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Engineering Strategies of Plant-Derived Exosome-Like Nanovesicles: Current Knowledge and Future Perspectives

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Abstract: Plant-derived exosome-like nanovesicles (PELNs) from edible plants, isolated by ultracentrifugation, size exclusion chromatography or other methods, were proved to contain a variety of biologically active and therapeutically specific components. Recently, investigations in the field of PELN-based biomedicine have been conducted, which positioned those nanovesicles as promising tools for prevention and treatment of several diseases, with their natural origin potentially offering superior biocompatibility and bioavailability. However, the inadequate targeting and limited therapeutic effects constrain the utility and clinical translation of PELNs. Thus, strategies aiming at bridging the gap by engineering natural PELNs have been of great interest. Those approaches include membrane hybridization, physical and chemical surface functionalization and encapsulation of therapeutic payloads. Herein, we provide a comprehensive overview of the biogenesis and composition, isolation and purification methods and characterization of PELNs, as well as their therapeutic functions. Current knowledge on the construction strategies and biomedical application of engineered PELNs were reviewed. Additionally, future directions and perspectives in this field were discussed in order to further enrich and expand the prospects for the application of engineered PELNs.

Keywords: plant-derived exosome-like nanovesicles, nanomedicine, engineered extracellular vesicles, drug delivery systems, targeted therapy

Introduction

Extracellular vesicles derived from animal cells, especially mammal stem cells, are being extensively investigated in nanomedicine as an alternative for combating diseases like myocardial infarction and inflammation due to their superior biocompatibility and potential therapeutic benefits, and several clinical studies have been conducted.[1–5] However, low yield and high cost pose challenges for the clinical translation of animal-derived exosomes.[6] Recent researches on milk-derived extracellular vesicles, which are characterized by the advantages of low cost, high yield, biocompatibility, tissue tropism, and good uptake, have encountered significant obstacles, including considerable inconsistencies in milk collection and processing batches, harsh storage conditions, as-yet-unexplored purification methods, and allergies to specific populations.[7] In contrast, plant-derived exosome-like nanovesicles (PELNs) from edible plants have made a useful exploration in the field of exosome biomedicine.[8,9] These lipid bilayer nanovesicles with a diameter of 50–1000 nm are highly analogous to animal-derived exosomes, exhibiting negative charges that range from −25 to −15 mV.[10–12] Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) analyses have demonstrated that plant-derived exosomes exhibit a cup-shaped morphology and a homogeneous structure.[13] Despite initial potential concerns due to cross-kingdom applications, PELNs were proved to contain a variety of biologically

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active and therapeutically specific components, including proteins, lipids and nucleic acids, and exerted protective effects in several models, with a higher yield, a lower cost, relatively small differences in source batches, and no significant toxicity observed in a series of pilot studies.[14–18] These properties indicate the potential for applications in antimicrobial, anti-inflammatory, anti-tumor, intestinal homeostasis regulation, and pro-regenerative medicine.[19] Nevertheless, the immunogenicity resulting from the species gap, coupled with the inadequate targeting, renders plant-derived exosomes susceptible to a short half-life and an insignificant therapeutic impact, thereby constraining their utility in nanomedicine advancement.[5,20,21]

Therefore, strategies aiming at bridging the gap by engineering natural PELNs have been of great interest. For instance, the use of membrane hybridization, physical and chemical modifications allow the capacity for targeted delivery and improved tissue uptake, thereby enhancing efficacy; encapsulating bioactive molecules, such as protein, expression vectors, siRNA, and DNA, promotes the therapeutic effects of PELNs. Dietary components have been demonstrated to regulate the cellular metabolism and function, and a bulk of gene expression in diseases, providing a rationale for the use of natural and engineered nanovesicles derived from edible plants as a similarly beneficial green synthetic strategy for utilizing food raw materials to promote health.[22] Herein, current knowledge on the biomedical application of natural PELNs and approaches to engineer PELNs to enhance their therapeutic efficacy were reviewed.[23–29] Additionally, future directions and prospective in this field were discussed in order to further enrich and expand the prospects for the application of engineered PELNs.

Overview of PELNs

Biogenesis and Compositions of PELNs

PELNs have been derived from edible plants and traditional herbs, including strawberries, amaranth, ginseng.[30–32] Generally, it is accepted that there are at least three biogenesis pathways, namely: 1) the multivesicular bodies (MVB) pathway; 2) the exocyst–positive organelle (EXPO) pathway; and 3) the vacuolar pathway.[6] The most frequently observed pathway is the MVB pathway, in which inward budding of the endosomal membrane forms the MVB and fusion of the MVB with the plasma membrane (PM) leads to the release of exosome-like nanovesicles.[33] In contrast, the remaining two pathways represent unconventional forms of secretion.

PELNs are characterized by a diversity of internal and surface components. Despite sharing similar isolation and characterization methods, as well as comparable therapeutic effects, animal-derived exosomes and PELNs exhibit structural and compositional differences, conferring PELNs with distinctive advantages. Some of the membrane surface molecules have been instrumental in the identification and characterization of PELNs, despite the lack of clarity the specific membrane surface markers.[34] In a study conducted by Zhang et al, it was demonstrated that the proteins present in ginger-derived exosome-like nanoparticles (GELNs) using HPLC/MS are predominantly cytoplasmic in nature, comprising actin and proteolysis enzymes. Additionally, the analysis revealed the presence of several membrane channel and transporter proteins, including aquaporin and chloride channels.[35] In light of the findings of such studies, engineering techniques that induce the coupling or expression of CD47, CD55, CD59 and CD200 on the membrane surface or enrichment of MHC-I can evade recognition and clearance by the immune system, thereby reducing the immunogenicity of PELNs. Study conducted by Sushrut Kamerkar revealed that the CD47 protein, in collaboration with the Signal Regulatory Protein α (SIRPα), renders PELNs-like membrane structures less susceptible to phagocytosis by the monocyte-macrophage system. This consequently resulted in a notable reduction in the clearance rate.[36]

In addition to proteins, lipids such as phosphatidic acid (PA), phosphatidylethanolamine (PE), phosphatidylcholine (PC), digalactosyl monoacylglycerol (DGMG), digalactosyl diacylglycerol (DGDG) and monogalactosyl diacylglycerol (MGDG), and nucleic acid, including DNA, mRNA, miRNA and non-coding RNA, form the basis of PELNs engineering.[37] Given the pivotal functions of miRNAs in regulating inflammation, the intestinal barrier, tumors and infantile immunological processes, it is perhaps unsurprising that they have been the subject of the most extensive research.[38] For instance, a study on grapefruit-derived nanovectors (GNVs) demonstrated that miR-18a impeded colon cancer liver metastasis by stimulating the generation of M1 macrophages via the Interferon (IFN)-γ/ Interferon Regulatory Factor (Irf) 2 pathway, which provides a potential therapeutic approach for colon cancer treatment.[39] It is

notable that the more extensive nucleic acid components of PELNs, as well as biologically active substances, have the potential for engineering applications. The diverse range of DNA and RNA (miRNAs, microRNAs, long-chain non-coding RNAs, cyclic RNAs, and small RNAs) endow PELNs with the capacity to communicate at the cellular level and regulate the state of target cells.[40] The distinctive bioactive constituents, including vitamin C, polyphenols, flavonoids, and carotenoids, endow PELNs with their efficacious therapeutic properties.[34,41]

Isolation and Purification of PELNs

In the pretreatment stage, isolation methods can be classified into two main categories: nanovesicles that occur naturally in plants and those isolated from plant roots, leaves, and fruits through destructive means.[42] After obtaining plant juice, ultracentrifugation and sucrose density gradient centrifugation are the main techniques employed in engineered PELNs.[43,44] The initial step involved grinding the plant material into a juice and filtering it. Subsequently, centrifugation was performed at varying centrifugal forces (1000–10,000×g) for 20–60 minutes to eliminate the presence of large sediments, cellular debris, and dead cells. The resulting supernatant was then subjected to ultracentrifugation (100,000–150,000×g) for 1–2 hours. To achieve the purification of PELNs, the precipitates were then resuspended and gently spread on sucrose density gradient layers, after which they were subjected to ultracentrifugation (100,000–150,000×g) for 1–2 h.[45,46] Under the influence of centrifugal force, the components of varying densities and sizes form distinct bands in each region of the density gradient.[43] The addition of a thin layer of high-density isotonic material as a buffer layer at the bottom of the centrifuge tubes allows the avoidance of impairment to the structural integrity of the PELNs that may otherwise result from the application of high centrifugal forces, thus preventing the formation of clumps.[47]

