Applications	References
Differential Ultrafiltration	Exosomes were pelleted from the solution via high-speed centrifugation.	Efficient; No additional reagents are needed to avoid chemical contamination.	Cost-prohibitive instrumentation; Shear stress-induced vesicle damage; Suboptimal recovery efficiency	Large scale extraction.	[20–22]
Gradient ultracentrifugation	Gradient ultracentrifugation (eg, sucrose/iodixanol) enables exosome enrichment at specific density layers.	High purity and recovery yield.	Non-specific adsorption may cause exosome loss/ contamination; low throughput.	Extraction of high-purity exosomes.	[23–27]
Size-exclusion chromatography	Size-based separation using gel-filtration chromatography (exosomes elute first)	Preserves exosome structural integrity and bioactivity	Requires specialized equipment; time-consuming and costly.	Exosome activity needs to be maintained.	[28–31]
Ultrafiltration	Size-exclusion filtration using membranes with defined pore sizes under pressure/centrifugation.	Simple operation and rapid processing; ideal for small-volume samples	Membrane fouling and compromised purity.	Rapid separation is required. Large-scale samples, It is suitable for preserving the vesicular structure and minimizing cosegregating contaminants.	[32,33]
Polymer-based precipitation	Polymer-based (eg, PEG) precipitation by dehydration effect.	Simple, rapid and cost-effective.	Low specificity; co-precipitation of contaminants (proteins/ nucleic acids).	Suitable for fast, large-scale extraction.	[34,35]
Immunoaffinity capture	Antibody-based isolation targeting exosome surface antigens	High specificity, purity, and operational simplicity	High antibody costs; requires prior marker knowledge; may miss non-specifically expressed subpopulations	Exosomes containing specific proteins.	[36]
Continuous tangential flow filtration	Tangential flow filtration combining shear force (reducing fouling) and size-exclusion (retaining exosomes)	High-volume processing capability	Requires sophisticated equipment and skilled operation	Isolation and purification of large-scale samples.	[37]
Electrophoresis with dialysis	Charge-based separation via electrophoretic mobility differences with dialysis membrane purification	Time-efficient and equipment-independent	Requires buffer replenishment every 30 min and polarity reversal (technically demanding)	Exosome subtype analysis.	[38]

regarded as the "gold standard" for plant exosome isolation, ultracentrifugation offers distinct advantages, including straightforward operational protocols and consistently high recovery rates. These characteristics facilitate the preliminary isolation and enrichment of extracellular vesicles from diverse biological matrices such as plant cell culture supernatants and tissue extracts. However, this technique presents notable limitations, primarily its extended processing time (typically requiring several hours for completion) and dependence on specialized ultracentrifugation equipment. Furthermore, several critical operational parameters, including gravitational field intensity, rotor configuration, sedimentation angle, and solution viscosity, have been demonstrated to significantly influence both the quantitative yield and qualitative

characteristics of isolated exosomes.[22] The application of high centrifugal forces may compromise vesicle structural integrity, potentially leading to membrane deformation or content leakage. Besides, the resulting isolates often contain co-precipitated contaminants, including protein aggregates and nucleic acids, which limit sample purity. Consequently, ultracentrifugation-derived preparations typically require subsequent refinement using density gradient centrifugation to achieve higher purification standards. Despite these limitations, the method remains well-suited for initial exosome enrichment and large-scale crude isolation, particularly in scenarios where rapid processing of voluminous samples is prioritized over absolute purity.

Gradient Ultracentrifugation (GUC)

Gradient ultracentrifugation enables the isolation and purification of PELNs through a stratified system composed of density-varying media such as sucrose and iodixanol.[23,24] This technique leverages differences in the buoyant densities of exosomes to achieve their selective concentration at specific gradient interfaces. In a typical sucrose density gradient protocol, layered solutions with progressively increasing concentrations (eg, 8%, 30%, 45%, and 60%) create a precisely calibrated density environment. As demonstrated by Seo et al, when samples are loaded onto the gradient surface and subjected to ultracentrifugation at 100,000×g for 2.5 hours, plant-derived extracellular nanovesicles (such as those from ginseng) become enriched at the 8–30% and 30–45% sucrose interfaces.[25] While this method significantly enhances exosome purity by leveraging density differentials, making it particularly valuable for precision isolation in functional studies of specific vesicle populations, it presents several technical challenges.[26] The protocol demands meticulous execution, often extending over two days, with substantial manual intervention. Two major limitations impact the utility of this technique for refined applications: the osmotic pressure of gradient media may compromise PELN recovery rates,[27] and a limited resolution for distinguishing vesicles of similar density. These limitations warrant careful consideration when selecting purification strategies for research applications requiring both high purity and representative vesicle yields.

Size-Exclusion Chromatography (SEC)

Size-exclusion chromatography represents a sophisticated separation technique that exploits differences in molecular dimensions for exosome isolation. This method employs porous gel filtration polymers as the stationary phase through which the starting biological fluid (eg, plant tissue extracts) passes as the mobile phase, achieving separation via molecular sieving effects. The porous architecture of the stationary phase facilitates differential elution based on particle size. Indeed, larger PELNs exhibit limited ability to penetrate the gel pores and thus migrate rapidly through interparticle voids, eluting first. In contrast, smaller vesicles partially enter the pores, resulting in delayed elution, while non-vesicular proteins and other small molecules permeate the pores completely, eluting last.[28] Researchers have successfully isolated structurally intact blueberry-derived exosomes using SEC, with subsequent nanoparticle tracking analysis and transmission electron microscopy confirming both morphological preservation and methodological feasibility.[29] In an illustrative application, Ramesh Bokka et al combined differential centrifugation with SEC for efficient separation and purification of microvesicles (MVs) and nanovesicles (NVs) from tomato fruits.[30] Their results demonstrated that incorporating SEC after differential ultracentrifugation significantly reduced protein contamination while enhancing nanovesicle purity, underscoring the technique's efficacy in eliminating co-isolated impurities. However, it should be borne in mind that SEC exhibits certain limitations, including incomplete removal of protein contaminants with size similarity to exosomes,[28,31] potential vesicle loss through stationary phase adsorption, and risks of column bed clogging. Besides, the requirement for specialized chromatography columns, filters, and trained personnel further increases operational costs. Notwithstanding these constraints, the SEC offers distinct advantages for functional studies requiring preserved vesicle integrity, particularly in investigations of PELN-mediated intercellular communication. The technique's ability to maintain biological activity while minimizing vesicle aggregation and structural damage, coupled with its superior purity outcomes compared to many alternative methods, establishes it as a valuable tool for sophisticated PELN characterization studies where biological relevance outweighs throughput considerations. The gentle separation mechanism makes SEC particularly suitable for downstream applications demanding functionally competent vesicles, though researchers must carefully consider the trade-offs between purity, yield, and operational complexity when designing isolation workflows.

