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REVIEW

Plant-Derived Nanovesicles for Ischemic Stroke Therapy via the Gut Microbiota-Gut-Brain Axis: A New Paradigm of Systemic Regulation

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Abstract: Ischemic stroke (IS) is a globally significant disease with complex pathological mechanisms. Traditional therapeutic strategies centered on central nervous system-targeted delivery face substantial limitations due to the presence of the blood-brain barrier (BBB) and the multifactorial nature of the disease. In recent years, the gut microbiota-gut-brain axis, which elucidates the multi-pathway dialogue between the gut and the brain, has provided a novel systemic intervention perspective for IS treatment. In this context, Plant-Derived Nanovesicles (PDNVs), a class of natural nanocarriers derived from plants, have emerged prominently due to their inherent multi-component synergistic properties, excellent biocompatibility, and cross-kingdom regulatory capabilities. Critically, IS itself rapidly induces gut dysbiosis and barrier disruption, creating a vicious cycle that amplifies neuroinflammation—a pathological feature shared with other inflammatory conditions such as colitis and Inflammatory bowel disease. In this context, PDNVs, a class of natural nanocarriers derived from plants, have emerged prominently due to their inherent multi-component synergistic properties, excellent biocompatibility, and cross-kingdom regulatory capabilities. Drawing on mechanistic insights from these related disease models, this article systematically discusses the multi-level integrated mechanism of PDNVs as novel "functional messengers", involving reshaping the gut microenvironment, mediating systemic metabolic-immune signals, and ultimately synergistically activating the central nervous repair network, thereby offering a new paradigm for IS therapy. This review not only summarizes the mechanisms of action of PDNVs but also systematically constructs a framework and strategy for their translation from experimental research to clinical application. Highlighting critical hurdles such as the need for standardized production and rigorous quality control to ensure batch-to-batch consistency.

Keywords: ischemic stroke, plant-derived nanovesicles, gut microbiota-gut-brain axis, systemic regulation, drug delivery, neuroinflammation

Introduction: From Localized Intervention to Systemic Regulation – The Evolution of the Therapeutic Paradigm for Ischemic Stroke

Ischemic stroke (IS), with its high incidence, disability, and mortality rates, imposes a heavy global disease burden.[1] This burden is expected to continue increasing with the intensification of population aging.[2] For decades, IS therapeutic research has primarily focused on early vascular recanalization (eg., thrombolysis, thrombectomy)[3] and neuroprotection.[4] However, neuroprotective strategies are often limited by the natural barrier of the blood-brain barrier (BBB), leading to low delivery efficiency for most candidate drugs.[5,6] Concurrently, the post-IS pathological process involves a complex interwoven network of mechanisms including excitotoxicity,[7] oxidative stress,[8] neuroinflammation,[9,10] and apoptosis,[11]

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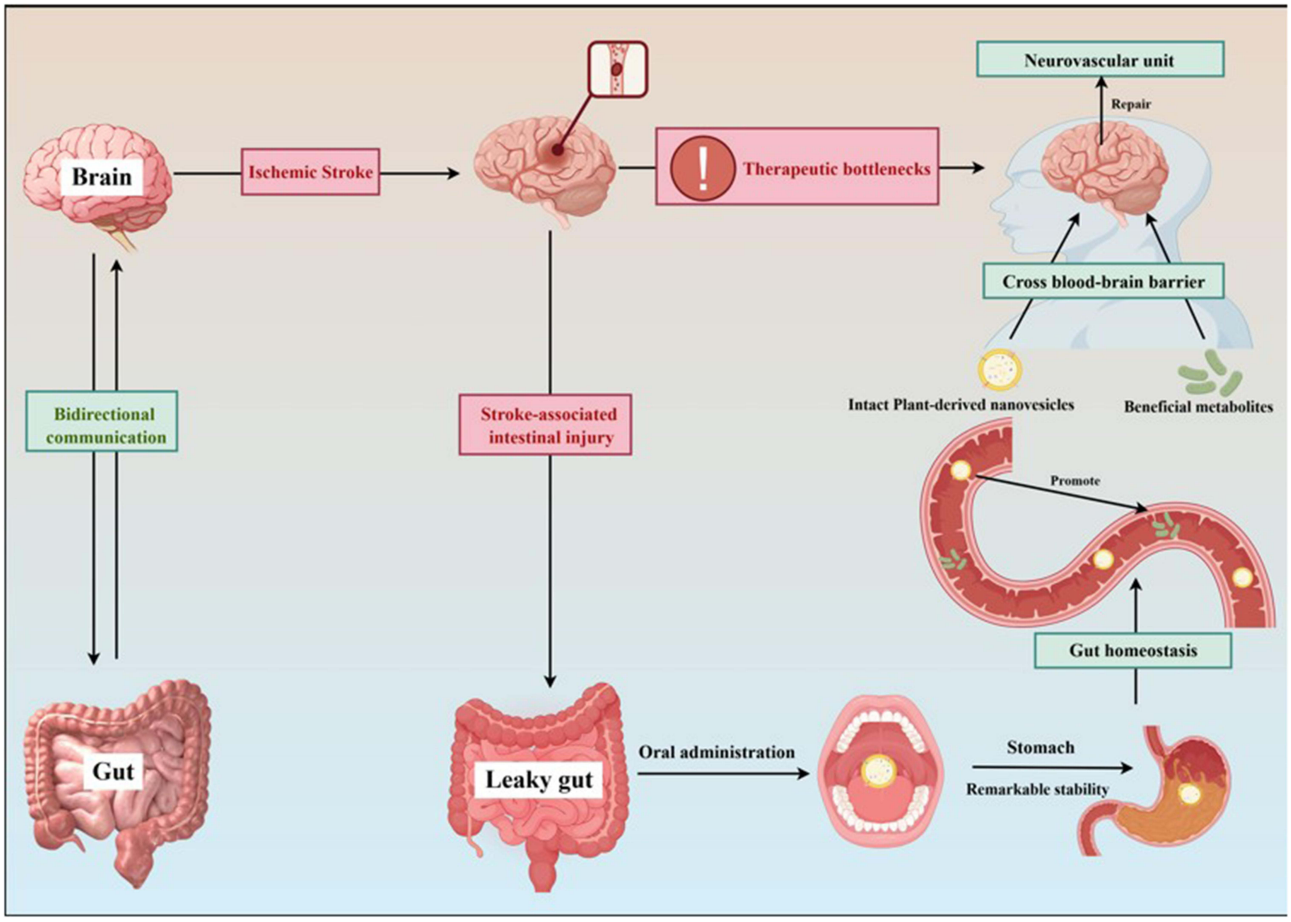
Graphical Abstract



rendering single-target interventions often minimally effective. Therefore, there is an urgent need for a new strategy capable of systemically and multi-targetedly regulating this disease network.

The burgeoning concept of the "gut microbiota-gut-brain axis" provides a theoretical breakthrough for this need,[12] particularly in the pathophysiology of neurological diseases like IS.[13] Critically, this axis is bidirectional. IS not only damages the brain but also rapidly induces gut dysbiosis and increased intestinal permeability, leading to a "leaky gut".[14–16] This, in turn, facilitates the translocation of bacteria and bacterial products into the systemic circulation, exacerbating peripheral and central neuroinflammation, thereby creating a vicious cycle that worsens stroke outcomes.[17,18] The gut, as the body's largest immune and endocrine organ, engages in continuous bidirectional communication with the Central Nervous System(CNS) through neural, endocrine, immune, and metabolic pathways.[19–21] The gut microbiota and its metabolites can profoundly influence systemic immune status and the structural and functional repair of the brain's neurovascular unit (NVU).[22,23] This suggests that intervening at the gut, a "peripheral hub," may remotely and systemically modulate the brain's pathological environment, opening a new front for IS treatment.

In recent years, Plant-Derived Nanovesicles(PDNVs), a class of natural nanocarriers derived from plants that are morphologically and functionally similar to mammalian exosomes, have emerged prominently. They are nanoscale lipid bilayer vesicles secreted by plant cells,[24–26] capable of crossing the BBB,[27] and naturally loaded with various bioactive components from their parent plants, including proteins, lipids, functional RNAs, and metabolites,[28,29] playing a key role in intercellular communication. Compared to synthetic nanocarriers, PDNVs possess inherent low immunogenicity, excellent biocompatibility,[30,31] and unique intrinsic bioactivity.[32–34] In contrast to mammalian exosomes, which face substantial

challenges in oral delivery due to their susceptibility to degradation in the harsh gastrointestinal environment,[35,36] PDNVs exhibit remarkable stability. Their robust lipid bilayer structure enables them to resist enzymatic digestion and maintain structural integrity under extreme pH conditions, facilitating effective transit through the stomach and intestine.[36,37] This inherent stability, combined with their nanoscale size and biocompatibility, positions PDNVs as particularly well-suited for oral administration—a critical advantage for engaging the gut microbiota-gut-brain axis in a non-invasive and sustained manner.[38] More importantly, their multi-component synergistic nature reflects the essence of traditional Chinese medicine's "holistic view" and "compound formula" multi-target synergistic treatment.[39,40]

Therefore, breaking through the bottlenecks in IS treatment requires an innovative strategy capable of systemic regulation, multi-target intervention, and effective BBB traversal. PDNVs, with their unique attributes, offer an ideal vehicle for achieving this systemic therapy via the "gut microbiota-gut-brain axis." This article aims to demonstrate that PDNVs are not merely drug "delivery tools" but are "functional messengers" capable of performing ordered, multi-level systemic regulation through the gut-brain axis. However, to argue for the rationale and urgency of this novel strategy, it is first necessary to fully understand the complexity of IS itself and the fundamental limitations of existing treatment paradigms.

IS: Disease Burden, Complex Mechanisms, and Therapeutic Bottlenecks

This section will systematically elaborate the urgency of developing new therapies and the potential entry points for PDNVs from three levels: epidemiology, pathophysiology, and current treatment dilemmas (Figure 1).

