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Review

Plant-derived nanovesicles: Promising therapeutics and drug delivery nanoplatforms for brain disorders

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ABSTRACT

Plant-derived nanovesicles (PDNVs), including plant extracellular vesicles (EVs) and plant exosome-like nanovesicles (ELNs), are natural nano-sized membranous vesicles containing bioactive molecules. PDNVs consist of a bilayer of lipids that can effectively encapsulate hydrophilic and lipophilic drugs, improving drug stability and solubility as well as providing increased bioavailability, reduced systemic toxicity, and enhanced target accumulation. Bioengineering strategies can also be exploited to modify the PDNVs to achieve precise targeting, controlled drug release, and massive production. Meanwhile, they are capable of crossing the blood-brain barrier (BBB) to transport the cargo to the lesion sites without harboring human pathogens, making them excellent therapeutic agents and drug delivery nanoplatform candidates for brain diseases. Herein, this article provides an initial exposition on the fundamental characteristics of PDNVs, including biogenesis, uptake process, isolation, purification, characterization methods, and source. Additionally, it sheds light on the investigation of PDNVs' utilization in brain diseases while also presenting novel perspectives on the obstacles and clinical advancements associated with PDNVs.

1. Introduction

Almost all living cells are capable of secreting vesicles, including plant cells. Plant vesicles are heterogeneous biological particles enclosed by phospholipid bilayers, which possess functions such as molecular transport and surface signaling, playing a crucial role in intercellular communication. There is currently no unified standard for the naming of plant vesicles. Plant exosome-like nanovesicles, plant-derived vesicles, plant-derived extracellular vesicles, plant-derived nanovesicles, etc., are described in different articles. In this article, we classify plant-derived nanovesicles (PDNVs) into two types: plant extracellular vesicles (EVs) and plant exosome-like nanovesicles (ELNs), according to the International Society for Extracellular Vesicles [1]. Plant EVs, naturally secreted from plants' apoplast, are engaged in cross-kingdom regulation in plants, animals, and microorganisms, as well as cell-to-cell communication [2]. They were first identified in carrot cells by Halperin et al. [3] in 1960, fifteen years earlier than the discovery of EVs in mammals. The extraction of EVs often involves interventions like gradient centrifugation and extrusion that disrupt their intact structure. Consequently, these methods yield extracted nanovesicles that are structurally incomplete and artificially damaged. Nevertheless, despite their compromised structure, these nanovesicles exhibit similarities to exosomes in terms of particle size and structure; hence they are referred to as ELNs. Plant ELNs can be extracted from freshly squeezed fruit and vegetable juice. In 2013, plant ELNs were extracted from grapes using differential centrifugation and exhibited favorable healing effects in colitis [4].

Current research directions are focused on the therapeutic application of vesicles in disease therapy, as well as investigations into their inherent properties, as PDNVs exhibit cross-kingdom regulatory properties. Owing to their massive production, low immunogenicity and toxicity, absence of human pathogens, as well as bioactive functions, they have been widely utilized in the field of biomedicine [5]. It is worth mentioning that PDNVs exhibit no detectable toxicity or immunogenicity and are devoid of human pathogens in contrast to mammalian-derived extracellular vesicles (MDEs) [6]. PDNVs are readily accessible and exhibit high productivity, as they can be obtained from numerous edible plants and herbs. Moreover, PDNVs are cost-effective, as EVs were initially regarded as cellular waste products resulting from cellular damage [7], thereby rendering them more extensively utilized compared to MDEs. However, there are certain limitations associated with

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MDEs. The study of PDNVs is still in its infancy, and the efficient isolation of PDNVs from plants lacks standardized methods. Additionally, identifying subtypes of PDNVs poses a challenge due to the absence of universal or specific markers for PDNVs [8]. Moreover, there is no systematic investigation into the compositional analysis of PDNVs. EVs and ELNs perform similar biological activities as their source plants. It has been confirmed that PDNVs possess the ability to inherit bioactive ingredients from their source plants, thereby endowing them with anti-inflammatory, antioxidant, and anti-cancer properties. For example, PDNVs extracted from traditional Chinese medicine (TCM), such as ginseng [9], Pu Gong Ying (PGY, *Taraxacum mongolicum*) [10], and Hong Jing Tian (HJT, *Rhodiola rosea* L.) [11], have outstanding biological activities, such as immunological regulation, anti-tumor, and anti-inflammatory functions. Similar to MDEs, PDNVs identified in various plants also exhibit drug loading capabilities [12]. Engineering modifications to PDNVs, such as lipid extraction for synthesizing nanoparticles of uniform size, drug loading, targeting modifications, or responsive release capabilities, can enhance the therapeutic efficacy and carrier functionality of PDNVs. PDNVs with active ingredients have emerged as therapeutics and drug delivery nanoplatforms that synergistically interact with medications to treat various diseases [13,14].

