

“Beyond Conventional Nanocarriers: Plant-Derived Extracellular Vesicles for Targeted Therapeutics and Biological Barrier Crossing”

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Abstract

The development of efficient, biocompatible and targeted drug-delivery systems remains an important objective in pharmaceutical research. Plant-derived extracellular vesicles (PDEVs) have recently attracted substantial attention as naturally occurring nanocarriers capable of transporting a variety of bioactive molecules. Derived from edible and medicinal plants, these vesicles contain lipids, proteins, nucleic acids, metabolites and other biologically active constituents that may contribute both to their carrier function and to their intrinsic therapeutic properties. Their biological origin, potential gastrointestinal stability, favorable biocompatibility and ability to interact with specific recipient cells make them attractive alternatives or complements to conventional synthetic nanoparticle systems. The present review discusses the development of PDEVs as multifunctional drug-delivery platforms, beginning with their biogenesis and molecular composition and extending to isolation, purification, characterization, administration and therapeutic applications. Conventional and emerging isolation techniques, including ultracentrifugation, density-gradient centrifugation, ultrafiltration, size-exclusion chromatography, polymer precipitation, immunoaffinity separation and microfluidic technologies, are compared with respect to yield, purity, processing time, scalability and preservation of vesicle integrity. Particular attention is given to the biological barriers that influence therapeutic delivery, including the gastrointestinal barrier, blood–brain barrier and cellular uptake barriers. The potential of PDEVs for oral, intravenous, inhalational and minimally invasive delivery is also discussed. Evidence from preclinical studies suggests that plant-derived vesicles may possess anticancer, anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, microbiome-modulating and tissue-regenerative properties. Their potential use as carriers for small molecules, nucleic acids and other therapeutic cargos further expands their pharmaceutical applications. However, translation from experimental research to clinical use is limited by several unresolved issues, including heterogeneity of vesicle populations, inconsistent nomenclature, source-dependent variation in composition, contamination during isolation, limited loading capacity, inadequate understanding of pharmacokinetics and biodistribution, storage-related instability and difficulties in large-scale reproducible manufacturing. In addition, standardized characterization methods, potency assays and regulatory frameworks for plant-derived vesicle-based therapeutics remain to be established. Continued multidisciplinary research integrating pharmacognosy, nanotechnology, pharmaceuticals, molecular biology and regulatory science may facilitate the development of PDEVs into clinically relevant natural nanocarriers.

Key words : Plant-derived extracellular vesicles (PDEVs), Natural nanocarriers, Drug delivery systems, Targeted drug delivery, Vesicle characterization, Biocompatibility, Therapeutic applications, Nanomedicine.

INTRODUCTION

Exosomes are nanoscale extracellular vesicles (40–150 nm) secreted by bacteria, animals, and plants; they have evolved from being viewed as cellular debris to becoming essential regulators of intercellular communication. In the past decade [fig-1](#), remarkable progress has been achieved in the exploration of the therapeutic potential of exosomes, especially as delivery vehicles for proteins, lipids, and nucleic acids, which mirror the genetic and proteomic signatures of their parent cells. The unique biological properties of exosomes, including their superior blood–brain barrier (BBB) penetration compared with synthetic nanoparticles, position them as dual-functional agents for diagnostic biomarker detection and targeted therapy.¹ Emerging evidence highlights exosome-mediated CRIPR-Cas9 delivery systems that achieve

neuron-specific gene editing with enhanced precision in preclinical models,³The inherent immune stealth characteristics of exosomes,²combined with their target specificity demonstrated in clinical trials, establish them as prime candidates for precision therapies, surpassing conventional delivery platforms. From immunoregulation to neural regeneration, exosome applications are revolutionizing therapeutic paradigms owing to their multifunctional biological capacity. Alongside exosomes, a diverse array of nanocarriers—such as gelatin, collagen, and zein-based platforms—have also shown tremendous promise in cancer therapy due to their biocompatibility, ease of surface functionalization, and tunable drug release properties. Recent studies have demonstrated that gelatin-based systems enable environment-responsive delivery, while zein nanoparticles offer effective mucosal adhesion and sustained release. These platforms are now increasingly engineered for tumor targeting, immune modulation, and combination delivery of chemotherapeutics and biologics, as evidenced in recent reports.



Fig-1

Exosomes are small nano-scaled membrane-bound vesicles secreted by eukaryotic cells and have gained significant attention for their potential as therapeutic vehicles, particularly in neurological disorders⁴. Plant-derived exosome-like nanovesicles (ELNs), which are naturally occurring nanoparticles secreted from plant cells, have emerged as a promising alternative to mammalian-derived exosomes and conventional nanocarriers. They have several benefits rendering them appeal for neurological therapy. Plant ELNs can cross the blood–brain barrier (BBB), have minimal immunogenicity, and are inherently rich in bioactive substances, including antioxidants and small RNAs. They exhibit biocompatibility, cost-effectiveness, and suitability for oral or nasal administration, which is atypical for traditional delivery methods. They are scalable and morally advantageous, overcoming manufacturing and safety limits associated with mammalian-derived vesicles. These characteristics establish plant ELNs as versatile tools for therapeutic administration and modulation of neuroinflammatory and neurodegenerative pathways. The biogenesis of these ELNs involves the formation of intraluminal vesicles within multivesicular bodies (MVBs), which are released into the extracellular environment upon fusion with the plasma membrane, encapsulating proteins, lipids, DNAs, RNAs, and metabolites. This makes them ideal for targeted drug delivery and intercellular communication. One of the key advantages of plant-derived ELNs is their superior biostability compared to mammalian exosomes, largely due to their robust lipid membranes, enabling them to withstand environmental stresses and maintain their integrity in gastric and intestinal fluids⁵. This stability is crucial for overcoming challenges such as the ability to cross the BBB, which often limits the delivery of therapeutic agents to the central nervous system (CNS), and to withstand gastric fluid. The microenvironment is the interstitial, tissue, organ, or humoral component of the cell, which is involved in constituting the environment of survival.