Ultrafiltration (UF)

Ultrafiltration is a size-based separation technique that employs membranes with defined molecular weight cut-offs to selectively retain larger particles, such as exosomes, while allowing smaller molecules to pass through under applied pressure.[32] During this process, the sample is fractionated into two components: the filtrate containing small molecules and the retentate enriched with larger vesicles. Subsequent washing of the membrane surface further purifies the retained exosomes by removing residual low-molecular-weight contaminants. Importantly, this method offers distinct advantages, including operational simplicity, elimination of specialized equipment (eg, ultracentrifuges), and capacity for high-volume sample processing, making it particularly suitable for rapid exosome isolation from complex matrices such as plant tissue homogenates. However, it is now understood that pressure-driven filtration can induce vesicle rupture, compromising structural integrity, especially for larger vesicles.[33] Besides, prolonged operation risks membrane fouling and pore clogging, which reduce filtration efficiency and prolong processing time. Given its limited purity in single-step applications, ultrafiltration is primarily employed as an initial enrichment step, often requiring supplementary purification techniques such as differential centrifugation or chromatography to achieve higher-grade exosome preparations. Ultrafiltration offers a balance of high throughput and acceptable risk to vesicle integrity, making it suitable for preliminary isolation. However, its utility in downstream applications depends on subsequent refinement to mitigate purity limitations and ensure sample quality.

Polymer-Based Precipitation (PBP)

Polymer-based precipitation represents a widely adopted technique for exosome isolation, leveraging the hydrophilic properties of polymers such as polyethylene glycol (PEG) to selectively precipitate vesicles from solution. The method operates by dehydrating the exosome surface through PEG-mediated water molecule sequestration, thereby reducing vesicle solubility and enabling low-speed centrifugal recovery.[34] For exosome precipitation, PEG, with a molecular weight of 6000–20,000 Da, is usually employed at concentrations between 5% and 12%.[35] Studies have shown that the recovery rate of exosomes can reach 60–90%, which is equivalent to the differential centrifugation method,[45] but the PEG method is more economical and efficient. A representative protocol for yam bean exosome isolation involves initial sample clarification to remove cellular debris, followed by incubation with 12% PEG-6000 at 4 °C overnight. Subsequent centrifugation at 10,000×g for 1 hour yields a pellet enriched with exosomes.[46] This approach is highly suitable for processing large-volume samples such as plant tissue homogenates or fruit extracts, owing to its minimal equipment requirements and procedural simplicity. However, the technique presents notable limitations, including nonspecific co-precipitation of contaminants (eg, immunoglobulins or viral particles) due to PEG's strong hydrophilicity. Furthermore, residual polymer may interfere with downstream analytical techniques. Consequently, polymer precipitation is primarily employed as an initial enrichment step, often requiring complementary purification strategies such as density gradient centrifugation or chromatographic separation to achieve the purity standards necessary for advanced characterization studies.

Immunoaffinity Capture (IAC)

Immunoaffinity capture represents a targeted isolation strategy that exploits the specific binding between antigens and antibodies to achieve highly selective enrichment of extracellular vesicles. This technique offers superior purity compared to conventional methods such as differential centrifugation or size-exclusion chromatography by minimizing the co-isolation of non-target vesicles through antibody-mediated specificity, proving particularly advantageous for isolating low-abundance exosome subpopulations. A seminal study by He et al demonstrated this approach through the development of native antibodies targeting the extracellular domain (EC2) of TET8, a marker protein of *Arabidopsis thaliana* exosomes.[36] By recombinantly expressing the TET8-EC2 domain and immunizing mammalian hosts, antibodies capable of specifically recognizing TET8-positive vesicles were generated. This immunoaffinity system enabled efficient isolation of TET8-positive extracellular vesicles from root secretomes, leading to the identification of functional cargos including RNA-binding proteins (eg, Argonaute 1, RNA helicases, Annexins) and small RNAs (eg, TAS1c-siR483, TAS2-siR453, miR166). Rigorous validation using TET8-knockout controls and

Western blotting confirmed antibody specificity, establishing a methodological paradigm for plant exosome marker studies. Despite its precision, immunoaffinity capture faces considerable challenges in PELN research. The scarcity of conserved, plant-specific exosome markers (with TET8 currently validated only in *Arabidopsis*) makes antibody development highly challenging, given that plant-specific glycosylation patterns may interfere with antigen-antibody interactions. Furthermore, the heterogeneity of exosome subpopulations necessitates multi-marker capture strategies, increasing technical complexity. Practical limitations include labor-intensive protocols, high costs, low throughput, and potential epitope masking effects, rendering the method unsuitable for large-scale processing. Accordingly, the optimal application of immunoaffinity capture lies in the high-purity isolation of specific exosome subsets for functional studies, particularly in studies involving well-defined surface markers and limited sample volumes. The technique's unparalleled specificity continues to render it indispensable for mechanistic research despite its operational constraints, provided that target antigens are sufficiently characterized and antibody performance is rigorously validated.

Continuous Tangential Flow Filtration (cTFF)

The staged tangential flow filtration/diafiltration process represents an innovative membrane-based separation technology that integrates hollow fiber modules for advanced extracellular vesicle purification. Operating on the principles of tangential flow filtration (TFF), this system facilitates simultaneous buffer exchange and fine purification of exosomes through continuous replenishment of fresh buffer and the removal of small molecular contaminants during sample recirculation. The underlying mechanism combines hydrodynamic shear forces, which minimize membrane fouling, with precise size-exclusion through membrane pores that selectively retain plant-derived exosomes while permitting passage of salts, metabolites, and other small molecules.

This technology has demonstrated successful application in isolating EVs from lemon juice,[37] establishing a novel pathway for the clinical translation of plant-derived EVs. While staged UF/DF provides an efficient and controllable solution for plant EV purification, its implementation requires careful consideration of the trade-off between technical complexity and cost-effectiveness. The method is particularly suitable for industrial-scale isolation and preclinical research scenarios involving medium-to-large sample volumes, where its advantages in scalability and process control outweigh the initial setup requirements. The continuous nature of the UF/DF process offers distinct advantages over batch methods, including improved product consistency and reduced processing time. However, achieving optimal performance depends on precise parameter control, including transmembrane pressure, cross-flow velocity, and concentration factor, to maintain vesicle integrity while achieving desired purity levels. These operational requirements position staged UF/DF as a promising, yet technically demanding approach for advancing plant-derived EV therapeutics toward clinical applications.

Electrophoresis with Dialysis (ELD)

ELD represents an emerging technique that combines electrophoretic migration with dialysis to achieve efficient separation of PELNs. This method leverages electrophoretic forces to drive charged particles across a semi-permeable membrane, harnessing differences in both surface charge and molecular size to selectively isolate PELNs from other macromolecular components. Subsequent dialysis further removes residual small-molecule impurities, yielding highly purified vesicle preparations. The technique has been successfully applied to isolate lemon-derived extracellular vesicles (LDEVs) from citrus juice.[38] Nanoparticle tracking analysis and transmission electron microscopy confirmed that ELD-derived vesicles exhibit comparable size distribution and structural integrity to those obtained via conventional ultracentrifugation. Notably, ELD requires no specialized instrumentation and can be readily implemented in standard biological laboratories, offering significant advantages in terms of accessibility and processing time. However, the method's separation efficiency is inherently constrained by the heterogeneous surface charge characteristics and size variability of exosomes. Furthermore, its generalizability across diverse plant species remains to be systematically validated.

Key Bioactive Components of PELNs

PELNs encompass a diverse array of bioactive constituents, including proteins, nucleic acids, lipids, and metabolites that exert pivotal roles in various biological processes. The potential functions of PELN components are discussed below and summarized in Figure 2.