Polyethylene glycol (PEG) precipitation method, Ultrafiltration centrifugation (UC), and Size-exclusion chromatography (SEC), on account of their distinctive characteristics, can also be subjected to further development and incorporated into the engineered nanovesicle preparation process.[10] Among these, precipitation with PEG exploits the property that PEG can competitively bind water molecules to separate PELNs from solution.[48] UC using microporous ultrafiltration membranes with specific pore sizes is used to separate nanoparticles of different sizes and dimensions; while SEC utilizes particle hydrodynamics and chromatography.[49,50] To enhance the productivity of engineered PELNs, Anagha Priya Suresh et al developed a low pH-based method to increase the yield of plant-derived nanoparticles from fresh ginger rhizomes. A polyethylene glycol (PEG6000)-based precipitation method for the isolation of ginger PELNs has recently been developed. It was demonstrated that the production of nanovesicles could be enhanced at pH levels below 7.0, specifically at pH 4 and 5.[51]

Despite the absence of standardized procedures for the isolation of PELNs, numerous studies have conducted comprehensive and valuable investigations into the merits and drawbacks of diverse methodologies (Figure 1). The current consensus in the field includes the following key perspectives: Ultracentrifugation, while straightforward and capable of high throughput, is costly, time-consuming, and potentially harmful to PELNs. Precipitation offers a simple and cost-effective approach but struggles with impurity separation, such as proteins and plant debris. Size exclusion chromatography (SEC) effectively maintains the integrity and consistency of PELNs, though it requires specialized equipment and may lead to the co-isolation of polysaccharide. Density gradient centrifugation (DGC) provides high purity and is cost-effective; however, its extended processing time and low yield make it unsuitable for scalable production. Tangential Flow Filtration (TFF) offers high purity, throughput, and structural integrity for PELNs at a scalable level, but is relatively expensive. Immunoaffinity capture enables precise separation of large, diluted samples with high purity, though it is time-consuming and costly.[52,53] The combination of separation methods has the potential to provide a solution to the aforementioned issues. For instance, recent studies have developed a gradient filtration method combined with high-speed centrifugation for the separation and purification of the PELNs. This engineered approach expands the means of producing PELNs on a large scale, as well as the source, while maintaining the therapeutic properties of PELNs, thereby enriching the prospective applications of PELNs.[54]

Characterization of PELNs

It is essential to employ a range of characterization techniques to identify the various subgroups of PELNs, as this constitutes a fundamental step in the development of PELNs-based therapeutics or drug nanocarriers. Morphological

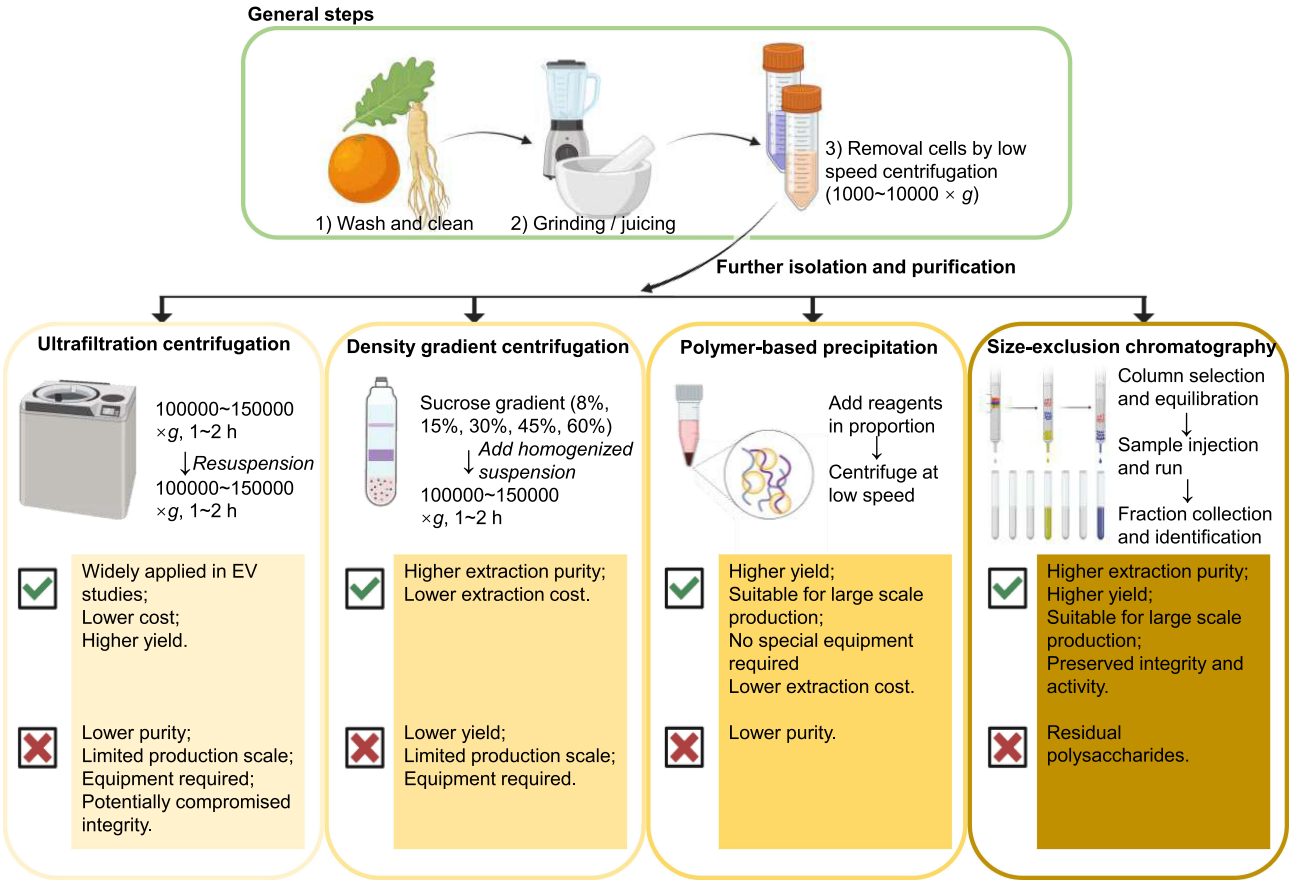

Figure 1 Widely-used isolation and purification approaches of PELNs and their advantages and limitations.

analysis of PELNs can be conducted using TEM, which is employed for ultrastructural examination of the subcellular state. Additionally, atomic force microscopy (AFM) can be utilized to elucidate the structural and dimensional characteristics of individual PELN.[23] By employing electron microscopy, Francesca Perut et al demonstrated that PELNs extracted from Fragaria exhibited a striking morphological homogeneity, with a diameter range of 30 to 191 nm. Moreover, they observed a distinct cup-shaped or rounded morphology, reminiscent of exosome-like nanovesicles derived from mammalian cells.[32] As the gold standard for the characterization of PELNs, concentration can be measured using Nanoparticle Tracking Analysis (NTA). Resistive Pulse Sensing (RPS) allows the size and concentration of PDENs in suspension to be determined without affecting the quantification of PELNs.[55] Flow cytometry represents a viable methodology for the detection of biomarkers associated with PELNs, which is capable of high-speed multi-channel analysis with low sample concentration.[56] Furthermore, the zeta analyzer can be employed to observe the repulsive nature against aggregation or dispersity and the membrane potential.[10–12] Techniques including small-angle X-ray scattering, NTA, dynamic light scattering (DLS), and tunable resistive pulse sensing have also been extensively employed for the characterization of the morphology of single nanovesicles.[57–59]

The identification of a uniform protein marker is a challenging endeavor, primarily due to the low protein content of PELNs in comparison to nanovesicles of animal origin, coupled with the significant inter-sample variability observed across different sources. Pinedo et al conducted a comprehensive analysis of surface proteins in PELNs, identifying Heat Shock Protein 70 (HSP70), Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), and S-adenosine-homocysteine lyase as the most prevalent protein families in PELNs. It is possible that these proteins may serve as surface markers for PELNs.[10] He et al demonstrated that Tetraspanin (TET) 8 co-localized with the Arabidopsis MVB marker Rab5-like GTPase, Ara6, inside the plant cells, suggesting that TET8-positive EVs are derived from MVBs and could be considered a marker of PELNs.[60,61] Exo70 and TET3 were also used for PELN characterization; however, their universality is still

worth exploring.[62] In conclusion, further research is required on the specific protein marker of engineered PELNs in order to facilitate a deeper understanding and modification of these systems.[34]

