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REVIEW

Plant-Derived Extracellular Vesicles for Nanomedicine in Cardiopulmonary Diseases: A Narrative Review

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Abstract: This narrative review summarizes research progress on plant-derived extracellular vesicles (PEVs) for nanomedicine in cardiopulmonary system diseases, based on key literature covering isolation, engineering, and disease mechanisms. PEVs possess high biocompatibility, low immunogenicity, broad source availability, and scalability. Their bioactive cargo (proteins, nucleic acids, lipids, secondary metabolites) regulates inflammation, oxidative stress, apoptosis, and fibrosis. This review systematically discusses PEV characteristics, large-scale isolation, and engineering approaches, with a focus on multi-target and cell-specific mechanisms in atherosclerosis, myocardial infarction, COPD, and pulmonary fibrosis. Although challenges in standardization, in vivo mechanisms, and translation remain, engineered PEVs hold promise as efficient and safe nanomedicines. The unique contribution of this review is to integrate PEV preparation and engineering with their disease-specific mechanisms, providing a coherent framework for future translational research in cardiopulmonary nanomedicine.

Keywords: plant-derived extracellular vesicles, nanomedicine, drug delivery, cardiovascular diseases, respiratory system diseases

Introduction

Cardiovascular diseases (CVDs) and chronic respiratory diseases are leading global causes of disability and mortality, exerting continuous pressure on socio-economic systems and public health.[1,2] Current mainstream pharmacological strategies, such as statins, bronchodilators, and anti-inflammatory drugs, often face bottlenecks including insufficient accumulation in target tissues, systemic side effects, and difficulty in reversing progressive tissue remodeling. Nanomedicine, through the design of intelligent delivery systems, offers a revolutionary approach to overcoming these barriers, aiming to achieve precise and controllable drug release.[3–5] The significance of nanomedicine for cardiovascular disease lies particularly in its capacity for targeted drug delivery, advanced imaging, and regenerative therapies, which collectively improve diagnosis, treatment, and patient outcomes. For example, nanocarriers can be engineered to home to diseased vascular endothelium or infarcted myocardium, enabling site-specific accumulation of therapeutic agents while minimizing systemic toxicity; nanoscale imaging probes allow real-time assessment of atherosclerotic plaque vulnerability; and regenerative nanoplatforms—including extracellular vesicles (EVs)—promote cardiomyocyte survival and angiogenesis.[6–8] For instance, a recent study demonstrates that an "ion cocktail" strategy delivered via nanoscale platforms can synergistically regulate both structural and electrical remodeling in the infarcted myocardium, highlighting the therapeutic potential of nanomedicine for myocardial infarction.[9] Among various nanocarriers, plant-derived extracellular vesicles (PEVs) are garnering unprecedented attention as an emerging natural nanoplatform. PEVs are lipid

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bilayer-enclosed vesicles actively secreted or released by plant cells under external stimulation, typically ranging from 30 to 1000 nm in diameter. Their internal lumen and membrane selectively encapsulate and enrich functional proteins, lipids, nucleic acids (eg, microRNAs), and pharmacologically active secondary metabolites from the source plant.[10,11]

Compared to synthetic nanoparticles (eg, liposomes, polymeric micelles) or mammalian-derived exosomes, PEVs possess a series of irreplaceable competitive advantages: their raw materials originate from renewable agricultural or food processing by-products, resulting in extremely low cost;[12] their plant origin grants them very low immunogenicity in mammals, avoiding the potential pathogen risks and immune rejection reactions associated with animal-derived carriers;[13] furthermore, many PEVs inherently possess "built-in" bioactivities such as anti-inflammatory and antioxidant effects.[14]

Mammalian-derived extracellular vesicles (eg, exosomes) have been extensively investigated as drug delivery systems and therapeutic agents, demonstrating advantages in biocompatibility and intercellular communication. However, their clinical translation is hampered by high production costs, low yields, immunogenicity concerns, and potential pathogen transfer.[15] In contrast, PEVs—also referred to in some literature as plant-derived nanovesicles (PDNVs) or plant exosome-like vesicles (PDEs)—have emerged as a promising alternative platform. Notably, unlike previous reviews that broadly discuss PEVs in general drug delivery or diverse disease contexts, the novel perspective of this review is its exclusive focus on cardiopulmonary diseases, integrating PEV preparation and engineering with disease-specific mechanisms to provide a coherent translational framework. Furthermore, like all biological nanocarriers, PEVs face regulatory challenges regarding standardization, scale-up characterization, and safety evaluation—issues that must be addressed early in their clinical development.

This narrative review is based on literature retrieved from PubMed, Web of Science, and Google Scholar up to December 2025, using search terms including "plant-derived extracellular vesicles", "plant exosomes", "nanomedicine", "cardiovascular diseases", "respiratory diseases", "engineering modification", and "drug delivery." Priority was given to studies reporting mechanistic insights, in vivo efficacy, and engineering strategies relevant to cardiopulmonary disease models. Given the narrative nature of this review, the selection emphasizes representative and high-impact findings rather than exhaustive coverage.

Despite growing interest, several key questions remain unanswered regarding the application of PEVs for cardiopulmonary nanomedicine: (1) What are the precise molecular mechanisms underlying cross-kingdom communication—such as PEV internalization, intracellular trafficking, and functional regulation of mammalian gene expression—in the context of the heart and lungs? (2) How do different engineering strategies (eg, surface modification, membrane hybridization, cargo loading) compare in terms of targeting precision, drug payload capacity, pharmacokinetics, and safety for specific cardiopulmonary indications? (3) To what extent do the endogenous bioactive molecules carried by PEVs (eg, plant miRNAs, polyphenols, lipids) contribute independently to therapeutic efficacy, and can they be leveraged synergistically with loaded therapeutics? Addressing these questions is critical for advancing PEVs from bench to bedside.

This review aims to systematically outline the preparation, characterization, and functional properties of PEVs. It will focus on elucidating the latest cutting-edge advances, multidimensional mechanisms of action, and translational challenges of PEVs serving as both natural therapeutic agents and engineered nanocarriers in treating core cardiopulmonary diseases, including atherosclerosis, myocardial infarction, chronic obstructive pulmonary disease (COPD), and pulmonary fibrosis. Finally, it will provide a prospective outlook on future interdisciplinary research directions.

PEVs: From Natural Vesicles to Engineered Nanoplatforms

Isolation, Characterization, and Native Functions

The isolation and purification of PEVs form the foundation for their research and application. Currently, differential centrifugation remains the most common initial method in laboratories, involving a series of centrifugation steps to gradually remove cell debris and large organelles. However, this method can easily cause vesicle aggregation and offers limited purity.[16] Size-exclusion chromatography (SEC) is highly favored for its gentle separation process and ability to yield high-purity PEVs, making it particularly suitable for subsequent functional studies.[17] For future large-scale

production, tangential flow filtration (TFF) and multimodal chromatography techniques are showing significant potential.[18]

However, plant cell walls impose unique isolation challenges. Unlike mammalian cells, plant-derived vesicles are largely trapped in the apoplastic space (between plasma membrane and cell wall), limiting their accessibility.[19] Conventional mechanical disruption (eg, grinding) enables bulk recovery but co-isolates intracellular debris, cell wall fragments, and viscous gels—especially in pectin-rich tissues—causing batch variability and membrane fouling.[19] Enzyme-assisted extraction (using cellulases/pectinases) partially digests the cell wall, facilitating controlled vesicle release with less contamination.[19] Yet excessive digestion risks intracellular leakage, and residual enzymes require additional purification.[19] Currently, no gold-standard method exists, and protocol heterogeneity hinders cross-study comparability; therefore, standardized reporting across different extraction methods is urgently needed.