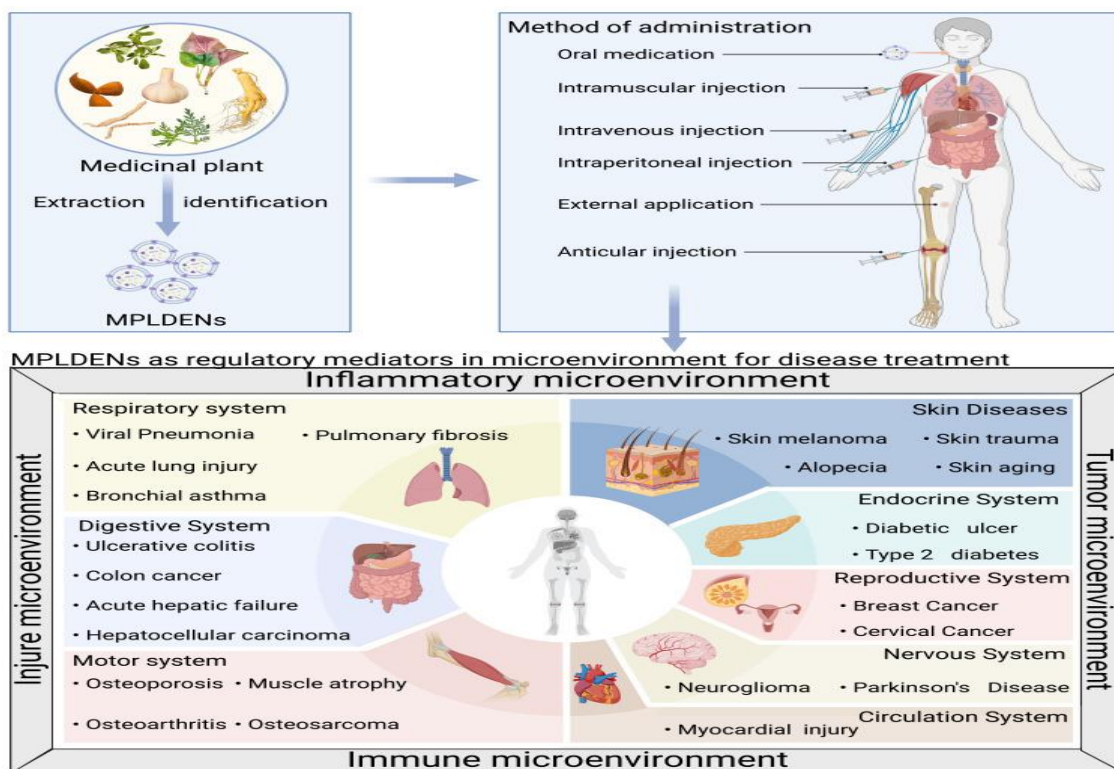


Fig-2

The stability of microenvironment is an important condition for maintaining normal physiological activities. Abnormal changes of microenvironment components can cause pathological changes in cells and tissues.⁶ Under normal physiological function, the microenvironment of organ tissues affects the normal physiological activities of organ tissues. The thymus microenvironment regulates the phenotype and number of hematopoietic cells by regulating chemokines,² bone marrow microenvironment has an important influence on the immune, hematopoietic and motor systems of the body. In addition, microenvironments exist in the lungs, pancreas, spleen and other organs, and play an important role in their physiology and pathology. In pathological conditions, the regulation of microenvironment also plays an important role in the occurrence and development of diseases, including tumor microenvironment, immune microenvironment, inflammatory microenvironment, and tissue damage microenvironment. In particular, the tumor microenvironment has become one of the targets of drug therapy in recent years.⁷ The regulation of microenvironment such as immunity, inflammation and tissue damage is also important for the occurrence and development of diseases, which directly or indirectly affects the disease process and the effect of drug therapy.

Medicinal plant is a plant used in medicine to prevent and treat diseases. The whole or part of Medicinal plant is used as Medicinal raw material, which is widely used in ethnomedicine. A lot of scientific research has been carried out on the monomer extracted from Medicinal plant. And widely used in clinical, such as paclitaxel, berberine and so on. In 1983, Pan BTd et al extracted exosomes for the first time from sheep reticulocytes in vitro.⁸ In 2009, Canal's research group found that the intercellular fluid in the leaves of sunflower seedlings contained plant-derived exosome-like nanovesicles, it was first demonstrated that plants can secrete plant-derived exosome-like nanovesicles (PLDENs) into cells. Medicinal plant-derived exosome-like nanovesicles (MPLDENs) are extracellular vesicles secreted by cells of medicinal plants and are rich in proteins, nucleic acids, lipids and other bioactive substances, it has anti-inflammatory, anti-tumor, immunomodulatory and anti-oxidative stress effects. In the past 15 years, research on the application and mechanism of MPLDENs in the improvement and treatment of various diseases has become a research hotspot. From the different biological characteristics of MPLDENs, it is discussed that MPLDENs, as a new type of natural nanomaterial, have high biocompatibility and a series of functions such as drug delivery.⁹ In the past 15 years, research on the application and mechanism of MPLDENs in the improvement and treatment of various diseases has become a research hotspot. From the different biological characteristics of MPLDENs, it is discussed that MPLDENs, as a new type of natural nanomaterial, have high biocompatibility and a series of functions such as drug delivery. Compared with other extracts or monomers of medicinal plants, MPLDENs, as natural biological nanovesicles, have rich endogenous substances and active ingredients, and

possess multiple therapeutic effects. Due to the biological characteristics of MPLDENS, MPLDENS have high lipid solubility. Therefore, MPLDENS significantly increase drug absorption and drug membrane permeability. Meanwhile, many related studies on MPLDENS have confirmed that MPLDENS are targeted to organs. The concentration of MPLDENS in the target organs is much higher than that in other organs, which is conducive to increasing the drug concentration in the target organs and can significantly enhance the therapeutic effect.

There have been significant advancements over the past decade in therapeutic strategies of cell-derived products, particularly extracellular vesicles (EVs). Exosomes, the smallest and most extensively studied EVs, can be produced by bacteria, animals, and plants to facilitate intercellular communication. There is accumulating evidence that exosomes are involved in the progression of diverse disease processes and possess multifaceted biological functions, such as antigen presentation, modulation of immune responses, and cytokine transport. Due to their favorable biocompatibility, low cytotoxicity, and minimal immunogenicity, exosomes have been applied in the diagnosis and treatment of various diseases¹⁰. Furthermore, exosomes exhibit specific targeting capabilities and the ability to traverse biological barriers, positioning them as promising carriers for drug delivery.

Exosomes Biogenesis

Exosomes originate from endosomal sorting within cells. Endocytosis and inward budding of the cellular membrane create endosomes, which develop into late endosomes that can undergo their own inward budding. These late endosomes transform into multivesicular bodies (MVBs) containing intraluminal vesicles (ILVs). MVBs play a crucial role in the sorting, cycling, release, and storage of cell contents. During the formation of ILVs, cargo exchange occurs between the MVB and the cytoplasm, Golgi bodies, and endoplasmic reticulum, allowing the regulation of cellular contents such as proteins and nucleic acids. MVBs have two potential fates: they can either fuse with the plasma cell membrane to release their ILVs as exosomes or be directed to the lysosome for degradation. The exact mechanisms determining which path an MVB will take remain largely unknown, although proteins on the MVB membrane and the content of ILVs have been proposed as influencing factors. When fusing with the plasma cell membrane, the ILVs are released into the extracellular space as exosomes. The formation of MVBs, ILVs, and exosome release is regulated through the endosomal sorting complexes required for transport (ESCRT) pathway.¹¹