Therapeutic Potential of PELNs

PELNs have garnered significant attention due to interspecies communication and are expected to serve as potent therapeutic agents for disease treatment.[63] Compared to single-component agents, PELNs have shown enhanced pharmacological efficacy attributed to their multi-component system.[64] PELNs are characterized by their natural lipid bilayer composition, which confers superior biocompatibility and bioavailability compared to synthetic delivery systems, enabling them to effectively evade immune phagocytosis in vivo and exhibit exceptional resistance to degradation. Besides, the ability of PELNs to effectively overcoming biological barriers such as the blood-brain barrier without triggering inflammation or necrosis has been revealed.[65–67] Recent studies have elucidated the pleiotropic functions of PELNs, encompassing anti-tumor, anti-inflammation, intestinal homeostasis regulation, anti-infection and regenerative promotion functions (Figure 2).[19,68–70]

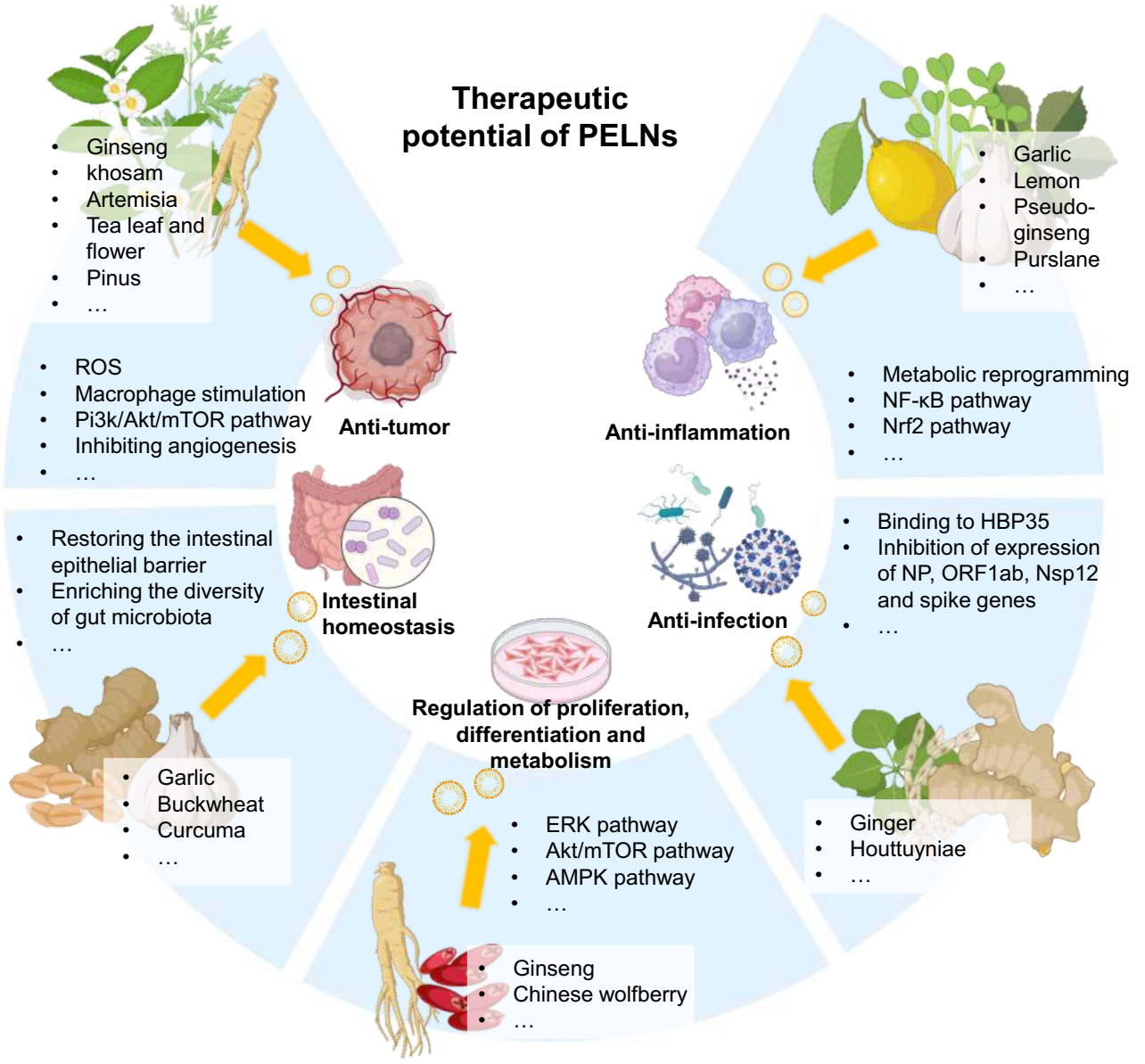

Figure 2 The sources, therapeutic potentials and underlying mechanisms of unmodified PELNs.

Anti-Tumor

PELNs have been shown to play a pivotal role in suppressing tumor growth. Mechanistic studies of PELNs derived from different plant sources suggest that PELNs not only exert direct influence on the physiological activities of tumor cells, including proliferation, apoptosis, metabolic processes, drug-resistance, but also indirectly remodel the tumor microenvironment, without significant influence on normal cells.[71,72] These multifaceted actions contribute to the mitigation of tumor progression and the reduction of its invasive characteristics.[73,74]

Chen et al discovered that tea flower-derived ELNs (TELNs) are rich in bioactive substances, which trigger oxidative stress in breast cancer cells, resulting in mitochondrial damage, cell cycle inhibition, and the subsequent cell apoptosis. Interestingly, in vivo studies have demonstrated that TELNs, intravenously or orally administrated, can effectively suppress the proliferation and metastasis of breast cancer.[71] Similarly, the level of reactive oxygen species (ROS) in breast cancer cells increased 2.5 times compared with untreated cells after 8-hour co-incubation with TELNs.[75] ELNs derived from *Raphanus sativus* L. var. *caudatus* Alef microgreens exhibit anti-proliferative effects in HCT116 colon cancer cells by inducing DNA damage, which alters the cellular biochemical compositions in the regions of nucleic acids and carbohydrates.[76] In a study by Yan et al, Brucea javanica-derived ELNs could deliver 10 functional miRNAs to 4T1 cells, effectively inhibiting breast tumor growth by targeting the phosphatidylinositol 3-kinase (PI3K) / Akt / mammalian target of rapamycin (mTOR) signaling pathway and promoting ROS/caspase-mediated apoptosis. Concurrently, they could also modulate the physiological functions of endothelial cells, thereby suppressing Vascular endothelial growth factor (VEGF)-mediated angiogenesis.[77] Immunomodulation in the tumor microenvironment (TME) are critical issues for improving cancer treatment,[78] and PELNs play a role in normalization of immune systems. Especially, the distribution and bioavailability of PELNs could be crucial for certain types of tumors. Some PELNs such as Ginseng-derived ELNs could exhibit efficient penetration through closely spaced epithelial cells and excellent targeting ability towards blood-brain barrier and glioma, promote M1 macrophage polarization within the TME, and facilitate the secretion of CCL5 and CXCL9.[8,79] This reprogramming facilitates the recruitment of CD8+ T cells into the tumor bed. The enhanced presence of these cytotoxic T cells synergizes with the PD-1 monoclonal antibody therapy, reinforcing the overall immunotherapeutic impact against cancer.