Comprehensive characterization of PEVs requires the integration of multiple techniques: Nanoparticle Tracking Analysis (NTA)[20] and Dynamic Light Scattering (DLS)[21] are used to determine particle size distribution and concentration; Transmission Electron Microscopy (TEM)[22] or Cryo-Electron Microscopy (Cryo-EM)[23] are employed to observe their typical cup-shaped or spherical morphology; Proteomics, lipidomics, and small RNA sequencing are utilized to decipher their complex molecular cargo[24] (Figure 1).

The core competitiveness of PEVs stems from their inherent biological properties:

(1) Excellent Biocompatibility and Physical Stability: Their lipid bilayer membrane is rich in plant-specific sphingolipids and phosphatidic acids, which not only confer good membrane stability but also enable effective fusion with mammalian cell membranes. Research by Sitong Zhang et al indicates that PEVs can survive the harsh degradative conditions of the gastrointestinal tract and reach the intestines. This unique functionality positions them as promising prebiotics in the health sector and as oral nanomedicines for treating intestinal diseases like inflammatory bowel disease.[14]

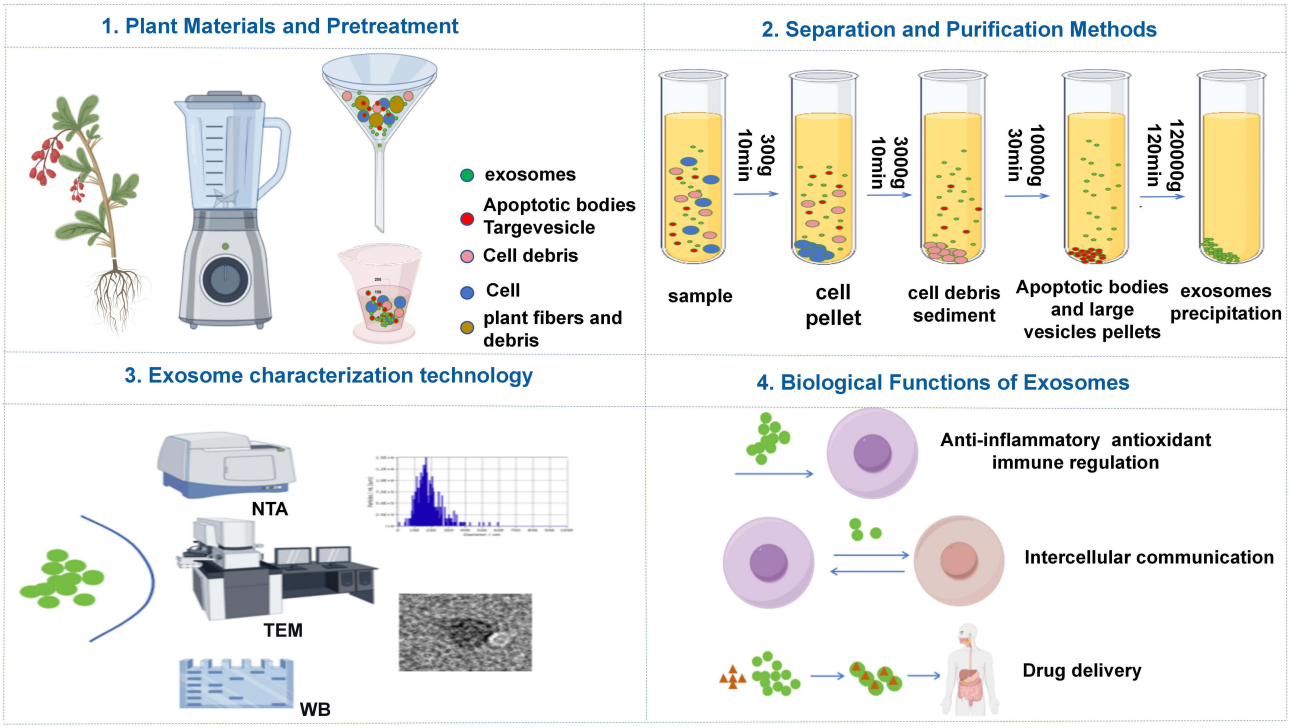

Figure 1 Schematic diagram illustrating the isolation, identification, and functional applications of plant-derived extracellular vesicles (PEVs). This figure consists of four key modules: (1) The natural sources and initial extraction workflow of plant exosomes; (2) Detailed purification procedures for PEVs using ultracentrifugation; (3) Comprehensive identification and characterization of PEVs via multiple techniques, including transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), and Western blotting (WB) for specific plant-derived marker proteins; (4) The core biological functions and therapeutic applications of PEVs, such as drug delivery, intercellular communication, targeted therapy, and biological regulation in cardiopulmonary diseases.

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(2) Intrinsic Pleiotropic Therapeutic Activity: PEVs are natural nanocapsules of plant bioactive components. Research by Stefania Raimondo et al found that lemon-derived extracellular vesicles contain flavonoids, limonoids, lipids, etc, which themselves possess potent anti-inflammatory (via inhibiting NF-κB and MAPK pathways) and antioxidant (via activating the Nrf2 pathway) capabilities.[25] More strikingly, CPA-MIR166E and ZMA-MIR166H-3P present in lavender exosome-like nanoparticles participate in DNA repair, oxidative stress response, and collagen synthesis pathways. They can prevent and treat UVB-induced skin photoaging, highlighting the potential of PEVs in dermatological applications.[24]

(3) Low Immunogenicity and Targeting Propensity: A significant advantage over animal-derived EVs is that PEVs do not carry human or animal pathogens, viruses, or MHC antigens, fundamentally avoiding such risks (as shown in Table 1). Studies in mice have found that after oral gavage of ginger-derived EVs (0.3 mg/mouse) for 7 days, the levels of pro-inflammatory cytokines remained largely stable, with no observed morphological or pathological changes;[26] after oral administration of grapefruit-derived EVs (10 mg/kg), no changes were detected in serum IFN-γ levels, liver enzymes, or AST/ALT ratios.[27] Interestingly, some PEVs exhibit natural targeting tendencies. For instance, lemon-derived PEVs are more readily taken up by intestinal cells,[28] while ginseng-derived PEVs show an affinity for macrophages.[29]

Engineering Modifications: From Natural Products to Intelligent Carriers

To overcome the limitations of natural PEVs in terms of drug loading capacity, targeting precision, and controllable therapeutic efficacy, a series of engineering modification strategies have been developed (Figure 2), aiming to transform them into "intelligent" precision medicine tools.

(i) Lumen Loading Strategies: Efficient encapsulation of therapeutic cargo is key. Passive incubation is simple but relatively inefficient.[30] Saponin-assisted permeabilization primarily uses permeabilizing agents like saponins, which form complexes with and deplete membrane cholesterol, creating pores in the membrane of PDNVs to increase membrane permeability and enhance the loading rate of small-molecule drugs (eg, curcumin, paclitaxel).[31] Electroporation is a straightforward loading technique. Its principle involves applying an electric field to a mixture of therapeutic agents and PDNVs, thereby creating transient pores in the lipid bilayer of the PDNVs.[32] For example, electroporation at 400 V (125 μF capacitance, two pulses) has been successfully employed to load exogenous dsRNA into citrus-derived exosome-like nanoparticles.[33] The optimal voltage depends on the specific electroporator system, cuvette gap distance, and sample composition, and the applied electric field strength (kV/cm) is a more critical parameter for determining membrane permeabilization.