Nucleic Acids

Nucleic acids represent one of the most functionally significant bioactive constituents of PELNs, comprising DNA, miRNAs, mRNAs, sRNAs, and various non-coding RNAs.[47,48] Among these, miRNAs represent the most critical molecular markers in exosomes, functioning as small non-coding RNAs (17–24 nucleotides) that regulate gene expression at the post-transcriptional level.[49] Through complementary base-pairing with target mRNAs, miRNAs induce translational repression or promote mRNA degradation, thereby modulating gene expression networks.[50] These RNA molecules serve as essential mediators of intercellular communication via exosomal mRNA and miRNA transfer. Within the nervous system, miRNAs participate in diverse regulatory pathways, including neuronal proliferation/apoptosis, neuroimmune responses, oxidative stress modulation, mitochondrial function, and synaptic plasticity.[51,52] Notably, miR-146a has been implicated in AD pathogenesis through its regulation of neuroinflammatory processes.[53]

Research has demonstrated that *Houttuynia cordata* Thunb-derived extracellular vesicle-like particles alleviate ischemia brain injury by delivering endogenous miR159a to target ACSL4, thereby regulating the ferroptosis pathway,

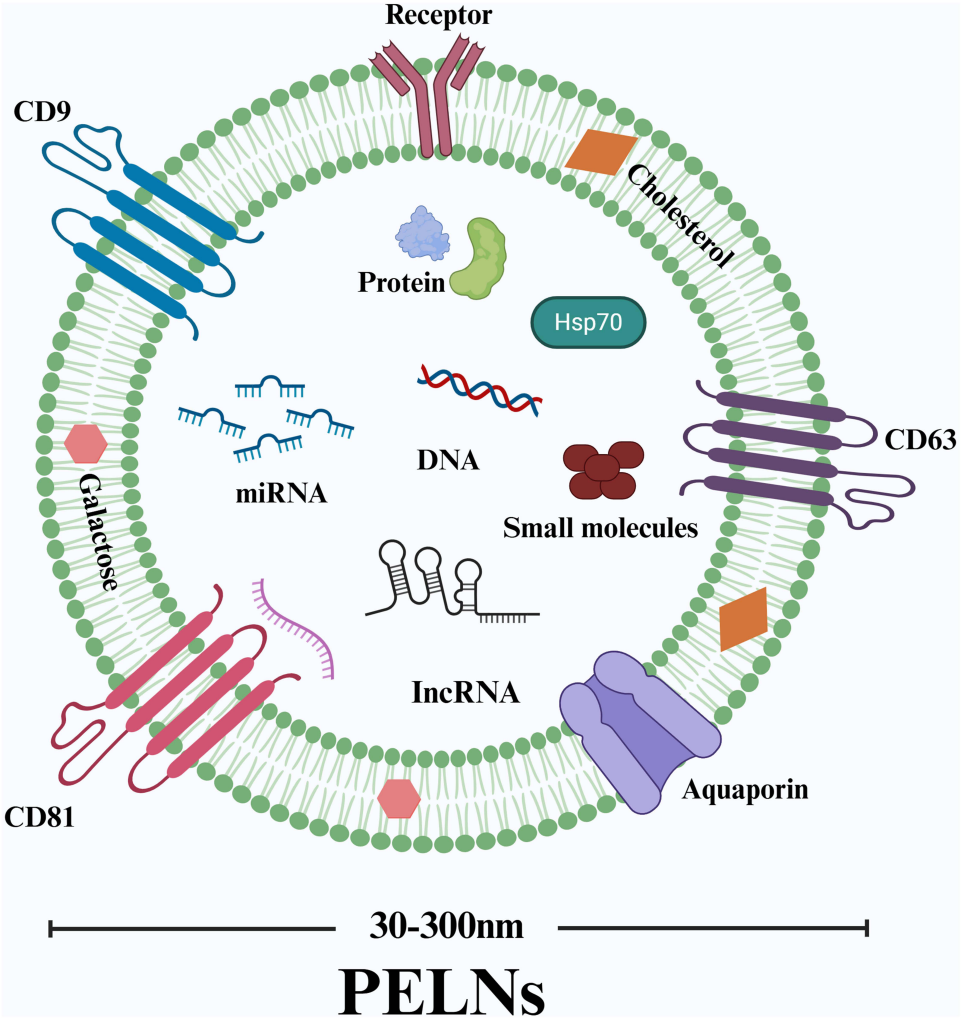

Figure 2 Structural characteristic of PELNs.

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inhibiting lipid peroxidation, and preserving mitochondrial function.[54] This multi-target regulatory capacity highlights the therapeutic potential of exosomal miRNAs across neurological disorders.[55,56] A study revealed that *Atropa belladonna* L.-derived aba-miRNA-9497 could specifically target and downregulate the expression of the transcription factor ZNF-691, which is enriched in the human brain, through a cross-species miRNA signaling mechanism.[57] This discovery not only reveals the role of plant miRNAs in the nervous system and their involvement in the actions of psychoactive substances, but also provides new insights into their potential functions in cross-species and cross-kingdom signaling.

In summary, PELN-miRNAs offer multi-target regulatory capacity, low immunogenicity, and cross-species bioactivity, positioning them as a promising "RNA drug" vector for neurotherapeutics. Nevertheless, sub-optimal in vivo delivery efficiency, heterogeneity in inter-species sequence conservation, and potential off-target toxicity remain critical bottlenecks that must be resolved to facilitate clinical translation.

Lipid

The lipid composition constitutes both the structural framework of PELNs and the core substance mediating their biological functions. It not only determines the physicochemical properties of the vesicles but also plays a critical role in modulating the central nervous system (CNS) microenvironment and intervening in disease pathogenesis. A deep understanding of the lipid characteristics of PELNs is essential for elucidating their therapeutic mechanisms and advancing translational applications. Current evidence suggests that lipids play key roles in maintaining membrane stability, promoting cellular uptake, and exerting bioactivities. The characteristic lipid bilayer structure is primarily composed of phospholipids, sphingolipids (such as ceramides), and sterols (such as cholesterol), which collectively regulate membrane fluidity, structural integrity, and fusion capacity with target cells. Among these, phospholipids represent the most abundant lipid class, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidic acid (PA), phosphatidylserine (PS), and digalactosyldiacylglycerol (DGDG). Moreover, PELNs from different plant sources exhibit significant differences in lipid composition and content. For instance, grape exosome-like nanoparticles are predominantly composed of PA (≈53%), with a high proportion of PE (≈26%), whereas PC constitutes only approximately 9%.[58] *Catharanthus roseus*-derived exosomes contain more than 30% ether phospholipids, a unique subclass of phospholipids that promotes neuronal differentiation, highlighting the species-specific lipid profiles of PELNs.[59] Besides, these lipids possess specific bioactivities; for example, PC and PE derived from ginger exosomes can inhibit NLRP3 inflammasome activation in bone marrow-derived macrophages.[60] Thus, distinct lipid compositions endow PELNs with diverse functions and targeting capabilities. Lipids derived from citrus exosomes exert cross-species anti-inflammatory effects by inhibiting the ERK1/2 and NF-κB signaling pathways,[61] thereby further expanding the regulatory scope of PELN lipids on the physiological functions of neural cells.