Anti-Inflammation

Inflammation is a protective mechanism initiated by external stimulation. However, an overreaction might cause acute or chronic diseases and tissue impairment. PELNs isolated from several sources are reported to modulate the expression of inflammation-related genes and ameliorate the inflammatory response.[10,37] For example, garlic ELNs are abundant in miR-396e, which exerts a significant influence on the metabolic reprogramming of macrophages by targeting 6-phosphofructo-2-kinase/fructose-2, 6-biphosphatase 3 (PFKFB3) and prevents obesity via macrophage-adipocyte cross-talk.[80] Further investigations suggest a similar role of garlic ELNs in enhancing lipid metabolism in hepatocytes through the macrophage-hepatocyte cross-talk.[81]

ELNs isolated from *Panax notoginseng* alleviate cerebral ischemia/reperfusion injury (I/R) by inducing M2 polarization of microglia, which is at least partly mediated by lipid components.[82] Portulaca oleracea-derived ELNs suppress pro-inflammatory cytokines, enhance IL-10, and ameliorate acute colitis in mice, with gut microbiota modulation potential for ulcerative colitis therapy.[31] Mechanistically, PELNs treatment alters the microbial metabolism and causes the reprogramming of conventional CD4+ T cells into double-positive CD4+CD8+ T cells. Ginger and lemon ELNs mediate the activation of nuclear factor erythroid 2-related factor 2 (Nrf2), which contributes to cytoprotective effects against peroxidation damage.[83]

Intestinal Homeostasis

The gut microbiota significantly influences physiological functions, with its dynamic balance closely tied to colonic health. Recent studies indicate that PELNs carrying miRNAs can be assimilated by intestinal bacteria, thereby altering the microbial metabolism and regulating the host's physiological processes.[84,85] The abundance and diversity of gut microbiota in Dextran Sulfate Sodium (DSS)-induced colitis mouse model were significantly improved treated by

turmeric-derived ELNs (TELNs).[69] Moreover, TELNs mitigate colitis-related symptoms by restoring the intestinal epithelial barrier, facilitating M2 macrophage polarization, and reshaping the immune microenvironment. Target gene analysis indicates that tartary buckwheat-derived ELNs can influence key genes in the physiological processes of *Escherichia coli* and *Lactobacillus rhamnosus*, which stimulate the proliferation and enrich the diversity of gut microbiota.[86] Consumption of garlic-derived ELNs (GENs) notably reduced the expression levels of toll-like receptor 4 (TLR4), myeloid differentiation primary response gene 88 (MyD88) and NF-κB, and decreased the secretion of pro-inflammatory cytokines in DSS-induced colitis. Another study has reported that Peu-miR2916-p3 enriched in GENs specifically promoted the growth of *Bacteroides thetaiotaomicron*, a beneficial intestinal bacterium known to ameliorate colitis symptoms, indicating synergistic effects exerted by the multiple components of PELNs.[87]

Regenerative Potential

The regenerative potential of PELNs has been widely investigated, encompassing wound healing, cell differentiation and tissue repair.[88,89] Ginseng ELNs can promote cell proliferation, migration, and angiogenesis via the extracellular signal-regulated kinase (ERK) and Akt/mTOR signaling pathway; and GELNs accelerate mouse skin wound healing and reduce inflammation in vivo.[90] *Rhizoma Drynariae*-derived nanovesicles are observed to facilitate human bone marrow mesenchymal stem cells (hBMSCs) proliferation and the expression of Estrogen Receptor α (ERα). Additionally, they can stimulate the differentiation of hBMSCs to osteogenic lineage, as evidenced by increased expression of bone morphogenetic protein 2 (BMP2) and runt-related transcription factor 2 (RUNX2)[91] Zhou et al found that Gouqi (Chinese wolfberry)-derived nanovesicles (GqDNVs) improve the cross-sectional area of quadriceps muscle and grip strength in dexamethasone-induced muscle atrophy model, mainly through the activation of AMP-activated protein kinase (AMPK). Furthermore, the energy-targeted metabolome analysis indicates that GqDNVs enhance the metabolism of oxidative phosphorylation, demonstrating their therapeutic capabilities for muscle regeneration.[92]

Anti-Infection

PELNs are known to confer resistance against various infections in plants, while the specific contributions of PELNs to antibiotic systems within the biomedical domain have not been extensively elucidated. miRNAs might play a crucial role in such cross-kingdom regulation. GELNs can be selectively internalized within the periodontal pathogen *Porphyromonas gingivalis* through a phosphatidic acid (PA)-mediated process, which involves specific interactions with the hemin-binding protein 35 (HBP35) present on the bacterial cell surface. Moreover, GELN-derived PA and aly-miR159a-3p significantly decreased the expression of type IX secretion system (T9SS), thereby affecting the virulence of the bacterium.[93] This is another example of synergy where cargo molecules interacted with multiple pathogenic factors in the recipient bacteria simultaneously. miR858a and miR858b enriched in *Houttuynia cordata*-derived exosome-like nanoparticles specifically target the neuraminidase (NP) gene within the H1N1 influenza virus. Similarly, miR166a-3p has been identified to target the open reading frame 1ab (ORF1ab) in the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), providing potential defense against viral replication and pathogenesis.[40] Nsp12, an activator of nuclear factor kappa-B (NF-κB) pathway and producer of a range of inflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1β, is inhibited by ginger ELNs containing aly-miR396a-5p, suggesting a therapeutic strategy for mitigating the impact of SARS-CoV-2 on lung cells.[94] Bacterial interaction may serve as another mechanism of their anti-infection effects. Lei et al reported that the treatment of lemon-derived exosome-like nanoparticles provide protection against *Clostridioides difficile (C. diff)* infection in a probiotic-dependent manner, which is mediated by the elevation of aryl hydrocarbon receptor (AhR) ligands indole-3-lactic acid (I3LA) and indole-3-carboxaldehyde (I3Ald) and subsequently induce the expression of IL-22. The observed increase in lactic acid production is implicated in the reduction of C. diff fecal shedding, ascribed to the inhibition of C. diff proliferation and the suppression of indole biosynthesis.[95]

Strategies for PELN Engineering

Similar to exosomes or exosome-like nanovesicles derived from other sources, PELNs exhibit the capacity to facilitate intercellular communication by selectively delivering bioactive substances to target cells, thus making them a promising

therapeutic modality. Nevertheless, their inherent defects, such as limited natural effects and the lack of targeting, have sparked interest in innovative engineering strategies, including surface modification, cargo encapsulation, and membrane fusion or coating, which have been developed to significantly enhance their therapeutic potential (Figure 3).[96] Reports on engineered nanovesicles prepared from animal cells, edible plants and other sources indicate that the choice of strategies is closely related to the intended objectives and anticipated benefits. Surface modification and membrane fusion are generally aimed at enhancing stability and targeting efficiency, while drug loading is typically intended to improve protective efficacy. Furthermore, although still in its infancy in PELN, the synergistic application of multiple engineering techniques is a promising research approach in terms of a series of studies on animal extracellular vesicles or artificial nanoparticles showing particularly encouraging results.[97–99]

Membrane Surface Modification

Biological modification is the most widely used strategy for nanovesicle membrane modification (Table 1), which is pivotal for increasing the circulation stability, improving biocompatibility, achieving active delivery to designated sites to reduce off-target effects, and exerting additional biological effects in certain special circumstances.[64,100,101] The modification strategies for animal cell-derived extracellular vesicles involve both parental cell engineering and direct manipulation. Parental cell-based modification is founded on the genetic alteration to exhibit the desired protein on the membrane which subsequently transfer to the surface of produced MEVs. Alvarez-Erviti et al firstly harnessed Lamp2b, an exosomal membrane protein, to fuse with the neuro-specific peptide rabies virus glycoprotein (RVG) for targeted

