

Plant-Derived Nanovesicles: A Next-Generation Therapeutic Platform for Targeted Drug Delivery in Neurodegenerative Disorders

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Abstract. Neurodegenerative disorders like Alzheimer, Parkinson and Huntington are becoming a growing health burden worldwide with few treatment options because of difficulties across the blood-brain barrier (BBB) and to deliver the therapeutic agent to the target location. Plant-derived nanovesicles (PDNVs) have recently become a new and promising nanocarrier due to their innate biocompatibility, reduced immunogenicity, and stability, as well as their natural encapsulation and transportation capacity of bioactive molecules. This is a review that empirically examines the current developments in isolation, characterization, and functionalization of PDNVs as a target-delivery vehicle in neurodegenerative diseases. Particular attention is given to their ability to cross-physiological barriers and react with neuronal cells and administer therapeutic agents in a high specificity and low toxicity. We also emphasize on the mechanisms of neuroprotection by PDNV, recent *in vitro* and *in vivo*, and existing limitations and challenges. Lastly, we suggest future research direction to bring PDNV-based therapeutics to the bench to the bedside, and in particular, scalable production, regulatory implications and clinical implementation.

Keywords. Plant-derived nanovesicles; Targeted drug delivery; Neurodegenerative diseases; Blood-brain barrier; Nanocarriers; Natural exosome mimetics.

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1 Introduction

Neurodegenerative diseases (NDs), including Alzheimer disease (AD), Parkinson disease (PD), Huntington disease (HD), and amyotrophic lateral sclerosis (ALS) are progressive and debilitating conditions that are characterized by the selective degradation of discrete cell subpopulations and the formation of pathogenic protein aggregates within the central nervous system (CNS) [1]. With aging of the world, the number of such disorders is expected to increase exponentially and hence create a stiff burden to the society and the economy.

WHO estimates that over 55 million people are currently affected by dementia in the world today- a figure that is expected to reach three times by the year 2050 -2]. The passage of therapeutic agents through the highly selective blood-brain barrier (BBB) has been listed among the primary challenges in the treatment of NDs. The BBB restricts the deepest intrusion of the majority of macromolecules, which restricts the curative power of many promising neuroprotective agents [3]. Traditional approaches to drug delivery systems, such as liposomes, synthetic nanoparticles and viral vectors, have been inconsistently effective in overcoming the BBB but difficulties exist in the fields of immunogenicity, cytotoxicity, lack of scalability, and regulatory limitations 4.

Plant-based nanovesicles (PDNVs), a subdivision of naturally occurring extracellular vesicles (EVs), have also received significant attention as a new generation delivery platform in this milieu. These are nano-sized vesicles that are extracted in edible plants, ginger, grapefruit, grape, and broccoli and share functional properties with mammalian exosomes, i.e. bilayered lipid membrane, vesicular morphology and cargo-carrying properties [5]. Compared to their mammalian equivalents, PDNVs have a number of salient features: they are inherently bio-compatible, have low or no immunogenicity and can be cost-effectively produced on a large scale [6]. Notably, PDNVs have been shown to possess natural therapeutic capabilities, namely, anti-inflammatory, anti-oxidant and anti-apoptotic, which makes them especially interesting in the neurological context, as the therapeutic agent of interest [7]. These bioactive qualities have been confirmed by independent research groups among diverse plant species and conditions of different diseases [8-10].

Recent preclinical studies have demonstrated the ability of PDNVs to transport small molecules, RNAs, or proteins to the brain parenchyma, through BBB penetration, providing new possibilities in the specific treatment of neurodegenerative diseases [11, 12]. Additionally, their derivation as a result of edible plants is also in line with the principles of green nanotechnology and contributes to increased regulatory acceptance, especially when it comes to the chronic and long-term delivery that is required by neurodegenerative diseases.

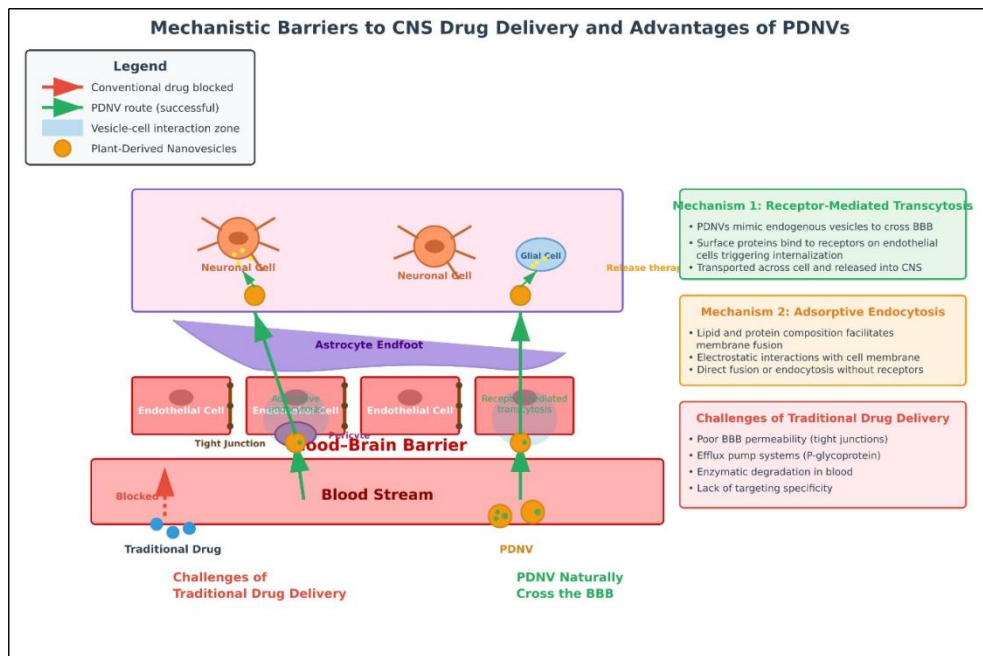
Following the increased body of literature in the field, the current review attempts to review the existing developments in the field of PDNV studies, especially as it pertains to their implementation in the specific treatment of NDs. Their biological sources and composition properties, isolation and characterization processes, loading strategies in therapy and neuroprotective properties will be reviewed and major challenges, technology gaps and translational potential in clinical practice will be outlined.