Brain diseases, including brain tumors, neurodegenerative diseases (NDs), neuropsychiatric diseases, and cerebrovascular diseases, impose a substantial economic and social burden on society [15]. Multiple comprehensive analyses worldwide have revealed a rapid increase in the incidence and prevalence of neurological diseases over the past three decades, establishing them as the primary cause of disability and mortality. While the blood-brain barrier (BBB) serves as a protective mechanism for preventing harmful substances from transiting from the blood to the brain, it also blocks adequate therapeutic conveyance. Therefore, the first consideration in selecting a drug for brain disorders lies in its ability to effectively cross the BBB. Based on the unique properties of PDNVs, such as biodegradability, capacity for encapsulating endogenous biologically active molecules, ability to traverse the BBB [16], and potential for large-scale production, PDNVs have demonstrated therapeutic promise in central nervous system (CNS) diseases. PDNVs have the potential to serve as therapeutics agents for modulating macrophages and glial cells, ameliorating brain inflammation, enhancing memory and promoting neural differentiation [13]. On the other hand, due to their structural resemblance to liposomes, PDNVs can encapsulate hydrophilic drugs within their aqueous core and lipophilic drugs within their lipid layer, thus improving drug stability and bioavailability.

Herein, this review aims to provide an overview of PDNVs as therapeutics and drug delivery platforms to treat brain disorders. Initially, we present a concise introduction to the biogenesis and uptake mechanisms of PDNVs. Their isolation, purification, and characterization methods are mentioned next. Then, we explore the functions of bioactive ingredients carried by PDNVs and highlight various bioengineering strategies that can enhance their therapeutic efficacy when combined with drugs. Recent applications of PDNVs for brain diseases, the challenges they face, and the prospects for clinical translation of PDNVs are also elucidated at last.

2. Biogenesis and uptake of PDNVs

As previously mentioned, PDNVs include two nanostructures, namely EVs and ELNs. While both are obtained from plants, they are divergent regarding isolation techniques and applications. Plant EVs are spontaneously released lipid bilayer structures that lack the ability to replicate [17]. Notably, EVs offer a promising avenue for comprehensive investigations into plant physiology and pathology, including intercellular communication and cross-kingdom regulation between plants, animals, and microbes [2]. As for plant ELNs, they are typically isolated from freshly squeezed fruit [18] and vegetable juices, such as grape [4], grapefruit [14], and ginger [19], whose production has undergone anthropogenic intervention. Given the distinction between EVs and ELNs, the subsequent introduction to the biogenesis will focus on EVs, while the isolation and characterization section will focus on ELNs. Although some progress has been made in understanding the biogenesis of plant EVs, further clarification is still needed.

Based on the research of plant EVs, three possible pathways are summarized: (I) The exocyst-positive organelle (EXPO) pathway is the unique biogenesis process in plants, which has been observed in *Arabidopsis* and tobacco cells to mediate cytosol to cell wall exocytosis [20]. (II) The multivesicular bodies (MVBs) pathway is the predominant mode in the biogenic pathway of plant EVs [21,22]. In *Arabidopsis* leaf tissue, vesicles are released after MVBs and paramural bodies merge with the plasma membrane following infection by barley powdery mildew fungus [23]. (III) The vacuolar pathway [24] is considered as a defense strategy for plants (Fig. 1a). Although plants lack immune cells, plant EVs play a crucial role in plant defense responses [25]. When encountering bacterial infection, central vacuoles fuse with plasma membranes to release secondary vesicles containing defense proteins and hydrolytic enzymes outside the cell, thus achieving anti-microbial effects and inducing bacterial death [26]. Although the presence of cell walls in plant cells may prevent the secretion of EVs, a few studies have suggested that PDVs contain substances such as cell wall remodeling proteins to aid their passage through the cell wall [27]. However, different plants may possess distinct biogenesis pathways for PDNVs whose patterns remain unknown, thus further investigation into their biogenesis processes is warranted.

After the formation of PDNVs, recipient cells may internalize them through various mechanisms: (I) Membrane fusion. (II) Receptor-mediated endocytosis. For instance, Lectin II on the surface of garlic-derived ELNs (GaELNs) interacts with CD98 receptor on liver cell surfaces to facilitate GaELNs uptake by liver cells [28]. (III) Lipid raft-mediated endocytosis. The lipid content of EVs may play a role in vesicular uptake, and sphingolipids have been shown to promote binding and endocytosis [29]. (IV) Clathrin-mediated endocytosis [30]. It is also a significant pathway of endocytosis in plant cells. Macropinocytosis and clathrin-dependent mechanisms allow intestinal macrophages to take in grapefruit-derived ELNs (GPDNs) [31]. (V) Other uptake mechanisms include cholesterol-dependent endocytosis for corn-derived nanoparticles, microtubule-dependent active transport and macropinocytosis for ginger-derived ELNs (GDNs), as well as caveolae-mediated endocytosis [32–34] (Fig. 1b).