From a strategic perspective, these lumen loading techniques can be categorized into passive and active approaches, each with distinct advantages and limitations. Passive loading (eg, direct co-incubation) relies on simple diffusion

Table 1 Characteristics Comparison Between PEVs and Animal/Human-Derived EVs

Feature	PEVs	EVs
Source & Cost	Plant biomass (fruits, leaves, etc.); abundant sources, extremely low cost, sustainable.	Cell culture supernatants or bodily fluids; expensive raw materials, significant challenges for large-scale production.
Immunogenicity Risk	Very low; no risk of animal-derived pathogen contamination; high potential for cross-species application.	Risk of homologous or heterologous immune reactions; donor screening is required.
Intrinsic Active Cargo	Plant-specific secondary metabolites (polyphenols, flavonoids), miRNAs, antioxidant enzymes.	Parent cell-specific proteome, miRNAs, signaling lipids.
Large-Scale Production	Easily extracted in large scale from agricultural waste, aligns with green production concepts.	Relies on cell expansion; process is complex, time-consuming, and costly.
Engineering Modification	Unique surface chemical properties; modification strategies are under exploration with great potential.	Well-defined surface proteins; mature modification strategies based on genetic engineering and chemistry.
Regulatory Pathway	As novel biologics or food-derived products, the regulatory framework is still being established.	Relatively mature regulatory experience from cell therapy products can serve as a reference.

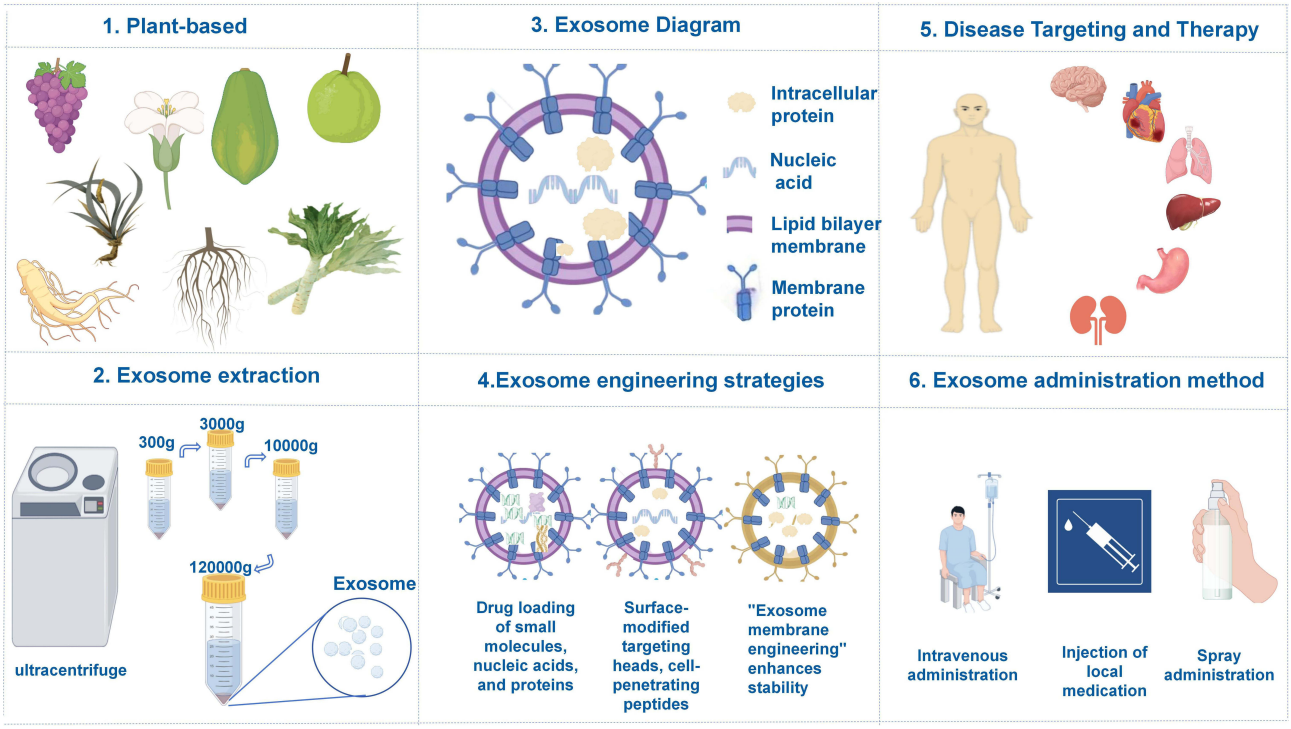

Figure 2 Schematic diagram of the sourcing, extraction, structure, engineering, targeted delivery, and administration routes of plant-derived extracellular vesicles (PEVs). This schematic comprises six integrated modules: (1) Common plant sources for PEV isolation, including fruits, vegetables, and medicinal plants; (2) Standardized extraction and purification procedures of plant-derived extracellular vesicles; (3) Detailed structural components of PEVs, including the lipid bilayer membrane, membrane proteins, and bioactive intraluminal cargos; (4) Engineering modification strategies of PEVs, such as drug loading, protein encapsulation, and nucleic acid delivery; (5) Targeted organ therapy enabled by engineered PEVs, with a focus on cardiopulmonary systems; (6) Diverse administration routes for PEVs, including intravenous injection and nebulized inhalation.

without disrupting the vesicle membrane; it is simple and preserves membrane integrity but suffers from low efficiency and strong dependence on drug hydrophobicity.[34,35] Active loading (eg, sonication, electroporation, extrusion, saponin-assisted permeabilization) physically or chemically permeabilizes the PEV membrane to achieve higher loading efficiency and broader cargo applicability (hydrophilic drugs, nucleic acids, proteins). However, active methods may compromise membrane integrity, cause vesicle aggregation, or introduce cytotoxicity (eg, saponin), thereby requiring careful optimization and post-loading purification.[34,36,37] A comparative summary of pros/cons for each method is provided in Table 2.

Table 2 Comparison of Passive and Active Loading Strategies for Engineering PEVs

Loading Strategy	Method	Pros	Cons
Passive	Direct Incubation	Simple, low cost, preserves membrane integrity, no special equipment	Very low efficiency, depends on drug hydrophobicity
Active	Sonication	High efficiency, good for hydrophobic drugs	May cause exosome aggregation, affects surface proteins
Active	Electroporation	Suitable for nucleic acids (siRNA/miRNA), widely used	Risk of RNA precipitation, vesicle aggregation, requires optimization
Active	Extrusion	High efficiency, uniform vesicle size	Mechanical force may alter membrane properties
Active	Saponin-assisted	High efficiency, preserves morphology	Saponin is difficult to remove, potential cytotoxicity and hemolysis
Active	Freeze-thaw	Simple, mild conditions	Low to moderate efficiency, induces aggregation

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(ii) Membrane Surface Engineering: Functional molecules can be displayed on the PEV membrane through methods such as hydrophobic insertion, chemical conjugation, or genetic engineering of the parent plant cells. For example, conjugating the c(RGDyK) cyclic peptide can confer the ability to target the αvβ3 integrin, which is highly expressed on activated endothelial cells or tumor cells.[38] Drawing inspiration from research on animal-derived EVs, future prospects also include modifying PEVs with ligands like single-chain variable fragments (scFv) targeting specific immune cell subsets to achieve more precise immune modulation.[39] Additionally, conjugating hydrophilic polymers like polyethylene glycol (PEG) is a common strategy for prolonging their in vivo circulation half-life.