Table 1: Global Prevalence and Projected Burden of Major Neurodegenerative Diseases [1]
 [2].

Disease	Current Global Prevalence (2024)	Projected Cases by 2050	Economic Cost (USD/year)
Alzheimer's Disease	~44 million	~139 million	>1.3 trillion
Parkinson's Disease	~10 million	~30 million	~80 billion
Huntington's Disease	~1 million	~1.5 million	Not available
Amyotrophic Lateral Sclerosis	~500,000	~800,000	~15 billion

A schematic (Figure 1) summarizes key physiological barriers in CNS drug delivery and highlights the specific mechanisms through which PDNVs overcome them, using annotated arrows and labeled pathways to guide interpretation

Fig. 1. Mechanistic overview of blood–brain barrier (BBB) transport routes and comparative advantages of plant-derived nanovesicles (PDNVs). PDNVs exploit receptor-mediated and adsorptive transcytosis to deliver therapeutic cargo across endothelial cells, overcoming limitations of conventional nanoparticles.



2. Plant-Derived Nanovesicles – Origin and Composition

Plant-derived nanovesicles (PDNVs) or plant exosome-like nanovesicles and plant extracellular vesicles (PEVs), are a class of naturally occurring lipid-bounded nanoscale vesicles that are secreted from various edible and medicinal plant species. These nanovesicles are now becoming established as being actively involved in biological signaling and are capable of mediating communication not only between different, conspecific species, but also between phylogenetically distant kingdoms of life. Recent investigations indicate that PDNVs may hold therapeutic potential especially in the field of drug delivery, immune modulation and tissue regeneration [13, 14].

2.1 Origin and Secretion

Plant-derived nanovesicles (PDNVs) are secreted from plant cells through endosomal trafficking processes that are similar to those used for the secretion of exosomes in mammals. They are formed mainly inside multivesicular bodies (MVBs) and by invagination of the plasma membrane in response to cellular events such as stress responses, pathogen defence and homeostatic regulation [15]. Although studies on the biology of plant vesicles are relatively new compared to the extensive literature on mammalian extracellular vesicles, recent studies have proven the ubiquitous nature of nanovesicles in a wide range of plant tissues including fruits, vegetables, roots and seeds .

To date the most extensively investigated plant sources are ginger, grapefruit, grape, carrot, broccoli, aloe vera and turmeric. Each species makes unique morphological and functional contributions . For instance, nanovesicles derived from ginger (Gingerol and shogaol) contain high concentrations of anti-inflammatories compounds gingerol and shogaol with well-characterised anti-inflammatory properties [10] whereas grapefruit-derived nanovesicles (GFNVs) show an efficient targeting of intestinal macrophages and sites of inflammation [10].

Table 2. Selected Edible Plants Used for PDNV Extraction and Their Therapeutic Attributes.

Plant Source	Size Range (nm)	Key Bioactive Compounds	Therapeutic Potential	Reference
Ginger	150–250	6-gingerol, shogaols	Anti-inflammatory, antioxidant	[10]
Grapefruit	100–200	Naringenin, vitamin C	Anti-cancer, macrophage targeting	[6]
Grape	50–100	Resveratrol, flavonoids	Neuroprotection, cardioprotection	[7]
Broccoli	100–150	Sulforaphane, isothiocyanates	Antioxidant, detoxifying	[14]
Aloe vera	200–400	Polysaccharides, aloin	Wound healing, anti-inflammatory	[6]

2.2 Structural and Molecular Composition

Plant-derived nanovesicles (PDNVs) are typically small spherical or slightly elongated, with diameters ranging from 30 to 400nm. Their size and shape depends on the plant source and isolation methodology used. Consistent with other extracellular vesicles, PDNVs have a phospholipid bilayer, which provides mechanical stability and enables differentiation of various molecular cargo [6].

The lipid components in PDNVs are phosphatidic acid, phosphatidylethanolamine, phosphatidylcholine and various glycolipids. These lipids are also found in mammalian exosomes; however, there are often subtle compositional differences that exist in vesicles from plants that affect their interaction and uptake by mammalian cells [6]. Many of these lipids are involved with the anti-inflammatories and anti-oxidants that are often associated with PDNVs.

Proteomic analyses have shown that PDNVs contain a heterogeneous set of proteins consisting of stress related proteins, proteins involved in vesicular trafficking such as HSP70 and annexins, as well as signal transduction molecules. Also, in addition to their protein cargo, PDNVs carry small RNAs and microRNAs, some of which can regulate gene expression in mammalian cells which contributes to the idea of restricted cross-kingdom

communication between plants and animals. Dietary plant microRNAs have been suggested in several studies to affect physiology in mammals, including lipid metabolism and immunity.

Apart from lipids, proteins and nucleic acids, PDNVs often contain plant-specific secondary metabolites - such as polyphenols, flavonoids, terpenoids, and alkaloids. These molecules might enhance the biological effects of the vesicles and might be responsible for their stability throughout digestion and systemic circulation[7].