3. Isolation and characterization methods of PDNVs

3.1. Isolation and purification methods

For pre-treatment of samples, succulent plants are typically subjected to squeezing, mixing or stirring in order to extract their juice while separating plant-origin vesicle. In cases where the juice is limited, it is commonly obtained by grinding the plants with PBS using a machine [35]. Dried plants are mixed with a vesicle isolation buffer and kept at room temperature for 24 h while gently agitated to reconstitute the ELNs [36]. Additionally, decoctions of TCM, including PGY, HJT, and *Astragalus membranaceus*, can also be used to extract ELNs. Subsequently, differential centrifugation is typically employed to eliminate particulate impurities and macromolecules, followed by further purification through sucrose density gradient centrifugation. Of course, these two methods are only the more common ones, and other separation methods are also used depending on the needs.

Isolation and purification are key steps in PDNVs research, but their extraction and isolation still pose significant challenges due to the absence of specific markers [37]. Plant EVs exclusively exist in extracellular apoplastic fluid, and the most common extraction method is vacuum infiltration-centrifugation [38]. Exactly as Lee's team [39] collected EVs from *Dendropanax morbifera* stems and leaves using a 100 K centrifugal filter. In another study, a discontinuous iodixanol density gradient was utilized to further purify EVs, determine their density, and sepa-

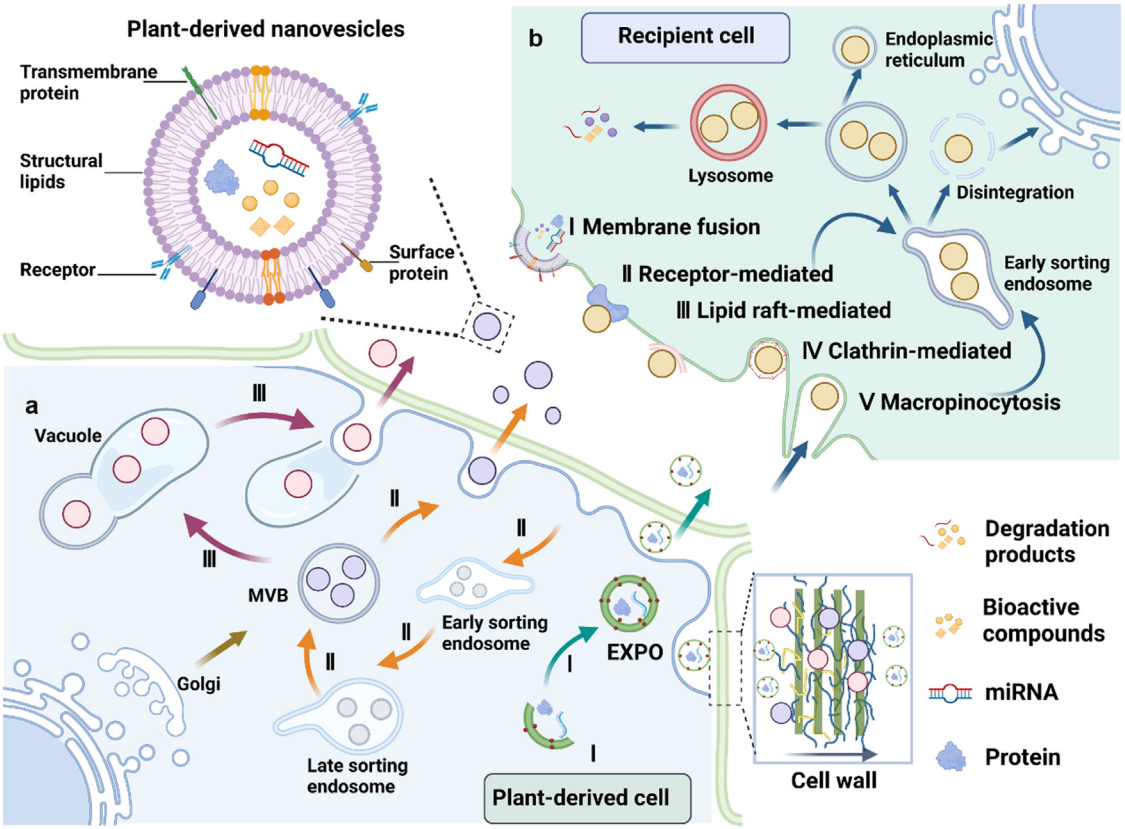

Fig. 1. Biogenesis and uptake process of PDNVs. (a) Biogenesis process of EVs in plant-derived cells. (I) EXPO pathway. (II) MVBs pathway. (III) Vacuolar pathway. (b) Uptake process of ELNs in recipient cells. (I) Membrane fusion. (II) Receptor-mediated endocytosis. (III) Lipid raft-mediated endocytosis. (IV) Clathrin-mediated endocytosis. (V) Macropinocytosis. This figure is created with biorender.com.