Global Epidemiology and Socioeconomic Burden

According to the latest report in The Lancet Neurology, among non-communicable diseases, stroke remains the world's second-leading cause of death and the third-leading cause of death and disability combined (measured in disability-adjusted life-years lost - DALYs). By 2021, the global number of stroke survivors reached 93.8 million, with 11.9 million new stroke cases.[42] It is estimated that the total global cost of stroke exceeds $890 billion, accounting for 0.66% of global GDP.[43] IS, as the most common form of stroke, constitutes approximately 65.3% (62.4–67.7%).[42] Currently approved vascular reperfusion therapies (eg., intravenous thrombolysis) not only have a narrow therapeutic time window but also carry risks of inducing cerebral hemorrhage and secondary injury to ischemic tissue, with significant variability in patients' long-term functional outcomes.[44] Given the time window limitations and the current lack of definitively effective interventions for ischemia/reperfusion injury or neuroprotective strategies, the therapeutic bottleneck for IS is stark.

The Complex Network Mechanism of Progressive Injury

IS initiates a complex, multi-stage pathological cascade. The immediate energy crisis—characterized by ATP depletion and ionic homeostasis disruption—triggers glutamate excitotoxicity and pathological Ca^{2+} overload.[7,45–48] This Ca^{2+} overload acts as a central signaling hub, simultaneously inducing mitochondrial dysfunction, reactive oxygen species burst, and endoplasmic reticulum stress, forming a self-amplifying "iron triangle" of organelle injury that drives the energy crisis toward inflammation and cell death.[49–55]

These molecular disturbances subsequently activate systematic neuroinflammation. Damage-associated molecular patterns released from necrotic neurons and damaged mitochondria are recognized by pattern recognition receptors on microglia and astrocytes, activating NF-κB and other pro-inflammatory pathways.[56–59] This drives glial polarization toward pro-inflammatory M1/A1 phenotypes and promotes the release of TNF-α, IL-1β, and other inflammatory mediators.[10,60–62] Simultaneously, peripheral immune cells infiltrate the ischemic brain,[63,64] forming a positive feedback loop with mitochondrial damage that perpetuates injury.[65,66]

A core consequence of this cascade is disruption of the BBB and NVU. Pro-inflammatory cytokines downregulate endothelial tight junction proteins, while activated microglia and infiltrating leukocytes secrete matrix metalloproteinases (MMPs) that degrade the basement membrane.[67–74] The resulting BBB breakdown induces vasogenic edema and allows uncontrolled entry of plasma proteins and inflammatory cells into the brain parenchyma, further exacerbating cell death and creating a positive feedback loop that amplifies injury.[70,75]

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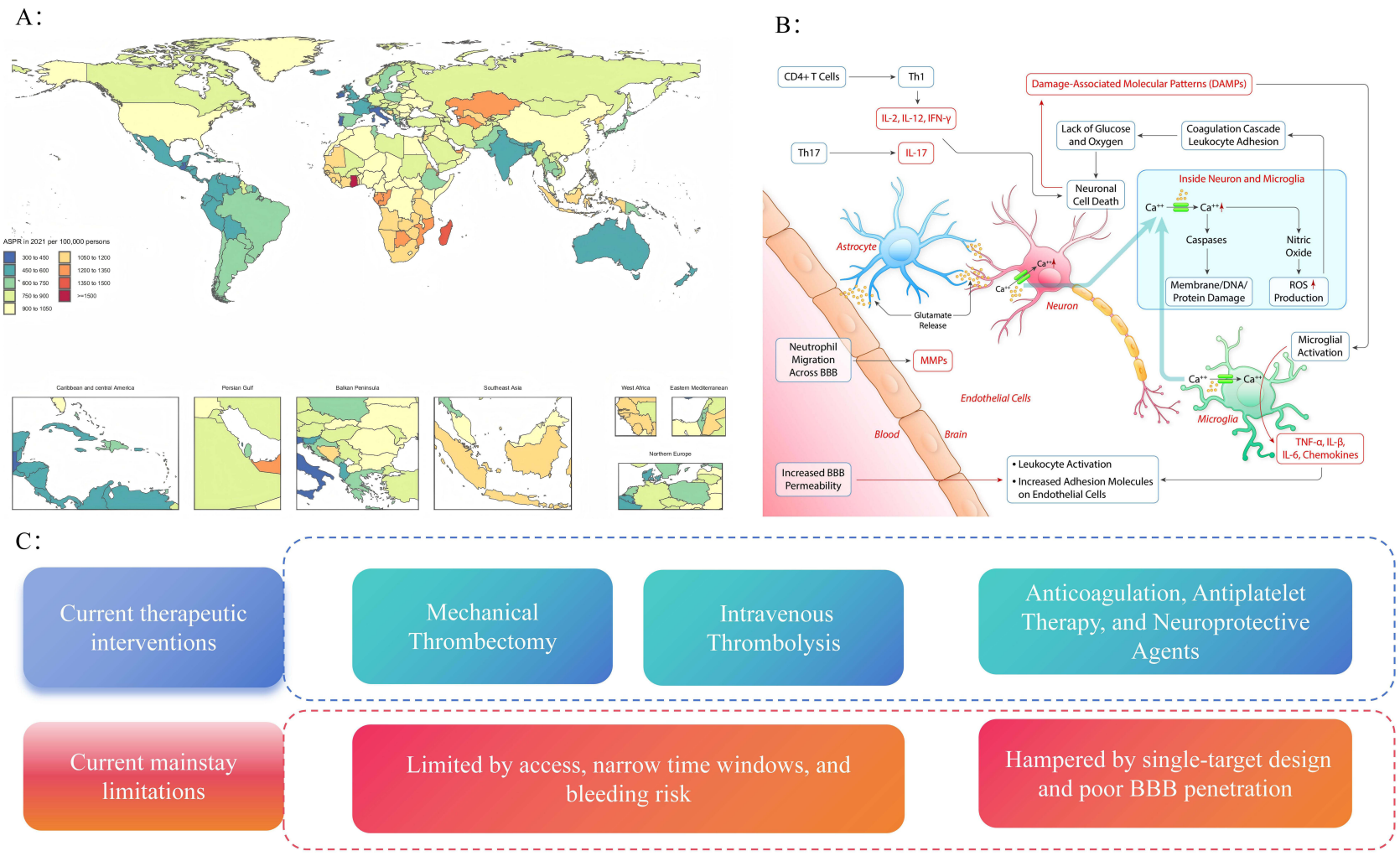

Figure 1 Global burden, complex mechanisms, and therapeutic bottlenecks of ischemic stroke (IS). (**A**) Geographical disparities in the global prevalence of IS, highlighting its pervasiveness as a major public health issue. Reprinted with permission from ref.[2] Copyright (2025) Springer Nature. (**B**) Core network of the "ischemic cascade" triggered by the energy crisis after IS, including multiple positive feedback loops such as excitotoxicity, oxidative stress, organelle dysfunction, neuroinflammation, and cell death. The simplified workflow illustrates the progression from energy failure to blood-brain barrier disruption. Reprinted with permission from ref.,[41] Copyright (2022) American Heart Association. (Black Arrows: Describe conventional biological processes and signal transduction. Red Arrows: Highlight pathological states. Cyan Arrows: Marking the pathological process of calcium overload).; (DAMP: damage-associated molecular pattern; IFN: interferon; IL: interleukin; MMP: matrix metalloproteinase; Th: helper T cell; TNF: tumor necrosis factor). (**C**) Inherent limitations of current mainstay treatments regarding time window, hemorrhage risk, BBB penetration, and multi-target regulation, emphasizing the urgency for developing a new paradigm.

Advances and Core Limitations of Current Treatment Modalities

Acute IS treatment plans are primarily formulated based on time since onset, severity of neurological deficit, and imaging findings. For eligible patients, standard treatments include intravenous thrombolysis and mechanical thrombectomy.[76] The most widely used thrombolytic drug, alteplase, has an extremely short half-life (4–6 minutes) and must be administered intravenously within 4.5 hours of symptom onset.[77,78] Although alteplase reduces the risk of disability at 3 months by approximately 30%, the recanalization rate with thrombolysis alone is less than 50%.[79] Although studies have explored combination therapies to mitigate risks, no clear breakthrough has yet been achieved.[80,81] Mechanical thrombectomy is the gold standard for large-vessel occlusion IS, but its application is similarly constrained by factors such as patient age,[82,83] time window, stroke location,[84] and post-procedural complications.[85–87]

Given the accessibility, time window limitations, and inherent bleeding risks of intravenous thrombolysis and thrombectomy, clinical IS management also includes medications such as anticoagulants, antiplatelets, and neuroprotective agents.[88] However, the BBB as a key barrier for drug delivery,[89] coupled with the inherent shortcomings of single-target strategies for complex network diseases,[90] are major reasons for the suboptimal efficacy of current drug therapies. In summary, the pathological mechanism of IS is a complex systemic network with multiple nodes and positive feedback loops. Any intervention targeting a single link is easily compensated for or bypassed by the network. Therefore, IS treatment urgently requires a new paradigm capable of overcoming BBB limitations and synergistically acting on multiple pathological aspects. Against this backdrop, PDNVs, which combine multi-route delivery, efficient BBB crossing, and systemic regulatory potential, are increasingly becoming a research focus, with their potential to revolutionize the therapeutic paradigm garnering widespread attention.[91–93]

Beyond Delivery: PDNVs as Multifunctional Therapeutic Messengers

Exosomes are a subclass of extracellular vesicles(EVs) secreted by eukaryotic cells, featuring a phospholipid bilayer structure and a diameter in the nanometer range.[94] They appear disc-shaped or cup-shaped under transmission electron microscopy.[95] Acting as intercellular communication mediators, they can transfer bioactive substances to recipient cells.[96,97] In recent years, the application of exosomes in brain drug delivery has advanced.[98] In contrast, PDNVs, widely studied only after 2009,[99] have demonstrated higher efficiency in intracellular substance delivery and transfer due to their low immunogenicity, lack of cytotoxicity, high delivery efficiency, and good biocompatibility.[100] PDNVs show therapeutic potential for neurological diseases by regulating multiple pathways including calcium signaling, anti-oxidation, neuroinflammation, and apoptosis,[101] and can also serve as novel carriers for delivering exogenous drugs.[100]. This chapter will delve into the unique biological characteristics of PDNVs compared to traditional synthetic nanocarriers, elucidating their potential as multifunctional therapeutic messengers (Figure 2).