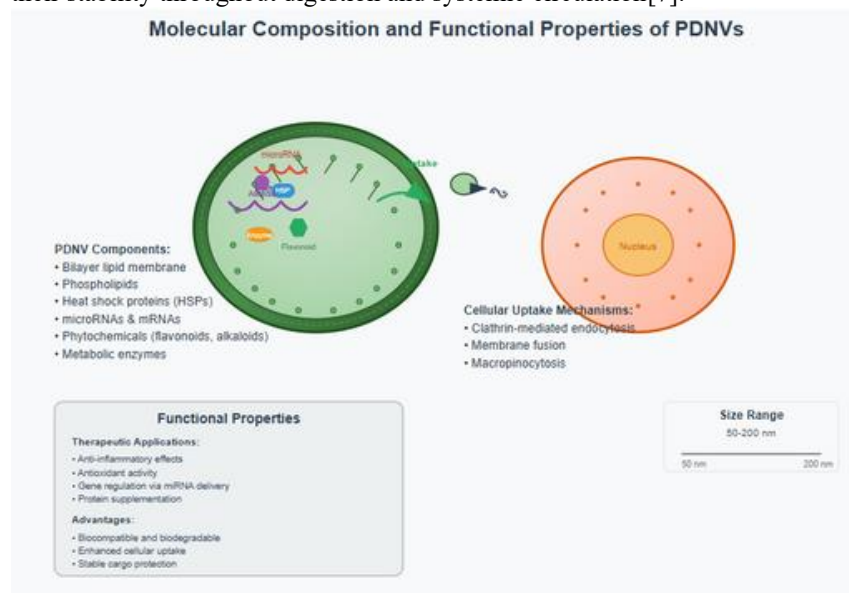


Fig. 2. Molecular Composition and Functional Properties of PDNVs.

2.3 Functional Implications of PDNV Composition

Plant-derived nanovesicles (PDNVs) obtain the functional characteristics mainly because of their inherent architecture and encapsulated constituents. Their structural conformation, in combination with the endogenous molecular cargo, eventually governs how long they remain in the system, with what spectrum of cellular interactions, and what bioresponses to expect after cellular uptake. For example, vesicles extracted from broccoli contain sulforaphane and in neuronal models the vesicles activate the Nrf2 signalling cascade, which enhances antioxidant forms and contributes to cellular tolerance to oxidative stress [11]. A similar investigation involving vesicles derived from ginger showed that some of the microRNAs contained in their cargo dampen pro-inflammatory signalling pathways both in the gastrointestinal tract and within components of the peripheral nervous system [11].

The lipidomic profile of PDNVs further modulates their functional properties. The lipid composition in particular provides efficient membrane fusion with target cells and reduces off-target cytotoxicity, which is still not the case for many of the synthetically made delivery systems [13]. Collectively, these observations highlight the therapeutic efficacy of PDNVs as dependent on their structural integrity as well as the heterogeneous biochemical milieu they carry. Owing to this combination PDNVs are showing a substantial versatility

as carrier for both native phytochemicals and exogenous incorporated pharmacophores. Importantly, PDNVs have been shown to cross restrictive physiological interfaces, notably the blood-brain barrier, and therefore expand the possibility of a neuro-centric indicating therapy.

3 Isolation and Characterization Techniques

PDNVs need to be isolated and their isolation verified to be therapeutic. purification processes needs to be constant and repeatable. It is considering that these vesicles are different. widely diverse in size, density and biochemical, but it is necessary to refine isolation strategies. get preparations that can be reproducibly pure and yielding and with biological activity. A variety of methods--mechanical homogenization, differential centrifugation and so on. Density-gradient ultracentrifugation, size-exclusion chromatography (SEC) and. microfluidic-based techniques- are used to purify nanovesicles in plant. tissues.

3.1 Isolation Strategies Isolation of PDNV

It is normally done in a sequence of steps:

1. Heterogenization of plant tissue.
2. Elimination of coarse debris and fibrous.
3. Separated vesicle fractions by different centrifugation or membrane filtration.
4. Last purification by gradient centrifugation or SEC-based.

3.1.1 Differential Ultracentrifugation

In this method, a clear and uniform suspension of the required protein is created and the contents are then subjected to a sequence of gentle centrifugation steps to fractionate the suspension into distinct components. It involves the formation of a transparent and homogeneous suspension of the desired protein, which is then subjected to a series of mild centrifugation steps to separate the suspension into separate parts. Differential ultracentrifugation is the most available protocol in the protocols at present. used and common technique of encryption of PDNVs. The process involves centrifugation at different increasing centrifugation speeds to eliminate cellular debris and concentrate nanovesicles, which are usually of the order 100,000 xg Despite its utility, it can be used to achieve co-isolation of protein aggregates or organellar fragments, which requires additional purification to make the use of vesicles specific Density Gradient Centrifugation.

3.1.2. Density Gradient Centrifugation

In order to achieve greater purity, density-gradient centrifugation of sucrose or iodixanol (OptiPreptm) was used. varying separations Vesicles can be separated by this method, which depends on their buoyancy density (typically). 1.13-1.19 g/mL for PDNVs) [12]. This is a labor-intensive method that results in high production of pure vesicles that are preclinical research and therapeutic grade.

3.1.3 Size-Exclusion Chromatography (SEC)

SEC is a chromatographic technique used to separate proteins according to mass. Size-exclusion chromatography provides a gentle scalable method of isolating PDNV which protects the integrity of the vesicles and inhibits aggregation. This approach has attracted publicity to manufacturing pharmaceutical grade preparations and reducing structural changes. Commercial qEV columns and the like SEC set-ups have now been modified to isolate PDNV, empowering more coherent downstream applications [13].

3.1.4 Emerging Techniques

New technologies of isolation, such as ultrafiltration, tangential flow filtration (TFF), and microfluidic based purification systems- have a potential benefit in scalability and processing efficiency [13]. These methods will help to decrease mechanical stress and improve recovery rates of vesicles, despite validation in various plant species and large-scale systems remains ongoing

Table 3. Comparative Summary of PDNV Isolation Techniques.