(iii) Membrane Biomimetics and Hybridization: To integrate the biological functions of natural vesicles with the design flexibility of synthetic carriers, membrane biomimetic and hybridization strategies have emerged. Using techniques like electroporation, freeze-thaw cycles, or spontaneous membrane fusion, PEVs can be fused with synthetic liposomes, lipid nanoparticles (eg, cubosomes), or other cell membranes to construct hybrid vesicles with customized functionalities. This strategy can effectively remove potentially unnecessary endogenous substances from PEVs while inheriting their natural homing and barrier-penetrating abilities.[39–41] Furthermore, by regulating the ratio and properties of the fusion components, precise control over the hybrid vesicle's drug-loading capacity, targeting specificity, and even in vivo metabolic behavior can be achieved, thereby creating advanced delivery systems with performance surpassing that of any single component.

Comparative Composition and Biological Impacts of PEVs from Different Plant Sources

PEVs derived from different plant sources—including fruits, vegetables, and medicinal plants—exhibit substantial compositional heterogeneity, which directly influences their biological activities and therapeutic potential.

Fruit-derived PEVs (eg, grapefruit, lemon, fig, blueberry) are typically rich in flavonoids, limonoids, and specific lipid species such as phosphatidylcholine. Lemon-derived PEVs inhibit ERK/NF-κB signaling and reduce pro-inflammatory cytokine release,[25] while grapefruit-derived PEVs exhibit strong intestinal macrophage-targeting capability and have been developed as oral delivery vehicles for anti-inflammatory drugs.[27] Fruit PEVs generally demonstrate excellent gastrointestinal stability and are preferentially suited for oral administration targeting intestinal and systemic inflammatory conditions.[14]

Vegetable-derived PEVs (eg, ginger, garlic, celery, broccoli) display distinct lipid and protein profiles, with high levels of phosphatidic acid and specific plant miRNAs. Ginger-derived PEVs can modulate gut microbiota and deliver nucleic acid therapeutics across the intestinal epithelium.[26] Garlic-derived PEVs have demonstrated anti-cancer effects via caspase-mediated apoptosis.[42] Celery-derived PEVs loaded with doxorubicin showed reduced cardiotoxicity and efficient tumor targeting.[43] Vegetable PEVs often exhibit intrinsic antiviral and metabolic regulatory activities, positioning them as promising carriers for oral RNA therapeutics.[44]

Medicinal plant-derived PEVs (eg, ginseng, tea, turmeric) are characterized by complex secondary metabolite profiles (ginsenosides, catechins, curcuminoids) alongside unique miRNA and protein cargo. Ginseng-derived PEVs reprogram tumor-associated macrophages from M2 to M1 phenotype, enhancing anti-tumor immunity.[29] Some medicinal plant PEVs have shown context-dependent dual effects, such as the antioxidant/pro-oxidant switching depending on the tumor microenvironment.[45] However, caution is warranted: certain medicinal plant PEVs, when administered intravenously, may exhibit complement activation or hepatotoxicity, highlighting the need for source-specific safety evaluation.[13]

In general, fruit-derived PEVs are favored for anti-inflammatory and antioxidant applications via oral routes; vegetable-derived PEVs excel in gut-targeted delivery, antiviral, and metabolic regulation; medicinal plant-derived PEVs offer superior anti-cancer and immunomodulatory activities but require more rigorous safety assessments. The choice of plant source should be guided by the intended disease target, administration route, and required bioactivity profile.

Storage Stability of PEVs: Current Knowledge and Challenges

The successful clinical translation and industrial application of PEVs critically depend on understanding and optimizing their storage stability. Emerging evidence indicates that PEVs exhibit source-dependent stability profiles under different storage conditions, including temperature, freeze-thaw cycles, and buffer/preservative composition.

Storage temperature is a primary determinant. For blueberry-derived EVs, short-term storage at 4 °C and long-term storage at −80 °C are recommended to maintain particle size, morphology, and bioactivity.[46] Leaf-derived EVs from Dendropanax morbifera stored at −20 °C and 4 °C for 4 weeks retained better size distribution and protein content compared to storage at 25 °C or 45 °C, where rapid degradation occurred.[47] However, temperature alone may not be sufficient. A study on Rehmannia-derived nanovesicles (RDNVs) found that after 2 months, significant aggregation occurred at all tested temperatures (4 °C, −20 °C, −80 °C). Notably, rapid freezing in liquid nitrogen prior to −80 °C storage better preserved the anti-migratory activity of RDNVs compared to direct −80 °C storage, suggesting that the freezing rate influences functional preservation.[48] A systematic review concluded that −80 °C is the most reliable temperature for long-term EV preservation, while 4 °C is suitable for short-term storage (up to 1 week).[49]

Repeated freeze-thaw cycles adversely affect PEV integrity. Multiple freeze-thaw cycles have been shown to increase particle size, promote aggregation, reduce protein and RNA content, and impair cellular uptake.[47,49] For RDNVs, even a single freeze-thaw cycle led to measurable changes in particle size and zeta potential, with progressive damage upon repeated cycles.[48] Therefore, preparing single-use aliquots to avoid repeated freezing is strongly recommended.

Buffer composition and cryoprotectants play a crucial role in preserving PEV stability. The addition of preservatives can significantly improve storage outcomes. Kim et al found that leaf-derived EVs formulated with TMO (a preservative blend containing Illicium verum extract, caprylyl glycol, and 1,2-hexanediol) exhibited higher stability at 4 °C compared to EVs stored without preservatives or with 1,3-butylene glycol.[47] Trehalose is widely recognized as an effective cryoprotectant that prevents vesicle aggregation and maintains cargo integrity during freezing and lyophilization.[50,51] Cloudberry-derived nanovesicles (CNVs) maintained structural integrity and functional properties after exposure to simulated gastrointestinal conditions, demonstrating the importance of intrinsic stability for oral delivery.[52] Furthermore, storage of EVs in native biofluids (eg, plasma, urine, milk) often yields better preservation compared to storage in purified PBS buffers, likely due to the protective effects of natural proteins and other biomolecules.[49]

In summary, current evidence recommends: (i) short-term storage at 4 °C and long-term storage at −80 °C, with rapid freezing preferred; (ii) minimizing freeze-thaw cycles by using single-use aliquots; (iii) supplementing storage buffers with cryoprotectants such as trehalose or preservatives like TMO to enhance stability. However, storage stability is highly source-dependent, and standardized protocols for PEV preservation remain an urgent unmet need. Although the above storage conditions were primarily discussed in the context of PEVs, similar considerations regarding stability, activity, and delivery efficiency apply to other EV-based systems, such as hydrogel-exosome platforms recently developed for diabetic wound healing.[53]

Distinction Between Cargo Protection and Enhanced Cellular Uptake

PEVs serve two distinct yet complementary functions in drug delivery: (i) protecting their payload from external degradation, and (ii) enhancing the intracellular delivery of that payload into target cells.

Cargo protection refers to the ability of the PEV lipid bilayer to shield encapsulated molecules from environmental stressors such as nucleases, acidic pH, and digestive enzymes. For example, ginger-derived PEVs (GELNs) loaded with siRNA remained functionally intact after exposure to RNase and simulated gastrointestinal fluids, whereas free siRNA was rapidly degraded.[26] Similarly, grapefruit-derived PEVs protected methotrexate (MTX) from acidic degradation, enabling effective oral delivery to the colon.[27] These examples demonstrate that the PEV membrane acts as a physical barrier, preserving cargo integrity.

Enhanced cellular uptake refers to the ability of PEVs to facilitate internalization of their cargo into target cells through specific endocytic pathways, thereby increasing intracellular bioavailability. Lemon-derived HRED nanodrugs entered doxorubicin-resistant cancer cells via caveolin-mediated endocytosis, macropinocytosis, and clathrin-mediated endocytosis, leading to >10-fold higher cellular uptake of DOX compared to free drug.[41] Ginger-derived PEVs were

preferentially internalized by intestinal epithelial cells and macrophages via lipid raft-dependent endocytosis.[26] Grapefruit-derived PEVs delivered siRNA to macrophages with high efficiency, achieving significant target gene silencing.[27] Thus, successful PEV-based delivery systems must optimize both cargo protection and cellular uptake.