In summary, PELN lipids possess inherent advantages for the treatment of neurological disorders, such as circumventing risks associated with synthetic carriers and enabling precise interventions, by virtue of their natural amphiphilic structure, species-specific composition, and dual functional characteristics. Current research limitations primarily revolve around the lack of standardized extraction and analytical techniques, unclear metabolic kinetics, and the absence of clinical translation processes.

Protein

Proteins are central to the execution of genetic functions and significantly contribute to the functionality of PELNs. Studies have demonstrated that EVs isolated from *Arabidopsis thaliana* leaves exhibit characteristics similar to mammalian exosomes, including a typical cup-shaped ultrastructure, conserved surface marker proteins (eg, CD9, CD63, and CD81), and endosomal sorting complex required for transport (ESCRT)-associated proteins such as TSG101 and ALIX.[22] These protein components facilitate cellular signal transduction and immune response regulation, suggesting that plant exosomes may have specialized roles in immune defense and stress adaptation. Furthermore, the exosomal proteome encompasses proteins associated with reactive oxygen species (ROS) signaling and membrane transport systems, indicating their potential physiological functions in maintaining ROS homeostasis, mediating ROS-based signaling cascades, and facilitating the molecular mechanisms of exosome biogenesis and secretion.[62]

Previous studies have confirmed that exosome-like nanoparticles isolated from grape (*Vitis vinifera*) callus cultures contain several evolutionarily conserved proteins, such as heat shock protein 70 (HSP70) and aquaporins, which exhibit significant overlap with the proteome of mammalian exosomes.[40] Notably, this finding corroborates earlier research in which HSP70 was successfully identified in exosomes derived from grape berries.[63] A hallmark pathological feature of neurodegenerative disorders such as AD and PD is the accumulation of toxic oligomers or amyloid fibrils formed by misfolded proteins. Notably, HSP70 has been demonstrated to regulate the metabolic processing of such proteins through multiple mechanisms, including facilitating their refolding, suppressing aggregation, and promoting the degradation of aberrant species.[64] Accordingly, the presence of HSP70 in PELNs provides a theoretical foundation for their potential application in treating neurological disorders.

In a groundbreaking study, EVs were isolated for the first time from *Davallia mariesii* (a traditional medicinal fern) using proteomic analysis, yielding 77 protein components, of which enzymes constituted a remarkable 47%. Pathway enrichment analysis revealed that key enzymes such as NAD(P)H-quinone oxidoreductase were significantly enriched in the oxidative phosphorylation pathway, a metabolic process conserved between plants and humans that is critically implicated in the oxidative stress pathology underlying AD, PD, and Huntington's disease.[65] These findings suggest that *D. mariesii*-derived EVs may serve as potential therapeutic agents for mitigating neurodegenerative pathologies, with NAD(P)H-quinone oxidoreductase likely playing a central regulatory role. However, the precise molecular mechanisms involved require further elucidation.

PELNs are enriched with stress- and immune-related proteins that are evolutionarily conserved in mammalian exosomes. They typically confer several therapeutic benefits, including intrinsic neuroprotective activity, high bioavailability across biological barriers, and low immunogenicity, offering a promising platform for the treatment of neurodegenerative diseases. However, their therapeutic potential is currently limited by the lack of in vivo functional validation and quantitative data on dosing and barrier penetration efficiency. Further studies are also warranted to elucidate the underlying molecular mechanisms.

Secondary Metabolites

Secondary metabolites are metabolites with therapeutic activity that can be encapsulated in PELNs. Emerging studies have revealed that PELNs naturally encapsulate various bioactive compounds derived from their parent plants. For instance, exosomes isolated from grape berries contain potent natural small molecules such as proanthocyanidins and polyphenols.[66] Similarly, ginseng-derived exosomes carry ginsenosides, a class of compounds renowned for their anti-inflammatory and anticancer properties.[10] Citrus exosomes are particularly rich in flavonoids, including naringin and hesperidin, which exhibit remarkable antioxidant activity.[67,68] Ginger, widely recognized for its multifaceted health benefits spanning antioxidant, anti-inflammatory, antimicrobial, and anticancer effects, demonstrates protective properties for the nervous, cardiovascular, and respiratory systems.[69] Notably, 10-gingerol, the bioactive compound responsible for fresh ginger's potent anti-neuroinflammatory capacity, has been shown to positively influence memory function, suggesting potential applications in managing and preventing neurodegenerative disorders. Ginger-derived exosomes have been confirmed to contain gingerols and shogaols, compounds with significant anti-inflammatory and anticancer effects.[12,70,71] Furthermore, exosomes isolated from *Cannabis sativa* reportedly contain cannabidiol at concentrations dependent on the plant's chemotype.[72] Those derived from *Solanum nigrum* berries are enriched with several bioactive compounds that demonstrate measurable anti-inflammatory activity in LPS-stimulated RAW 264.7 cells.[73] Curcumin, the principal active component of turmeric, has been identified within turmeric-derived exosome-like nanoparticles and contributes to their anti-inflammatory properties.[74] Similarly, broccoli-derived nanovesicles have been shown to encapsulate sulforaphane,[75] with high-performance liquid chromatography (HPLC) analysis revealing significantly higher enrichment of this compound in nanoparticles compared to microparticles, while broccoli extracts contain minimal free sulforaphane. Besides, grapefruit-derived nanovesicles have been found to incorporate naringenin.[76]

While the metabolites in PELNs have shown promise for the prevention of neurological diseases, their specific molecular mechanisms, in vivo metabolic pathways, targeting specificity, and long-term effects remain incompletely elucidated.

Therapeutic Applications of PELNs in Neurological Disorders

PELNs exhibit multi-dimensional therapeutic potential for neurological disorders, with mechanisms of action encompassing key pathways such as anti-inflammation, neuroprotection, blood-brain barrier penetration, antioxidant stress, apoptosis inhibition, and synaptic plasticity modulation. Table 2 and Figure 3 summarizes the key mechanisms of PELNs in the treatment of neurological disorders and their corresponding effects. These effects are synergistically mediated by bioactive cargoes carried by PELNs, including miRNAs, lipids, proteins, and small-molecule compounds. Specifically, PELNs alleviate neuroinflammation by suppressing the NLRP3 inflammasome and modulating cytokine networks, while their nanoscale size and biocompatibility facilitate blood-brain barrier penetration. Besides, they exert neuroprotective effects through free radical scavenging, inhibition of neuronal apoptosis, and promotion of synaptic remodeling. The following sections elaborate on these mechanisms in detail.

Ischemic Stroke (IS)

IS remains a prevalent cerebrovascular disease characterized by vascular occlusion-induced hypoxia and hypoperfusion in localized brain regions, leading to ischemic neuronal damage or necrosis.[16] Currently, intravenous thrombolysis and mechanical thrombectomy constitute the gold standard therapy for IS,[88] enabling early vascular recanalization. However, these methods have limitations such as a high risk of bleeding, a narrow therapeutic window (due to the short half-life and poor targeting of tissue plasminogen activator), and restricted applicability to a small subset of patients with large vessel occlusion.[89]

The complexity of reperfusion injury and the ischemic cascade, coupled with the poor target selectivity of drugs for the ischemic penumbra, has contributed to the paucity of truly effective treatments for IS. In recent years, exosome-like nanoparticles derived from natural medicines have demonstrated unique advantages in IS treatment due to their excellent biocompatibility and targeting capabilities.