Natural Multi-Component Synergistic System

PDNVs are rich in biomolecules, including lipids, nucleic acids, proteins, and plant active ingredients,[102] with some biological functions resembling those of animal-derived exosomes.[103] Oral administration is a common and ideal route for PDNVs, offering advantages such as non-invasiveness, safety, and high compliance, but its bioavailability is often limited and variable due to the complex gastrointestinal environment.[104] The excellent physicochemical properties of PDNVs (small particle size, negative charge, lipid bilayer membrane, and hydrophilic surface) enable them to effectively evade physiological barriers in the gastrointestinal tract such as extreme pH,[105,106] digestive enzymes,[107] and the mucus barrier,[106] safely target lesion sites, prolong residence time in the gut,[108,109] and achieve responsive delivery.[110]

Specifically, the lipid bilayer of PDNVs effectively protects their carried miRNAs from degradation by ribonucleases and harsh gastrointestinal conditions.[27] The inherent 2′-O-methylation modification of miRNAs also confers acid resistance and stability, allowing them to maintain long-term activity in the human intestinal environment.[111] The protein composition of PDNVs is mainly intracellular, including enzymes related to cell wall remodeling with potential anti-pathogen activity,[112] and metabolic enzymes conferring antioxidant capacity.[113] Their peripheral and transmembrane proteins may be involved in vesicle formation and specific targeting.[114] The RNA in PDNVs, especially miRNAs, is believed to play roles in intercellular and even cross-species gene regulation.[115] Beyond their structural role, specific lipid species in PDNVs actively mediate interactions with host cells and gut microbes, determining tissue tropism and cellular uptake efficiency.[116–118] For instance, phosphatidic acid,

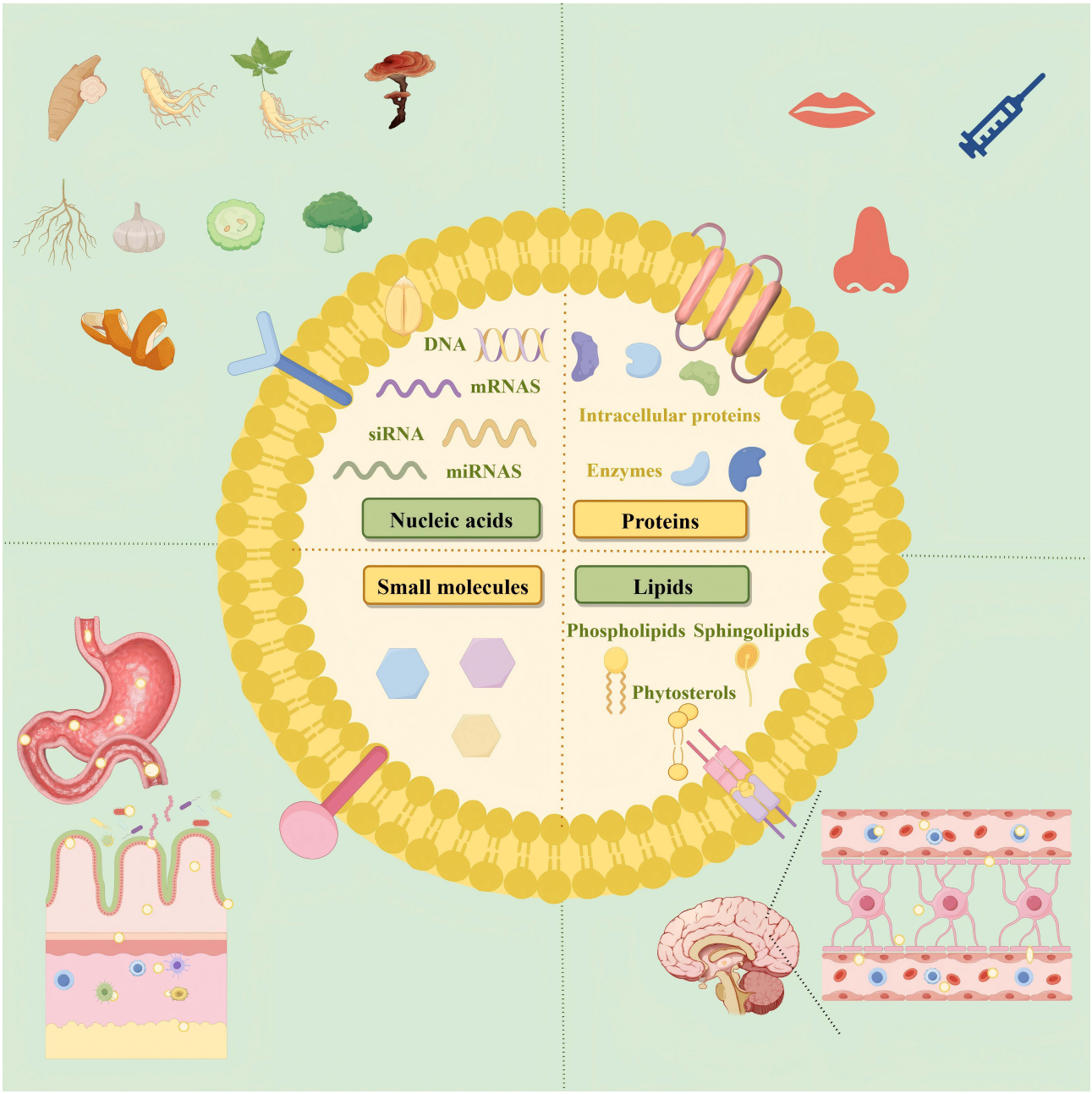

Figure 2 Plant-derived nanovesicles (PDNVs) as natural multifunctional nano-therapeutic messengers. PDNVs are nanoscale lipid bilayer vesicles extracted from various plant cells. They are naturally loaded with a variety of bioactive components including proteins, lipids, nucleic acids (eg., miRNAs), and plant active metabolites, constituting a multi-component synergistic system. Their unique composition confers superior stability. Upon oral administration, PDNVs resist gastrointestinal digestion and are efficiently taken up by intestinal epithelial cells, immune cells, and even gut microbiota via multiple endocytic pathways. More importantly, PDNVs exhibit good biocompatibility and low immunogenicity, and can effectively cross the blood-brain barrier, enabling targeted delivery and regulation from peripheral sites (eg., the gut) to the central nervous system.

a key lipid component in ginger-derived PDNVs, is preferentially recognized and internalized by *Lactobacillaceae* in a lipid-dependent manner, facilitating targeted delivery of vesicle-encapsulated miRNAs to specific bacterial taxa.[117] Similarly, galactolipids such as digalactosyldiacylglycerol, abundant in oat PDNVs, play a crucial role in their interaction with microglial cells and contribute to their anti-neuroinflammatory effects after crossing the BBB.[119] Grapefruit-derived PDNVs, which are rich in phosphatidylcholine, preferentially accumulate in the liver and spleen following systemic administration, demonstrating the role of lipid composition in dictating tissue tropism.[120] These examples illustrate that lipid composition is not merely a structural determinant but actively dictates PDNV targeting, uptake, and biological function.

Furthermore, differences in the lipid composition of PDNVs determine their different functional and distribution tendencies. For example, specific phospholipids may facilitate their accumulation in the intestine or promote transport to the liver and exert varying attraction to different gut microbes.[116–118] Homologous plant active molecules contained in PDNVs can also be delivered into the body to produce corresponding biological effects.[121,122] These biomolecules are not randomly combined but form a therapeutically functional unit with intrinsic connections.

Source-Determined Functional Programming

The functional properties of PDNVs exhibit significant "parental memory." Compared to plant crude extracts or single compounds, PDNVs are rich in numerous endogenous active components, possess higher lipid solubility, can significantly promote drug absorption and membrane permeability, increase drug concentration in target organs, thereby enhancing efficacy.[123] PDNVs have been confirmed to possess various effects such as anti-inflammatory, anti-tumor, immunomodulatory, and antioxidant stress.[124,125] Simultaneously, PDNVs from different sources demonstrate specific therapeutic effects on different disease systems.[123,126] For example, ginseng, known for its anti-inflammatory, antioxidant, and anticancer properties, yields PDNVs reported to improve symptoms related to dementia, diabetes, respiratory infections, and cancer[127,128] (more examples summarized in references).[123,126,129] This aligns closely with the traditional efficacy classification of Chinese medicinal herbs, providing a rational basis for screening and applying specific PDNVs based on therapeutic goals.[130]

Excellent Biocompatibility, Stability, and BBB-Crossing Ability

The excellent biocompatibility of PDNVs is primarily manifested as low immunogenicity and potential natural targeting. They are free of zoonotic or human pathogens,[131] suitable for oral administration, non-toxic to healthy tissues, and possess outstanding biocompatibility.[109,132] Their potential natural targeting is often attributed to their unique phospholipid bilayer structure and specific biomolecules they carry, which may confer tissue tropism, ensuring therapeutic components accumulate at intended sites.[129,133] For instance, grape PDNVs rich in phosphatidylcholine preferentially accumulate in liver tissue;[134] ginger PDNVs rich in phosphatidic acid can be preferentially taken up by *Lactobacillaceae* in a lipid-dependent manner and induce IL-22 production via miRNA delivery, alleviating colitis.[117] Ceramide lipids in ginseng PDNVs can promote macrophage M2 polarization via the TLR4/MyD88 pathway, inhibiting tumor growth.[135,136] Garlic PDNVs express lectins that specifically bind to the CD98 receptor on hepatocytes, promoting targeted uptake and anti-inflammatory responses.[114] Coffee PDNVs exert therapeutic effects on chronic liver disease through their miRNA-mediated regulation of related genes.[137] Broccoli PDNVs rich in the secondary metabolite glucoraphanin can activate AMP-activated protein kinase (AMPK) and target dendritic cells (DCs), thereby alleviating colitis.[121] Further elaboration on the natural targeting potential of PDNVs can be found in reference.[138]

The particle size of PDNVs typically ranges from 10 to 1000 nanometers, with morphology similar to animal exosomes.[100] Compared to the latter, PDNVs demonstrate superior biostability, mainly due to their robust lipid membrane, enabling resistance to enzymatic degradation and maintenance of structural integrity in gastric and intestinal fluids, achieving long circulation.[139] Studies show that ginger PDNVs can maintain integrity in extreme pH environments.[140] Their nanoscale size also grants them inherent potential to penetrate the BBB, intestinal barrier, and skin barrier.[141] This stability is crucial for overcoming the challenges of drug delivery via the gastrointestinal tract and BBB traversal.[142,143] Recent studies report that PDNVs from various sources, such as Panax notoginseng,[144] grapefruit,[145] and celery seed,[146] can effectively penetrate the BBB, increase their distribution in the brain, and exert neuroprotective effects. Table 1 (located at the end of the text) compiles the effects of different administration routes of representative PDNVs on their BBB-crossing efficiency and mechanisms of action.