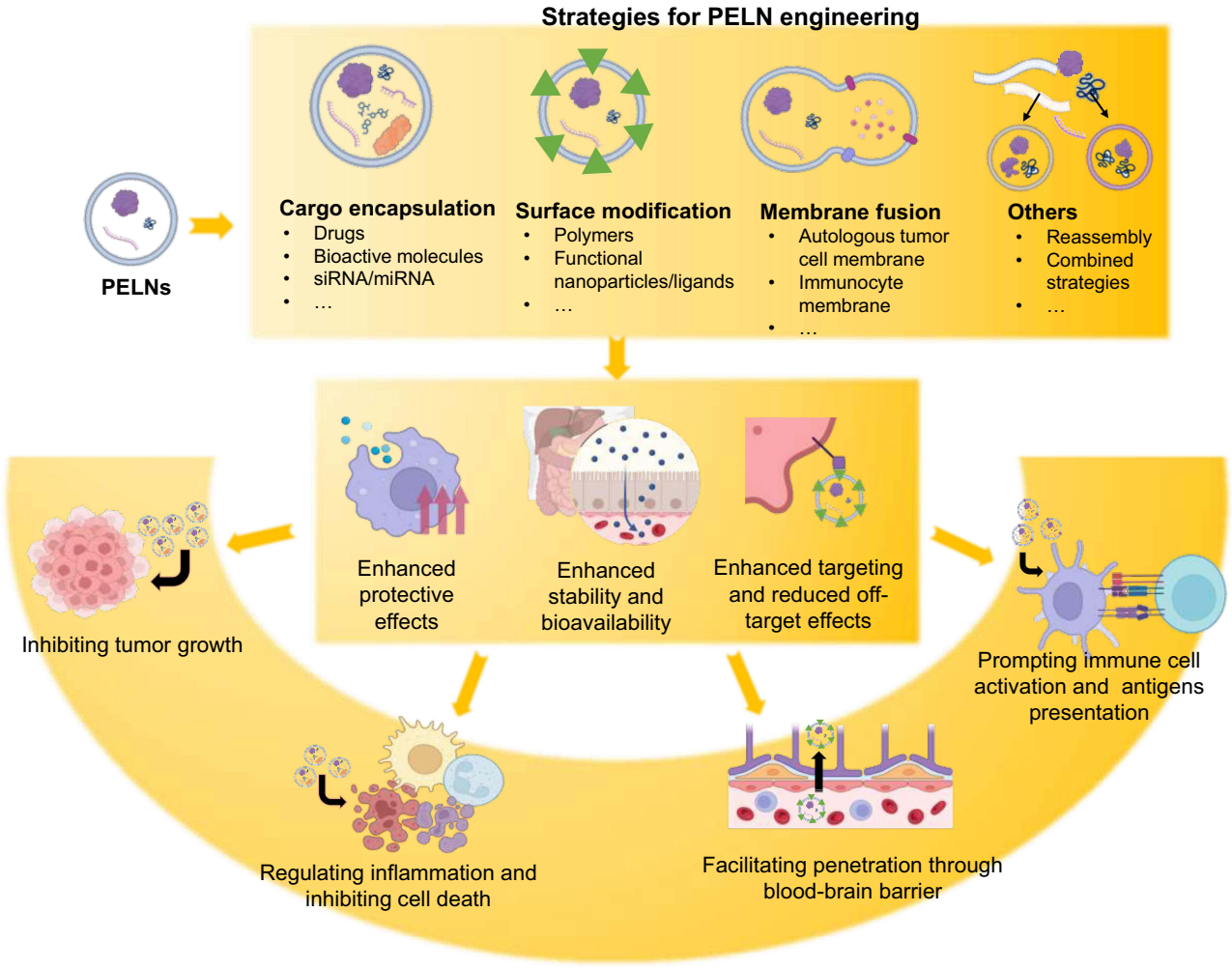

Figure 3 Strategies for PELN engineering and corresponding benefits.

Table 1 Membrane Surface Modification of PELNs and Enhanced Therapeutic Effects

Plant	Isolation and Purification Method	Fabrication Method	Disease Model	Modification Effect	Therapeutic Effect or Function	Ref.
Lemon	Ultracentrifugation and density-gradient centrifugation	cRGD modification and reassembly through ultrasonic emulsification	Glioblastoma	Facilitating penetration through blood–brain barrier/blood–brain tumor barrier, and inducing higher accumulation and cellular internalization	Targeted delivery of DOX and suppression of tumor growth	Chen et al[106]
	Ultracentrifugation and density-gradient centrifugation	Heparin-cRGD modification and DOX loading	DOX-resistant ovarian cancer	Enhanced tumor targeting, long-term retention time and good biosafety	Apoptosis promotion, anti-proliferation and anti-angiogenesis effect, endocytosis-triggered energy dissipation and decreased ATP production	Xiao et al[104]
Ginger	Membrane filtration, ultracentrifugation and density-gradient centrifugation	Cholesterol-conjugated FA-3WJ decoration	KB tumor xenograft model	Lower cytotoxicity, targeting delivery of survivin siRNA and gene knockdown effect	Suppression of tumor growth	Li et al[107]
	Ultracentrifugation and density-gradient centrifugation	Pd-Pt nanosheets modification	*S. aureus*-infection	Sustained generation of ROS at infection sites, synergistic electrodynamic and photothermal therapy	Lower expression level of TNF-α and IL-6, efficient anti-bacterial therapy	Qiao et al[108]
	Ultracentrifugation and density-gradient centrifugation	Folic acid coating and DOX loading	Colon cancer	Decreased systematic toxicity and prolonged circulation time	Suppression of tumor growth	Zhang et al[109]
Orange	Ultracentrifugation and density-gradient centrifugation	cRGD-targeted DOX-loaded nanoparticles modification	Ovarian cancer	Increasing accumulation and penetration at tumor sites, reducing degradation and inflammation	Anti-proliferation and anti-angiogenesis effects on tumor	Long et al[103]

(*Continued*)

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Table 1 (Continued).

Plant	Isolation and Purification Method	Fabrication Method	Disease Model	Modification Effect	Therapeutic Effect or Function	Ref.
Grapefruit	Ultracentrifugation	Insertion of DSPE-PEG2000-Mal and aptamer R11-3	-	Enhanced targeting at the brain endothelial cell line and cellular uptake	-	Moon et al[110]
	Ultracentrifugation and density-gradient centrifugation	Heparin-targeted DOX-loaded nanoparticles modification	Glioma	Increased drug loading capacity, penetration through blood–brain barrier/blood–brain tumor barrier, prolonged circulation time	Enhancing drug absorption of tumors and anti-angiogenesis	Niu et al[111]
	Sonication and homogenization	Aptamer HA1 conjugation	$HER2^+$ breast cancer	Enhanced cellular uptake, targeted distribution in tumor	Suppression of tumor growth	Tang et al[112]
	Ultracentrifugation and density-gradient centrifugation, lipid reassembly	Aptamer LA1 and Psi-LA1 conjugation	Multidrug resistant colon cancer	Targeted distribution in tumor, good biosafety	Suppression of tumor growth	Yan et al[113]
	Ultracentrifugation and density-gradient centrifugation, lipid reassembly	Folic acid coating and miR-17 loading	Brain tumor	Enhanced efficiency in targeting brain tumor cells	Inhibiting the expression of MHC-1 on GL-26 tumor cell and triggering NK cell activation	Zhuang et al[114]
Asparagus cochinchinensis	Ultracentrifugation and density-gradient centrifugation	PEG modification	HepG2 tumor xenograft model	Improved blood circulation period the tumor-targeting ability	Increased level of AIF, Bax, and Bak and activation of caspase-9, suppression of tumor growth	Zhang et al[17]
Pueraria lobata	Ultracentrifugation and filtration	DSPE-PEG-RVG ligand modification	Parkinson's disease (PD)	Enhanced blood-brain barrier penetration and dopaminergic neuron targeting	Improving autonomic behaviors of PD mice, mitigating neuron deterioration by modulating PINK1-Parkin-mediated mitophagy and activating mitochondrial respiratory chain complexes I and V	Xu et al[115]
Aloe	Ultracentrifugation	DSPE-PEG-RGD ligand modification, indocyanine green and doxorubicin loading	Breast cancer	Enhanced loading efficiency of competitive drugs, stability and leakproof capacity, high tumor targeting ability	Inhibition of tumor cell growth and migration, inducing increased drug accumulation at tumor sites	Zeng et al[149]

Abbreviations: Dox, doxorubicin; FA, folic acid; siRNA, small interfering RNA; ROS, reactive oxygen species; TNF, tumor necrosis factor; IL, interleukin; DSPE, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine; PEG, polyethylene glycol; Mal, maleimide; HA1, hemagglutinin 1; HER2, human epidermal growth factor receptor 2; Psi, P-gp siRNA; MHC-1, major histocompatibility complex class I; AIF, apoptosis-inducing factor; Bax, BCL-2-associated X protein; Bak, BCL2 homologous antagonist/killer; RVG, rabies virus glycoprotein; PD, Parkinson's disease; PINK1, PTEN induced putative kinase 1.

delivery of siRNA.[102] However, the unique process of PELNs isolation and undetermined membrane markers often renders parental cell-based genetic methods difficult to realize, thereby many researchers have focused on direct modification through physical or chemical approaches, which means combining specific substances (ligands, homing peptide, polymers or other small molecules) with the surface membrane of PELNs through special binding methods. Covalent bonding is considered the subject of investigation for engineering EVs surface modification.[65] For instance, the cyclic RGD peptide (cRGD), a family of peptides which endow natural exosomes with high affinity with αvβ3 integrin receptors overexpressing on the surface of tumor cells or tumor vascular endothelial cells, has been applied in engineering PELNs. Lemon and orange ELNs are endowed with tumor-targeting ability after functionalization with heparin-cRGD, with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) as catalysts.[103,104] Aptamers are short oligonucleotides or peptides with specific structures, which could target tumor cells with high affinity and specificity.[105] Conjugation with HA1, an aptamer specific for Human epidermal growth factor receptor 2 (HER2)-overexpressing breast cancer cells endows GNVs with HER2+ breast cancer cell-targeting ability.