Isolation Method	Advantages	Limitations	Suitability
Differential Ultracentrifugation	High yield, established protocol	Time-consuming, co-isolation of contaminants	Research-grade
Density Gradient Centrifugation	High purity	Labor-intensive, low throughput	Preclinical studies
Size-Exclusion Chromatography	Gentle on vesicles, scalable	May require pre-concentration	Clinical-grade
Ultrafiltration/TFF	Rapid, scalable	Membrane fouling, potential vesicle loss	Industrial scale
Microfluidic Devices	Precision, automation	Still under optimization for PDNVs	Lab-on-chip studies

Table 3A: Minimal Characterization Parameters for PDNV Quality Control

Parameter	Analytical Method(s)	Rationale for Inclusion	Acceptable Benchmark / Indicator
Particle size and distribution	Dynamic Light Scattering (DLS), Nanoparticle Tracking Analysis (NTA)	Confirms homogeneity and defines vesicle class (30–400 nm)	Mean size < 400 nm; PDI < 0.3
Morphology and integrity	TEM or Cryo-TEM	Verifies bilayer structure and absence of aggregation	Intact, spherical vesicles with clear bilayer
Surface charge (ζ-potential)	Electrophoretic light scattering	Reflects colloidal stability and potential for uptake	–20 to –35 mV indicates good dispersion
Protein profiling	SDS-PAGE, Western blot, LC–MS/MS	Confirms vesicular identity and purity	Presence of HSP70/annexin-like proteins; absence of chloroplast or cytosolic contaminants
RNA/miRNA quantification	qRT-PCR, Bioanalyzer	Evaluates nucleic acid cargo for biological activity	Detectable small RNAs; integrity confirmed by RNA profile
Lipid composition	LC–MS, lipidomics	Ensures consistency of plant lipid signatures affecting uptake	Comparable phospholipid profile to source species
Purity index	Protein-to-particle ratio, density gradient profiling	Differentiates vesicles from co-purified debris	< 1×10^9 particles/ μ g protein (EV purity guideline)

The suggested minimum characterization standards (Table 3A) are meant to be a guiding principle to standardize the practices of PDNV research. By including such measures in subsequent reports, it will be easier to conduct reproducibility, conduct meta-analyses, and provide regulatory validation when developing translational PDNV-based therapeutics.

3.2 Characterization of PDNVs

PDNVs can be characterized reliably to ascertain identity, quality and functional properties. Most of the methods of mammalian extracellular vesicles studies have been applied to plant vesicles, although with certain alterations to consider their unique characteristics.

3.2.1 particle Size and Distribution

Particle size and distribution are determined by measuring the diameter of the particle and assessing the particle size distribution distribution (Baumrind, 2014). Particle size and

distribution Particle size and distribution are measured by measuring the diameter of the particle and evaluating the distribution of particle size distribution (Baumrind, 2014). The most commonly used methods of measuring the size distribution of PDNV, as well as particle concentration, are dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA). PDNVs in other plant sources are typically within the range of 30-400 nm of nanoscale, depending on the type of plant and the isolation technique [7]. This size scale is in agreement with their capacity to interact effectively with the biological membranes and cellular receptors and remain in colloidal suspension.

3.2.2 Morphological Analysis

The bilayered, spherical morphology of the PDNVs is visualized by use of high-resolution implication methods which include transmission electron microscopy (TEM) and cryogenic TEM (cryo-TEM). These methods verify that there is no vesicular rupture or aggregation and structural distortion. Also, scanning electron microscopy (SEM) or atomic force microscopy (AFM) might be used to determine surface topology to determine uniformity and nanoscale features [13].

3.2.3 Surface Charge and Stability

This section presents the surface charge and stability of the nanoparticles. The measurements of zeta potential are important to give important details on the surface charge of vesicles and stability. The negative zeta potentials of most of the PDNVs are between -20 and -35 mV, which indicates the repulsion of the vesicles due to electrostatic repulsion [12]. This negative charge is credited to the fact that their membranes contain phosphatidic acid and other anionic lipids, which increases dispersion and increases the suspension stability in aqueous media.

3.2.4 Molecular Profiling

Molecular characterization of PDNVs involves the analysis of protein, RNA, and lipid components of PDNVs. Vesicle-related proteins like annexins and heat shock proteins (HSP70) are typically identified by protein profiling using the SDS-PAGE and Western blotting techniques, which prove the vesicular origin. qRT-PCR or next-generation sequencing is used to measure RNA and microRNA cargo, which gives information concerning their gene-regulatory potential [7]. Lipidomic and metabolomic studies also indicate the plant-specific lipid species and phytochemicals that are introduced into PDNVs to enhance their biological activities and stability [14].

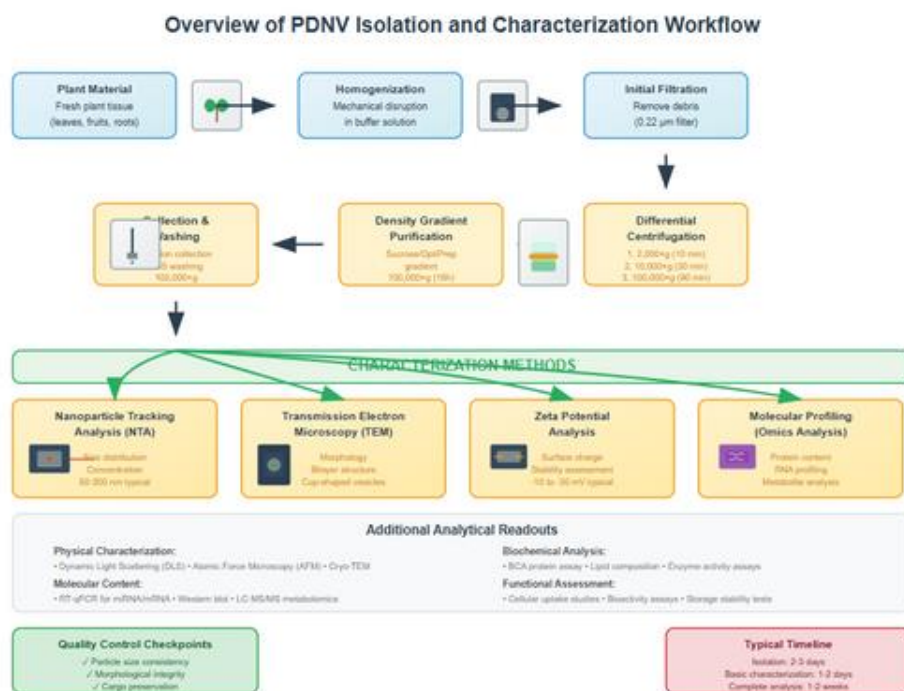


Fig. 3. Overview of PDNV Isolation and Characterization Workflow.