Exosome-like nanoparticles derived from Panax notoginseng (PDNs) can efficiently cross the blood-brain barrier and mitigate cerebral ischemia-reperfusion injury (CI/RI) by modulating microglial polarization. PDN treatment significantly reduces infarct volume and upregulates the phosphorylation ratios of PI3K (p-PI3K/PI3K) and Akt (p-Akt/Akt), indicating their role in activating the PI3K/Akt signaling pathway to shift microglial polarization. Lipidomics revealed that the lipid components of PDNs were primarily responsible for their anti-inflammatory effects. These findings collectively demonstrate the neuroprotective potential of PDNs, suggesting their therapeutic utility against CI/RI.[5] Furthermore, miR-5266 enriched in *Momordica charantia*-derived exosome-like nanoparticles (MC-ELNs) has been identified as a functional component capable of suppressing matrix metalloproteinase-9 (MMP-9) expression, providing experimental evidence for the application of MC-ELNs in ischemic stroke therapy.[77]

Building upon the above findings, PELNs demonstrate significant therapeutic potential for IS by modulating microglial polarization, suppressing MMP-9 expression, and activating neuroprotective pathways such as PI3K/Akt. Their excellent biocompatibility and innate targeting capabilities offer novel strategies to overcome reperfusion injury and disrupt the ischemic cascade.

Alzheimer's Disease (AD)

AD is a progressive neurodegenerative disorder marked by cognitive decline, memory loss, and behavioral changes, driven by Aβ plaques, tau tangles, neuroinflammation, and neuron loss. Current treatment strategies, such as acetylcholinesterase inhibitors (eg, donepezil) and NMDA receptor antagonists (eg, memantine), only alleviate symptoms. Recently developed anti-Aβ antibodies (eg, aducanumab) typically show limited efficacy due to poor BBB penetration and side effects, underscoring the need for more effective therapies.

Onion-derived exosomes exhibit remarkable anti-inflammatory properties by suppressing pro-inflammatory cytokines and interfering with key signaling pathways,[78] demonstrating neuroprotective potential against inflammation-mediated damage in AD therapy. Exosome-like nanovesicles derived from citrus lemons (EXO-CLs) possess an average particle size of 93.77 nm and demonstrate in vitro antioxidant activity comparable to that of ascorbic acid. These nanovesicles are capable of traversing the blood-brain barrier and confer protection to SH-SY5Y neuroblastoma cells against β-amyloid-

Table 2 Summary of Mechanisms Involved in the Treatment of CNS Disorders with PELNs

PELNs Sources	Isolation Methods	Characterization Method	Particle Diameter (nm)	Zeta (mV)	Shape	CNS Disease	Mechanism	References
Panax notoginseng	sgUC	DLS, TEM	151.3	−8	Spherical	IS	PDN mitigates CI/R injury by shifting microglial polarization from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype.	[5]
Momordica	sgUC	TEM, NTA	120	–	–	Gliomas	MCELNs inhibited glioma cell migration and invasion by modulating EMT and MMP9, penetrated the BBB, and suppressed tumor growth and metastasis in vivo.	[77]
Onion	SEC	NTA, TEM	150	−9.96	Spherical	Chronic inflammation	Onex exerts anti-inflammatory effects by inhibiting the phosphorylation of the NF-κB signaling pathway.	[78]
Lemon	DC	DLS, TEM	93.77 ±12.31	−3.46 ±1.45	Spherical	AD	EXO-CLs ameliorate Alzheimer's disease symptoms by reducing β-amyloid-induced oxidative stress and neurotoxicity through their antioxidant activity and blood-brain barrier penetration capability.	[67]
Lycium ruthenicum	PEG6000	TEM, DLS	50–450	–	Spherical	AD	LELN mitigates Aβ aggregation, alleviates cholinergic dysfunction, mitochondrial damage, and oxidative stress by activating the DAF-16 pathway.	[79]
Miltiorrhiza hairy roots	dUC, SEC	NTA, SEM, TEM	80–220	–	Spherical	PD	HR EVs inhibited the autoxidation of 6-OHDA and significantly reduced the accumulation of its oxidative products.	[80]
Pueraria lobata	DC	DLS, TEM	125.0±9.7	−5.0 ±0.7	Cup-shaped	PD	Pu-Exos ameliorate Parkinson's disease symptoms by eliminating dysfunctional mitochondria through PINK1-Parkin-mediated mitophagy and restoring the activities of mitochondrial respiratory chain complexes I and V to improve ATP production.	[6]
Ginger	sgUC	TEM, NTA	130.57 ±7.71	−28.74 ±1.28	Double-layered discs	PD	Exo@tac ameliorates Parkinson's disease symptoms by modulating gut bacteria associated with the microbiota-gut-brain axis, suppressing inflammatory factor production, reducing neuroinflammation and apoptosis, promoting neurotransmitter and dopamine precursor synthesis, and decreasing α-synuclein deposition.	[81]
Carrot	SEC	NTA, TEM, DLS	143.9	−10.2	Spherical	–	Carex effectively suppresses ROS generation and cellular apoptosis induced by oxidative stress.	[82]
Petasites japonicus	DC	DLS, TEM	122.6	–	Spherical	MS	PJ-EVs induce dendritic cell maturation by activating MAPK and NF-κB signaling pathways, thereby promoting Th1 immune responses and CD8+ T cell activation.	[83]

(*Continued*)

Table 2 (Continued).

PELNs Sources	Isolation Methods	Characterization Method	Particle Diameter (nm)	Zeta (mV)	Shape	CNS Disease	Mechanism	References
Grapefruit	–	DLS, TEM	135 ± 5	–	Spherical	Gliomas	EV-DNs is efficiently internalized by glioma cells via αvβ3 receptor-mediated endocytosis and membrane fusion, with demonstrated blood-brain barrier (BBB) penetration capability.	[84]
Grapefruit	sgUC	TEM	87.2±11.3	−13.9	–	Brain Tumor	GNVs exert therapeutic effects against brain tumors by selectively delivering miR-17 to target and suppress GL-26 expression in tumor cells, while concurrently activating NK cells to mediate tumor cell killing.	[85]
Ginseng	–	DLS, TEM	144.1±2.8	−27.4 ±0.45	Cup-shaped	Neural injury and neurodegenerative diseases	G-Exos promote the neural differentiation of BMSCs through activation of the PI3K signaling pathway.	[86]
Oat	OptiPrep gradients	TEM	–	–	–	Chronic brain inflammation	OatN targets microglia by leveraging the interaction between β-glucan and HPCA to inhibit the Dectin-1 signaling pathway, thereby reducing the expression of inflammatory factors, protecting neuronal cells, and improving memory function.	[87]