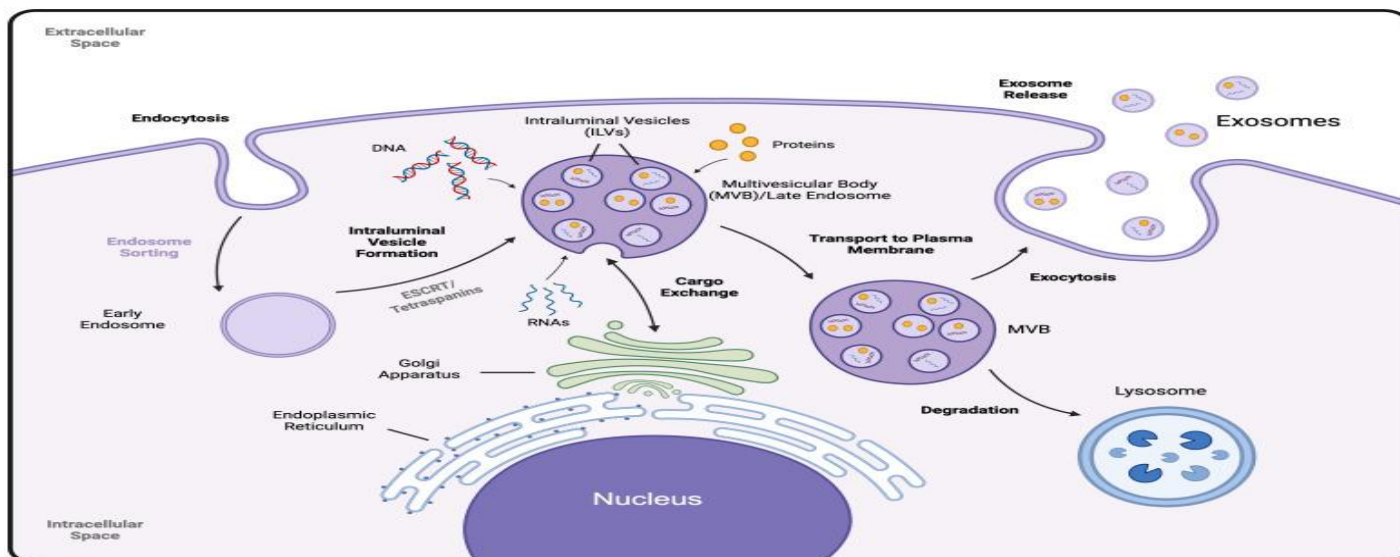


Fig-3

PLNs are increasingly recognized as functionally active extracellular vesicles sharing structural similarities with mammalian exosomes. However, these nanoparticles are generated via unique biosynthetic pathways. Three primary mechanisms are responsible for PLN formation: the multivesicular body (MVB) pathway, the exocyst-positive organelle (EXPO) pathway, and vacuole–plasma membrane fusion, and each contributes distinctively to vesicle formation and cargo loading. Among the proposed pathways, the MVB pathway is the principal route for PLN biogenesis ([fig-3](#)).¹² This process is initiated by inward budding of the plasma

membrane, which results in the formation of early endosomes. These endosomes mature and interact with the trans-Golgi network, leading to MVB development. Within MVBs, inward invagination of the endosomal membrane generates intraluminal vesicles (ILVs), which serve as carriers for selectively loaded biomolecules, including RNAs, DNAs, lipids, and proteins. Upon fusion of MVBs with the plasma membrane, ILVs are released into the extracellular milieu as PLNs and contribute to intercellular and interorganismal communication. The MVB pathway, which is well established in mammalian systems, also operates in plants and has been explicitly demonstrated in *Arabidopsis thaliana* by identifying TET8-positive vesicles. These plant vesicles are functional analogs of mammalian CD63-positive exosomes and are particularly induced during pathogen attacks, facilitating the secretion of small RNAs and immune regulatory molecules crucial in plant defense responses.¹³

The EXPO pathway represents a plant unconventional secretion mechanism that bypasses the classical endoplasmic reticulum–Golgi–endosome system. It involves the formation of double-membraned vesicles that directly fuse with the plasma membrane, resulting in the release of their contents into the apoplast. Unlike vesicles derived from the MVB pathway, EXPOs do not associate with canonical endosomal markers, which underscores their independence from endosomal trafficking routes. The EXPO pathway plays a critical role in the mediation of the rapid extracellular trafficking of defense-related proteins and signaling molecules in response to abiotic stresses, such as drought, salinity, and oxidative stress.¹⁴ These findings mark a notable shift in our comprehension of the functional landscape of plant extracellular vesicle biogenesis, which extends the relevance of EXPO pathway beyond developmental signaling and pathogen response. In *Arabidopsis thaliana*, stress-induced activation of EXPO vesicle secretion enhances apoplastic reactive oxygen species scavenging and salt tolerance through targeted delivery of antioxidant enzymes and chaperones. This outcome supports a novel model in which EXPOs act as first responders to environmental extremes, which rapidly reprograms the extracellular milieu. Furthermore, EXPO-derived plant-derived exosome-like nanoparticles, PLNs have shown an increasing potential as therapeutic nanocarriers. Given their origin in stress-adapted pathways, these vesicles exhibit enhanced stability under physiological conditions and can encapsulate stress-induced metabolites and RNAs with pharmacological relevance. They can bypass conventional secretion, which makes them especially valuable for the delivery of hydrophobic or structurally labile therapeutic agents. Recent engineering approaches have explored how the EXPO pathway can be harnessed or mimicked synthetically to design plant-based delivery systems for human applications, including targeted immunotherapy and metabolic disorder modulation.¹⁵ These advances underscore the EXPO pathway not only as a unique secretion mechanism but also as a biotechnological gateway for stress-responsive bioactive compound delivery. The third pathway involves direct fusion between vacuoles and the plasma membrane, predominantly under biotic stress. This fusion event results in the extracellular release of hydrolytic enzymes and defense-related proteins, which provides immediate local defense mechanisms against pathogens and environmental stressors.¹⁶

Methods for Isolating Exosomes

EVs are lipid bilayer structures derived from the plasma membrane that are secreted by cells. They play a vital role in facilitating intercellular communication and shaping the tumor microenvironment. However, exosomes are often mixed with various contaminants in complex biological fluids, so how to effectively isolate and enrich relatively pure exosomes has become a key to research. This article analyzes seven widely used methods for exosome separation and enrichment that have been implemented in recent years. These techniques include conventional ultracentrifugation, density gradient centrifugation, polymer precipitation, molecular exclusion chromatography, ultrafiltration, immunoaffinity chromatography, and microfluidic approaches. Furthermore, emerging technologies such as commercial kits, rinse separation methodologies, and fluidic systems are being explored to enhance the efficiency and speed of EV separations¹⁷.

1. Ultracentrifugation

Ultracentrifugation (UC) has become widely acknowledged as the benchmark technique for the isolation of exosomes, accounting for 45.7% of the methodologies employed for exosome separation. This technique operates on the principle of particle sedimentation in a solution, applying varying centrifugal forces to segregate sample components based on their density, size, and morphology. The sample is first subjected to processing based on its specific characteristics, during which larger biological particles are removed via low-speed centrifugation (e.g., 300× *g*). This is succeeded by a series of sequential centrifugation steps, employing centrifugal forces that range from 2000× *g* to 100,000× *g*. These steps are designed to efficiently eliminate cellular components, debris, apoptotic vesicles, and various contaminants, ultimately facilitating the isolation

and purification of exosomes. This methodology is not only straightforward but also allows for large-scale exosome preparation using existing liquid concentration technologies. However, ultracentrifugation may result in vesicle disruption, thereby affecting membrane structure and its biological activity ¹⁸.

2. Density Gradient Ultracentrifugation

Density gradient ultracentrifugation represents an enhanced separation methodology grounded in ultracentrifugation, predominantly utilized for the extraction and purification of exosomes. This methodology centers on creating a density gradient by employing two or more separation media with differing densities, such as sucrose and iodixanol. The standard protocol initiates with the removal of larger impurities through low-speed centrifugation, subsequently introducing the sample at the top of the density gradient medium, followed by a prolonged ultracentrifugation process that typically lasts over 16 h. This sequential operation facilitates the aggregation of distinct particles in precise locations throughout centrifugation, predicated on their density disparities and relative densities to the medium, thereby yielding separations with elevated purity. Given that exosome densities generally span 1.1 to 1.2 g/mL, this technique facilitates the effective isolation of exosomes from other extracellular components. Despite the clear advantages of density gradient centrifugation in achieving relatively pure exosome separation, the procedure is cumbersome and time-consuming. Additionally, it does not effectively distinguish between vesicles and exosomes with similar densities ¹⁹.