3.3 Quality Control and Standardization Challenges

Despite significant advancements, establishing standardized protocols for plant-derived nanovesicles (PDNVs) remains a considerable challenge. Variations in plant species, growth conditions, isolation methods, and yield introduce inconsistencies in vesicle characteristics across laboratories [14]. The International Society for Extracellular Vesicles (ISEV) has underscored the necessity for developing guidelines tailored specifically to PDNVs to ensure reproducibility and eventual clinical translation.

To address these issues, researchers are working toward **Good Manufacturing Practice (GMP)-compatible isolation systems** and identifying **plant-specific vesicle markers** for authentication and quantification [14][15]. These measures are vital for transitioning PDNVs from experimental models to therapeutic applications.

For reproducible and clinically relevant PDNV research, a **minimal set of physicochemical parameters** must be consistently evaluated following each isolation. Key indicators include particle size distribution, morphology, surface charge, protein and RNA cargo content, and compositional integrity. These metrics collectively reflect vesicle stability, purity, and functional performance. Furthermore, purity indices—such as the protein-to-vesicle ratio and the absence of large contaminants—should be documented to differentiate genuine vesicles from co-isolated plant debris or protein aggregates. Implementing standardized reporting will improve scientific rigor, enable cross-study comparability, and accelerate the development of GMP-compliant production pipelines.

4 Mechanisms of Pdnv Uptake and Blood–Brain Barrier Penetration

The neurodegenerative diseases and, in particular, their therapeutic efficacy of the plant-derived nanovesicles (PDNVs) depends on the capacity of these nanoparticles to cross the blood-brain barrier (BBB) and transport bioactive molecules to neural tissues. The BBB, which is composed of endothelial cells connected together through tight junctions and supported by astrocytes and pericytes, is an extremely important defense mechanism in ensuring homeostasis of the central nervous system (CNS). However, it poses a significant challenge to delivering drugs. Due to their nano-dimensions, bilayer composition, and membrane proteins, PDNVs have become potential natural carriers that can penetrate the BBB [15][3].

4.1 Cellular Uptake Mechanisms of PDNVs

After administration, plant-derived nanovesicles (PDNVs) can penetrate cell membranes through several different mechanisms similar to those used by mammalian extracellular vesicles such as:

- **Clathrin-mediated endocytosis:** Internalization via clathrin-coated pits, frequently observed in endothelial and epithelial cells. Ginger- and grape-derived PDNVs are known to exploit this pathway [3].
- **Caveolin-mediated endocytosis:** A lipid raft-dependent mechanism prevalent in vascular endothelium and neuronal cells [15].
- **Macropinocytosis and phagocytosis:** Energy-dependent uptake routes in immune cells, enabling PDNVs to regulate neuroinflammation in disease states [10].

Vesicle surface charge and lipid composition as well as patterns of membrane proteins modulate the uptake efficiency. PDNVs containing phosphatidic acid, digalactosyldiacylglycerol and phytosterols have a greater cellular fusion and endosomal escape, and thus increase bioavailability.

4.2 Strategies Enabling Blood–Brain Barrier (BBB) Penetration

The most critical barrier in the creation of central nervous system-directed therapies is translocation across the blood-brain barrier. Polydisperse nanovesicles (PDNVs) a variety of measures are used to overcome this barrier:

4.2.1 Receptor-Mediated Transcytosis (RMT)

Passive drug-nanovesicles have the ability to utilize receptor-mediated transcytosis by expressing endogenous or synthetic ligands selective to endothelial receptors, e.g. transferrin receptors, low-density lipoprotein receptors or insulin receptors (47).

As an example, grapefruit vesicles have shown blood-brain barrier penetration through low-density lipoprotein receptor-mediated endocytosis (44).

4.2.2 Trojan Horse Mechanism

Alternatively, the PDNVs might use immune cells, especially monocytes and macrophages, to internalise vesicles in the blood and then cross the blood-brain barrier and release their cargo into the brain parenchyma. This is an indirect but efficient transport pathway which is done by a cell-mediated process [4].

4.2.3 Paracellular and Adsorptive-Mediated Transport

PDNVs have the capacity to temporarily alter tight junction proteins and therefore open paracellular spaces that permit passage of nanoscale structures. Simultaneously, transcytosis by adsorptive interaction between negatively charged vesicle membranes and positively charged blood-brain barrier surfaces occurs [14]. The two processes play a role in providing context-dependent mechanisms of blood-brain barrier penetration.

Table 4: Summary of Mechanisms for PDNV Uptake and BBB Penetration.

Mechanism	Description	Relevance to PDNVs	References
Clathrin-mediated endocytosis	Internalization via clathrin-coated pits	Common in epithelial and endothelial cell types	[7]
Caveolin-mediated endocytosis	Endocytosis via lipid raft domains	Observed in vascular endothelium	[13]
Receptor-mediated transcytosis	Vesicle movement mediated by ligand-receptor binding across the BBB	PDNVs engineered with targeting ligands like Tf, LDL	[1]
Trojan horse strategy	Uptake by immune cells and transport across BBB	Allows indirect BBB crossing via immune cell migration	[11]
Adsorptive-driven transport	Electrostatic interactions drive non-specific vesicle uptake	Dependent on surface zeta potential of PDNVs	[12]