To further leverage the "intervention + delivery" advantages of PDNVs, drug delivery systems based on PDNVs have become a research hotspot.[153] Compared to synthetic lipid carriers, PDNVs are not only non-cytotoxic but are also taken up by cells more rapidly.[120] Even at high doses, their cellular uptake efficiency remains high.[154] In summary, as a natural bionanosystem shaped by evolution, the core advantage of PDNVs lies in their ability to intelligently respond to complex internal microenvironments, achieving protective delivery, targeted enrichment, and conditional release of their cargo. This "friendly and intelligent" interactive ability with host biological systems constitutes the physical and biological foundation for their effectiveness as "functional messengers" capable of traversing multiple biological barriers and implementing multi-level, systemic regulation via the gut-brain axis.

Table 1 BBB-Crossing Efficiency and Main Mechanisms of Action of Representative PDNVs

PDNVs Source	Administration Route	Animal Model	Main Peripheral Organ Accumulation	GI Tract Residence Properties	BBB-Crossing Efficiency	Mechanism
Panax notoginseng[144]	Intravenous (tail vein)	Focal cerebral ischemia/ reperfusion	Liver	Not addressed	Detected at 8h	Induces microglial polarization via PI3K/Akt pathway
Momordica charantia[147]	Intravenous (tail vein)	Focal cerebral ischemia/ reperfusion	Not addressed	Not addressed	Begins at 6h, significant at 24h	miR-5266 inhibits MMP-9 expression, maintains tight junction proteins, protects BBB
Houttuynia cordata Thunb[148]	Intravenous (tail vein)	Focal cerebral ischemia/ reperfusion	Liver, Kidneys	Not addressed	24h	miR159a targets ACSL4 to inhibit ferroptosis, alleviating brain injury
Ligusticum sinense chuanxiong	Intravenous	Photothrombotic stroke	Liver, Spleen, Kidney	Not addressed	Peak at 12h	Reprograms 30 key metabolites across arginine-proline, methionine, purine, and tyrosine metabolism; activates VEGF signaling to promote neurovascular repair
Salvia miltiorrhiza	Intravenous	Traumatic Brain Injury	Liver	Not addressed	Detectable at 6h, peaks at 12h	Suppresses microglial activation and proinflammatory M1 phenotype; reduces oxidative stress; preserves dendritic structure and promotes functional recovery via modulation of purine metabolism
Ginseng[128]	Intravenous (tail vein)	Glioma	Liver, Spleen, Lungs, Heart	Not addressed	Efficiently crosses within 1h	Enhances BBB and tumor targeting, modulates tumor microenvironment by recruiting M1 macrophages
Pueraria lobata[149]	Intravenous (tail vein)	Parkinson's disease	Heart, Liver, Spleen, Lungs, Kidneys	Not addressed	Enriched at 6h	Delivers plant miRNA to neurons, regulates target genes, reduces apoptosis, improves dopaminergic neuron survival
Pueraria lobata[149]	Intranasal	Parkinson's disease	Not addressed	Not addressed	Enriched at 12h	Delivers plant miRNA to neurons, regulates target genes, reduces apoptosis, improves dopaminergic neuron survival
Grapefruit[150]	Intranasal	Brain tumor-bearing	Not addressed	Not addressed	Rapid entry at 1.5h, significant at 12h	miR17 rapidly enters brain and is selectively taken up by tumor cells, inhibiting tumor growth
Ganoderma lucidum[151]	Intranasal	Alzheimer's disease	No liver/renal toxicity	Not addressed	Rapid entry at 2h	Crosses BBB, attenuates Aβ aggregation and inflammation, alleviates behavioral deficits
Oat[119]	Oral	Chronic encephalitis	Liver	Stable aggregation	Enters brain at 1h, peaks at 4h	Regulates microglia via synergy of β-glucan and DGDG, inhibits neuroinflammation, restores memory
Garlic[152]	Oral	High-fat diet-induced obesity	Liver, Small intestine, Large intestine	Stable aggregation	24h	Preferentially taken up by microglia, reduces pro-inflammatory factors (IFN-γ, TNF-α), inhibits brain inflammation

Note: Most studies in this table used intravenous or intranasal administration. However, orally administered plant-derived nanovesicles also reach the brain. Representative examples include oat-derived nanovesicles and garlic-derived nanovesicles. The mechanisms of oral absorption and blood-brain barrier crossing are discussed in the "Stage 2" section.

However, despite these compelling advantages, several limitations must be acknowledged. The targeting efficiency of unmodified PDNVs to specific brain lesions remains modest, and their multi-component nature, while synergistic, also raises the possibility of off-target effects from bioactive molecules that are not therapeutically relevant. Furthermore, the challenges in large-scale, good manufacturing practice-compatible production and batch-to-batch consistency, as discussed later, currently hinder their clinical translation.[129,138,155–157]

Targeting the "Gut Microbiota-Gut-Brain Axis" for IS Therapy: The Systemic Therapeutic Pathway of PDNVs

Within 24 hours after IS onset, gut dysbiosis and increased intestinal mucosal permeability (ie., "leaky gut") can occur, subsequently inducing endotoxemia and bacterial translocation.[16,158–160] Concurrently, the dysregulated microbiota and compromised gut barrier jointly alter the signaling pattern of microbial metabolites to the brain. These metabolites are

now regarded as potential biomarkers and important pathophysiological mediators for IS.[161,162] Based on this, this chapter constructs a three-stage continuous action model of PDNVs treating IS via the "gut microbiota-gut-brain axis," systematically elaborating this new paradigm of multi-level, holistic regulation (Figure 3).

To better illustrate the unique advantages of PDNVs, Table 2 provides a comparative analysis with synthetic liposomes and mammalian exosomes across key parameters including cost, stability, scalability, BBB crossing efficiency, and clinical translation status.

Stage 1: Remodeling the Gut Microenvironment – Establishing the "Bridgehead"

Studies show that when PDNVs are administered intravenously or intraperitoneally, they primarily accumulate in the liver and spleen; whereas upon oral administration, they preferentially distribute to the gastrointestinal tract.[135] After oral dosing, their fluorescent signals can be detected in multiple regions of the intestine, confirming effective delivery to the target site.[166] PDNVs are not passively decomposed in the gastrointestinal tract but actively engage in multi-level, complex interactions with the gut microenvironment, leveraging their nano-size, stability, and surface properties. This interaction begins with efficient internalization by different cells within the gut,[167] such as intestinal epithelial cells[139,168] and lamina propria immune cells,[139,169,170] via various endocytic pathways. These pathways include: clathrin-mediated endocytosis (a classic ligand-specific uptake pathway),[171] caveolin-mediated endocytosis (potentially involved in trans-epithelial transport of PDNVs and can evade lysosomal degradation, protecting their bioactive cargo),[172] macropinocytosis (nonspecifically and efficiently internalizing large amounts of PDNVs and surrounding extracellular fluid),[173] and phagocytosis (primarily the way professional phagocytes like gut macrophages take up PDNVs, directly related to their subsequent immunomodulatory function).[154,174] The specific pathway depends on cell type, PDNVs surface composition, and receptor-ligand interactions.[175] Upon internalization, PDNVs release their loaded active components, achieving cross-kingdom intercellular regulation.[176]

Particularly important is that PDNVs can also be directly recognized and selectively internalized by gut microbes.[118] Mechanistically, PDNVs reshape the gut microbiota through two distinct but potentially synergistic modes of action. First, PDNVs can function as "prebiotic-like" agents, serving as a metabolic substrate that selectively promotes the growth of beneficial bacterial taxa. For example, garlic PDNVs restore the abundance of beneficial *Lachnospiraceae* while suppressing pro-inflammatory *Helicobacter genus*;[177] lemon PDNVs enhance the intestinal survival of *Lactobacillus rhamnosus* and *Streptococcus thermophilus* [169]. Second, and more remarkably, PDNVs can act as "cross-kingdom regulators" by delivering functional small RNAs (eg., miRNAs) that are internalized by specific bacteria, where they can modulate bacterial gene expression. One hypothesized mechanism involves direct fusion of their lipid bilayer with the bacterial cell membrane, a process dependent on the specific lipid composition of the PDNVs.[175] For example, labeled garlic PDNVs were taken up by major phyla like *Bacteroidetes* and *Firmicutes* within 3 hours of oral administration.[178] Ginger PDNVs can be preferentially taken up by *Lactobacillaceae* in a lipid-dependent manner and deliver miRNAs targeting *Lactobacillus rhamnosus* genes.[117] Furthermore, PDNVs and their components can serve as "ecological substrates," metabolized and transformed by microbes into more active secondary products, representing a third layer of microbiota modulation.