Mechanisms and Applications of PEVs in Cardiopulmonary Disease Treatment

PEVs intervene in the complex pathological networks of cardiopulmonary diseases through multiple synergistic mechanisms. Their core value lies in serving as a programmable "multi-effect nanotherapeutic platform".

Cardiovascular Disease Treatment: From Plaque Stabilization to Myocardial Regeneration

As illustrated in Figure 3, engineered PEVs exert interventional effects at multiple pathological stages of CVDs through the following integrated mechanisms:

(1) Anti-Inflammation and Endothelial Function Repair:

Beyond their cargo of flavonoids which can directly promote endothelial nitric oxide synthase (eNOS) activation and increase nitric oxide (NO) production to improve vasodilation, the cross-kingdom regulatory role of plant-derived functional small RNAs (eg, miRNAs) encapsulated within PEVs is particularly crucial.[54] During the initiation of atherosclerosis, activated endothelial cells are the focal point of inflammation. Studies show that PEVs can significantly reduce monocyte adhesion and recruitment to the vascular wall by delivering their endogenous miRNAs. More importantly, the protective effects of PEVs are not limited to endothelial cells. For example, miR164a/b-5p, which is

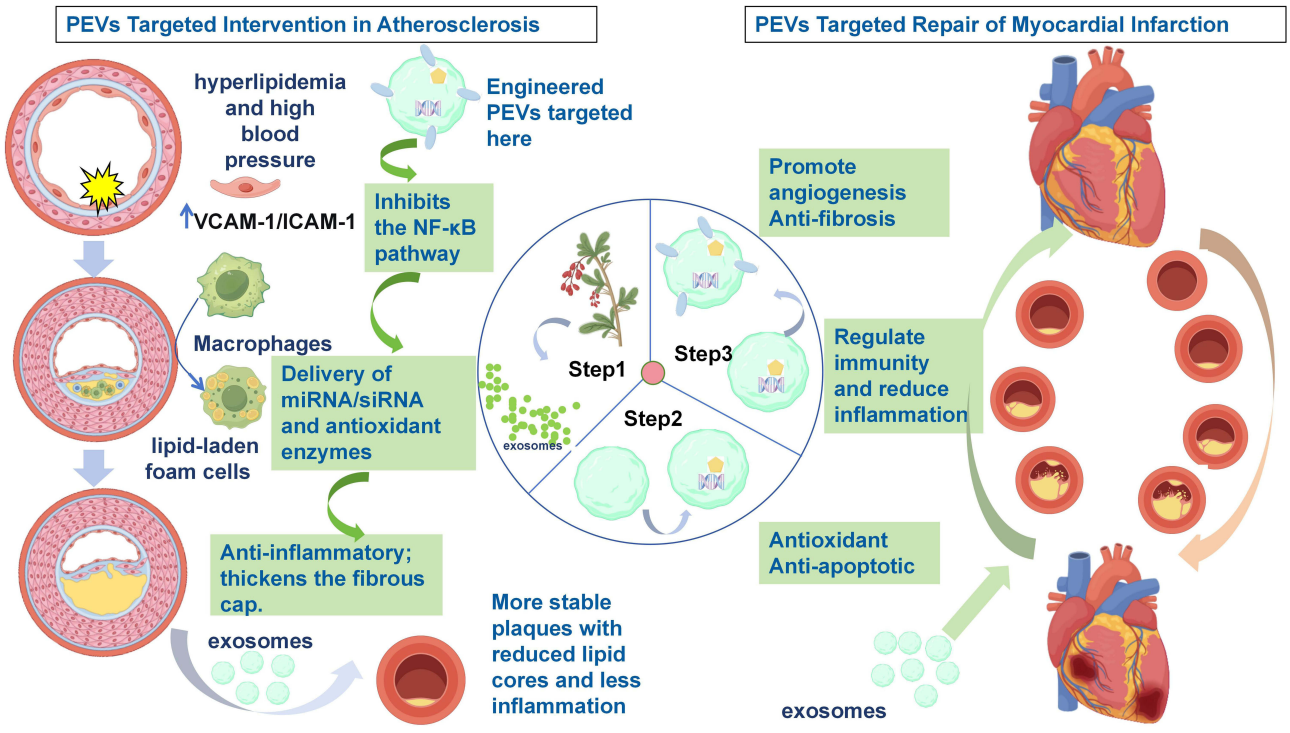

Figure 3 Schematic diagram illustrating the therapeutic mechanisms of plant-derived extracellular vesicles (PEVs) in atherosclerosis and myocardial infarction. This figure depicts the dual therapeutic effects of PEVs in two major cardiovascular disorders: (1) Atherosclerosis intervention: Under hyperlipidemia and hypertension, endothelial injury upregulates VCAM-1/ICAM-1, triggering macrophage recruitment, foam cell formation, and plaque progression. Engineered PEVs inhibit the NF-κB pathway and deliver therapeutic cargos (miRNAs/siRNAs, antioxidant enzymes) to macrophages, reducing inflammation, limiting lipid accumulation, thickening the fibrous cap, and promoting stable plaque formation. (2) Myocardial infarction repair: PEVs exert multi-modal cardioprotective effects, including promoting angiogenesis, reducing fibrosis, regulating immunity/inflammation, and providing antioxidant/anti-apoptotic support to ischemic cardiomyocytes, thereby mitigating damage and improving cardiac remodeling. The central workflow illustrates the general pipeline for PEV-based therapy, including plant sourcing, purification, and engineering modification to enhance efficacy. Graphical symbols are defined as follows: upward arrows indicate increased expression or upregulation of molecules/pathways; inhibitory arrows indicate suppression of signaling pathways.

enriched in exosome-like nanoparticles (SL-ELNs) from *Solanaceae* plants, can be taken up by vascular smooth muscle cells (VSMCs). This miRNA promotes nuclear translocation of Nrf2 by reducing the mRNA level of Keap1, thereby activating the expression of a series of antioxidant genes, effectively alleviating cellular oxidative stress, and consequently inhibiting the proliferation, migration, and phenotypic switching of pathological VSMCs.[44] This indicates that by delivering specific miRNAs, PEVs can simultaneously target endothelial cells (inhibiting inflammation) and smooth muscle cells (inhibiting proliferation/migration) within atherosclerotic lesions, achieving synergistic regulation across multiple cellular components to maintain vascular homeostasis and delay disease progression.

(2) Antioxidant Effects and Plaque Stabilization:

Oxidative stress permeates the entire course of CVDs. PEVs are not only inherently rich in antioxidants like vitamins C and E but also serve as natural protective carriers for protein enzymes such as catalase (CAT) and superoxide dismutase (SOD). In atherosclerosis models, CAT-loaded PEVs have been reported to accumulate within plaques and scavenge the large amounts of hydrogen peroxide (H_2O_2) produced by macrophages and foam cells, inhibit lipid peroxidation, and reduce the release of pro-inflammatory factors. Notably, the antioxidant mechanisms of PEVs extend far beyond delivering exogenous antioxidants. Recent research reveals that PEVs such as apple-derived nanovesicles (ADNVs) can interact with mammalian cells to trigger intracellular calcium (Ca^{2+}) signaling, subsequently activating downstream endogenous antioxidant defense pathways and remodeling the cell's own antioxidant status.[55] The diverse plant-specific active components they carry (eg, specific miRNAs, plant proteins with antioxidant potential, and abundant bioactive lipids) may play synergistic roles in this process. This indirect antioxidant strategy, achieved by modulating cellular second messenger systems, provides a new explanatory dimension for PEVs' antioxidant function. Additionally, PEVs can enhance plaque stability by downregulating the expression of matrix metalloproteinase-9 (MMP-9) in plaque macrophages, thereby increasing collagen content in the fibrous cap.