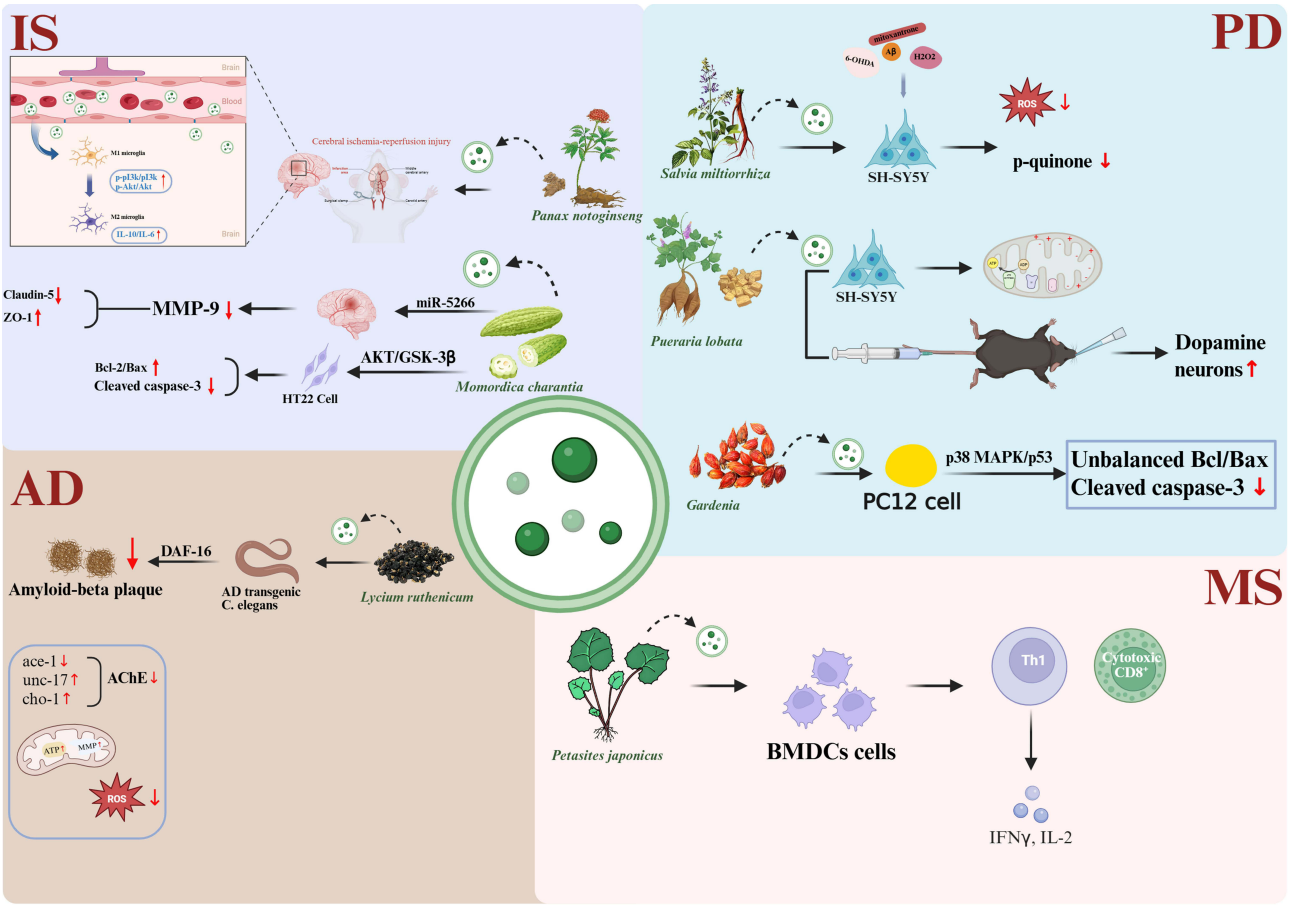

Figure 3 Summary of mechanisms involved in the treatment of IS, PD, AD and MS with PELNs.

induced toxicity, achieving a cell survival rate exceeding 80%.[67] Besides, recent studies have revealed that *Lycium ruthenicum*-derived exosome-like nanoparticles (LELNs) demonstrate significant therapeutic potential against AD pathology through multifaceted mechanisms. These bioactive nanoparticles effectively mitigate Aβ-induced AD-like symptoms by activating the DAF-16 pathway, leading to a substantial reduction in both Aβ oligomers and monomers.[79] The primary therapeutic mechanisms of LELNs involve the amelioration of cholinergic dysfunction through acetylcholinesterase activity inhibition, restoration of mitochondrial function via recovery of membrane potential and adenosine triphosphate production, and attenuation of oxidative stress and inflammatory responses. Furthermore, these nanoparticles demonstrate potent antioxidant effects in Aβ-exposed HT22 cells by orchestrating nuclear translocation of Nrf2 with concomitant upregulation of heme oxygenase-1 (HO-1) and NAD(P)H quinone oxidoreductase 1 (NQO1) expression, thereby mitigating Aβ-induced oxidative damage.[90] These results collectively suggest the potential of LELN as a promising therapeutic strategy for the treatment of AD.

A critical challenge in treating AD is the efficient delivery of therapeutics across the BBB. While antioxidant compounds such as vitamins C and E have shown neuroprotective potential, their efficacy is severely limited by poor BBB permeability. PELNs provide a promising solution, given that their innate nanoscale size and biomimetic properties enable efficient BBB crossing, overcoming this fundamental delivery bottleneck. By encapsulating vitamins C and E, PELNs not only protect these compounds from degradation but also ensure their targeted delivery to the brain. Further enhancement can be achieved through surface modification with targeting ligands (eg, folate or RGD peptides), which promote receptor-mediated uptake into brain cells, maximizing therapeutic effects against AD pathology.[91]

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Therefore, PELNs provide a multi-mechanistic neuroprotective strategy for the treatment of AD by inhibiting inflammatory cytokines, activating the DAF-16/Nrf2 pathway, ameliorating cholinergic dysfunction and mitochondrial activity, as well as enhancing the brain delivery of antioxidant vitamins.

Parkinson's Disease (PD)

PD is a progressive neurodegenerative disorder marked by the selective degeneration of dopaminergic neurons in the substantia nigra, leading to striatal dopamine deficiency. The disease pathogenesis involves α-synuclein aggregation, oxidative stress, and mitochondrial dysfunction. Current therapies are limited by poor drug delivery and side effects. PELNs demonstrate remarkable neuroprotective potential through their capacity to deliver diverse antioxidant molecules, including polyphenolic compounds and antioxidant enzymes, which effectively scavenge intracellular ROS and mitigate oxidative damage to neurons. Recent studies have demonstrated that EVs isolated from *Salvia miltiorrhiza* hairy roots (HR EVs) exhibit potent and multifaceted neuroprotective effects in Parkinson's disease cell models. These HR EVs display comprehensive therapeutic properties encompassing neurotoxicity suppression, mitochondrial function modulation, metabolic regulation, and neutralization of neurotoxic molecules, highlighting their potential as a novel therapeutic approach for neurodegenerative disorders.[80] A groundbreaking study by Professor Peng Lihua's team at Zhejiang University[6] revealed that *Pueraria lobata*-derived exosomes (Pu-Exos-PR) serve as an efficient intranasal delivery platform for transporting bioactive miRNAs into the brain, exhibiting significant anti-PD effects. These plant-derived nanovesicles enhance PINK1-Parkin-mediated mitophagy while simultaneously boosting the activity of mitochondrial respiratory chain complexes I and V, thereby restoring ATP synthesis and ameliorating mitochondrial dysfunction in SH-SY5Y cells. The therapeutic efficacy of Pu-Exos-PR manifests through multiple mechanisms: promoting dopaminergic neuron survival, reducing cellular damage and oxidative stress responses in PD conditions, and alleviating both motor and non-motor symptoms, thereby establishing a novel nanotherapeutic platform for PD treatment. Kim et al further demonstrated that carrot-derived exosomes significantly suppress H_2O_2- and 6-OHDA-induced oxidative stress by activating the Nrf-2/HO-1/NQO-1 antioxidant pathway, effectively reducing ROS generation and attenuating cellular apoptosis.[82] Owing to their potent antioxidant capacity, low cytotoxicity, and scalable production characteristics, these plant-derived exosomes exhibit considerable potential as promising therapeutic agents for PD treatment. The study highlights the dual advantage of these natural nanovesicles in simultaneously targeting multiple pathological mechanisms while maintaining favorable biosafety profiles for potential clinical translation.