Through the above composite network, PDNVs synergistically remodel the gut microenvironment from three core dimensions: First, precise modulation of gut microbiota: Different PDNVs exhibit regulatory capabilities toward specific microbes. For instance, garlic PDNVs restore the abundance of beneficial *Lachnospiraceae* and reduce pro-inflammatory *Helicobacter* genus abundance;[177] their miRNA can also enhance *Bacteroides thetaiotaomicron* abundance.[179] PDNVs from sources like ginseng,[180] fresh Rehmanniae Radix,[181] lemon,[182] pueraria lobata,[183] and tea[184] all demonstrate the ability to modulate microbial structure, promote beneficial bacteria, and suppress harmful ones. Second, repair and enhancement of the intestinal physical barrier: PDNVs directly promote barrier repair by acting on intestinal epithelial cells. Grape PDNVs can stimulate intestinal stem cell proliferation.[134] PDNVs from aloe,[185] allium tuberosum,[186] etc., can upregulate tight junction protein (ZO-1, Occludin) expression; tea PDNVs can promote antimicrobial peptide secretion;[187] mulberry root bark PDNVs protect epithelial cells via the aryl hydrocarbon receptor (AhR) pathway.[188] PDNVs from ginseng,[189] turmeric,[190] folium artemisiae argyi,[191] honeysuckle,[192] etc., also repair or enhance the intestinal barrier through different mechanisms. Furthermore, regulating intestinal immune homeostasis is another key aspect. PDNVs shift the gut immune state toward anti-inflammatory and reparative by influencing various immune cells within the gut-associated lymphoid tissue (GALT), including macrophages, DCs, and T cells. A core function is driving macrophages toward the M2 phenotype,[193] as seen with PDNVs from Centella asiatica,[175] ginger,[194] grapefruit,[122] and

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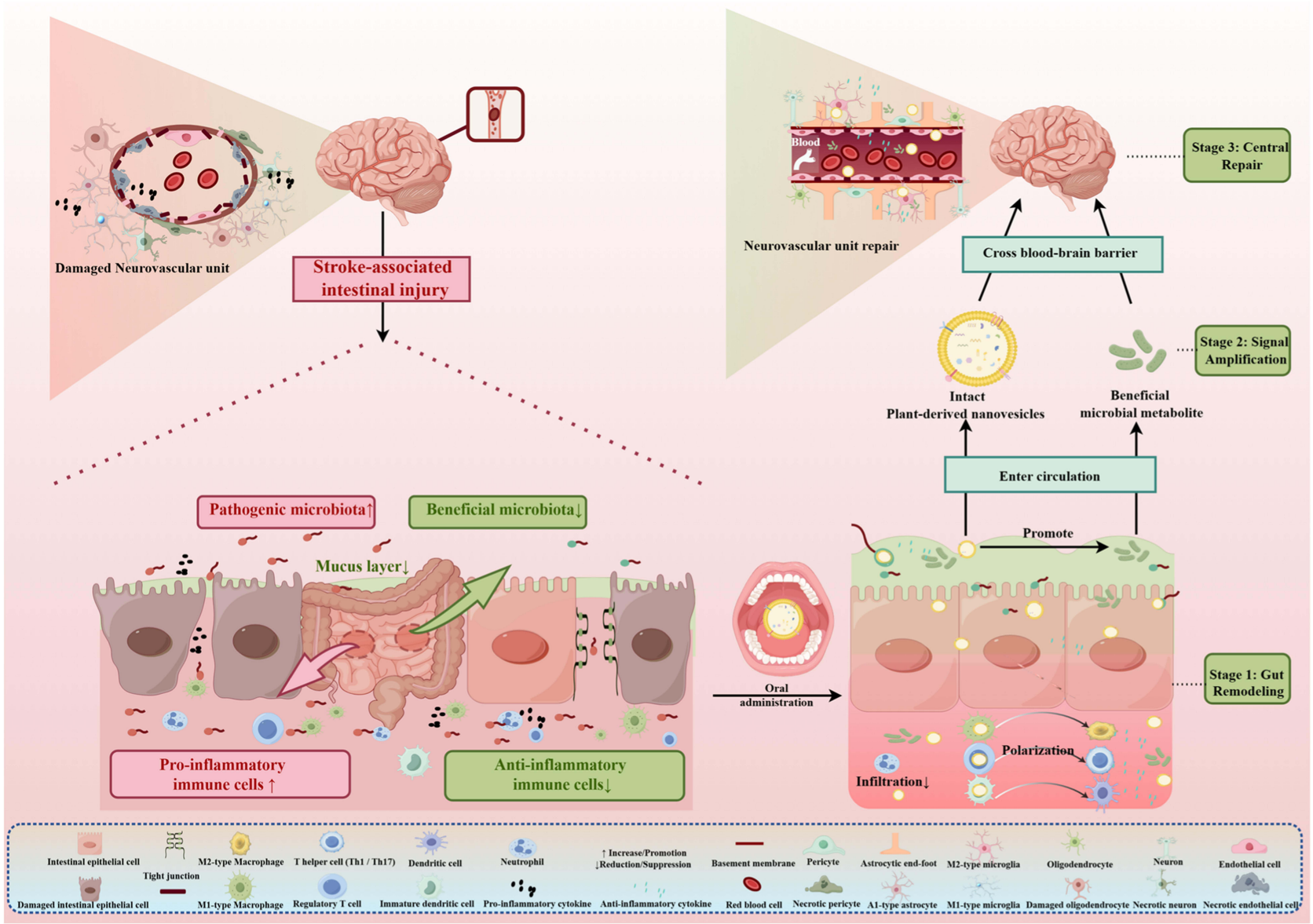

Figure 3 Three-stage model of PDNV-mediated systemic therapy for ischemic stroke via the gut-brain axis. Stage 1 (Gut Remodeling): Orally administered PDNVs are taken up by intestinal epithelial cells, immune cells, and specific microbes. They collectively reshape a stable, anti inflammatory gut microenvironment "bridgehead" by repairing tight junctions, modulating immune cell phenotypes (eg., macrophage M2 polarization), and precisely modulating microbial structure (increasing beneficial bacteria, suppressing harmful ones). Stage 2 (Signal Amplification): Two parallel pathways operate. Direct pathway: a fraction of intact PDNVs are absorbed via the mesenteric lymphatic system and portal vein, enter the systemic circulation, cross the blood-brain barrier, and act as "direct messengers" delivering native bioactive cargo (eg., miRNAs, proteins, lipids) to brain parenchyma. Indirect pathway: most PDNVs remain in the gut, where they promote the production of short-chain fatty acids, bile acid metabolites, indole derivatives, and DHA, which enter the bloodstream as a "humoral signal tide" and act as "indirect modulators" without requiring the vesicles themselves to reach the brain. Stage 3 (Central Repair): The above signals synergistically reprogram brain microglia/macrophages toward the M2 phenotype (anti-inflammatory), shift astrocytes toward the neuroprotective A2 type, support oligodendrocyte precursor cell survival and remyelination, restore blood-brain barrier integrity, and enhance neuronal survival and synaptic plasticity. This ultimately achieves the structural and functional holistic reconstruction of the neurovascular unit.

Table 2 Comparative Analysis of PDNVs with Synthetic Liposomes and Mammalian Exosomes

Feature	Plant-Derived Nanovesicles	Synthetic Liposomes	Mammalian Exosomes
Source	Edible/medicinal plants (eg., ginger, garlic, grapefruit)	Chemically synthesized lipids	Cell cultures (eg., mesenchymal stem cells, human embryonic kidney 293 cells)
Production Cost	Low to moderate	High (for good manufacturing practice grade)	Very high
Scalability (Industrialization Potential)	Potentially high, dependent on plant source	High, batch-to-batch consistency achievable	Low, limited by cell culture expansion
Stability	High (robust lipid bilayer, resistant to gastrointestinal degradation)	Moderate to low (can be engineered for enhanced stability)	Low (prone to degradation, requires careful storage)
Inherent Bioactivity	High (carries native plant bioactives: miRNAs, lipids, proteins)	Low (requires additional loading of therapeutic cargo)	Moderate (carries native cellular signals, eg., miRNAs, proteins)
Immunogenicity	Very low (generally recognized as safe)	Variable (can be immunogenic depending on composition)	Low, but may carry donor-specific surface antigens
BBB Crossing Efficiency	Demonstrated (inherent property, can cross intact)	Requires specific surface modification (eg., targeting ligands)	Demonstrated, but efficiency varies by source and modification
Cargo Loading	Naturally loaded with multi-component synergistic cargo	Requires active or passive loading	Can be engineered, but loading efficiency can be challenging
Ethical Concerns	Minimal (plant-derived, no animal involvement)	Minimal (chemically synthesized)	Moderate (related to cell source, culture conditions, and donor variability)
Clinical Translation Status	Emerging (preclinical stage, ongoing optimization)	Established (multiple Food and Drug Administration-approved formulations)	Early stage (several clinical trials, but challenges remain)

Notes: Data presented in this table are synthesized from the literature cited throughout the main text. Key supporting references include: for plant-derived nanovesicle stability and oral delivery;[37,142,163] for plant-derived nanovesicle blood-brain barrier crossing;[144,145,147] for plant-derived nanovesicle low immunogenicity and generally recognized as safe status;[31,131] for synthetic liposome properties; [164,165] for mammalian exosome properties.[31,163]

Houttuynia cordata.[167] Simultaneously, PDNVs can modulate DC function; Petasites japonicus PDNVs promote their maturation,[195] whereas broccoli[121] and Boehmeria japonica[196] PDNVs induce a tolerogenic phenotype, subsequently modulating T cell responses. Importantly, these locally modulated immune cells in the GALT do not remain confined to the gut. Following PDNV-mediated education, they can migrate via the mesenteric lymphatics and thoracic duct into the systemic circulation, eventually trafficking to the brain where they contribute to the resolution of neuroinflammation.[117,178] This process represents a critical link between peripheral immune modulation and central repair.