(3) Anti-Apoptosis and Promotion of Angiogenesis:

Myocardial ischemia/reperfusion injury leads to massive cardiomyocyte apoptosis and tissue necrosis, requiring both cytoprotection and vascular reconstruction for effective repair. PEVs demonstrate potential advantages as a multifunctional platform in this process. On one hand, they serve as highly efficient and biocompatible carriers, overcoming the delivery challenges of traditional phytochemicals (eg, flavonoids, terpenoids) related to hydrophobicity and instability. On the other hand, they are also ideal delivery systems for nucleic acid therapeutics. During the repair phase, PEVs loaded with vascular endothelial growth factor (VEGF) mRNA can be taken up by cardiac-resident progenitor cells and endothelial cells, leading to efficient intracellular translation of functional protein, thereby promoting capillary neovascularization in the infarct border zone, and improving perfusion and myocardial remodeling. Notably, the functions of PEVs extend beyond being mere "delivery vehicles." Recent studies show that certain PEVs themselves contain photosensitive phytochemicals (eg, hypericin) capable of generating reactive oxygen species (ROS) *in situ* under specific conditions to modulate the microenvironment for adjuvant therapy.[56] Furthermore, their endogenous plant miRNAs and proteomes have been shown to cross-kingdom regulate mammalian cell signaling pathways, directly participating in processes like anti-apoptosis and pro-proliferation.[57] This suggests that PEVs can synergistically deliver exogenous therapeutics while exerting endogenous bioactivity, achieving multi-mechanism synergistic therapeutic enhancement through an integrated "carrier-drug" strategy in scenarios involving anti-apoptosis, promotion of angiogenesis, and broader tissue repair.

(4) Inhibition of Myocardial Fibrosis:

The excessive activation and aberrant transformation of cardiac fibroblasts are central processes leading to ventricular stiffness and functional deterioration during heart failure progression. PDEs offer a nano-strategy with dual advantages for intervening in this pathological process. On one hand, PDEs can serve as precisely engineered delivery vehicles. For instance, surface modification can confer them with properties targeting activated fibroblasts. Such "precision-guided" systems enable the targeted delivery of gene-silencing drugs, efficiently blocking downstream phosphorylation and nuclear translocation, thereby inhibiting the transformation of fibroblasts into hyper-secretory myofibroblasts at the source and reducing excessive deposition of type I and III collagen. On the other hand, unlike purely synthetic carriers, PDEs themselves encapsulate a rich array of plant-derived bioactive molecules such as lipids, proteins, and microRNAs. These endogenous components may inherently possess auxiliary anti-fibrotic potential, such as regulating extracellular

matrix metabolism, anti-inflammatory, or antioxidant effects.[58] Therefore, PDEs-based delivery systems merge the precision of exogenous genetic regulation with the synergy of endogenous bioactivity, collectively delaying or even reversing pathological myocardial remodeling, suggesting their potential value and application prospects in the precision treatment of cardiac fibrosis.

Beyond their well-documented antioxidant activity, emerging evidence suggests that the redox-modulating effects of PEVs are context-dependent. A recent conceptual framework proposes a "dualistic yin-yang" model in which PEVs can switch between antioxidant and pro-oxidant functions depending on the pathological microenvironment.[45] Specifically, in non-tumor diseases such as inflammation, ischemia, and degenerative disorders, PEVs exert antioxidant effects by scavenging ROS, activating the Nrf2/Keap1 pathway, and disrupting inflammatory-oxidative feedback loops. In contrast, in the context of cancer, certain PEVs (eg, from tea or bitter melon) can induce mitochondrial depolarization and ROS overload, leading to DNA damage and tumor cell apoptosis—an effect that can be therapeutically harnessed.[45] This duality underscores that PEVs are not inherently beneficial antioxidants but intelligent redox regulators whose net effect depends on recipient cell status. While such pro-oxidant actions have not been directly studied in CVDs, they caution against overgeneralizing PEVs as universally protective and highlight the need for disease- and context-specific evaluation.

Respiratory Disease Treatment: Nebulized Delivery and Pulmonary Microenvironment Modulation

As shown in Figure 4, nebulized inhalation provides an ideal route for achieving local high-concentration delivery of PEVs to the lungs with low systemic exposure. Their role in diseases such as chronic obstructive pulmonary disease (COPD), asthma, and pulmonary fibrosis is reflected in the precise remodeling of the alveolar microenvironment:

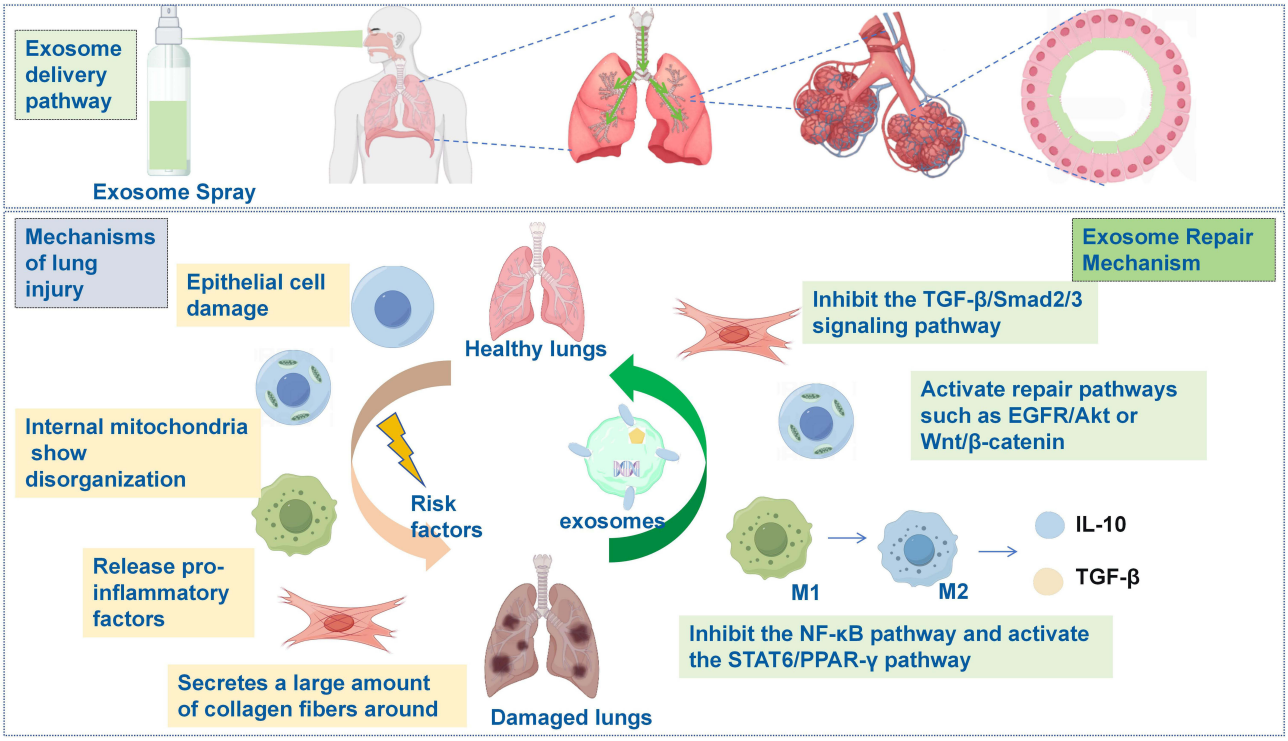

Figure 4 Schematic diagram illustrating the therapeutic mechanisms of inhaled plant-derived extracellular vesicles (PEVs) in pulmonary diseases. This figure depicts the delivery pathway and multi-modal repair mechanisms of PEVs in the lung: (1) Inhalation delivery: PEVs are administered via nebulized spray, enabling direct delivery to the respiratory tract, lung parenchyma, and alveolar epithelium for targeted therapy. (2) Lung injury mechanisms: Risk factors trigger pathological changes including epithelial cell damage, mitochondrial disorganization, pro-inflammatory factor release, and excessive collagen deposition, leading to damaged lungs. (3) PEV-mediated repair mechanisms: PEVs exert protective effects by: inhibiting the TGF-β/Smad2/3 pathway to reduce fibrosis; activating repair pathways (eg, EGFR/Akt, Wnt/β-catenin) to restore epithelial integrity; and regulating macrophage polarization from pro-inflammatory M1 to anti-inflammatory M2 phenotypes via NF-κB inhibition and STAT6/PPAR-γ activation, thereby alleviating inflammation and promoting lung repair.

(1) Reprogramming Alveolar Macrophages:

Alveolar macrophages are central to pulmonary immune defense and inflammatory regulation. PEVs can be effectively endocytosed by them and reprogram their functions multidimensionally through the delivery of bioactive substances. As discussed in Section 3.1(1), PEVs inhibit NF-κB and MAPK signaling to exert anti-inflammatory effects. In the pulmonary context, this translates into the reprogramming of alveolar macrophages. Specifically, gingerols and specific miRNAs within PEVs drive macrophages toward an anti-inflammatory, pro-repair M2 phenotype, upregulating IL-10 and downregulating TNF-α, thereby alleviating airway inflammation. Notably, the regulation of macrophages by PEVs reveals a more complex cross-kingdom synergistic mechanism in antiviral defense. Recent studies show that specific plant miRNAs (eg, miR858a/b, miR166a-3p) enriched in exosome-like nanoparticles from plants like *Houttuynia cordata* (HELNs) can, after uptake by macrophages, simultaneously target viral genomes (eg, the NP gene of influenza virus, the ORF1ab gene of SARS-CoV-2) to inhibit viral replication and regulate host cell signaling pathways (eg, suppressing MAPK3, AKT1 expression via miRNAs like miR168a-3p).[43] This "killing two birds with one stone" mechanism allows PEVs to both mitigate virus-induced excessive inflammation (cytokine storm) by regulating pathways like NF-κB and MAPK and directly interfere with the viral life cycle. Therefore, by reshaping the phenotype and function of alveolar macrophages, PEVs play a key immunomodulatory role in both chronic inflammatory lung diseases and acute viral respiratory infections, suggesting potential for further exploration as natural nano-immunomodulators.

(2) Repairing the Alveolar Epithelial Barrier:

The integrity of the alveolar epithelial barrier is fundamental for maintaining lung homeostasis and gas exchange function. In various acute and chronic lung diseases, including viral infections like COVID-19, alveolar epithelial cells (particularly type II cells, AECII) often suffer damage and apoptosis, leading to loss of barrier function. On one hand, PEVs can enable direct repair and regeneration of the epithelium. On the other hand, in the context of viral infection, the reparative effects of PEVs closely synergize with antiviral and anti-inflammatory mechanisms. Research indicates that in SARS-CoV-2 infection, viral proteins (eg, Nsp12, Nsp13) can be delivered to macrophages via exosomes released by infected host lung epithelial cells, activating the NF-κB pathway and triggering a storm of inflammatory factors like TNF-α, IL-6, IL-1β, which in turn exacerbates epithelial cell apoptosis and barrier damage. Plant miRNAs carried by ginger-derived exosome-like nanoparticles (GELNs), such as aly-miR396a-5p, can both directly reduce viral replication and cytopathic effects by inhibiting the expression of these viral proteins and downregulate the resulting excessive inflammation, thereby indirectly protecting alveolar epithelial cells from apoptosis and creating a favorable microenvironment for epithelial repair.[42] Thus, PEVs not only promote epithelial regeneration by directly delivering reparative components but also, in specific pathological states like viral infection, block the damage cascade through combined antiviral and anti-inflammatory actions, synergistically achieving structural and functional restoration of the alveolar epithelial barrier.

(3) Direct Intervention in the Fibrotic Process:

The pathological core of diseases like idiopathic pulmonary fibrosis (IPF) lies in the excessive activation of myofibroblasts and pathological deposition of extracellular matrix. PEVs offer a dual-advantage strategy combining precise targeting and biocompatibility for intervening in this process. On one hand, through engineering to display antibodies targeting fibroblast activation protein (FAP) on their surface, they can precisely deliver small-molecule antifibrotic drugs (eg, nintedanib) or siRNA targeting CTGF (connective tissue growth factor) to the lesion site, effectively inhibiting fibroblast proliferation, migration, and collagen synthesis by suppressing the TGF-β/Smad pathway. On the other hand, the unique value of PEVs lies in their nature as "natural nanocarriers." Emerging perspectives suggest PEVs should not be viewed merely as passive delivery vehicles for exogenous drugs; they themselves are important dietary bioactive entities. They naturally encapsulate various phytochemicals (eg, polyphenols, lipids, miRNAs), and these endogenous components may inherently possess the potential to regulate tissue renewal, inhibit abnormal activation, or alleviate local inflammation.[59] Therefore, a prospective antifibrotic strategy is: utilizing PEVs derived from specific medicinal plants, where the synergistic action of their endogenous active components and targeted delivery capability, or their use as "green" carriers loaded with high concentrations of natural antifibrotic compounds, enables

"dual natural intervention" at fibrotic foci, may offer new possibilities for developing highly effective and low-toxicity antifibrotic therapies.

(4) Immunomodulation and Antioxidant Defense:

PEVs exhibit multi-target immunomodulatory effects in the lung beyond macrophage reprogramming. For instance, PEVs can induce dendritic cells (DCs) toward a tolerogenic phenotype, promoting regulatory T cell (Treg) expansion and activation, which suppresses type 2 immune responses and alleviates airway hyperresponsiveness in asthma models.[60]

The antioxidant mechanisms of PEVs in the respiratory system are consistent with those detailed in Section 3.1(2) (Nrf2/Keap1 pathway activation, direct ROS scavenging by GPx/SOD, and calcium-signaling-mediated endogenous defense). Therefore, rather than reiterating these molecular pathways, we focus here on their functional integration with pulmonary immunomodulation: by simultaneously reducing oxidative stress and promoting an anti-inflammatory microenvironment (eg, via M2 macrophage polarization and Treg expansion), PEVs help maintain alveolar redox balance and immune homeostasis. This dual action supports their potential application in chronic inflammatory and oxidative stress-driven lung diseases such as COPD and pulmonary fibrosis.

Despite these promising findings, cross-kingdom regulation by plant miRNAs remains controversial. Huang et al fed mice with corn miRNAs or corn powder for two weeks and, using an improved detection method that eliminated false positives, found no significant increase in corn miRNA levels in blood or tissues. An in vitro digestion system further showed that corn miRNAs were extensively degraded, with less than 1% recovered after oral and gastric phases.[61] Similarly, Dickinson et al highlighted the substantial biological barriers—nuclease degradation, membrane impermeability, and rapid clearance—that limit oral bioavailability of nucleic acids, noting that limited success has been achieved despite considerable efforts.[62] These findings do not entirely rule out cross-kingdom regulation, especially when PEVs serve as protective carriers, but they caution that many positive reports may be confounded by detection artifacts or insufficient controls. Rigorously controlled, blinded, and independently replicated studies are needed before definitive conclusions can be drawn.