Additionally, decorations with several polymers and small molecules have also been reported. For instance, Moon et al extracted ELNs from grapefruit, inserted 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE) into the membrane and conjugated aptamer R11-3 onto the surface using click chemistry, which facilitated penetration through blood-brain barrier and brain cellular uptake.[110] Zhuang et al developed GNVs hybridized with polyethyleneimines (pGNVs) and combined with folic acid (FA). These modified GNVs were capable of delivering miR-17 efficiently intranasally to the mouse brain tumor that overexpresses folic acid receptor (FR).[114,117] Similarly, Li et al applied arrow-shaped RNA to display FA on ginger ELNs membrane and successfully achieved targeted delivery of siRNA survivin to KB cancer cells. Furthermore, in vivo distribution of ELNs can be monitored through fluorescent dye labeling on the membrane surface.[64,118] Targeted delivery of siRNA to duodenal epithelium via folate-modified ginger nanovectors presents a feasible strategy for alleviating iron burden.[119] Besides, polyethylene glycol (PEG) decoration could significantly prolong systemic half-life and reduce non-specific immune clearance of *Asparagus cochinchinensis* ELNs.[17] Qiao et al constructed a biomimetic nanoparticle combining electrodynamic Pd-Pt nanosheets and ELNs derived from ginger. With high biocompatibility and stability in vivo, the platform presented efficient targeting and accumulation at infection sites, coupled with sustained generation of ROS to achieve synergistic electrodynamic and photothermal anti-bacterial therapy.[108] Chen et al explored extracellular vesicle-engineered structural droplet drugs (ESDDs) functionalization strategies by programming the self-assembly of lemon-derived ELNs on the doxorubicin (DOX)@squalene–PBS surface, optimizing the delivery of DOX across the blood-brain barrier for improved glioblastoma chemotherapy and demonstrating prolonged circulation time.[106] Additionally, such strategy is proved to yield synergistic benefits by modifying with bioactive molecules. For instance, Ruan et al reported that surface modification of stromal cell-derived factor 1 (SDF-1) enhanced the migratory effects of EVs on neural stem cells (NSCs) without compromising their ability to promote NSCs differentiation.[101] However, covalent functionalization of PELN with ligands or bioactive therapeutics have not been explored yet; to date, there have been few attempts to modify the surface of PELN using non-covalent modification method based on charge and hydrophobicity, positioning them as a potential research directions. Conclusively, surface modifications of PELNs enable enhanced targeting capability and improved pharmacokinetics profiles.

Cargo Encapsulation

In addition to transporting endogenous agents to target cells, the lipid layer and internal aqueous phase of PELNs endow them with the capability to encapsulate exogenous hydrophilic or hydrophobic molecules, such as chemotherapeutic drugs, mRNAs, miRNAs, siRNAs, proteins, while shielding them from degradation (Table 2).[120–123] This attribute renders PELNs an exemplary option for drug delivery systems (DDS). In comparison with artificially synthesized nanoparticles for DDS, like liposomes or micelles, PELNs have proven to be a better choice in certain conditions in terms of higher stability, lower immunogenicity, higher efficacy and stronger cellular uptake.[64,73,124,125]

To accomplish optimal delivery, several factors need to be addressed: enhancing encapsulation efficiency, ensuring the structural integrity of the nanoparticle, and maintaining the drug molecule's bioactivity. Multiple techniques can be employed to encapsulate drugs within PELNs, encompassing co-incubation, electroporation, sonication, click chemistry,

Table 2 Cargo Encapsulation of PELNs and Enhanced Therapeutic Effects

Plant	Isolation and Purification Method	Cargo	Disease Model	Therapeutic Effect or Function	Ref.
Grapefruit	Ultracentrifugation	DOX	–	Sustained releasing of DOX	Moon et al[110]
	Ultracentrifugation	BSA, HSP70	–	Dose-dependent cell protection from the etoposide-induced cytotoxicity	Garaeva et al[121]
	Ultracentrifugation and density-gradient centrifugation	Sodium thiosulfate	Vascular calcification	Attenuating vascular calcification through promotion of M2 macrophage polarization, inhibition of inflammation, and suppression of the bone-vascular axis, better biocompatibility	Feng et al[126]
	Ultracentrifugation	Anti-luciferase siRNA	-	Suppress luciferase expression	S. Itakura et al[127]
	Ultracentrifugation and density-gradient centrifugation, lipid reassembly	PTX	CT26 colon cancer model and SW620 colon cancer model	Suppression of tumor growth	Wang et al[128]
Watermelon	Ultracentrifugation and density-gradient centrifugation	miR146a-5p	Ovarian cancer	Regulation of IRAK1 and SERPINE1 expression to produce anti-angiogenic effect	Corvigno et al[129]
Broccoli	Ultracentrifugation size exclusion chromatography	miR159a, miR159b-3p, miR166b-3p, miR403-3p	-	-	L. Del Pozo-Acebo et al[120]
Kiwifruit	Ultracentrifugation and density-gradient centrifugation	Sorafenib	HepG2 tumor xenograft model	Reduced dose-associated toxicity, hepatic targeting and uptake	Fang et al[130]
Grape	Ultracentrifugation	Metformin, doxorubicin, tamoxifen	Breast cancer	Increasing tumor intracellular ROS level and facilitating cell apoptosis and necrosis	Farheen et al[131]
Citrus reticulata Blanco	PEG precipitation	Tangeretin	-	Enhancing antioxidant effect for LPS-induced inflammation	Li et al[132]
Green tea	Ultracentrifugation and density-gradient centrifugation	Anta-HAAPIR piRNA	Aortic dissection	Decreasing blood vessel width and alleviating aortic dissection through myocyte enhancer factor 2D (Mef2D) and matrix metallopeptidase 9 (MMP9) pathways	Liu et al[133]
Celery	Ultracentrifugation and density-gradient centrifugation	DOX	A549 subcutaneous tumor model	Lower toxicity and better anti-tumor efficacy	Lu et al[24]

Tomato	Ultracentrifugation and density-gradient centrifugation	Curcumin	-	Inhibiting the expression of IL-1β and IL-6 on LPS-treated THP-1 Cell Line	R. Mammadova et al[122]
Orange	Ultracentrifugation	mRNA	COVID-19	Delivering mRNAs into macrophages and expressing N, S1 and FS proteins, inducing a specific humoral and cell-mediated immune response in vivo	Pomatto et al[134]
Avocado	Centrifugation and filtration	Ginkgetin, berberine	Atherosclerosis	Suppressing activation of NF-κB and NLRP3, inhibiting expression of Cd36, TNF-α, IL-1β, IL-6 and oxidized low-density lipoprotein -induced macrophage foam cell formation	Sharma et al[135]
Acerola	Ultracentrifugation, Affinity column	miR-340	-	Gene downregulation	Umezu et al[136]
Ginger	Ultracentrifugation and density-gradient centrifugation, lipid reassembly	siRNA-CD98	Ulcerative colitis	Specifically targeting colon and mediating CD98 gene inhibition	Zhang et al[123]

Abbreviations: Dox, doxorubicin; BSA, bovine serum albumin; HSP, heat shock protein; siRNA, small interfering RNA; DSS, dextran sodium sulfate; TNF, tumor necrosis factor; IL, interleukin; PTX, paclitaxel; IRAK1, interleukin-1 receptor-associated kinase 1; SERPINE1, serine proteinase inhibitor, Clade E1; ROS, reactive oxygen species; LPS, lipopolysaccharide; piRNA, Piwi interacting RNA; Mef2D, myocyte enhancer factor 2D; MMP9, matrix metallopeptidase 9; NLRP3, nucleotide-binding domain and leucine-rich repeat related family, pyrin domain containing 3.