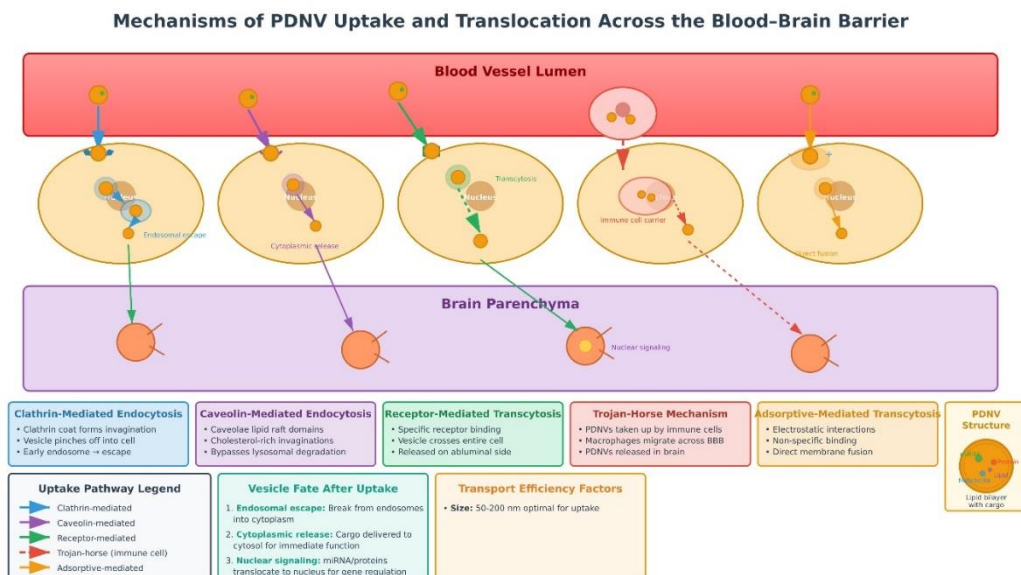


Fig. 4. Graphical illustration of the key processes through which plant-based nanovesicles (PDNVs) cross the bloodbrain barrier (BBB). The scheme outlines endocytosis, which is mediated by clathrin and caveolin, receptor-mediated transcytosis, adsorptive transcytosis and immune cell-mediated Trojan-horse delivery. The arrows show the direction and the nature of vesicular movements and cargo release in neuronal and glial compartments.

4.3 Evidence of BBB Crossing in Preclinical Studies

A number of vesicle systems derived by plants have been experimentally demonstrated to be permeable across the blood-brain barrier (BBB):

- **Ginger-derived nanovesicles (GDNVs):** Demonstrated the ability to deliver doxorubicin and siRNA into the mouse brain, exhibiting neuroprotective and anti-inflammatory effects [15].
- **Grapefruit-derived nanovectors:** Engineered to display **RVG peptides** have successfully crossed the BBB and delivered miRNAs and chemotherapeutic agents [3].
- **Citrus-derived vesicles:** Inhibited glioma progression in murine models after systemic administration, confirming BBB penetration and tumor targeting [4].

These findings underscore the potential of **PDNVs** as non-immunogenic, scalable, and customizable carriers for **central nervous system (CNS)** drug delivery.

4.4 Engineering PDNVs for Enhanced Brain Targeting

To further enhance BBB permeability and specificity, PDNVs can be **surface-engineered** using several biofunctionalization approaches:

- **Ligand peptides** (e.g., RVG, TGN) targeting CNS receptors.
- **Polymeric coatings** (e.g., PEGylation) to improve circulation time and reduce immune clearance.
- **Fusion proteins or monoclonal antibodies** for receptor-specific binding and transcytosis [15].

These bioengineering approaches give PDNVs greater neuronal tissue tropism, greater serum stability, and greater accumulation in pathological areas of the brain, overcoming species-specific and anatomical delivery barriers.

5 Therapeutic Applications of Pdnvs in Neurodegenerative Diseases

Neurodegenerative diseases (NDs) like **Alzheimer's disease (AD)**, **Parkinson's disease (PD)**, **Huntington's disease (HD)** and **amyotrophic lateral sclerosis (ALS)** are all characterised by progressive neuronal loss, oxidative stress and neuroinflammation. The complexity of these conditions in conjunction with the restrictive environment of the BBB prevents the efficacy of conventional therapeutics. Plant-derived nanovesicles (PDNVs) - due to their natural origin, intrinsic bioactivity and biocompatibility - have become promising therapeutic nanocarriers with the capacity to modulate neuropathological processes at the molecular/cellular levels [15][15].

5.1 Antioxidant and Anti-inflammatory Action of PDNVs

Oxidative stress and inflammation play a major role in the progression of neurodegenerative. PDNVs enriched with flavonoids, phenolics and microRNAs have shown inherent antioxidant and anti-inflammatory properties.

- **Ginger-derived nanovesicles (GDNVs): 6-gingerol and shogaol**, inhibit the generation of reactive oxygen species (ROS) and inhibit the activation of **NF- κ B** signaling that leads to proinflammatory cytokines in neural tissues [15][10].
- **Grapefruit-derived vesicles:** Regulate macrophage polarization, promoting **M2 (anti-inflammatory)** phenotypes over **M1 (pro-inflammatory)** ones—an essential mechanism in mitigating neuroinflammation associated with PD and ALS [3].

Even in the absence of exogenous cargo, PDNVs have shown an intrinsic therapeutic efficacy. In addition, other studies have documented anticancer, wound healing and antioxidant activity of PDNVs, consolidating their wide translational potential [10].

5.2 PDNVs as Vehicles for Transport of Therapeutic Cargoes for CNS Disorders

PDNVs are good vehicles for various therapeutic agents such as small molecules, nucleic acids, and peptides due to their phospholipid bilayer and biomolecular protection, which provide protection against enzymatic degradation and premature elimination of loaded drugs.

5.2.1 Delivery of Nucleic Acids

PDNVs have been successfully employed to transport **siRNA**, **miRNA**, and **DNA-based** therapeutics targeting neurodegeneration-associated genes:

- **siRNA targeting BACE1 (β -secretase):** Encapsulated in **ginger-derived PDNVs**, significantly reduced **amyloid- β ($A\beta$)** accumulation in Alzheimer’s models [15].
- **Plant microRNAs (miR168a, miR156):** Naturally present in PDNVs, modulate mammalian **inflammatory** and **apoptotic** signaling pathways, supporting cross-kingdom gene regulation [10].