rate apoplastic vesicles from transgenic GFP-PEN1 plants [40]. However, since current isolation and purification methods often result in cell rupture, leading to extracted ELNs instead of EVs, we choose to summarize the isolation and characterization techniques for ELNs (Table 1). Differential centrifugation remains the most popular and reproducible technique for isolating PDNVs. It offers advantages such as removal of large plant tissues and cellular debris, high yield with low risk of contamination, as well as suitability for large sample sizes [41]. Additionally, selection of appropriate speed, time duration, and rotor type depends on different plant sources and desired particle size range [42]. Density gradient centrifugation is frequently combined with differential centrifugation for additional purification purposes [19,32,43], with sucrose gradient methods being widely used. This approach provides high separation efficiency with minimal distortion rates. However, introduction of a density medium can interfere with subsequent tests, making it unsuitable for large-scale applications [19,32]. Ultracentrifugation is a reproducible technique that requires minimal consumables and has negligible impact on ELNs [44]. Co-precipitation method, although simple and high-yield, exhibits low-purity and incurs significant expenses. Moreover, there are other emerging technologies such as electrophoresis with dialysis [45], Aqueous Two-Phase System [46], combined size exclusion chromatography with ultrafiltration for achieving high yields and purity [47], or field flow separation techniques in combination with other methods for precise separation of PDNVs and functional studies of them are desired.

3.2. Characterization methods

3.2.1. Physical characterization of PDNVs

By performing physical characterization, it is possible to ascertain the successful extraction of PDNVs and evaluate whether certain modifications preserve membrane integrity. Particle size distribution, surface charge, and morphology are usually physical characteristics for assessing PDNVs. Standard methods include nanoparticle tracking analysis (NTA), dynamic light scattering (DLS), and electron microscopy (EM). DLS is suitable for identifying single-component particles and enables rapid measurement of their average hydrodynamic diameter [53,54], but its resolution is poor [55]. NTA, an advanced particle tracking method, provides data on particle size and concentration based on the random Brownian motion of nanoparticles in solutions [39]. In comparison to DLS, NTA can obtain concentration data with more excellent resolution while reflecting the actual state of particles and being less susceptible to high-intensity scattering from large particles [2]. It is suitable for measuring particle size and concentration in complex multiphase systems. For example, the average size of cabbage-derived PDNVs obtained through different extraction methods was determined using NTA to be 148.2, 134.2, and 98.8 nm, respectively. Then, by combining NTA with a standard colorimetric protein assay, the purity of cabbage-derived PDNVs was measured to be 10×10^9, 0.242×10^9, 0.432×10^9 particles μg^{-1} protein [56]. PDNVs isolated from six species of *Allium* were measured using NTA, revealing particle sizes ranging from 113 nm to 153 nm [57]. In addition, the Zetasizer Nano is also commonly utilized for measuring the potential of nanoparticles [32], where most of the PDNVs exhibited potentials within the range of −20 to −40 mV.

EM is another standard method for measuring PDNVs, including transmission electron microscopy (TEM), scanning electron microscopy (SEM) and cryo-electron microscopy (Cryo-EM). The morphology and interior structure of particles can be seen by TEM [19], while SEM provides surface structural information [41]. In addition, TEM offers superior resolution [58], but the sample must be dehydrated and fixed before measurement to prevent distortion. PDNVs isolated from six species of *Allium* were observed to have suitable spherical particles by SEM, and

Table 1
Comparison of PDNVs isolation techniques.

Isolation methods	Mechanisms	Advantages	Limitations	Ref.
Differential centrifugation	Differences in density between PDNVs	• Low pollution risk • Suitable for extensive sample processing	• Loss of a large number of nanovesicles • Retention of protein aggregates contaminates EVs • Low recovery • Lengthy duration	[19,48]
Density gradient centrifugation	Differences in density between PDNVs	• High purification • Separation of a subpopulation of exosomes	• Limited preparation scale • Low volume processability • High equipment requirement • Lengthy duration	[19,49]
Ultrafiltration	Differences in the size and molecular weight of PDNVs	• Low-cost equipment • Fast running time • Great portability	• Moderate purity • Possible deformation and breaking up of PDNVs caused by force • Clogging and contamination of filter membranes • Low yields	[39]
Immunoaffinity enrichment	Specific interaction between membrane-bound antigens (receptors) of PDNVs and immobilized antibodies (ligands)	• Excellent for the isolation of specific PDNVs and their isoforms • Great enrichment • Simple operation	• High cost • Commercial antibody-dependent • Low capacity • Complexity of mass production	[50]
Polymer precipitation	Reducing EVs hydration to cause precipitation	• Simple operation • Low equipment requirement • Suitable for both small and large sample volume • High efficiency	• Contaminants of protein aggregates, other EVs, and polymeric contaminants • Extended processing time • Complicated clean-up steps required • Lack specificity • Downstream analysis and quantification influenced	[49,51]
Size-exclusion chromatography	The size difference between PDNVs and other particulates for isolation	• High purification • Wide variety of eluents • Fast preparation • Keep the integrity and biological activity of exosomes • Great reproducibility • Capable of handling all types of samples	• Lengthy duration • Difficulty in scaling • Dedicated equipment required • An additional method for exosome enrichment required • Lack of specificity	[49,52]
Field flow separation	Differences in the size and molecular weight of the sample in the vertical direction and the differences in flow rate in the horizontal order for the separation	• Lower system pressure • Fewer shear effects • Less effect on PDNVs • Broad separation range	• Fractionation equipment required • Lengthy duration	[50]