3. Ultrafiltration

Ultrafiltration (UF) is a separation method predicated on molecular dimensions, recognized as one of the most efficient and uncomplicated approaches for the isolation of exosomes. This methodology employs filter membranes characterized by diverse pore sizes or molecular weight cut-offs (MWCO) to remove contaminants from the sample; impurities exceeding the MWCO are retained, whereas exosomes that are smaller than the MWCO are permitted to traverse the membrane. Ultrafiltration can be applied using various driving forces, including pressure, centrifugation, and electrical charge. In contrast to conventional ultracentrifugation methods, ultrafiltration markedly decreases the time required for exosome isolation and does not necessitate costly specialized equipment, making it a highly advantageous alternative. However, this method is often constrained by the membrane's lifespan and transmembrane pressure, making it challenging to preserve exosomes in their native state ²⁰.

4. Size Exclusion Chromatography

Size exclusion chromatography (SEC) is a separation methodology that capitalizes on variations in molecular size and is extensively employed for the extraction and purification of exosomes. This technique facilitates separation by introducing the sample into a column filled with porous media (such as Sephadex, Sepharose, and Sephacryl) and leveraging the hydrodynamic characteristics of these particles. Unlike ultracentrifugation, SEC does not rely on strong centrifugal forces, but rather separates by gravity or low-speed centrifugation, which helps maintain the biological function and integrity of exosomes. In recent years, the SEC has been recognized as the most effective method for isolating morphologically and functionally intact exosomes from plasma ²¹. However, exosomes isolated by SEC columns may exhibit a wide size distribution, especially in the smaller size range, and contaminants similar in size to exosomes may be present.

5. Polymer Precipitation

Polymer precipitation (PP) is an exosome separation technique based on the principle of superhydrophilic polymers (e.g., polyethylene glycol, PEG), which has gained popularity in exosome extraction in recent years. The technique facilitates the aggregation of exosomes through low-speed centrifugation by attaching the polymer to the water molecules encasing the exosomes, thereby diminishing the solubility of the solute. This polymer precipitation approach is straightforward, does not necessitate sophisticated apparatus, is appropriate for processing substantial sample volumes, and effectively preserves the biological functionality of exosomes. However, the purity of exosomes isolated through polymer precipitation methods is relatively low, primarily due to the co-precipitation of soluble non-exosomal proteins, viral particles, and other contaminants. This contamination may lead to inaccuracies in exosome quantification, thereby affecting subsequent functional analyses.

6. Immunoaffinity

Immunoaffinity chromatography (IAC) is a separation technique based on antigen–antibody-specific reactions, which is widely used for exosome extraction and purification. The method utilizes the highly specific binding between specific proteins on the surface of exosomes (e.g., tetraspanin and ESCRT complex-associated proteins) and the corresponding antibodies, and realizes the selective capture of exosomes by immobilizing the antibodies on magnetic beads, chromatographic substrates or microfluidic devices. The

immunoaffinity method can significantly improve the purity of exosome isolation, and the isolated exosome has high specificity and good biological activity, but its operation process is relatively time-consuming, and the specificity and quality of the antibody are highly required. And the commonly used antibodies are mostly non-specific, which may lead to non-specific adsorption of exosomes. The above reasons limit its application in large-scale studies.

7. Microfluidics

Microfluidics is considered to be a promising approach to enable miniaturization, integration, and high-throughput capabilities by integrating processes such as sample handling, analysis, and detection into a chip. The technology enables the label-free isolation of exosomes by combining their physical and chemical properties (e.g., surface antigen, density, and size) with common separation methods. In addition, microfluidic devices can utilize external forces such as acoustic, magnetic or electric fields to further improve separation efficiency, and are becoming highly efficient platforms. In microfluidics, separation strategies based on physical properties have become a powerful approach. For example, exosome total isolation chip (ExoTIC) and deterministic lateral displacement (DLD) techniques have been developed for high-throughput exosome separation. Furthermore, immunoaffinity microfluidic devices allow for the selective isolation of specific exosomes via antigen–antibody interactions, a technique that emphasizes the importance of choosing suitable antibodies to enhance microscale mass transfer and improve particle-surface interactions. Despite the advantages of microfluidics, such as low sample consumption and short separation time, it still faces challenges such as sample clogging, low output, and high equipment cost.

A comprehensive review of current methodologies for exosome isolation and purification is lacking in the systematic inclusion of novel, engineered separation strategies. One such technology, a novel high-throughput, label-free inertial microfluidic device (ExoArc), has been reported for the isolation of platelet-free plasma from blood for RNA and EV analysis. ExoArc has also been coupled with SEC to isolate EVs within 50 min, with a yield 10-fold higher than ultracentrifugation. Furthermore, recent publications have described the use of an iodixanol–sucrose composite gradient system for density gradient centrifugation to isolate exosomes ²².

Challenges

Despite significant progress in the field of exosome research in the past, the biogenesis of exosomes is still not clearly explained, especially the cargo sorting mechanism of RNA and DNA, the factors and mechanisms affecting exosome secretion. These basic studies on exosomes are essential for our comprehensive understanding of exosomes and for further research on their applications.. PLNs represent an exciting frontier in nanomedicine because they offer inherent biocompatibility, low immunogenicity, and bioactive compound delivery. These challenges span technical, biological, and regulatory domains, including scalability, standardization, loading efficiency, and the lack of a comprehensive regulatory framework.

1. Stability and Immunogenicity

Barrier is the physicochemical stability of PLNs during storage and after administration. Factors such as temperature, pH, oxidative degradation, and mechanical stress can affect PLN integrity, which leads to reduced encapsulation efficiency and premature drug release. Some therapeutic cargos, such as hydrophobic small molecules or siRNAs, can further destabilize vesicle membranes or aggregates during storage. Although plant-derived vesicles generally exhibit lower immunogenicity than mammalian exosomes, their behavior in human hosts is not fully understood. Variables such as the genetic background, gut microbiota composition, and co-administered agents can influence immune activation. Although initial studies have indicated that PLNs are well-tolerated, systematic immunotoxicity testing, allergenicity profiling, and long-term biodistribution tracking are necessary before their clinical use.

2. Storage

PLNs are sensitive to temperature, freeze-thaw cycles, and moisture, which makes cold chain logistics a potential bottleneck. Lyophilization and cryoprotectant strategies are being explored to increase shelf life without compromising bioactivity. Preserving PELNs presents great challenges, particularly regarding storage conditions. Conventionally, freezing PELN samples below their reactivity threshold is a standard practice in research sample preservation. Storing ginger-derived PELNs at -80°C has proven effective in inhibiting IL-1 β secretion and Casp1 self-cleavage, thereby facilitating prolonged preservation. However, the recurrence of freeze-thaw cycles may result in PELN aggregation. Thus, the incorporation of cryoprotectants such as glucose, sucrose, and trehalose forms a hydrating shield around PELNs during freeze-drying. While

this approach holds promise for improving PELN quality during storage and transportation, its significance and effectiveness warrant further investigation.