Challenges, Translational Strategies, and Future Perspectives

Despite encouraging preclinical results, translating PEVs to the clinic faces a series of scientific and technical challenges that must be overcome: (1) Contradictory findings and under reporting of negative evidence. Several often-overlooked negative or inconclusive findings exist in the literature: ① Cross-kingdom miRNA regulation remains controversial – orally administered corn miRNAs showed no significant increase in blood or tissues, with >99% degradation in an in vitro digestion system; ② The redox-modulating effects of PEVs are dualistic – they can switch between antioxidant and pro-oxidant functions depending on the pathological microenvironment, indicating that PEVs are not universally beneficial; (2) Production Standardization and Quality Control: Currently, there is a lack of a unified "gold standard" isolation method. The future requires establishing standardized processes based on cGMP principles, including agricultural control of the starting plant material, scalable and gentle extraction processes (eg, TFF combined with affinity purification), and a multi-parameter quality control system covering particle size, density, marker proteins/lipids, exogenous contaminants (eg, pesticide residues), and endotoxin levels. (3) In-depth Pharmacokinetic and Mechanistic Studies: There is an urgent need to systematically investigate the *in vivo* distribution, metabolic clearance pathways, and biodistribution of PEVs under different administration routes (intravenous, oral, nebulized) using technologies like live imaging and isotopic/fluorescent labeling. Simultaneously, advanced models such as CRISPR screening and organoid co-culture are needed to precisely identify the key active molecules within PEVs that exert therapeutic effects and their exact targets in human cells. (4) Re-evaluation of Engineering Efficiency and Safety: There is a need to develop loading techniques with higher biocompatibility (eg, pH gradient methods) and to identify gentle surface modification methods that do not compromise the inherent targeting of PEVs. For engineered PEVs, rigorous long-term toxicity, immunogenicity (especially with repeated dosing), and potential tumorigenicity assessments are essential. To facilitate clinical translation of PEV-based nanomedicines, we propose a roadmap (Table 3) outlining key milestones from preclinical development to clinical trials.

Looking forward, the following directions merit focused attention: developing stimulus-responsive smart PEVs that can trigger drug release in the specific pH, enzymatic, or redox environment of the lesion site; exploring PEVs as

Table 3 Proposed Roadmap for Clinical Translation of PEV-Based Nanomedicines in Cardiopulmonary Diseases

Phase	Estimated Timeline	Key Milestones	Regulatory Interactions
Preclinical (Discovery)	0–2 years	– In vitro efficacy and mechanism in cardiopulmonary cell models – In vivo proof-of-concept in animal models (atherosclerosis, MI, COPD, PF) – Preliminary safety and biodistribution	Pre-IND meeting with FDA/NMPA
Preclinical (Development)	2–4 years	– GMP-compliant production (scalable isolation, eg, TFF + SEC) – Toxicology (rodent and non-rodent), PK/PD studies – Stability and formulation development	IND submission
Clinical Phase I	4–6 years	– First-in-human (FIH): safety, tolerability, PK in healthy volunteers or mild patients – Single and multiple ascending dose (SAD/MAD)	Phase I completion report
Clinical Phase II	6–8 years	– Proof-of-concept (PoC) RCT in target cardiopulmonary patients – Optimal dose selection, biomarker assessment	Phase II meeting
Clinical Phase III	8–11 years	– Large-scale, multicenter RCT to confirm efficacy and safety – Long-term follow-up	NDA/BLA submission
Post-market (Phase IV)	Ongoing	– Pharmacovigilance, real-world evidence – Label extension for additional indications	Regulatory approval

pulmonary delivery vehicles for mRNA vaccines or CRISPR-Cas gene-editing tools for the prevention and treatment of infectious or genetic lung diseases; investigating the interaction between PEVs and the gut microbiome to explore their potential for exerting systemic immunomodulatory effects via the "gut-lung axis"; and fostering close collaboration between industry, academia, and medicine to design and conduct early-stage clinical studies evaluating the safety, tolerability, and preliminary efficacy of PEV-based therapies in patients with specific cardiopulmonary diseases. This roadmap (Table 3) provides a reference for researchers and regulatory agencies to accelerate the clinical adoption of PEV-based therapies.

Conclusion

In summary, this review highlights several key takeaways regarding PEVs for nanomedicine in cardiopulmonary diseases:

(1) PEVs represent a natural, low-immunogenicity, and scalable nanoplatform with intrinsic therapeutic activity. Unlike synthetic nanoparticles or mammalian exosomes, PEVs can be sourced from abundant plant biomass at low cost, carry endogenous bioactive molecules (eg, miRNAs, polyphenols, lipids), and exhibit excellent biocompatibility. This positions PEVs as a uniquely accessible and safe foundation for next-generation nanomedicine.

(2) Engineering strategies (cargo loading, surface modification, and hybridization) enable PEVs to serve as customizable and targeted delivery systems. By incorporating synthetic drugs, nucleic acids, or targeting ligands, engineered PEVs overcome the limitations of native vesicles in terms of drug loading capacity and precision. This versatility allows PEVs to be tailored for specific cardiopulmonary indications, from acute myocardial infarction to chronic pulmonary fibrosis.

(3) PEVs exert multi-target, context-dependent effects on key pathological processes in cardiopulmonary diseases, including inflammation, oxidative stress, apoptosis, and fibrosis. As summarized in Figures 1–4, PEVs can simultaneously regulate multiple cell types (endothelial cells, smooth muscle cells, macrophages, fibroblasts) and pathways (Nrf2, NF-κB, TGF-β/Smad), may offer a synergistic therapeutic advantage over single-target drugs. Notably, their redox-modulating effects can switch from antioxidant to pro-oxidant depending on the microenvironment, cautioning against overgeneralization.

(4) Significant challenges and unresolved controversies remain, including the lack of standardized isolation methods, the ongoing debate over cross-kingdom miRNA regulation, and insufficient long-term safety data. Acknowledging these negative and contradictory findings—such as the poor oral bioavailability of plant miRNAs and the source-dependent variability in efficacy—is essential for a balanced view. Future research must prioritize transparent reporting of both positive and negative results, rigorous independent replication, and scalable cGMP-compliant production.

Although significant hurdles remain, and despite some contradictory findings that caution against overoptimism, next-generation nanomedicine strategies based on PEVs may eventually offer safer and more effective treatment options for cardiopulmonary diseases, provided that standardization, safety, and reproducibility challenges are systematically addressed. A cautious but forward-looking perspective is warranted at this stage.

Abbreviations

PEVs, Plant-derived extracellular vesicles; CVDs, Cardiovascular diseases; COPD, Chronic obstructive pulmonary disease; SEC, Size-exclusion chromatography; TFF, Tangential flow filtration; NTA, Nanoparticle Tracking Analysis; DLS, Dynamic Light Scattering; TEM, Transmission Electron Microscopy; Cryo-EM, Cryo-Electron Microscopy; miRNA, MicroRNA; EVs, Extracellular Vesicles; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; VSMCs, vascular smooth muscle cells; CAT, catalase; SOD, superoxide dismutase; MMP-9, matrix metalloproteinase-9; VEGF, vascular endothelial growth factor; ROS, reactive oxygen species.

Data Sharing Statement

This is a review article. All data discussed or analyzed in this manuscript are included in this published article and the reference list.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

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