Beyond macrophages and DCs, PDNVs also directly modulate T cell populations within the GALT. For instance, Portulaca oleracea L PDNVs promote *Lactobacillus reuteri* growth to produce indole derivatives, activating the aryl hydrocarbon receptor (AhR) in $CD4^+$ T cells and driving their reprogramming into regulatory $CD4^+CD8^+$ T cells, which exhibit potent immunosuppressive functions.[197] Honeysuckle PDNVs modulate T cell immune responses by restoring the Treg/Th17 balance, thereby alleviating both local and systemic inflammation.[198] These PDNV-educated T cells, once mobilized into the circulation, can suppress systemic inflammatory tone and limit the infiltration of pro-inflammatory immune cells into the brain. Furthermore, PDNVs can influence neutrophil dynamics, a key player in post-stroke neuroinflammation. Although direct evidence in IS models is emerging, studies in colitis models indicate that PDNVs can reduce neutrophil infiltration into inflamed tissues by downregulating chemokine expression and inhibiting neutrophil extracellular trap formation.[167,177] This suggests that PDNVs may similarly limit neutrophil-mediated damage in the ischemic brain by modulating the gut-neutrophil axis.

A particularly compelling example that bridges the two arms of the gut-brain axis comes from Momordica charantia (bitter melon)-derived PDNVs. Intravenous administration of these vesicles has been directly shown to protect against ischemic brain injury by preserving BBB integrity and inhibiting neuronal apoptosis via the AKT/GSK3β pathway.[147] Concurrently, oral administration of the same PDNV source effectively alleviates ulcerative colitis by inhibiting macrophage inflammation, scavenging reactive oxygen species, and protecting mitochondrial integrity in the gut.[199] This parallel evidence—demonstrating both direct neuroprotection and oral gut-remodeling efficacy for PDNVs from a single plant source—provides unique support for the feasibility of oral PDNV-based IS therapy via the gut-brain axis.

These three dimensions of remodeling constitute a highly synergistic, self-reinforcing network: barrier repair reduces harmful substance translocation, providing a stable environment for beneficial microbes; beneficial microbes and their metabolites support epithelial health and enhance immune tolerance; a balanced immune microenvironment in turn

safeguards barrier repair and microbial homeostasis. Through this network, PDNVs reshape the post-IS dysregulated gut into a structurally intact, micro-ecologically balanced, and immunologically tolerant robust "bridgehead," laying the foundation for the generation and dissemination of subsequent systemic therapeutic signals. Representative studies are summarized in Table 3 (located at the end of the text).

Table 3 Summary of Representative Studies on PDNVs Remodeling the Gut Microenvironment

PDNVs Source	Mechanism of Remodeling Gut Microenvironment	Disease Model
Garlic[177]	Inhibits TLR4/MyD88/NF-κB pathway, reduces TNF-α, IL-6 secretion; restores *Lachnospiraceae* abundance, reduces *Helicobacter genus* abundance	Inflammatory bowel disease (IBD)
Ginseng[180]	Inhibits NF-κB, reduces pro-inflammatory cytokine secretion; lowers Firmicutes/Bacteroidetes ratio	IBD
Fresh Rehmanniae Radix[181]	miR-7972 inhibits pro-inflammatory factors, ROS, and NO secretion via GPR161-Hedgehog pathway, promotes macrophage M2 polarization; targets and inhibits *E. coli biofilm* formation, reducing its abundance	LPS-induced lung inflammation
Lemon[182]	Enhances bile resistance and intestinal survival of *Lactobacillus rhamnosus* and *Streptococcus thermophilus*	*Clostridioides difficile infection*
Pueraria lobata[183]	Increases *Lactobacillus* genus abundance, reduces *Proteobacteria* phylum proportion, ameliorates intestinal and lung inflammation	IBD with lung inflammation
Tea[184]	Decreases Firmicutes/Bacteroidetes ratio and *Oscillibacter genus* abundance, increases *Bifidobacterium, Lachnospiraceae, Akkermansia*; promotes IL-10 secretion from macrophages	IBD
Grape[134]	Crosses mucus layer to act on intestinal stem cells, stimulates $Lgr5^+$ stem cell proliferation via Wnt/β-catenin pathway, repairs intestinal structure	Colitis
Tea[187]	Promotes IL-22 production, increases antimicrobial peptide Reg3g secretion, protects intestinal barrier; miRNAs miR-44 and miR-54 have similar effects	Colitis
Mulberry root bark[188]	Induces antimicrobial peptide generation, protects intestinal epithelial cells via AhR/COP9/COPS8 pathway	Colitis
Aloe[185]	Significantly upregulates ZO-1, Occludin expression, improves intestinal permeability	Colitis
Turmeric[190]	Repairs intestinal barrier, modulates microbiota, remodels macrophage phenotype, exerts anti-inflammatory effects	Ulcerative colitis
Ginseng[189]	Activates autophagy inducing M2 macrophage polarization and IL-10 secretion, reduces intestinal epithelial NO levels; alleviates inflammation, repairs barrier via IKK/IκB/NF-κB pathway	IBD
Ginger[172]	Specifically internalized by intestinal cells, downregulates NF-κB, IL-6, IL-8, TNF-α expression, counteracts LPS inflammatory damage	Intestinal Caco-2 cells
Folium Artemisiae Argyi[191]	Enhances tight junction protein expression, protects intestinal epithelium and mucus layer, restores barrier integrity; reverses microbial dysbiosis	Ulcerative colitis
Allium tuberosum[186]	Downregulates pro-inflammatory factors (SAA, IL-1β, IL-6, TNF-α), upregulates ZO-1, occludin, and IL-10; restores microbial structure (lowers Firmicutes/Bacteroidetes ratio and *Proteobacteria* abundance), promotes acetate production, increases *Lactobacillus* genus	Colitis
Honeysuckle[192]	Crosses damaged intestinal barrier to target liver, inhibits hepatic immune inflammation; modulates microbiota, restores intestinal barrier, reduces intestinal LPS translocation to liver	Acute liver failure
Centella Asiatica[175]	Targets delivery of miRNA to alleviate inflammation, promotes microbiota reconstruction (reduces pathogens like *Salmonella*), modulates immune cell gene expression	Ulcerative colitis
Solanum nigrum L. berries[174]	Internalized by RAW264.7 macrophages, significantly reduces IL-6 expression, exerts anti-inflammatory activity	LPS-stimulated macrophages
Grapefruit[122]	Upregulates heme oxygenase-1, inhibits IL-1β and TNF-α production by intestinal macrophages	Colitis
Houttuynia cordata[167]	Disrupts NLRP3 inflammasome signaling, reduces IL-1β, IL-18; modulates microbiota (promotes SCFA-producing bacteria, inhibits LPS-producing bacteria), restores homeostasis	Colitis
Petasites japonicus[195]	Increases DC surface molecule (CD80, CD86, MHC-I/II) expression and Th1-polarizing cytokine (TNF-α, IL-12p70) production, enhances antigen-presenting capacity, induces DC maturation	In vitro bone marrow-derived DC model
Broccoli[121]	Taken up by DCs, activates AMPK pathway, induces tolerogenic DC generation; inhibits pro-inflammatory factors, promotes IL-10, modulates T cells toward anti-inflammatory phenotypes	Colitis
Boehmeria japonica[196]	Promotes mature DC production of IL-10, reduces pro-inflammatory factors and surface molecule levels; attenuates ability to stimulate Th1/Th17 cell activation, promotes regulatory T cell activity	Colitis
Portulaca oleracea L[197]	Promotes *Lactobacillus reuteri* growth and increases indole derivative levels, activates AhR in $CD4^+$ T cells, driving their reprogramming into regulatory $CD4^+CD8^+$ T cells	Colitis
Honeysuckle[198]	Increases beneficial bacteria, reduces pathogens, elevates SCFAs, promotes bile acid absorption and modulates their metabolism, improves Treg/Th17 balance, protects intestinal barrier, alleviates local and systemic inflammation	Ulcerative colitis

Notes: Although the disease models listed are primarily colitis, IBD, and related inflammatory conditions, they share key pathological features with the post-ischemic gut—namely dysbiosis, barrier disruption, and local inflammation. Therefore, the mechanisms by which PDNVs remodel the gut microenvironment in these models are highly relevant to IS therapy and provide a robust mechanistic foundation for oral PDNV-based strategies targeting the gut-brain axis.

Stage 2: Generation and Amplification of Systemic Signals – Initiating "Humoral Communication"

Following the successful establishment of the gut "bridgehead," therapeutic signals need to be efficiently transmitted systemically and centrally. This process is achieved primarily through two parallel and synergistic pathways: direct delivery of intact vesicles and indirect mediation by gut-derived active molecules, together constituting a powerful systemic "humoral communication" network.

On the one hand, a fraction of structurally intact PDNVs are absorbed from the gut—likely a small but functionally significant portion, depending on the plant source and vesicle surface properties.[38,147] Consistent with the observation that orally administered PDNVs predominantly localize to the gastrointestinal tract,[135,139] the majority of these vesicles remain within the gut lumen and mucosa, where they exert their primary remodeling effects. Nevertheless, a detectable fraction enters systemic circulation via the mesenteric lymphatic system and portal vein, and crosses the BBB by virtue of their nanoscale size and biomembrane properties.[119,152] Studies have confirmed that intravenously injected PDNVs from sources like Panax notoginseng,[144] grapefruit,[145] and Momordica charantia[147] can efficiently reach the brain. More importantly, orally administered PDNVs from oats and garlic have also been detected in the brain, demonstrating that they can act as intact "therapeutic messengers" reaching brain lesions after intestinal absorption.[119,152] In this context, PDNVs act as "direct messengers", delivering their native bioactive cargo (eg., miRNAs, proteins, lipids) directly to the brain parenchyma, where they can be internalized by neurons, microglia, and other NVU cells.[144]