3. Purity, Contamination, and Regulatory Issues

PLN preparations are often contaminated with cellular debris, lipoproteins, and other non-vesicular particles. Furthermore, their bioactive content and physicochemical properties can vary considerably depending on the plant species, cultivar, growth conditions, and extraction processes. This lack of formulation uniformity poses a direct threat to clinical reproducibility and safety. The cellular uptake of PLNs, their absorption, distribution, metabolism, and excretion characteristics remain largely unexplored. Without clear PK data, dosing regimens and assessment of long-term biodistribution or prediction of off-target effects—factors that are essential for any clinical-grade therapeutic product—become extremely challenging. Although agencies such as the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), have commenced formulating guidelines for mammalian-derived exosomes, plant-based nanovesicles currently lack clear classification and approval pathways.

4. Scalability and producibility

One of the most critical and persistent barriers to the clinical application of PLNs is the development of scalable, reproducible, and economically sustainable manufacturing platforms that meet the rigorous demands of pharmaceutical production. Traditional techniques used to isolate extracellular vesicles, such as ultracentrifugation, SEC, and immunoaffinity capture, although effective in small-scale research settings, are unsuitable for industrial-scale Good Manufacturing Practice (GMP) production due to their high cost, time consumption, and poor yield reproducibility. In ultracentrifugation-based protocols, for example, small deviations in speed, duration, or rotor type can significantly impact vesicle recovery and structural integrity. SEC, while providing better separation resolution, scales poorly due to column capacity limitations and often requires trade-offs between throughput and purity. Immunoaffinity methods offer specificity but are cost-prohibitive and difficult to standardize for large-volume processing. Compared to synthetic nanoparticles, PLNs are often considered more cost-effective due to their natural abundance and plant-based origin, which reduce raw material expenses. However, this cost advantage can be significantly offset by the complexity and inefficiency of current purification and isolation methods. Synthetic nanoparticles benefit from well-established, scalable, and automated production processes, while PLN production still suffers from low yields, labor-intensive protocols, and batch-to-batch variability. Therefore, when considering total production costs—including downstream processing and quality control—PLNs may not currently offer a significant economic advantage over synthetic alternatives. To address these issues, the field is increasingly exploring emerging technologies such as tangential flow filtration (TFF), microfluidics-based continuous separation, and automated high-throughput isolation platforms. TFF, in particular, is gaining momentum for its compatibility with GMP environments and ability to process large volumes while maintaining vesicle integrity. However, industrial adoption remains limited by technical bottlenecks such as membrane fouling, vesicle retention, and shear stress effects. Moreover, plant cell suspension cultures and controlled-environment agricultural systems (eg, vertical farming) have been proposed for the standardization of PLN production across species and batches. These systems offer better control over environmental variables and enable continuous harvesting, which is crucial for batch consistency and regulatory compliance. However, widespread industrialization will require more than technological solutions—it will also necessitate the establishment of process analytical technologies (PAT), validated quality control assays for vesicle identity and purity, and real-time monitoring protocols compatible with GMP standards. Lessons learned from other biologics (eg, vaccines, monoclonal antibodies, enzyme therapies) suggest that early integration of scalable manufacturing design with regulatory compliance pathways is critical to reduce translational delays and downstream reengineering costs.

5. Contents in exosome

Plants have diverse components and functionalities in various parts, including roots, stems, leaves, flowers, bark, fruits, seeds, and dried aerial portions. Evident differentiations in miRNA content and disease-targeting profiles exist between the underground roots and above-ground stems/leaves of *houltuynia cordata*-derived PELNs. Additionally, discernible dissimilarities manifest between PELNs extracted from dried and fresh plant samples. Dry plants may present greater difficulties in obtaining PELNs compared to fresh counterparts, and the cargo of PELNs derived may also exhibit variability. Furthermore, the cargo content of PELNs sourced

from mature and immature plant samples also varies noticeably. For instance, the miRNA abundance in mature coconut-derived PELNs surpasses that in immature coconut-derived PELNs

6. Administration of PELNs

Currently, the most prevalent method of administration of natural and modified PELNs currently under investigation is oral, and has been demonstrated to be efficacious. In addition to this, intravenous, intraperitoneal, nebulized inhalation and microneedles have also been explored as potential delivery modes. While PELNs delivery modalities such as intravenous injection demonstrate some targeted delivery capability, they also face significant challenges, including degradation and immune clearance of the drug in the body's circulation. These challenges result in limited therapeutic efficacy. Nebulized inhalation allows for direct delivery to the alveoli, thereby optimizing drug concentration for pulmonary application. In contrast, microneedling represents an emerging and efficient delivery strategy for PELNs, combining minimally invasive, localized drug delivery with the pro-angiogenic, pro-tissue regeneration and anti-inflammatory properties of PELNs, offering a promising avenue for clinical application.

Differences between milk-derived and plant-derived exosomes

Function	Milk-Derived	PDENs
Cellular communication	They mediate cell-cell communication via transferring molecular signals such as proteins, lipids, and RNAs	They facilitate cellular signaling by transferring lipids, proteins, and RNAs between different cells during stress responses or development processes.
Biological response regulation	They are involved in immune response including antigen presentation and the modulation of immune cell activity	They are reported to regulate cellular responses to drought, salt stress, and immunity.
Disease modulation	They play an important role in cancer progression. Tumor derived exosomes might facilitate metastasis.	They regulate plant pathogen immune responses by transferring immune related molecules like plant specific small RNAs.

Promising Developments in Biomedical Applications of PDENs

1. Anticancer Effect of Plant-Derived Exosomes

Numerous studies have shown PDENs' potential to fight a wide range of diseases (Fig-4). One of the most interesting and exciting areas is the anticancer properties of exosomes. For instance, PDENs isolated from *Asparagus cochinchinensis* were found to inhibit hepatocellular carcinoma cell proliferation, induce cellular apoptosis, and upregulate factors related to apoptosis in both in vitro and in vivo studies without causing any adverse side effects. Exosomes derived from the juice of fresh tea leaves were co-incubated for 5 h with breast tumor cells; afterwards, it was discovered that more than 80% of the cancer cells absorbed the nanoparticles. An increased amount of reactive oxygen species induced by absorbed exosomes has led to the damage of mitochondria, cell cycle arrest, and, finally, the apoptosis of cancer cells. Additionally, the next in vivo experiments have demonstrated no toxicity in the oral administration of tea leaf nanoparticles. Nanoparticles were successfully absorbed by the small intestine to modulate microbiota and regulate tumor gene expression profiles, achieving the desired therapeutic effect in the fight against breast cancer. It was possible to achieve outstanding results in the fight against gastric cancer using exosomes isolated from lemons. Their anticancer activity is associated with the ability to promote an increase in ROS, which upregulates GADD45a gene expression, ultimately leading to S-phase arrest and apoptosis. The biosafety of lemon-derived exosomes was also evaluated. A comparison of the organs for the morphological normality of the histological sections of the main organs was made. The obtained results suggest that the use of these nanoparticles does not harm the body²³.