their membrane structure was further observed by TEM [57]. In contrast, Cryo-EM does not require fixation and staining, but it requires analysis at extremely low temperatures s to maintain sample integrity [59]. SEM and TEM images depict cup-shaped EVs and ELNs, whereas Cryo-EM images show them as nearly spherical structures [60]. In addition to the above, atomic force microscopy (AFM) exhibits superior imaging capability and resolution in mechanical measurements; it can also be employed to improve understanding of the structural and size-related aspects of certain ELNs and provide 3D surface information without preprocessing [61,62].

3.2.2. Biochemical analysis and biomarker confirmation

The biochemical analysis of PDNVs is indispensable. Analyzing the composition of PDNVs, including proteins, lipids and nucleic acids, constitutes the initial step in elucidating PDNVs' properties. Moreover, a comprehensive comprehension of the chemical composition, physical structure, and pharmacological effects of PDNVs will enhance their utilization as exceptional drug delivery vehicles and potent drugs with optimal efficacy. Currently, broad-spectrum analysis of the biomolecules of PDNVs has only been reported for a few plant species, and more proteins or lipids need to be markers.

3.2.2.1. Lipids

In the study of lipids contained in *Arabidopsis*, total lipids were extracted from EVs in *Arabidopsis* leaves for analysis, revealing a significant amount of sphingolipids. The researchers then examined the relative quantities of the four types of EVs sphingolipid and found that glycosylinositolphosphoceramides constituted 99.9% of the total. These lipids play an essential role in plant EVs formation, signaling within EVs membranes, and distinguish EVs from other cell membranes [63].

During lipidomic analysis, distinct differences were observed in the lipid species and content of various ELNs. ELNs typically have higher concentrations of phosphatidic acid (PA), phosphatidylcholines (PC), phosphatidylethanolamine (PE), monogalactosyldiacylglycerol, and digalactosyldiacylglycero in their exosomal membranes. Teng and colleagues [64] examined comparative lipid profiles produced by mass spectrometry analysis. They discovered that PC was the predominant lipid in GPDNs and GaELNs, while PA was enriched in GDNs and turmeric-derived ELNs. PA facilitated the internalization of GDNs by specific intestinal bacteria *Lactobacillus rhamnosus* GG (LGG), while PC correlated with the uptake of intestinal Ruminococcaceae. Another study demonstrated that GDNs selectively absorbed *Porphyromonas gingivalis* through heme-binding protein 35 in a PA dependent manner [65].

Choline obtained from PC facilitated the translocation of ELNs from the intestine to the liver and protected the large intestine's cell wall by forming cell clumps in the cell membrane [64]. The most abundant lipid in grape-derived ELNs is PA, which plays a role in cell proliferation and signal transduction. It is followed by PE, which regulates the curvature of cell membranes and is essential for cell division and fusion [4].

3.2.2.2. Proteins

Relatively limited proteomic analyses of PDNVs are currently available. Comparing analysis of proteins in apoplastic EVs derived from olive pollen grains [66], *Arabidopsis thaliana* leaves [40], sunflower in seedling [30], *Nicotiana benthamiana* leaves [22], ELNs derived from squeezed lemons [67] and citrus species [67], high-speed blended grapefruits [31] and pressed grapes [4] has been conducted. Researchers have identified 5 protein families frequently found in plants [1]: heat shock protein 7, S-adenosyl-homocysteinase, glyceraldehyde 3 phosphate dehydrogenase, glutathione S-transferase, and annexin families, which are also present in MDEs proteins. Interestingly, the existence of aquaporins have been discovered in citrus [68], grape [4], and broccoli-derived PDNVs [69]. Furthermore, it has been demonstrated that the aquaporin family in broccoli is associated with the stability of PDNVs. Referring to the proteomics database, Yu et al. identified various proteins in GaELNs, such as plasma membrane proteins like ATPase and pleiotropic drug resistance protein, and cytoplasmic proteins such as lattice proteins with heavy and light chains, which supported the vesicle-like properties of GaELNs [57].