On the other hand, the majority of orally administered PDNVs remain localized within the gastrointestinal tract.[135,139] There, they engage in extensive interactions with the gut microbiota, intestinal epithelial cells, and GALT. These interactions lead to the remodeling of the gut ecosystem, including modulation of microbial composition, repair of the intestinal barrier, and induction of tolerogenic immune responses.[117,167,177] The beneficial effector molecules generated from this gut-level remodeling converge into a broad "humoral signal tide" absorbed into the bloodstream, exerting systemic regulation on distal organs without requiring the PDNVs themselves to reach the brain. Specifically, PDNVs can effectively regulate levels of key metabolites: (1) By promoting the abundance of specific SCFA-producing bacteria (eg., Lachnospiraceae, Bifidobacterium, and Lactobacillus),[117,177,186] PDNVs significantly increase intestinal production of SCFAs (eg., acetate, butyrate, and propionate).[167,186] These SCFAs are absorbed into the bloodstream, cross the BBB via monocarboxylate transporters, and exert dual protective effects: they enhance BBB integrity by upregulating tight junction proteins (eg., occludin, claudin-5, ZO-1),[200,201] and they promote microglial polarization from the pro-inflammatory M1 phenotype toward the anti-inflammatory, neuroprotective M2 phenotype, thereby reducing neuroinflammation and enhancing synaptic plasticity;[201,202] (2) Promoting bile acid absorption and modulating their metabolism;[198] (3) Increasing levels of immunomodulatory indole derivatives by promoting Lactobacillus reuteri growth;[197] (4) Through delivered aly-miR159a-3p inhibiting bacterial phospholipase C expression, leading to accumulation of the potent neuroprotective agent docosahexaenoic acid (DHA) in the body.[118] In this scenario, PDNVs act as "indirect modulators".[117,178]

The balance between these two pathways—direct vesicle delivery versus indirect gut-derived signaling—likely depends on factors such as the PDNV source, surface lipid composition, particle size, and the integrity of the host gut barrier. Nonetheless, both pathways converge to create a systemic anti-inflammatory and pro-regenerative environment conducive to central nervous system repair.

Stage 3: Synergistic Activation of the CNS Repair Network – Achieving "Ultimate Repair"

When therapeutic signals arrive in the brain, their ultimate task is to reverse ischemic damage and initiate orderly repair. PDNVs and the systemic signals they mediate, through synergistic regulation of key cell populations within the NVU, initiate an endogenous, multi-level repair program aimed at systemically restoring the structural and functional integrity of the NVU.

Core Regulation: Phenotypic Reprogramming of Microglia/Macrophages

Activation of the repair network begins with phenotypic reprogramming of the brain's core immune cells—microglia and infiltrating macrophages. Research shows components of orally administered PDNVs can be transported to the brain and preferentially taken up by microglia, lowering brain levels of pro-inflammatory factors like IFN-γ and TNF-α and inhibiting neuroinflammation.[178,203] In vitro experiments confirm PDNVs can be internalized by microglial cell lines, suppressing downstream inflammatory mediator release by downregulating receptors like TLR4.[204–206] In a traumatic brain injury model, intravenous injection of PDNVs inhibited excessive microglial activation and M1 polarization while reducing astrocyte activation, neuronal oxidative stress and apoptosis, and protecting dendritic structures.[207] This process can also benefit from gut-derived signals (eg., SCFAs); circulating SCFAs can cross the BBB, synergistically promoting microglia/macrophage polarization from M1 to M2 phenotypes, playing a central role in combating neuroinflammation and enhancing synaptic plasticity.[202]

Synergistic Support: Reparative Responses of Astrocytes and Oligodendrocytes

The anti-inflammatory and neurotrophic microenvironment created by M2-type microglia/macrophages provides key conditions for repair in other supportive cells of the NVU. In this environment, activated astrocytes are more inclined to shift toward the neuroprotective A2 type, thereby enhancing metabolic and trophic support for neurons.[201] Metabolites like SCFAs may participate in regulating astrocyte activation via pathways such as SGK1/IL-6. Simultaneously, this favorable microenvironment also promotes the survival, proliferation, and differentiation of oligodendrocyte precursor cells, helping to reduce their loss post-ischemia, thereby supporting remyelination and remodeling in the ischemic penumbra, laying a structural foundation for restoration of neural conduction function.[208]

Integrated Outcome: Structural and Functional Reconstruction of the NVU

Building upon the synergistic action of the above cellular events, repair ultimately manifests as the holistic reconstruction of the NVU. On one hand, neuronal apoptosis in the penumbra is effectively inhibited, and synaptic plasticity is enhanced. On the other hand, PDNVs and the systemic signal molecules they elevate (represented by SCFAs) can act on cerebrovascular endothelial cells, promoting angiogenesis in the ischemic region, and restoring and consolidating BBB integrity by upregulating tight junction protein expression, among other means.[147,200] This establishes a virtuous cycle of reduced harmful substance leakage, improved local microcirculation, and support for neural regeneration, creating a sustainable homeostatic environment for the functional recovery of the entire NVU.

In summary, through direct and indirect signals transmitted via the gut microbiota-gut-brain axis, PDNVs initiate within the CNS a cascade repair network centered on immune reprogramming, supported by multi-cellular synergistic responses, and culminating in holistic NVU repair, marking the completion of their therapeutic strategy's systemic leap from peripheral intervention to central functional reconstruction. The multi-level actions of PDNVs along the gut-brain axis are systematically summarized in Table 4.

Toward the Clinic: Translational Challenges and Prospective Strategies

Although the prospects of PDNVs for IS therapy via the gut-brain axis are exciting, the path to clinical translation remains fraught with challenges. These are not mere technical optimization issues but involve systemic hurdles spanning production paradigms, regulatory frameworks, and therapeutic concepts. This chapter aims to clarify the translational challenges of PDNVs from laboratory research to clinical application, analyze core bottlenecks, and propose forward-looking yet feasible coping strategies based on existing scientific research and regulatory developments.

Core Challenge One: The Standardization Leap from "Phytochemistry" to "Nanodrug"

The complexity of PDNVs is both their strength and the primary obstacle to industrialization.[209] Their production currently heavily depends on plant source (variety, part, origin, growth conditions), extraction method, and post-processing techniques, leading to significant batch-to-batch variability in product characteristics such as particle size distribution, vesicle yield, biomolecular composition, and functional activity. Among these variables, the lack of

Table 4 Overview of Plant-Derived Nanovesicles Mechanisms of Action Along the Gut-Brain Axis

Plant Source	Active Cargo	Target in the Axis	Axis Link/ Mechanism Direction	Primary Therapeutic Effect
Garlic[177]	microRNA-3630-5p, 26 lipids, 61 proteins	Toll-like receptor 4 pathway; Gut microbiota (*Lachnospiraceae*↑, *Helicobacter*↓)	Gut → brain (indirect)	Ameliorates colitis, upregulates barrier proteins, inhibits pro-inflammatory cytokines
Garlic[178]	Lipid (phosphatidylcholine 16:0); bacterial Amuc-1100 and P9 proteins	*Akkermansia muciniphila*; Microglia via gut-brain axis	Gut → brain (trains gut bacteria to release outer membrane vesicles)	Reverses type 2 diabetes, increases glucagon-like peptide-1, reduces brain inflammation
Garlic[152]	Lipid (phosphatidic acid 36:4)	Microglia (brain acid soluble protein 1/ c-Myc/cyclic GMP-AMP synthase pathway)	Gut → brain (oral, crosses blood-brain barrier)	Reverses obesity, improves memory and insulin sensitivity
Ginseng[128]	Lipids (28.8% phosphatidylcholine), 98 miRNAs, 86 proteins, ginsenosides	Gut microbiota (*Firmicutes*/*Bacteroidota* ratio); Macrophage M2; Blood-brain barrier	Gut → brain (bidirectional)	Anti-glioma (crosses blood-brain barrier); alleviates colitis (supports gut-brain axis)
Houttuynia cordata[148]	microRNA-159a, flavonoids (luteolin), amino acids, lipids	Brain (acyl-CoA synthetase long-chain family member 4, ferroptosis); Blood-brain barrier	Direct brain protection (intravenous)	Reduces infarct volume, protects blood-brain barrier, inhibits ferroptosis in ischemic stroke
Houttuynia cordata[167]	Flavonoids, amino acids, alkaloids, terpenes	Gut microbiota (*Lactobacillus*↑); NOD-like receptor family pyrin domain containing 3 inflammasome; Gut barrier	Gut → brain (indirect)	Alleviates colitis, restores intestinal barrier, modulates gut microbiota
Oat[119]	Beta-glucan, digalactosyldiacylglycerol	Microglia (hippocalcin/dectin-1/Rab11a pathway); Blood-brain barrier	Gut → brain (oral, crosses blood-brain barrier)	Inhibits alcohol-induced brain inflammation, restores memory
Momordica charantia[147]	microRNA-5266 (targets matrix metalloproteinase-9), exosomal marker proteins	Brain (matrix metalloproteinase-9, zonula occludens-1, claudin-5); AKT/ glycogen synthase kinase 3 beta pathway	Direct brain protection (intravenous)	Reduces infarct volume, protects blood-brain barrier, inhibits neuronal apoptosis
Momordica charantia[199]	345 proteins (antioxidant enzymes), lipids	Gut macrophages (reactive oxygen species, mitochondria); nuclear factor kappa-B pathway	Gut → brain (indirect)	Alleviates colitis, reduces oxidative stress, protects mitochondria

a standardized, scalable, and good manufacturing practice-compatible isolation protocol represents a particularly critical roadblock. Currently, the most commonly employed methods—ultracentrifugation, size-exclusion chromatography, and polymer-based precipitation—each present distinct advantages and limitations that directly impact the final product.

Ultracentrifugation, the most widely used method in research settings, offers high purity but suffers from low yield, potential vesicle aggregation, and poor scalability.[163,209] Size-exclusion chromatography preserves vesicle integrity and maintains native bioactivity but often yields dilute samples that require concentration, increasing processing time and cost.[142] Polymer-based precipitation is simple and scalable but frequently co-precipitates non-vesicular contaminants such as proteins and lipoproteins, compromising purity and potentially introducing confounding biological effects.[109,140] These methodological differences result in PDNV preparations with divergent physicochemical properties (size, zeta potential, yield) and biomolecular cargo profiles (lipid, protein, and RNA composition), which in turn affect functional outcomes—making direct comparison between studies challenging and hampering the establishment of reproducible efficacy profiles necessary for clinical development.[210] This heterogeneity not only affects experimental reproducibility but is also the "Achilles' heel" for clinical studies and mass production.