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freeze–thaw, osmotic shock, etc.[124] For instance, due to the highly hydrophobic property, the bioavailability of curcumin, a lipophilic polyphenol serving as an anticancer, antibiotic and anti-inflammatory agent, is low which still prevents its clinical application.[122] Ramila Mammadova et al constructed curcumin-loaded tomato-derived ELNs by extrusion, direct loading by co-incubation and sonication, discovering that sonication enhanced the inherent anti-inflammatory properties.[122] Shoko Itakura et al adopted a novel method for loading siRNAs into grapefruit-derived nanoparticles by optimizing pressure conditions in the microfluidic device, which presented in vitro gene knockdown effects.[127] Luiza Garaeva et al combined passive and active loading strategies, and demonstrated that ELNs derived from grapefruit serve as potent vehicles for delivery of bovine serum albumin (BSA) and heat shock protein 70 (HSP70) into both human peripheral blood mononuclear cells and colon cancer cells to exert cytoprotective activity.[121] Numerous strategies are explored to achieve superior loading efficiency. Zhuang et al explored an innovative strategy for the intranasal delivery of therapeutic miR-17 to brain tumor cells, utilizing GNVs as carriers. Notably, GNV-coated polyethylenimine (PEI)/RNA complex not only increased the miR-17 loading efficiency from 5.91±0.6% to 86.2±5.7%, but also mitigate the cytotoxic effects typically associated with PEI.[114] Similar paradigm has been reported where enhanced biomedical effects were observed in drug-loaded PELNs comparing with free drug.[109,125,133,137] Recently, green tea-derived PELNs were reported to deliver an antagomir targeting heart-apoptosis-associated piwi-interacting RNA (HAAPIR) to the lesion of aortic dissection.[133] The nanosystem effectively regulates vascular remodeling, mitigating AD occurrence and progression through the myocyte enhancer factor 2D (Mef2D) and matrix metallopeptidase 9 (MMP9) pathways. It's interesting to note that PELN and the antagomir could be synergistic since PELN itself showed limited but significant effects. Bitter melon-derived extracellular vesicles (BMEVs) demonstrated significant therapeutic benefits when combined with 5-Fluorouracil (5-FU) for the treatment of oral squamous cell carcinoma (OSCC), outperforming the single-agent administration. This combination therapy was associated with a downregulation of the NOD-like receptor family pyrin domain containing 3 (NLRP3) expression, potentially attenuating the drug resistance of OSCC to 5-FU.[138] As natural transporting platforms with low toxicity and high absorption rates, PELNs have an intriguing potential for delivering vaccine mRNA, which accelerates their application from bench to bedside. In addition, antigen-incorporated PELN vaccine ensures enduring immunity without the risks associated with vaccine-induced virulence reversion or pre-existing immunity.[10,116] ELNs derived from orange juice have been identified as effective carriers for the delivery of SARS-CoV -2 mRNA vaccines via oral and intranasal routes. This approach has been shown to effectively immunize mice, eliciting a targeted humoral and cellular immune response.[134]

Nevertheless, the challenge of achieving efficient cargo loading without altering membrane integrity and contents remains significant. Matricellular contents restrict the loading of exogenous drugs, and the delivery of hydrophilic compounds is constrained by lipid bilayer membranes. This ultimately limits the potential of exosomes as carriers.[139] As a result, technologies such as electroporation have thrived,[140] but might lead to the aggregation and fusion of EVs, which can result in changes to their surface potential. On the other hand, ultrasonic treatment offers a simple and rapid alternative, markedly improving the loading efficiency of active ingredients into the vesicles.[65] Besides, A standardized approach to assess the loading efficiency of PELNs is yet to be established, due to the impact of the loading technique, the duration of the loading process, the ratio of drug to carrier, and the origin of the carrier itself.

Membrane Hybridization and Coating

Although membrane surface modification endows traditional PELNs with unique characteristics not originally present, their applications are still constrained by the singular functionality of the ligands and intricate preparation methods, such as weak accumulation capacity.[106] However, harnessing the inspiration of bionic design principles, the direct construction of biomimetic nanocarriers using cellular components from biological autologous sources holds the potential to endow PELNs with lower immunogenicity, extended circulation times, and enhanced targeting capabilities (Table 3).[141,142] Hybrid membrane nanovesicles (HMNVs), synthesized by homologous or heterologous membrane origins, allow for the efficient and large-scale fabrication, and can be tailored to possess multiple functionalities within a single vesicle type. Furthermore, HMNVs derived from the fusion of two distinct cell types exhibit a combination of biological functions.[143] Zhang et al developed an in situ cancer vaccine by fusing bacteria-derived outer membrane vesicles with thylakoid nanovesicles of spinach. Such bacteria-plant hybrid vesicles (BPNs) increase homing to tumor tissues, prompt immune

Table 3 Membrane Hybridizing of PELNs and Enhanced Therapeutic Effects

Plant	Isolation and Purification Method	Fabrication Method	Disease Model	Modification Effect	Therapeutic Effect	Ref
Spinach	Sonication and extrusion	Hybridized with *Escherichia coli* MG1655-derived outer membrane vesicles	Colon Cancer	Enhancing targeting of tumor cells and to initiate an immune response	Enhancing cancer immunotherapy	Zhuang et al[144]
Ginseng	Differential centrifugation	Hybridized with the membrane originated from the resected autologous tumors	Breast Cancer	Enhance the phagocytosis of autologous tumor antigens by dendritic cells (DCs) and facilitate DCs maturation through TLR4, ultimately activating tumor-specific cytotoxic T lymphocytes (CTLs)	Strengthening specific immune responses to suppress tumors recurrence and metastasis	Wang et al[145]
	Differential centrifugation	Electroporation and coated with neutrophil membrane	Acute lung injury	Target regulation of NOX4/Drp-1/NLRP3 signal pathway	Downregulating the inflammatory response and ameliorating acute lung injury	Ma et al[146]
Grapefruit	Extrusion	Coated with inflammatory chemokine receptor enriched membrane fraction of activated T cells	Colon and breast cancer, inflammation	Enhancing for homing to inflammatory tumor tissues	Inhibiting of tumor growth and inhibiting the inflammatory effects of DSS induced mouse colitis	Wang et al[147]

Abbreviations: DC, dendritic cell; TLR4, Toll-like receptor 4; CTL, cytotoxic T lymphocyte; NOX4, nicotinamide adenine dinucleotide phosphate hydrogen oxidase 4; Drp-1, dynamin related protein 1; NLRP3, nucleotide-binding domain and leucine-rich repeat related family, pyrin domain containing 3; DSS, dextran sodium sulfate.

cell activation and tumor-associated antigen presentation, eliciting potent CD8+ T lymphocyte responses. Additionally, BPNs mitigate the immunosuppressive tumor microenvironment and enhance the overall immune response.[144] Employing this concept, functional hybrid cancer vesicles are designed by fusing ginseng-derived ELNs extracellular vesicles-like particles (G-EVLPs) with membranes of autologous tumors. G-EVLPs facilitate dendritic cell phagocytosis and tumor-specific cytotoxic T lymphocyte activation, potentially preventing tumor recurrence and metastasis.[145]

As mentioned above, HMNVs can exhibit the respective biological characteristics of different origins, which provides inspiration for precision of targeting. When transitioning to a state of "camouflage", the cell membrane-coated nanoparticles (CMNPs) acquire the characteristics of the donor cells and the capability to evade immune detection.[141] For instance, The recruitment ability into inflamed sites was imparted to grapefruit nanovectors after coating with enriched membranes of activated leukocytes, and inflammatory related receptor C-X-C Motif Chemokine Receptor 2 (CXCR2) and Leukocyte Function-associated Antigen 1 (LFA-1) played a key role.[147] Neutrophil-camouflaged *Panax ginseng* root-derived ELNs target lung inflammation, and loaded miRNA-182-5p mitigates the symptom of sepsis.[146] More research is needed to investigate the pleiotropy of PELNs interacting with heterogeneous membranes and the application of this bionic nanoparticle in more disease models.