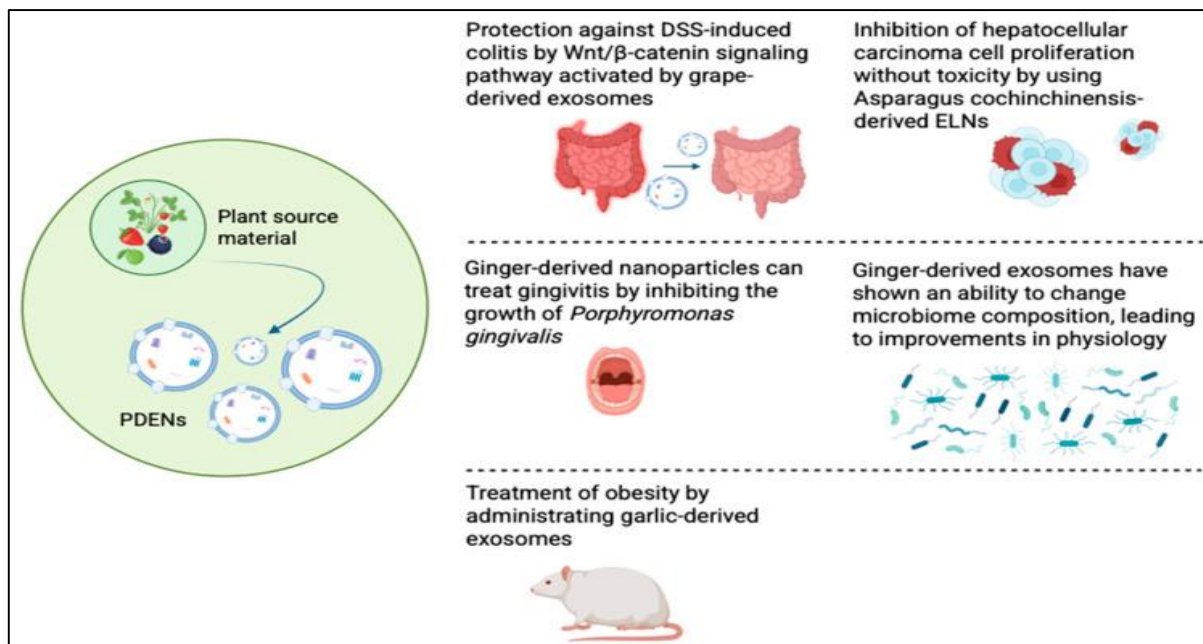


Fig-4

2. Treatment of Periodontitis

Periodontitis is a chronic, multifactorial inflammatory condition linked to the buildup of dental plaque. It is marked by the progressive destruction of the structures that support the teeth, such as the periodontal ligament and alveolar bone. The Gram-negative anaerobic bacteria *Porphyromonas gingivalis* is the main pathogen that influences the risk of periodontitis infection and its progression. It is capable of creating comfortable conditions for the colonization of oral surfaces, the degradation of periodontal tissues, the induction of destructive immune responses, and growth in a peptide- and hemin-rich inflammatory microenvironment. A specific ratio of phosphatidic acid in ginger-derived nanoparticles has been found to interact with proteins of *Porphyromonas gingivalis*, namely, bind to HBP35 protein on the surface of bacteria, leading to the inhibition of bacterial growth and a decrease in the incidence of gingivitis ²⁴.

3. Alteration in Microbiome Composition

PDENs have great significance in digestive system diseases. They are highly stable and can persist in the digestive system, exhibiting their therapeutic properties and successfully resisting digestion by various enzymes. The results of the study conducted by Teng et al. revealed that exosome-like nanoparticles isolated from ginger were mainly taken up by Lactobacillaceae in a lipid-dependent GELN-mediated manner and contained microRNAs that target various genes of *Lactobacillus rhamnosus*. One such microRNA, mdo-miR7267-3p, suppresses the *ycnE* gene, causing the bacteria to induce production of indole-3-carboxaldehyde (I3A). This substance can activate a special receptor (AHR), which leads to the production of the protein IL-22, consequently resulting in strengthening the protective barrier of the intestine. Additionally, the co-incubation of ginger exosome-like nanoparticles with bacteria from the *Lactobacillus* family showed that they promoted the growth of beneficial strains *L. reuteri* and *L. murinus* ²⁵.

4. Treatment of Obesity

Treating obesity caused by chronic inflammation is a major challenge. One of the features of this disease is the accompanying inflammation of the brain, which is transmitted through the gut–brain axis. The ability of exosomes to penetrate the BBB and circulate in both directions makes them promising in the treatment of inflammation. Thus, it was discovered that garlic-derived exosomes reversed HFD-induced brain inflammation and obesity in a mouse model of HFD-induced obesity via oral administration. The brain inflammation process was suppressed through the IDO1-mediated AHR pathway and the c-Myc-regulated c-GAS/STING inflammatory pathway when garlic-derived exosomes were taken up by microglial cells. Pang et al. also found that kidney-bean-derived exosome-like nanovesicles were able to alter gut microbiota and thereby improve symptoms of high-fat-diet-induced obesity. The effect of the nanoparticles was reflected in the growth of beneficial bacteria such as *Lactobacillus*, *Romboutsia*, *Bifidobacterium*, and *Ruminococcus*. A significant decrease in the excess of Proteobacteria and Desulfobacterota, bacteria whose high presence is often associated with intestinal diseases, was also noted ²⁵. Pang et al. suggested that legumes may have antihyperlipidemic, antiobesity, and antidiabetic activity due to the selective enrichment of bacteria.

5. Treatment of Colitis

The therapeutic potential of grape-derived exosome-like nanoparticles has been studied in the treatment of colitis. It was found that the oral administration of grape exosomes to mice resulted in a significant reduction in colonic inflammation induced by dextran sodium sulfate (DSS). The decrease was due to the induction of Lgr5^{hi} intestinal stem cells. Treatment with grape-derived exosomes activates the Wnt/ β -catenin pathway and induces the expression of genes that regulate the growth of stem cells. It is assumed that grape nanoparticles are absorbed through the intestinal wall and deliver bioactive molecules that promote stem cell proliferation and tissue regeneration ²⁵.

6. Anti-Aging Effects

Melanocytes are neural-crest-derived cells that are localized in several organs in humans, including the epidermis, eye, inner ear, and soft meninges. In recent years, plant-derived products have emerged as potential alternatives to chemical products due to their high antioxidant content and low side effects. Lee et al. investigated the antimelanin effect of ELNs extracted from the leaves and stems of *Dendropanax moribifera* and found that leaf-derived ELNs and stem-derived ELNs reduced the melanin content and tyrosinase (TYR) activity in a concentration-dependent manner in a B16BL6 mouse melanoma cell line. ELNs from certain plants may be novel natural substance candidates for use as antimelanin agents in cosmeceutical preparations.

7. Prevention and Treatment of Gingivitis

Periodontitis is an infectious oral disease that causes the destruction of periodontal tissue and tooth loss. Although great progress has been made in the treatment of periodontitis, effectively treating periodontal tissues invaded by periodontitis is still an urgent problem to be solved. Therefore, finding new treatment methods for periodontitis is an urgent issue to be addressed. Natural products have good antibacterial, anti-inflammatory, antioxidative, and osteoprotective properties on periodontal tissues. Some scholars have proven that a specific ratio of PA in ginger-derived nanoparticles (GELNs) can bind to the HBP35 protein on the surface of *Porphyromonas gingivitis*, thereby inhibiting the growth of bacteria and significantly reducing the incidence of gingivitis. Ginger-derived ELNs are expected to be developed as a drug for the prevention and treatment of chronic periodontitis.