Establish a "Quality by Design" (QbD) production framework: Drawing on experience from biologics and advanced therapy medicinal products (ATMPs), define the Critical Quality Attributes (CQAs) of PDNVs, such as: (1) Physical attributes (particle size, Zeta potential, concentration); (2) Chemical composition signature (characteristic lipid, protein, plant functional RNA, or metabolite marker profiles); (3) Functional activity (eg., ability to regulate specific cytokine secretion, selective promotion or inhibition of target microbes). Critical Process Parameters during production (eg., homogenization intensity, centrifugation force, purification column packing) must be tightly controlled to ensure consistency of CQAs.

Develop multi-omics-based characterization and quality control standards: Single physicochemical characterization is insufficient to guarantee functional consistency. Lipidomics, proteomics, small RNA sequencing, and metabolomics should be integrated to establish a "multi-omics fingerprint" for each therapeutic PDNVs, serving as the core basis for batch release. Simultaneously, develop in vitro potency assays related to in vivo efficacy (eg., testing the ability to regulate specific immune cell phenotypes in a simulated gut environment), linking chemical consistency to biological functional consistency.

Advance synthetic biology and cultivation technologies: To break free from dependence on natural plant raw materials, long-term exploration should focus on utilizing plant cell suspension cultures for controlled PDNVs production, or reconstructing "artificial plant vesicles" with specific therapeutic functions in model microorganisms via synthetic biology, enabling fully controllable design of composition and function.

Core Challenge Two: A New Regulatory Science Paradigm – How to Define and Evaluate a "Living" Nanosystem

PDNVs are neither single chemical entities nor traditional botanical drug crude extracts, but functional nanosystems with intrinsic bioactivity, complex and potentially interacting components. Existing drug regulatory frameworks (eg., FDA/EMA/NMPA) with their core review logic based on defined active ingredients are ill-suited. The fundamental question facing regulators is: when the active ingredient is a "whole" composed of hundreds of dynamically interacting molecules, how do we define its identity and assess its quality, safety, and efficacy?

Advocate for an "evidence-based batch" and "totality of evidence" assessment philosophy: Learn from regulatory experience with ATMPs (eg., stem cell products, CAR-T cells) and botanical drugs (eg., FDA's Botanical Drug Guidance). The core of regulatory submission should not be exhaustive characterization of all components, but providing sufficient evidence demonstrating: (1) Stable manufacturing processes yielding "quality-similar" batches consistently (via multi-dimensional quality control); (2) Plausible mechanism of action, even if complex, elucidated through systems biology methods (network pharmacology, multi-omics analysis) for main pathways and networks; (3) Clear and reproducible clinical efficacy.

Construct a risk-based tiered regulatory pathway: Differentiate non-clinical and clinical study requirements based on PDNVs source (edible vs. medicinal plant), route of administration (oral vs. intravenous), degree of modification (natural vs. engineered), and therapeutic area (life-threatening disease vs. chronic management). For example, orally administered PDNVs from edible plants, based on their Generally Recognized as Safe (GRAS) background, might have simplified toxicology requirements, with focus on newly discovered pharmacological actions and long-term safety.

Strengthen early dialogue with global regulatory agencies: Researchers and industry should proactively engage in Pre-Investigational New Drug (Pre-IND) meetings with innovative drug review departments of agencies like the FDA, EMA, and NMPA to seek scientific advice on key issues such as product classification, Chemistry, Manufacturing, and Controls (CMC) requirements, and non-clinical study strategies, jointly promoting the formation of regulatory guidelines adapted to such innovative products.

Core Challenge Three: Personalized Medicine – from "One-Size-Fits-All" to "Tailor-Made"

The core of the "gut microbiota-gut-brain axis" mechanism lies in its high degree of individualization. Patients' baseline gut microbiota composition, immune status, and metabolic phenotype vary greatly, inevitably leading to differential therapeutic responses to the same PDNVs preparation. Ignoring this heterogeneity may result in clinical trial failure or mediocre efficacy.

Discover predictive biomarkers for patient stratification: Systematically collect patients' baseline gut metagenomic, metabolomic, and immunomic data in preclinical and early clinical studies, correlating them with treatment response (eg., improvement in neurological function scores, imaging-based repair, decrease in inflammatory markers). The goal is to identify biomarker combinations predictive of PDNVs efficacy (eg., specific microbial signatures, metabolite profiles) for precise patient enrollment in later-stage clinical trials.

Develop "enterotype-guided" PDNVs matching strategies: Based on gut microbiota composition, populations can be broadly categorized into different "enterotypes." Future exploration could establish an "enterotype-PDNVs functional profile" correspondence. For instance, for patients with a Prevotella-dominant enterotype, match PDNVs that specifically promote their beneficial metabolic functions; for patients with extremely low microbial diversity, consider PDNVs with stronger microbiota-reshaping capabilities or combination with prebiotics.

Explore modular engineering and combination therapies: Leveraging the ease of engineering PDNVs, develop "chassis vesicles." After clarifying core mechanisms, surface modification (targeting peptides) or cargo loading (specific siRNA, neuroprotectants) of natural PDNVs can enhance their intervention capability for specific patient subgroups or disease-critical nodes. Simultaneously, explore synergistic effects of PDNVs with existing therapies (eg., antiplatelets, statins) to form combination regimens that enhance efficacy and reduce side effects.

Future Directions: Toward Oral PDNV Formulations for IS Therapy

While the current evidence establishes a strong mechanistic foundation for PDNVs in IS therapy, direct validation of orally administered PDNVs in IS models remains a key priority. Encouragingly, recent studies have demonstrated that orally administered nanoparticles can effectively treat IS via gut-brain axis mechanisms, providing compelling proof-of-concept for this strategy. Yang et al showed that oral administration of Lactobacillus plantarum-derived EVs inhibited neuronal apoptosis in a mouse mouse Middle Cerebral Artery Occlusion(MCAO) model by delivering miR-101a-3p that targets the c-Fos/TGF-β axis.[211] This study represents the first demonstration that orally administered, non-mammalian EVs can treat IS through gut-brain axis signaling. More recently, Weng et al reported that oral administration of Tianma Gouteng decoction-derived nanoparticles maintained blood-brain barrier integrity, reduced infarct area, and improved neurological function in a mouse MCAO model by regulating the S1PR1/ERK/MEK signaling axis.[212] These findings collectively validate the feasibility of oral nanoparticle-based gut-brain axis targeting for IS therapy. Future research should prioritize the development of orally delivered PDNV formulations optimized for gastrointestinal stability, targeted gut microbiota modulation, and efficient systemic signaling to the brain.

Conclusion

PDNVs represent an innovative fusion between the "holistic view" of traditional herbal medicine and the "precision" of modern nanomedicine. Through the systems biology pathway of the gut microbiota-gut-brain axis, they operate in a multi-component synergistic, multi-target regulatory, multi-level progressive manner, dynamically reshaping pathological microenvironments and activating endogenous neural repair networks, thereby offering a novel therapeutic paradigm for complex systemic diseases like IS with intertwined mechanisms. A pivotal advantage underpinning their clinical potential is their origin from edible plants, which confers a "generally recognized as safe" status. Unlike synthetic nanoparticles, which often raise concerns regarding long-term toxicity and immunogenicity, PDNVs exhibit inherent low immunogenicity and excellent biocompatibility, positioning them as a particularly attractive candidate for clinical translation.[30,31,131] Looking ahead, research in this field should focus on the following key directions: (1) Deepening mechanistic understanding: Utilizing multi-omics, approaches, including transcriptomics, metabolomics, and metagenomics, along with single-cell technologies and spatiotemporal kinetic analyses, to finely map the dynamic network of interactions between PDNVs multi-components and the host/microbiota, elucidating the molecular and cellular basis of their systemic efficacy. (2) Advancing translational research: Concurrently developing QbD-based standardized production processes, innovative regulatory science evaluation systems, and biomarker-based personalized application strategies, building a complete translational pathway from basic discovery to clinical application. (3) Expanding the therapeutic horizon: Validating and exploring the therapeutic potential of PDNVs in other neurological diseases characterized by neuroinflammation, barrier damage, and gut-brain axis dysregulation (eg., Alzheimer's disease, Parkinson's disease, multiple sclerosis, depression).

Ultimately, through interdisciplinary collaboration and an integrated innovation system spanning basic mechanisms, standardized processes, preclinical evaluation, and regulatory science, there is potential to successfully advance this innovative strategy—merging traditional wisdom with modern technology—to the clinic, not only providing transformative treatment options for IS patients but also opening new avenues for systemic intervention against other complex diseases.

Abbreviations

IS, Ischemic Stroke; BBB, Blood-Brain Barrier; PDNVs, Plant-Derived Nanovesicles; NVU, Neurovascular Unit; CNS, Central Nervous System; TNF-α, Tumor Necrosis Factor-alpha; IL-1β, Interleukin-1 beta; MMPs, Matrix Metalloproteinases; SCFAs, Short-Chain Fatty Acids; DHA, Docosahexaenoic Acid; EVs, Extracellular vesicles; ATMPs, Advanced Therapy Medicinal Products; QbD, Quality by Design; CQAs, Critical Quality Attributes; CMC, Chemistry, Manufacturing, and Controls; GRAS, Generally Recognized As Safe; Pre-IND, Pre-Investigational New Drug (Meeting); FDA, Food and Drug Administration (U.S.); EMA, European Medicines Agency; NMPA, National Medical Products Administration (China); GALT, gut-associated lymphoid tissue.

Data Sharing Statement

No datasets were generated or analysed during the current study.

Author Contributions

All other authors have made substantial contributions to the reported work (in conception, research design, execution, data acquisition, analysis, and interpretation, or in all of these areas), participated in drafting, revising, or critically reviewing the article, given final approval of the version to be published, agreed to submit the article to this journal, and agreed to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing interests.

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