Proteins are frequently employed as biomarkers to identify whether PDNVs have been successfully extracted. However, the absence of particular markers in plants poses significant challenges for their identification. Correspondingly, it is imperative to discover specific markers for PDNVs and develop commercialized antibodies for PDNVs study. The syntaxin PEN1, the ABC transporter PEN3, and the Tetraspanin-8 (TET8) exhibit potential as biomarker candidates [40,70]. Syntaxin PEN1 is present in both *Arabidopsis* and *Nicotiana benthamiana* proteomes, with western blot analysis demonstrating its highest abundance in native EVs [40,71]. Although PEN3 has been identified in the *Arabidopsis* natural EVs proteome, its detection by western blot has yet to be investigated. TET8 shares a structural resemblance with mammalian tetraspanin CD63, and therefore it is proposed as a genuine marker for a subtype of MDEs [17,71-73].

3.2.2.3. Nucleic acids

Through gel electrophoresis, Teng's team revealed a large number of small-sized RNAs (less than 300 nucleotides) in GDNs. As an RNA transport vehicle, PDNVs possess several advantages over synthetic nanoparticles, including high cellular internalization, gastrointestinal stability and low immunogenicity [19]. Orange-derived EVs (OEVs) loaded with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) mRNA were developed as vaccines and demonstrated the ability to induce humoral and cellular immune responses after delivery to target cells [74]. Recent studies have highlighted the extensive involvement of PDNVs in cross-regulation of miRNA, which not only relates to the defense process of plants but also involves the regulation of related pathophysiological processes after PDNVs enter animals or humans. Jin's group [8] discovered that Botrytis cinerea gene expression was silenced due to small RNAs (sRNAs) transported into the fungus by EVs released from host *Arabidopsis* cells. Peng et al. conducted a study to identify miRNAs in various ginseng-derived ELNs (GSDNs) batches, revealing that 44 of these miRNAs aligned completely with the ginseng genome database [75]. Subsequently, upon uptake of GSDNs by bone marrow-derived mesenchymal stem cells (BMSCs), it was observed that 20 out of the 59 miRNAs present in BMSCs originated from GSDNs. Furthermore, the treatment of GSDNs resulted in the up-regulation of the phosphatidylinositol 3-kinase signaling pathway, thereby inducing the differentiation of BMSCs. Using TargetScan prediction analysis, Xiao' team [35] discovered the existence of miRNAs in 11 distinct PDNVs and discovered several highly expressed miRNAs controlling the expression of mammalian genes associated with inflammation and tumor response. In another research, Zhou et al. [76] found that honeysuckle decoction contains miR2911, which directly targeted a variety of influenza viruses (H1NI, H5N1, and H7N9) after oral administration in mice, effectively repressing virus replication.

Active small molecular components of homologous plants have also been detected in PDNVs, such as naringin and naringenin in GPDNs [31], citric acid and limonin in lemon-derived ELNs (LELNs) [77], shogaol and capsaicin detected in GDNs [32], ascorbic acid in strawberry [78], and so on. Some active molecular compounds have also been found in TCM, such as ginsenoside Rg3 [9] in GSDNs, flavonoids, and polyphenols in tea leaves-derived ELNs [79], and curcumin in turmeric-derived ELNs [80]. These findings indicate that the delivery of PDNVs is accompanied by the transport of small molecule compounds, but their exact location in PDNVs and efficacy remains unclear.

4. PDNVs as nanotherapeutics

4.1. PDNVs derived from edible plant sources

Recently, PDNVs have shown remarkable potential as clinical biotherapeutics and drug carriers due to their desired morphologies, ease of large-scale production, and inherent therapeutic effects in treating specific diseases. Researchers have extracted PDNVs from grape, lemon, cabbage, and so on. They are exploited therapeutically as natural products to treat different diseases (Table 2).

Growing evidence indicates that PDNVs possess anti-inflammatory properties and are involved in various mechanisms. (I) Blueberry-derive ELNs (BELNs) achieve anti-inflammatory effects by regulating genes implicated in inflammatory responses and inhibiting the formation of reactive oxygen species (ROS) generated by tumor necrosis factor (TNF) [81]. (II) GaELNs can dose-dependently repress pathways downstream of nucleotide-binding domain and leucine-rich repeat-related family, pyrin domain containing 3 (NLRP3) inflammasome activation [57]. Both cabbage [56] and GDNs [19] exhibit anti-inflammatory activity through inhibiting the expression of pro-inflammatory factors interleukin IL-6 and IL-1β, while GDNs increase the expression of anti-inflammatory factors (IL-10, IL-22) at the same time [19]. (III) Mu's team [82] showed that intestinal Wnt/TCF4 activation and macrophage nuclear factor (erythroid-derived 2)-like 2 (Nrf2) nuclear translocation are critical anti-inflammatory responses induced by carrot-derived ELNs. (IV) Broccoli-derived ELNs enhance adenosine monophosphate-activated protein kinase (AMPK) signaling pathway activation, potentially reinforcing the induction of AMPK-activated anti-inflammatory factors [83].