Other Modification Strategies

Confronted with the restrictions of single-site modifications for PELNs, researchers have devised a solution through dual or multifaceted modification strategies. These encompass a synergistic application of surface ligand modifications, cargo

loading, and membrane fusion techniques to develop multifunctional vesicles. For example, ginger-derived nanovectors modified with FA facilitated the precise delivery of DOX to Colon-26 tumor cells, resulting in stronger suppression of tumor growth.[109] Huang et al constructed fused nanovesicles (FV@CX5461) from grapefruit and gingiva-derived mesenchymal stem cells, encapsulating them with immunosuppressant CX5461. The C-C-Motif Receptor 6 (CCR6) activity increased homing to inflammation tissue and mediated the effective immune microenvironment reconfiguration of FV@CX5461.[148] Meanwhile, hydroxyapatite crystal binding peptide (ESTP) modified grapefruit ELNs can be used for vascular calcification-targeted delivery of sodium thiosulfate.[126] Zeng et al introduced an optimal method for drug carrying that leverages π-π stacking interactions to augment the combined therapeutic impact of DOX and Indocyanine green (ICG). Integrin-targeted peptide covalent conjugation Aloe-Derived Nanovesicles further enhanced their targeting specificity towards breast cancer cells.[149]

Other engineering approaches have also been reported in several studies. Wang et al first fabricated nanovectors from lipids extracted from grapefruit (GNVs), utilizing a high-pressure homogenization technique for reassembly. They further verified GNVs' capability to encapsulate and transport chemotherapeutic agents or siRNAs, and explored various modifications to enhance the targeting specificity for improved therapeutic delivery.[114,128] In the context of facilitating wound healing, utilizing a composite hydrogel system for EVs loading presents a superior approach for the protection and controlled release of EVs. This method circumvents issues like rapid degradation and depletion, ensuring a sustained therapeutic effect.[150] ELNs derived from *Olea europaea* leaves integrated with a hyaluronic acid and tannic acid hydrogel have demonstrated efficacy in mitigating ultraviolet-induced skin damage and promoting skin regeneration.[151] However, more investigations are required for the benefits and clinical transition potentials of those novel methods.

Perspective and Current Challenges

PELNs have shown great promise for the advancement of drug delivery, messaging, and tissue repair applications due to their environmentally friendly nature, excellent biocompatibility, superior gastrointestinal tolerance, and absence of human pathogens. Nevertheless, the current understanding of the biogenesis, signature markers, and substance transport of PELNs remains inadequate. Targeted delivery, yield, high heterogeneity, and lack of standardized GMP have also become pivotal issues that require immediate attention in the context of the current clinical translation and large-scale utilization of PELNs.[14,136,152] Four specific pressing issues are listed here as examples: comparison among sources, storage and administration, endosomal escape and the effects of protein corona.

Comparison Among Sources

Engineered PELNs present distinctive features in comparison to exosomes derived from animal cells and milk-derived extracellular vesicles. While there is general consensus in the scientific community on the fundamental methodology for obtaining nanovesicles, significant variations remain in processing extracted materials, largely due to source differences. For PELNs, a primary challenge lies in breaking down and clearing cell walls. In terms of nanovesicle characterization, animal cell-derived exosomes and milk-derived extracellular vesicles display well-defined protein markers, including transmembrane/lipid-binding proteins (eg, CD63, CD9) and cytoplasmic proteins (eg, TSG101), whereas PELNs lack robust evidence for such markers. Regarding purification, exosomes from animal cells often contain impurities like cellular debris and vesicles of organelle or nuclear membrane origin, and milk-derived extracellular vesicles are characterized by high protein and lipid particle concentrations. Batch variability of nanovesicles due to instability between cell passaging cultures and animal-derived individuals has limited the engineering development of both of these nanovesicles. PELNs, however, may contain small cellular debris unique to their plant origin, posing specific challenges for purification. Furthermore, the biological activity of nanovesicles typically relates to their surface molecules and contents. Unlike the animal-derived bioactive compounds in cell-derived exosomes and milk-derived vesicles, PELNs offer plant-derived nucleic acids and small molecule compounds with a broad therapeutic potential.[7,10,153,154] A comparative analysis of the characteristics of PELNs will facilitate the identification of future research avenues and the urgent need for further investigation. Due to previous reports that different PELNs exert complex biological effects, careful selection of sources is crucial.

Storage and Administration

Methods to prevent degradation, damage and inhomogeneous composition of engineered PELNs during storage and preparation represent an additional avenue of research that is worthy of continued investigation.[155] Currently, the most prevalent method of administration of natural and modified PELNs currently under investigation is oral, and has been demonstrated to be efficacious.[6] In addition to this, intravenous, intraperitoneal, nebulized inhalation and microneedles have also been explored as potential delivery modes.[156] 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.[18,157] Nebulized inhalation allows for direct delivery to the alveoli, thereby optimizing drug concentration for pulmonary application.[158] 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.[156]

Endosomal Escape

Endolysosomal trapping of PELNs and endosomal escape strategies contribute to the enhancement of PELNs activity upon entry into the cell, thus allowing for the more effective exertion of the inherent biological effects.[159] As a lipid complex, PELNs have the potential to escape through a mechanism called flip-flop. Recent research has begun to employ methods used by viruses and bacteria for endosomal escape. To date, a number of endosomal escape agents have been purified or synthesized from various sources, including bacteria-derived agents like listeriolysin O (LLO), virus-derived agents such as influenza-derived fusion peptide diINF-7, plant-derived agents like ricin, human/animal-derived agents such as epidermal growth factor receptor (EGFR), and other synthetic chemical agents.[160] By employing a specific class of ionizable lipids, the researchers were able to fabricate lipid nanoparticles for the purpose of facilitating cytoplasmic nucleic acid transfer, such as 1.2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA), DLin-KC2-DMA, and DLin-MC3-DMA. In an acidic environment (endosomes), this class of lipid complexes exhibits a positive charge, which results in membrane fusion and endosome rupture. This process allows the lipid complexes to escape into the cytosol. This type of technology also has the potential to modify PELNs, thereby enhancing efficacy and ensuring a certain level of delivery.[161,162]

Effects of Protein Corona

A further study has demonstrated that the protein corona formed when PELNs are cultivated or administered under specific conditions has a deleterious impact on nanomedicine in numerous instances. This is evidenced by the disabling of PELNs targeting, the activation of immune responses and the rapid clearance of the nanoparticles. Following injection, PELNs interact with components of the circulation, resulting in the formation of a protein corona. This process alters the properties of the nanoparticles, influencing their subsequent fate. Despite the incomplete understanding of the formation mechanism and the imperfect characterization technique, the significant advantages of this engineered modification include enhanced targeting of PELNs, prolonged circulation time and reduced aggregation embolism.[163] Jun-Yong Wu et al developed and utilized angiopep-2 (Ang) modified multifunctional exosome mimics (Ang-EM) for the purpose of controlling protein corona. The findings revealed that Ang-EM exhibited diminished protein corona formation due to its capacity to absorb fewer serum proteins. Furthermore, Ang-EM demonstrated an enhanced ability to target glioblastoma and a notable suppression of glioblastoma growth in mice through its mediated brain delivery of docetaxel (DTX).[164] Besides, safety of PELNs is a prerequisite for extensive research and clinical translation. The ability of all types of PELNs to reach target cells without any toxicity and exert possible biological effects remains to be further explored. Meanwhile, clinical translation is limited by some unreported side effects. The potential cytotoxicity of some PELNs and the underlying mechanisms remain unclear.[165] Additionally, establishing of transgenic plants may prove an attractive option as endotoxin-free bioreactors for engineered nanomedicine, with the capacity to express human-compatible recombinant proteins and other biologically active components with therapeutic potential, thus enhancing the effects of PELNs.[166] More investigations are required on those topics.

It is incontestable that natural and engineered PELNs offer considerable advantages. A more comprehensive approach to research and optimization, coupled with a systematic consideration of the current state of research and challenges, could facilitate the further expansion of the prospects for the application of PELNs as drugs and drug delivery systems. The full realization of the potential of engineered PELNs in clinical therapy will necessitate interdisciplinary collaboration and sustained efforts.

Conclusion

In conclusion, PELNs represent a promising frontier in nanomedicine, offering a viable alternative to animal cell-derived extracellular vesicles due to their superior biocompatibility, low immunogenicity, and capacity for large-scale production. Their unique properties have positioned PELNs as a promising tool in the arsenal against various diseases. In addition, Strategies for PELN engineering have been instrumental in enhancing the therapeutic efficacy by improving their targeting specificity, bioavailability, and cellular uptake. The innovative engineering techniques have also paved the way for the encapsulation of a wide range of therapeutic molecules, including chemotherapeutic drugs, nucleic acids, and proteins, further expanding the utility of PELNs as drug delivery systems. To facilitate the translation of PELNs from bench to bedside, issues such as heterogeneity and the lack of standardized manufacturing practices need to be addressed. Additionally, the optimization of PELN isolation and purification methods is crucial for ensuring the purity and stability of these nanovesicles. With challenges abound, ongoing research and interdisciplinary collaboration hold the key to unlocking the full potential of PELNs, paving the way for their integration into clinical practice and the advancement of personalized medicine.

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Disclosure

The authors report no conflicts of interest in this work.

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