8. Treatment of Liver Diseases

Several studies have reported on the role of exosomes in hepatitis B. Ginger-derived nanoparticles protect against alcohol-induced liver damage. Cannabis bud-derived ELNs, as hepatoprotective agents, attenuate liver fibrosis. In addition, ELNs derived from *Pueraria lobata* can effectively alleviate alcohol intoxication, improve alcohol metabolism, inhibit iron death, and protect the liver from alcohol damage. ELNs derived from garlic exhibit excellent anti-inflammatory effects, inhibiting NLRP3 inflammatory vesicle activation and inducing autophagy during liver injury. Beta vulgaris-derived ELNs alleviate chronic doxorubicin-induced cardiotoxicity by inhibiting ferroptosis.

Exosomes as Delivery Tools to Overcome Biological Barriers

The antitumor effects of exosomes with therapeutic payloads also depend on the ability to deliver cargo to the intracellular action site. Approaches to enhance intracellular delivery for exosomes with encapsulated cargo are discussed, as the cell membrane is an existing barrier to the anti-tumor efficacy. In addition, the ability of exosomes to cross biological barriers, including the blood–brain barrier (BBB) and the gastrointestinal (GI) tract, inspire growing areas of research interests utilizing exosomes for drug delivery.

Enhanced Intracellular Delivery

Cells can take up exosomes by various means including direct membrane fusion, pinocytosis, and endocytosis. In order to facilitate exosome entry into cells, one approach is to equip exosomes with cell-penetrating peptide (CPPs), such as arginine-rich CPPs, which enhance exosome internalization by stimulating cell micropinocytosis. Another approach is by increasing the chance of membrane fusion. Biophysical studies suggest that increasing membrane rigidity by enriching sphingolipid and cholesterol improves fusion efficiency between exosomes and the recipient cells. In addition, exosomes with a surface display of G protein of vascular stomatitis virus (VSV-G) exhibit increased intracellular delivery of payloads by promoting exosome-cell membrane fusion. Integration of exosomes with connexin 43 (Cx43), a membrane protein that assembles to form hexameric channels, allows exosomes to dock at the gap junction pore embedded in the plasma membrane of recipient cells, and therefore provides another route for direct cytoplasmic transfer of exosome payload. As for internalized exosomes following the endosomal traffic route, the ability of the cargo to escape before lysosomal degradation is another challenge. Exosomes complexed with cationic lipids together with a pH-sensitive fusogenic peptide, GALA, increased exosome-endosome

fusion and, therefore, aided in the cytosolic release of cargo. Currently, specific mechanisms for exosome internalization by cells remain to be elucidated. The inclusion of targeting moieties on exosomal membrane also impact the uptake of exosomes, as well as exosome distribution among cell organelles. For example, cell uptake of HEK293-derived naive exosomes is similar to the uptake of cholera toxin subunit B (CtxB), a marker for lipid raft-associated cell uptake. On the other hand, exosomes with a surface display of anti-Her2 ScFv increased overall cell uptake, with a greater extent of colocalization with transferrin and dextran, markers for clathrin-mediated endocytosis and micropinocytosis, respectively. The uptake mechanism also differs depending on recipient cells. The observed contributing fractions by caveolae-dependent endocytosis of exosomes vary among cell lines, which is partly subject to caveolin-1 expression levels. As the research community is starting to appreciate the diversity of exosomes in terms of size and originated cell organelles, trafficking routes other than the putative endosome destination, such as the nucleus, have also been described. Thus, exosomes may serve as potential tools to confer cell organelle-targeted delivery once the intracellular fates of exosomes are fully elucidated²⁶.

BBB

The BBB prevents the entry of toxic compounds into the brain and, consequently, restricts the delivery of most drugs for treatment of brain-associated diseases. Circulating exosomes are able to cross the BBB. Therefore, exosomes are considered as promising tools for drug delivery to the central nervous system (CNS). Exosomes derived from brain endothelial bEND.3 cells are able to deliver anticancer drugs across the BBB in zebrafish embryos due to the homotypic effect of exosomes. Another study suggests that unmodified exosomes from macrophages are sufficient to confer BBB penetration in the context of brain inflammation, where the expression level of adhesion molecule 1 (ICAM-1) on endothelial cells increased the interaction with lymphocyte function-associated antigen 1 (LFA-1) on macrophages-derived exosome membranes. Exosomes modified with rabies viral glycoprotein (RVG) peptide, a CNS-specific peptide bound to acetylcholine receptor, also showed the ability to cross the BBB in vivo with minimal liver accumulation.²⁷

GI Tract

Oral drug delivery is still a challenging task for many therapeutic biologics. Exosomes derived from milk or edible plants are able to cross intestinal barriers and, therefore, are considered as potential drug vectors for oral delivery. An interesting finding is the discrepancy of vector effect; milk exosomes were mainly distributed into the liver and spleen, whereas the encapsulated miRNA accumulated in the spleen and brain. The authors speculated that the distinct tissue distribution of payload was subject to a relayed substance transfer from foreign exosomes to endogenous exosomes in the endosomal traffic routes in cells, therefore allowing the cargo re-distribution to the brain.²⁸

Therapeutic Use of PDENs

PDENs have demonstrated a range of therapeutic properties, including antioxidant, anti-inflammatory, anticancer, and antibiotic effects. By analogy with milk exosomes, PDENs could hypothetically be used either as transport vesicles or as independent therapeutic substances. Interestingly, the structural and functional biomolecules they contain could also be useful in clinical applications. To date, PDENs have been obtained from a variety of edible plants, including grapes, grapefruit, ginger, lemon, broccoli, carrots, coconuts, and apples. Importantly, it is safe to assume that they may have beneficial therapeutic effects due to natural biochemicals in the source plants. PDENs derived from grapes, blueberries, and strawberries exhibited inherent biological activity (antioxidant, cytoprotective, anti-inflammatory, anti-proliferative) in vitro and, moreover, have the ability to deliver various molecules in a targeted fashion. There is no evidence of inflammation or toxicity associated with the use of PDENs, and they are considered to be relatively safe. Studies have demonstrated that PDENs derived from fruits like grapes and blueberries exhibit antioxidant and anti-inflammatory activities in vitro, highlighting their potential as therapeutic nanocarriers³⁰.

Although research on PDENs has only recently begun, there are several earlier researchers who have described the biogenesis and mechanisms of PDENs. Early evidence suggests that plants produce PDENs in response to multiple biotic and abiotic environmental stresses, including infection. PDENs have been shown to be taken up by intestinal macrophages and are capable of exhibiting cross-species communication by inducing multiple cytokines. Some studies have shown that PDENs may provide health benefits, so it would be interesting to apply them as a new functional food ingredient. However, the stability of PDEN in food digestion, processing, and storage must be clearly demonstrated before they can be used in functional foods.