In terms of intestinal structure restoration, grape-derived ELNs [4] and GDNs [84] have shown remarkable therapeutic effects. They promote the multiplication of intestinal stem cells and contribute to the remodeling of intestinal tissue, thereby treating colitis. Corn-derived ELNs induce the release of TNF-α and activate immune cells, exerting inhibitory effects on colon26/fluc cells multiplication through direct and indirect mechanisms [33]. With regard to anti-oxidative stress modulation, BELNs maintain cellular redox balance by suppressing reactive oxygen species (ROS) production while positively regulating heme oxygenase (decycling) 1 and nuclear respiratory factor 1 to restore antioxidant capacity [81].

Furthermore, research on PDNVs in tumor treatment has also been expanded. LELNs induce ROS generation, leading to upregulation of growth arrest and DNA damage 45α protein expression, resulting in S-phase arrest of gastric cancer cell cycle as well as concentration-dependent induction of apoptosis [45]. *Momordica charantia*-derived ELNs (MC-ELNs) inhibit the proliferation of oral squamous cell carci-

Table 2
PDNVs as biotherapeutics to treat a variety of disorders.

Plant origin	Disease	Components	Functions	Mechanisms	Outcomes	Ref.
Grape	DSS-induced colitis	PA, PE, miRNA	Remodeling intestinal tissue, anti-inflammation	Target $Lgr5^+$ intestinal stem cells, enable intestinal epithelial cell proliferation, and directly promote the generation of stem cell-like organs.	Regulate the renewal process of intestinal tissues.	[4,92]
Grapefruit	DSS-induced colitis	PE, PC, naringin	Anti-inflammation, anti-oxidant	Upregulate heme oxygenase-1 (HO-1) expression and inhibit IL-1β and TNF-α production in intestinal macrophages.	Attenuate inflammatory responses.	[31]
	Melanoma	Organic acids, amino acids, others	Anti-cancer	Promote cell cycle arrest, downregulate cell cycle proteins B1 and B2, and upregulate CDKN1.	Reduce the invasiveness of melanoma, which holds significant promise for limiting the likelihood of metastasis in cancer treatment.	[93]
	Wound healing	Proteins	Anti-oxidant	Increase cell viability and migration while dose-dependently decreasing intracellular ROS generation in HaCaT cells.	Accelerate and improve the wound healing process.	[46]
Ginger	Alcohol-induced liver damage	Lipids, 6-shogaol	Anti-inflammation	Induce the expression of Nrf2, inhibit the production of ROS and LPS-induced inflammation by TLRs mediated pathway.	Protect mice against alcohol-induced liver damage.	[32]
	IBD	6-gingerol, 6-shogaol	Anti-inflammation	Block the production of destructive pro-inflammatory cytokines and enhance the generation of pro-healing anti-inflammatory cytokines.	Reduce acute colitis, improve intestinal healing, and defend against chronic colitis and cancer linked to colitis.	[19]
	SARS-CoV-2 Nsp12-induced lung inflammation	microRNAs (miRNA aly-miR396a-5p)	Anti-inflammation	Aly-miR396a-5p- and rlcv-miR-rL1–28–3p-mediated suppression of Nsp12 and spike genes expression.	Inhibit SARS-CoV-2-induced cytopathic effects and are a potential therapeutic agent for COVID-19.	[91]
	Periodontitis	PA, miRNAs	Anti-inflammation, immunomodulation	Interact with hemoglobin-binding protein 3 on the surface of *P. gingivalis* to target *P. gingivalis* and inhibit virulence factors expression.	Decrease *P. gingivalis*-induced alveolar bone loss and affect the immune response.	[65]
Ginseng	Melanoma	DGMG, PE, Cer, proteins	Anti-cancer	Induce M1-type macrophage polarization via TLR-4 and MyD88 signaling pathways, promote ROS production, and increase M1-related cytokines and chemokines.	Utilize an immunomodulatory effect on mouse macrophages to reduce tumor development.	[9]
Broccoli	DSS-induced colitis	Sulforaphane	Anti-inflammation	Increase the expression of IL-10, reduce the IFN-γ, IL-17A, and TNF-α in colon tissue, and mediate activation of AMPK in dendritic cells.	Play a critical role in maintaining intestinal immune homeostasis through communication with gut dendritic cells.	[83]
Lemon	Cancer	Proteins	Anti-cancer	Increase the pro-apoptotic gene's expression, inhibit the anti-apoptotic gene's expression, activate TNF-related apoptosis-inducing ligand-mediated apoptotic cell process, and inhibit the secretion of pro-angiogenic factors.	Inhibit CML tumor growth *in vivo*.	[67]
	Gut microbiota	Polysaccharides	Improvement of probiotic function	Enhance the bile resistance of LGG by limiting the production of Msp1 and Msp3, thereby reducing the accessibility of bile to the cell membrane.	Protect gut bacteria from bile damage.	[91]