Therefore, PELNs exhibit multi-target interventional potential for PD treatment by enhancing mitophagy, regulating antioxidant pathways (Nrf2/HO-1), reducing ROS generation, and inhibiting neuronal apoptosis. Their low cytotoxicity and scalable production characteristics support promising clinical translation prospects.

Multiple Sclerosis (MS)

MS is an autoimmune-mediated neurodegenerative disorder of the central nervous system characterized by inflammatory demyelination accompanied by axonal transection, leading to neurological dysfunction.[92] The inflammatory response, a core pathological mechanism in MS, typically arises from physical injury or pathogenic infection, triggering abnormal immune system activation and subsequent release of inflammatory mediators. Current oral therapeutic approaches remain limited by peripheral side effects and marginal clinical benefits, highlighting the need for more targeted treatment strategies.

PELNs have emerged as a research focus in MS prevention and treatment due to their unique biological properties and therapeutic potential. Notably, nanovesicles (exosomes) isolated from *Petasites japonicus* have demonstrated immunomodulatory capabilities by regulating both Th1 cell and cytotoxic T cell activities.[83] These plant-derived exosomes carry specific bioactive molecules that effectively suppress Th1 cell hyperactivation and reduce proinflammatory cytokine secretion, thereby mitigating neuroinflammatory responses. Furthermore, *P. japonicus*-derived exosomes were found to enhance cytotoxic T cell function, promoting their protective effects on damaged neural cells while concurrently exhibiting anti-inflammatory and neurorestorative properties through multifaceted mechanisms.

Therefore, PELNs offer a dual strategy of immunomodulation and neuroprotection for the treatment of MS by regulating Th1 cell and cytotoxic T cell functions, suppressing neuroinflammatory responses, and promoting neural repair.

Applications of PELNs as Natural Nanocarriers for Drug Delivery

PELNs have emerged as ideal drug delivery vehicles due to their innate nanostructure and superior biocompatibility. These versatile nanocarriers have demonstrated remarkable capacity for co-delivering both hydrophilic and hydrophobic therapeutics, significantly enhancing drug stability and bioavailability. Their exceptional ability to cross mammalian biological barriers without eliciting inflammatory responses or necrosis makes them particularly valuable for transporting diverse therapeutic agents, including small interfering RNAs, and poorly water-soluble natural compounds like curcumin. Experimental evidence highlights their therapeutic potential: potato-derived exosomes loaded with the anti-inflammatory drug dexamethasone exhibit enhanced efficacy in mitigating microglial inflammation compared to either treatment alone.[93] Similarly, curcumin-encapsulated exosomes obtained through co-incubation with macrophages have demonstrated targeted delivery to ischemic brain tissue, effectively suppressing ROS-mediated mitochondrial apoptosis and substantially reducing cerebral ischemia-reperfusion injury.[94] Researchers have successfully isolated exosome-like nanovesicles from ginger and subsequently loaded them with antibacterial nucleic acids to create the formulation Exo@tac. This formulation, through modulation of the gut microbiota and the microbiota-gut-brain axis, has been shown to significantly enhance motor function and ameliorate pathological features in a murine model of PD.[81] A groundbreaking study by researchers at Zhejiang University revealed that ginseng-derived exosomes serve as highly efficient and safe carriers for delivering bioactive miRNAs to bone marrow-derived mesenchymal stem cells.[86] These exosomes not only facilitate neural differentiation of stem cells but also promote functional neural regeneration and conduction repair in both in vitro and in vivo models. This finding provides compelling evidence for the cross-species transfer of plant-derived miRNAs via PELNs, establishing their potential as novel nanoplatforms for neural differentiation therapies. A study developed a novel EV-DNs complex by integrating doxorubicin (DOX)-loaded heparin-based nanoparticles (DNs) with natural grapefruit-derived EVs. The DNs were designed with pH-sensitive hydrazone bonds for DOX conjugation and modified with cRGD to enhance targeting specificity. The EVs facilitated efficient drug delivery by leveraging their innate membrane fusion and transcellular transport capabilities, successfully overcoming the BBB. This strategy offers a promising approach for EV-based treatment of brain tumors and demonstrates potential for clinical translation.[84] Collectively, these findings underscore PELNs' remarkable capacity for targeted blood-brain barrier penetration and position them as promising nanotherapeutics for treating neurological disorders. Their unique ability to facilitate cross-species communication while maintaining excellent safety profiles represents a significant advancement in drug delivery technology.

PELNs possess inherent advantages for cross-species drug delivery, including exceptional targeting ability and high biocompatibility. However, their clinical translation remains hindered by several critical challenges: low drug-loading capacity, significant batch-to-batch variability, poorly understood in vivo behavior, and unresolved risks of immunogenicity and phytotoxin exposure.

Challenges and Limitations of PELNs

Despite the considerable promise exhibited by PELNs in the treatment of neurological diseases and drug delivery, current researches encounter numerous challenges and limitations. These issues significantly impede their translation from basic research to clinical application, necessitating targeted advancements in future studies.

At present, the isolation of PELNs predominantly utilizes techniques, such as DUC, GUC, and SEC. Nevertheless, variables including the plant source, extraction batch, harvest season, freshness, storage conditions, and inconsistencies in operational parameters across laboratories (such as centrifugation speed, gradient medium concentration, and chromatography column type) result in substantial variability in the purity (eg, levels of protein and nucleic acid impurities), yield (eg, extraction quantity per gram of plant tissue), and biological activity of the isolated PELNs. For example, when employing density gradient centrifugation to isolate PELNs from ginseng and ginger, reported yields can differ by a factor of 2–3 across various studies (eg, 168 mg protein/kg ginseng versus 500 mg protein/kg ginseng).[10,95]

Furthermore, the field currently lacks a standardized quantification framework for PELNs, with divergent quantification units employed across studies, thereby complicating the cross-comparison and replication of research findings. Therefore, a key priority is to establish standardized preparation protocols and unified quantification units to ensure the reproducibility and consistency of PELNs from different sources.

The identification and validation of specific markers for PELNs continue to pose significant challenges. The development of universal or plant species-specific biomarkers for PELNs would not only establish a precise foundation for targeted isolation and purification—thereby reducing impurity interference and enhancing separation efficiency—but also significantly advance the comprehensive analysis of their biological functions and elucidate their interaction mechanisms with target cells. Although some studies have identified potential markers, such as the TET8 protein in ELNs derived from Arabidopsis thaliana, the applicability of this marker to PELNs from other plant species remains inadequately verified.[22] Additionally, the existence of core PELN markers common across different species is yet to be determined. Addressing these gaps necessitates systematic investigations involving large sample sizes and multiple plant species.