Studies have recently shown that, when taken orally, PDENs penetrate into the colon and, depending on the source of the PDENs, remain in the colon area, facilitating intestinal function, providing an anti-inflammatory

effect, or penetrating into liver cells, exerting a hepatoprotective effect there. These results demonstrate the effectiveness of PDENs as ingredients in functional foods to alleviate various diseases. PDENs are currently being investigated for their suitability as an alternative to milk exosomes as drug carriers. In terms of large-scale production capabilities, PDENs have great potential for applications in disease therapy as well as for the development of drug nanocarriers that are capable of delivering different doses depending on their physiological, chemical, and biological characteristics. The yield of PDENs that can be extracted from plants is much higher than that of exosomes from mammalian cell cultures, indicating their cost-effectiveness as nanofactories. For example, Wang et al. first developed PDENs as nanovectors for delivering therapeutic agents to brain tumors. They showed that PDENs accumulate in certain tissues in vivo and circulate for a long time in the peripheral blood due to the high stability of the nanovectors.

Plant biomolecules have attracted much attention due to their ability to improve health and provide protection against various diseases. PDENs are naturally generated and carry harmless components from parent cells, some of which have been shown to possess therapeutic effects. In addition, PDENs can be intrinsically localized to target tissues, one of the most important features of a targeted delivery system. PDENs exhibit intrinsic targeting capabilities owing to their specific lipid and protein composition, including surface molecules that facilitate selective uptake by intestinal epithelial cells and macrophages. However, PDEN is a new concept in nanomedicine, and not all its aspects have been fully identified and described. There remains a need to expand the use of innovative approaches to drug development to more effectively and efficiently treat various diseases.

Research on PDENs is still mostly carried out at the in vitro level, which opens up more opportunities for comprehensive research, especially regarding their biological activity, isolation, handling, and standardized mass production. Previous studies have assessed, under preclinical conditions, the effects of polyphenol concentrates from cranberries, lingonberries, bilberries, blueberries, and grapes in vitro and in vivo on several models of age-associated pathology. According to research data, polyphenolic extracts of grapes and blueberries had the most pronounced therapeutic potential. This circumstance initiated further research into the possibility of increasing the biological activity of polyphenols through the use of recently discovered exosome-like nanoparticles of plant origin.

Limitations of the Current Studies

1. While current studies have demonstrated the numerous functions of PELNs, there is still much to be learned about their mechanisms. For example, it is unclear how plant exosomes interact with recipient cells and how they specifically modify those cells. Additionally, further investigations are required to elucidate the endocytic pathways involved in the uptake of exosomes derived from plants.
2. Plant exosomes contain a variety of biomolecules that may cause unknown side effects. Therefore, it is crucial to conduct extensive clinical trials to investigate pharmacokinetics, pharmacodynamics, safety, stability, and efficacy prior to implementation.
3. Most animal experiments involving plant exosomes have focused on oral administration, with only a few choosing intravenous injection. No experiments using local injection or coating administration have been found. Oral administration has advantages in simplicity, convenience, and relative safety, but first-pass elimination through the liver after oral administration significantly reduces the concentration and dose of the drug in the body circulation. Furthermore, slow absorption rates seriously affect treatment efficiency. Alternatively, intravenous administration provides faster and longer-lasting effects, but it comes with greater risks of adverse drug reactions. Thus, investigating different routes of administration and comparing their effectiveness can help find an optimal route of administration for exosomes from plants.
4. To date, there is no unified plan for the separation and extraction of exosomes. Different methods can obtain varying content and purity of exosomes, leading to heterogeneity and affecting experimental results. Ultracentrifugation and sucrose density gradient centrifugation are commonly used due to their simplicity and ability to obtain a large number of exosomes. However, both methods require repeated centrifugation, which is time-consuming and may damage exosomes. Developing a complete exosome

extraction method that shortens extraction time while ensuring quantity and purity and does not affect exosome activity is desired.

5. Proteomic analysis can greatly improve the understanding of plant exosomes, but research on exosome-tagged proteins of plants is limited. This limitation hinders the characterization of PELNs.
6. The utilization of PELNs as carriers for drug delivery carriers exhibits promising development prospects, but current research faces three main challenges: improving the load of active components of exosomes, improving their specificity for tissues to accurately deliver drugs to the desired sites, and improving the efficiency of exosome drug release. Studies using genetic or chemical methods to change the composition of exosomes derived from animal cells have led to engineered exosomes that exhibit improved biological functions. We expect similar approaches will yield positive results for exosomes from plants.
7. Seasonal and geographical factors must be considered when studying plant exosomes because some plants only grow in specific seasons or regions. This factor complicates the study of plant exosomes, and researchers may face additional burdens if they consider artificial cultivation of some plants with long growth cycles. Tissue culture technology is a viable solution to these issues, but it comes with relatively high economic costs.
8. A review of published literature on plant exosomes shows that most research focuses on the digestive system, tumor system, respiratory system, and circulatory system diseases. Research on haematological system, endocrine system, nervous system, and motor system diseases is lacking, indicating significant gaps in research on plant exosomes.

Recommendations for Future Research

1. Further investigation into the mechanisms underlying the therapeutic effects of PELNs is necessary to gain a deeper understanding of PELN and promote its development.
2. It is essential to conduct studies on the pharmacokinetics, pharmacodynamics, safety, and stability of plant extracellular vesicles as potential therapeutics to fully understand their clinical applications.
3. Exploring different administration routes for PELN, such as local injection and topical application, can help identify the optimal delivery method for different diseases.
4. A consistent focus on identifying the optimal method for extracting plant extracellular vesicles with maximum yield and purity while minimizing extraction time without compromising vesicle activity is critical.
5. More research on specific protein markers for PELNs is needed to enhance the characterization of these important biological structures.
6. Exploring strategies to enhance the cargo capacity of PELNs to enhance their tissue specificity for accurate drug delivery to desired sites, and to improve drug release efficiency is also crucial.
7. Further research on the therapeutic effects of PELNs for diseases related to the blood system, endocrine system, nervous system, musculoskeletal system, and other systems will provide us more potential clinical applications.

Conclusion

Plant-derived exosomes, also known as plant-derived extracellular vesicles (PDEVs/PDENs), have emerged as promising natural nanocarriers in modern nanomedicine. Their nanoscale size, biocompatibility, stability, and ability to carry bioactive molecules make them attractive for therapeutic applications. The study of their biogenesis and isolation methods provides an understanding of how these vesicles can be obtained and developed for biomedical use. Plant-derived exosomes can also help deliver therapeutic substances across biological barriers, which may improve the effectiveness and targeted delivery of drugs.

The therapeutic potential of plant-derived extracellular vesicles (PELVs/PDENs) includes applications in drug delivery, cancer therapy, inflammation control, tissue repair, and other biomedical areas. Compared with milk-derived exosomes, plant-derived exosomes offer advantages such as plant-based availability, potentially lower production costs, and good biocompatibility, although both types have unique properties and applications. Recent developments indicate that PDENs could become valuable tools for personalized medicine and advanced drug delivery systems.

However, challenges such as standardization, isolation and purification, characterization, stability, scalability, loading efficiency, targeting, and regulatory approval still need to be addressed. Therefore, future research should focus on developing standardized isolation and characterization protocols, improving large-scale production, enhancing targeted delivery, understanding their safety and mechanisms of action, and conducting more extensive preclinical and clinical studies.

Overall, plant-derived exosomes represent a promising and innovative class of natural nanoparticles with significant potential in biomedical applications. With continued research and technological development, they may contribute to safer, more efficient, and more sustainable therapeutic delivery systems in the future.

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