5.2.2 Delivery of Synthetic or Natural Drugs

PDNVs have been employed to enhance the solubility, stability, and BBB penetration of **neuroprotective phytochemicals** and synthetic drugs:

- **Curcumin-loaded PDNVs** derived from turmeric exhibited superior brain accumulation and prolonged half-life, resulting in reduced **tau phosphorylation** and cognitive decline in AD models [15].
- **Resveratrol-loaded grape vesicles** demonstrated improved antioxidant delivery in hippocampal neurons, restoring mitochondrial function and enhancing neuroprotection in vitro [4].

Table 5: Examples of Therapeutic Applications of PDNVs in Neurodegenerative Models

PDNV Source	Therapeutic Cargo	Target Disease	Mechanism of Action	Outcome	Reference
Ginger	siRNA-BACE1	Alzheimer’s Disease	$A\beta$ inhibition, anti-inflammatory	Reduced plaque burden and memory loss	[10]
Grapefruit	Resveratrol	Alzheimer’s Disease	Antioxidant, mitochondrial repair	Neuroprotection in hippocampal neurons	[15]
Citrus limon	TRAIL peptides	Parkinson’s Disease	Induction of apoptotic pathways in damaged neurons	Enhanced survival of dopaminergic neurons	[12]
Turmeric	Curcumin	Alzheimer’s Disease	Anti-tau phosphorylation, BBB penetration	Improved cognition in transgenic mice	[15]

Grape	Native vesicles (no cargo)	ALS / PD	Macrophage modulation, signaling	miRNA	Reduced inflammation and improved motor control	[13]
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Table 6: Pathway comparison for BBB penetration

Mechanism	Representative numeric result (brain uptake)	Notes & reference
RMT — Angiopep-2 / LRP1 (example: ANG1005 / ANG-drug conjugates)	~0.1% ID (ANG1005) → ~0.62 nmol/g parenchyma at 30 min in mice; reported as ~0.11% of injected dose in one study.	ANG1005 (Angiopep-2–paclitaxel conjugate) delivered measurable parenchymal drug; shows RMT can move small-molecule cargos and conjugated NPs across the BBB. [15]
RMT — TfR / antibody/ligand targeted NPs	Range varies widely; examples include ~0.06% → up to several %ID/g in optimized models; some targeted constructs report ~3.1% ID/g (8D3 antibody targeting) in mouse models vs lower for other ligands.	TfR strategies can give large fold-improvements vs untargeted controls but depend on ligand, affinity, valency and model. [7][10]
AMT — cationic (adsorptive) carriers	Large fold increases vs control (e.g., 10–20× for cationic albumin in classic studies); absolute %ID varies by construct.	AMT gives high capacity and high endothelial binding, but is non-specific and may increase peripheral/off-target uptake and cytotoxicity. [12]
Cell-mediated (Trojan horse)	Demonstrated delivery to tumours/lesions; quantitative %ID/g often lower/variable and disease-dependent.	Effective in inflammatory / tumour contexts where monocytes/macrophages traffic into brain; less predictable for intact BBB in chronic neurodegeneration. [8]

Carrier-mediated transport (CMT: GLUT, LAT, MCT)	Generally ineffective for intact vesicles; data show transporters favor small molecules (no reliable %ID/g for whole PDNVs).	Transporters are optimized for small metabolites; whole vesicles rarely use CMT productively. [11]
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5.3 Comparative Advantage Over Synthetic Nanocarriers

Compared to synthetic liposomes or polymeric nanoparticles, PDNVs exhibit:

- **Greater biocompatibility and safety profile**, with minimal systemic toxicity or immune response [7].
- **Natural targeting capabilities**, including membrane proteins that facilitate tissue-specific tropism.
- **Intrinsic bioactivity**, offering dual roles as delivery vehicles and therapeutic agents.

Such advantages make PDNVs ideal candidates for sustained and personalized drug delivery in complex CNS pathologies.

Table 7 : PDNV vs Synthetic nanocarriers

Feature	PDNVs (plant-derived nanovesicles)	Synthetic nanocarriers (liposomes / polymeric / LNPs)
Scalability	High potential — can be produced from abundant plant biomass and food-industry waste; several reports show large-yield isolations from fruits/roots and simple upstream (juice/leaf) processing.	Mature scalable processes exist (microfluidics, high-shear mixing, nanoprecipitation) with industrial examples for liposomes/LNPs — but scale-up requires specialized, controlled equipment and GMP facilities. [13]
Cost	Often lower raw-material cost (plants are cheap); isolation can be inexpensive at lab scale, and using agri-waste may reduce costs — but standardized, GMP-grade PDNV production and purification methods are still being developed (costs	Higher materials and process costs (synthetic lipids, polymers, solvents, equipment); well-defined manufacturing pipelines exist but can be costly, especially for clinical/GMP batches. Cost predictable but not cheap.

	uncertain at clinical scale). [12]	
Availability	Very good — many edible plants (ginger, lemon, grape, etc.) produce vesicles; seasonal/geographic variation possible but supply can be large and diversified.	Good — synthetic components are manufactured globally and standardized; supply chain for clinical lipids/polymers is established (but some specialty lipids can be constrained). [6]
Safety / biocompatibility / immunogenicity	Favorable in preclinical studies: low toxicity and low immunogenicity reported (plants are edible), and several studies show minimal inflammation in animal models — but human clinical safety data are limited so far.	Mixed: many synthetic carriers (approved liposomal drugs) have acceptable safety profiles when well-formulated; however, some polymers, inorganic NPs or poorly purified formulations can cause toxicity or immunogenicity — toxicity profiles are well-studied for common platforms.
Ease of targeting (intrinsic + engineering)	Some intrinsic tropisms reported (gut stability, cellular uptake patterns), and PDNVs can be loaded/modified, but targeted-engineering methods (ligand conjugation, controlled PEGylation) are less standardized than for synthetic carriers. Promising for oral/GI targeting.	Highly tunable: well-established chemistries to add targeting ligands, PEG, stimuli-responsive components; reproducible and modular for precise targeting strategies (tumor, receptor-mediated). [6]

6 Challenges, Limitations, and Future Directions for Clinical Translation

Despite their promise in neurotherapeutics, the clinical translation of plant-derived nanovesicles (PDNVs) remains in its infancy. Several scientific, technical, and regulatory challenges hinder their broad application in targeted drug delivery for neurodegenerative diseases. The solution to these shortcomings is found in the interdisciplinary research and regulatory harmonization which is critical to PDNVs taking a step forward in bringing it to the bench to the bedside.