Additionally, the stability of PELNs during storage is profoundly affected by the interplay of temperature, time, and protective agents. In a study by Kim et al, a comparative analysis was conducted to evaluate the impact of different storage temperatures (−20°C, 4°C, 25°C, and 45°C) and freeze-thaw cycles on LEVs.[96] The results demonstrated that during short-term storage (0 to 2 weeks), the size and protein content of PELNs remained relatively stable at 4°C. Conversely, protein levels showed a significant decline after one week at −20°C. Regardless of the storage temperature, PELNs were found to undergo fusion, aggregation, and a reduction in surface potential over prolonged storage durations. Notably, after two weeks at 4°C, PELNs exhibited substantial fusion and aggregation, with a shift in the particle size distribution peak towards larger sizes and an increase in surface potential. Similarly, after 1–2 months at −20°C, there was a gradual increase in particle size and a slight rise in surface potential. Although storage at −80°C or rapid freezing in liquid nitrogen followed by transfer to −80°C effectively preserved the dispersity of RDNVs for up to one month, significant aggregation was observed after two months.[97] Maintaining low-temperature conditions during handling or transport poses significant challenges, as repeated freeze-thaw cycles can lead to vesicle aggregation and a subsequent decrease in bioactivity.

The International Society for Extracellular Vesicles advises the storage of exosomes in phosphate-buffered saline (PBS) at −80°C.[98] However, PBS is inadequate in fully preventing lipid oxidation and cargo hydrolysis, which results in membrane protein instability and a notable reduction in miRNA levels after 30 days. PELNs, which are rich in unsaturated fatty acids and polyphenols, are theoretically at a higher risk of oxidation; nonetheless, comprehensive data on their specific oxidation kinetics are lacking and necessitate systematic investigation. The most clinically pertinent formulation involves lyophilization with protectants, such as trehalose.[99,100] However, the reconstitution efficiency and safety of PELNs remain to be validated. Given the diverse plant sources, PELNs exhibit considerable variability in lipid and protein composition, which leads to differing tolerances to freezing and lyophilization. There is also a deficiency in systematic comparisons of protectants and standardized protocols. Furthermore, the safety of formulations containing protectants, the additional processing steps required prior to in vivo application, the high rates of vesicle rupture during room-temperature transportation of PELNs, and the lack of effective cold-chain alternatives pose significant challenges to their clinical translation and application.

PELNs have demonstrated significant preclinical potential in the treatment of neurological disorders, primarily due to their distinctive ability to cross the blood-brain barrier and offer multi-target neuroprotection. However, the field is still in the early stages of transitioning from laboratory research to clinical application, with a marked scarcity of clinical trial data. Additionally, the natural origin of PELNs results in a complex composition and batch-to-batch variability, posing challenges for the design of clinical studies. On an international level, regulatory frameworks for PELNs as therapeutic agents or drug delivery systems—covering classification definitions, approval processes, and quality control standards—remain underdeveloped, leaving enterprises and research institutions without clear policy guidance for clinical translation. It is crucial to establish standardized dosing metrics based on the content of active ingredients, such as specific microRNAs, rather than relying on total particle count. Moreover, systematic pharmacokinetic and toxicological studies are essential to develop clinical dosing regimens.

Summary and Prospects

Neurological disorders pose significant threats to human health, imposing substantial burdens on both society and affected families. Conventional therapeutic approaches face multiple limitations in managing these conditions, including poor BBB penetration, suboptimal efficacy, and undesirable side effects. Accordingly, PELNs have emerged as a promising therapeutic strategy, offering novel opportunities for neurological disease intervention through their unique biological properties and multimodal mechanisms of action. PELNs exhibit considerable advantages for biomedical applications due to their abundant natural sources, cost-effective production, and favorable biological characteristics, including excellent biocompatibility, low immunogenicity, and cholesterol-free composition. These attributes minimize the risk of immune reactions upon administration while ensuring good safety profiles and efficient bioabsorption. As such, PELNs represent an attractive platform for developing novel biotherapeutics and drug delivery systems.

The therapeutic potential of PELNs stems from their diverse bioactive cargo, comprising nucleic acids, lipids, proteins, and secondary metabolites. These components mediate neuroprotective effects through multiple mechanisms: mitigating neuroinflammation, facilitating BBB traversal, counteracting oxidative stress, inhibiting apoptotic pathways, and modulating synaptic plasticity. This multifaceted pharmacological profile positions PELNs as a transformative approach for preventing and treating neurological disorders.

Despite growing interest, the current PELN research landscape is marked by pronounced disparities and inherent limitations, as identified by a comprehensive literature review. While extensive studies have focused on their applications in digestive, oncological, and circulatory systems, investigations targeting neurological disorders remain disproportionately scarce, highlighting a significant knowledge gap in this field. Several technical and translational challenges hinder PELN research and clinical implementation. Notably, existing isolation protocols predominantly adapt methods designed for animal-derived extracellular vesicles, lacking standardized procedures for separation and characterization, which compromises result comparability across studies. Besides, the broad size distribution of PELNs poses substantial challenges for efficient isolation across the full particle spectrum, while high centrifugal forces risk inducing nanoparticle aggregation and membrane structural damage that impair biological activity. Moreover, current techniques frequently yield preparations contaminated with cellular organelles or impurities, reducing both purity and therapeutic efficacy. Indeed, the clinical translation of PELNs is impeded by a scarcity of human trials, underdeveloped regulatory frameworks, and a paucity of well-characterized targeting biomarkers. Notably, while edible plant-derived exosomes have demonstrated consistent anti-inflammatory and cytoprotective properties, research on exosomes from medicinal herbs remains in its nascent stages, representing an underexplored area with considerable therapeutic potential.

From a clinical translation perspective, the large-scale application of PELNs still faces multiple challenges that require resolution. First, extraction processes need further optimization to improve both the yield and purity of PELNs to meet clinical-scale demands. Second, the bioactive components and their mechanisms of action remain incompletely characterized, necessitating more in-depth fundamental research. Regarding safety assessment, while animal studies have preliminarily demonstrated the biocompatibility of PELNs, their long-term safety in humans requires systematic verification. Besides, critical pharmacokinetic parameters, including in vivo stability, biodistribution patterns, and metabolic pathways, must be thoroughly investigated to optimize drug delivery efficiency and therapeutic outcomes.

Looking ahead, as research progresses, we anticipate the identification of more PELN-specific biomarkers, refinement of extraction and large-scale production technologies, establishment of standardized manufacturing protocols and quality control systems, and development of stable long-term storage methods. These advances will accelerate the translation of PELNs from laboratory research to clinical applications, ultimately providing safe, effective, and innovative solutions for treating neurological disorders to benefit a broader patient population. Indeed, while this review adopts the terms "exosomes" or "extracellular vesicles" as used in the literature, the PELNs derived from fruit and vegetable juicing extracts likely represent heterogeneous mixtures of plant cell-derived vesicles. This inherent characteristic should be carefully considered in future research and applications to ensure accurate interpretation of results and proper therapeutic implementation.

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Disclosure

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