6.1 Standardization and Production Problems on a Large Scale

The lack of standardized isolation and purification procedures is one of the greatest challenges in the development of the PDNV as it leads to the heterogeneity of the size, yield and bioactivity across the various studies and plant sources[13]. Factors (age of the plant, species, growth conditions and the method of extraction (e.g. ultracentrifugation or precipitation) may dramatically affect the composition and reproducibility of the vesicles. Moreover, increasing the production of PDNV on large scale to be used clinically without affecting its integrity and efficacy is a challenge. Though there are food-grade sources (e.g., ginger, grapefruit), which promise to be processed at the industrial scale, quality control and the ability to obtain batch-to-batch consistency are associated with the need to use the solutions of the advanced bioengineering.

Table 8: Major Technical Barriers in PDNV Development and Potential Solutions.

Challenge	Description	Proposed Solution
Lack of standard isolation protocols	Variable yield and purity across studies	Development of GMP-grade, automated purification platforms
Scalability	Difficulties in consistent large-scale production	Bioreactor-based plant cell culture systems
Stability during storage	Lipid degradation and loss of activity	Freeze-drying or cryopreservation using protective excipients
Batch heterogeneity	Variation in size, cargo content, and zeta potential	Quality control via NTA, DLS, and proteomic profiling

6.2 Mechanistic and Translational Limitations

The PDNVs have been proven to be biocompatible in that they can target the brain despite being brain-targeting. mechanism of accurate control over their contact with mammalian cells, biodistribution, and clearance are not understood adequately. Vesicle surface proteins and their role are associated with the role of vesicle surface proteins and their role in vesicle biology. Further requirements on glycoproteins in transiting the BBB or cell-specific uptake include. elucidation [15]. In addition, inter-species miRNA dialogue is debatable. While some miRNAs have been reported to be taken up in mammalian cells and regulate genes in plant miRNAs [11] and others. contend that miRNAs in plants can be digested or lost in the blood, doubted their medical applicability [11]. The controversy of cross-kingdom control of dietary plant miRNAs is mostly. is due to variation in the detection and functional validation across investigations. Early studies indicated that certain plant miRNAs including miR168a and miR159 were able to. penetrate into mammalian circulation and regulate target gene expression [11]. However, follow-up independent studies with a better

sequencing depth, increased contamination. These miRNAs were not always detected with controls, and exogenous spike-in standards, giving attribution to earlier results as being due to technical artifact or to dietary pollution as opposed to real absorption [11]. Such methodological aspects like the lack of relevant negative controls, differences in the efficiency of RNA extraction, and PCR over-amplification can also be a result of conflicting outcomes. Moreover, the inconsistency of the vesicle isolation and quantification procedures makes it difficult to determine miRNAs protection or degradation during PDNVs digestion. To settle this controversy strictly it will be necessary to have harmonized analytical pipelines, functional validation and confirmation of uptake by inclusion of stable isotope-labeled tracers. by loss- and gain-of-function experiments in controlled models in animals.

6.3 Safety, Immunogenicity and Regulatory Barriers

The chronic biosafety of PDNVs especially with repeated dosing is still not clear, well characterized. Though PDNVs are not considered to be toxic, they have some subtle effects. There are immunomodulatory actions or unwanted off-target gene actions that cannot be discounted. [1]. Also, the regulatory status of PDNVs, be it as biologics, botanicals, or nanomedicines--is unclear, cation of PDNVs, whether they are biologics, botanicals, or nanomedicines--is still hesitant, so it is difficult to be clinically approved and limiting assimilation into the pharmaceutical systems.

6.4 Future Directions and Research Priorities

6.4.1 Engineering and Functionalization

- Development of surface-modified PDNVs with targeting ligands (e.g., transferrin, peptides) to enhance BBB specificity [15].
- Encapsulation of **gene-editing tools** like CRISPR/Cas9 for precision therapy of monogenic neurodegenerative disorders.

6.4.2 Artificial or Hybrid PDNVs

- Production of synthetic mimetics or hybrid vesicles through fusion of plant vesicles with synthetic liposomes in order to enhance stability and delivery of the vesicles [15].

6.4.3 Tracking and in vivo imaging Advanced

- Fluorescent and radioactive labeling to learn more about the in vivo trafficking, half-life, and organ distribution of PDNVs.

6.4.4 Personalized Medicine Interaction

- The mechanisms behind individual patient-specific biomarkers of neurodegeneration rationally design PDNV-based therapies on-the-fly using omics-based methods (transcriptomics, proteomics, metabolomics).

7 Summary and Conclusion

The application of nanovesicles derived from plant (PDNVs) as a platform for targeted drug delivery is a promising frontier in neurodegenerative diseases (NDs) management. These vesicles, as a natural secretion of edible and medicinal plants, which are biocompatible, biodegradable, and having the ability to transport bioactive molecules across barriers such as the blood-brain barrier (BBB). Their inherent therapeutic cargo combined with potential

for exogenous drug loading and surface modification, makes them a promising tool in nanoneurotherapeutics.

During the last decade, significant progress has been made in understanding PDNV origin, composition, isolation and function. Preclinical studies have been done using vesicles derived from ginger, grape, broccoli and grapefruit have shown efficacy in mitigating oxidative stress, inhibition of neuroinflammation and enhancement of disease-related outcomes in Alzheimer's, Parkinson's, Huntington's and ALS models. However, key challenges still remain such as protocol standardization, reproducibility, validation for mechanistic reasons, and regulatory approval. Collaborative, multidisciplinary research will be crucial for bringing PDNVs from bench to bedside

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