(*continued on next page*)

Table 2 (*continued*)

Plant origin	Disease	Components	Functions	Mechanisms	Outcomes	Ref.
Carrot	Myocardial infarction, PD	Proteins	Anti-oxidant	Inhibit oxidative stress-induced apoptosis by effectively inhibiting the reduction of antioxidant proteins.	Suppress oxidative stress levels in models of myocardial infarction and PD.	[94]
Cabbage	–	Proteins	Anti-inflammation	Inhibit the production of pro-inflammatory molecules in cells and the activation of caspase-3.	Inhibit inflammation and apoptosis.	[56]
Garlic	HFD induced obesity	PA	Anti-inflammation	Suppress the synthesis of a variety of inflammatory cytokines and regulate the IDO1-AHR signaling pathway.	Inhibit systemic and brain inflammation and reverse obesity generated by a high-fat diet in mice.	[95]
Garlic chive	Acute liver injury	Lipids	Anti-inflammation	Inhibit the NLRP3 inflammasome.	Attenuate NLRP3 inflammatory vesicle-mediated inflammation in chemically induced acute liver injury.	[57]
Momordica charantia	OSCC	microRNA	Anti-cancer, anti-inflammation	Induce S-phase cell cycle arrest and death, downregulate NLRP3 expression, and reduce 5-Fluorouracil resistance in OSCC.	Exert a significant synergistic therapeutic impact of 5-Fluorouracil against OSCC both *in vitro* and *in vivo*.	[85]
Edible tea flower	Breast cancer	EGCG, EC, ECG	Anti-cancer	Reduce metastatic breast cancer by regulating ROS production and microbiota.	Accumulate at breast tumor and lung metastasis sites, prevent growth and metastasis of breast cancer and modulate intestinal microbiota.	[43]
Turmeric	Ulcerative colitis	Curcumin	Anti-inflammation, pro-resolving bioactions	Regulate the expression of pro-inflammatory cytokines and antioxidant gene HO-1β.	Accelerate the regression of colitis.	[80]
Asparagus cochinchinensis	Liver cancer	Polysaccharide	Anti-cancer	Inhibit the proliferation of Hep G2 cells.	Inhibit tumor growth without side effects.	[96]
Apple	Gut microbiota	miRNA	Intestinal function	Modulate expression of OATP 2B1 in Caco-2 Cells.	Affect intestinal function.	[97]
Strawberry	–	Vitamin C	Anti-oxidant	Improve adipose-derived mesenchymal stem cells' survival and reduce ROS levels.	Produce anti-oxidative stress effects.	[78]
Watermelon	Fetal growth restriction	microRNA, proteins	Regulation of intestinal secretion groups	Modify the intestinal secretome and bioinformatic analyses when actively absorbed by human intestinal epithelial cells *in vitro*.	Modify intestinal communication with distal tissues, including the placenta.	[98]
Petasites japonicus	–	–	Immunomodulation	Induce dendritic cells maturation through increasing expression of surface molecules, producing Th1-polarizing cytokines and antigen-presenting ability.	Trigger the activation of the adaptive immune response.	[58]
Mulberry bark	DSS-induced colitis	Protein, lipid, RNAs	Protection against DSS-induced colitis	Promote heat shock protein family A member 8 -mediated activation of the AhR signaling pathway.	Protect against colitis in a mouse model.	[99]
Nut	Obesity	Lipids, miRNAs	Anti-inflammation	Reduce Tnfrsf1a protein and dampen the TNF-α signaling pathway in adipocytes.	Attenuate cellular inflammation and reverse insulin resistance.	[100]
Oat	Brain inflammation	Proteins, PC, DGDG, polysaccharides	Anti-inflammation	Selectively taken in by brain microglia and protect neuronal cells from microglia-mediated brain damage.	Improve brain memory function in alcohol-fed mice.	[13]

(*continued on next page*)

Table 2 (*continued*)

Plant origin	Disease	Components	Functions	Mechanisms	Outcomes	Ref.
Tartary buckwheat	Gut microbiota	miRNA	Modulation of gut bacteria	Target functional genes in *Escherichia coli*.	Promote the intestinal microorganisms' ability to absorb and utilize nutrients, and serve as a possible nutritional factor to controlling the host's intestinal health.	[101]
Shiitake mushroom	Fulminant hepatic failure	RNA, proteins, lipids	Anti-inflammation and protection against acute liver injury	Suppress the activity of the NLRP3 inflammasome.	Protect mice from acute liver damage caused by GalN/LPS.	[102]

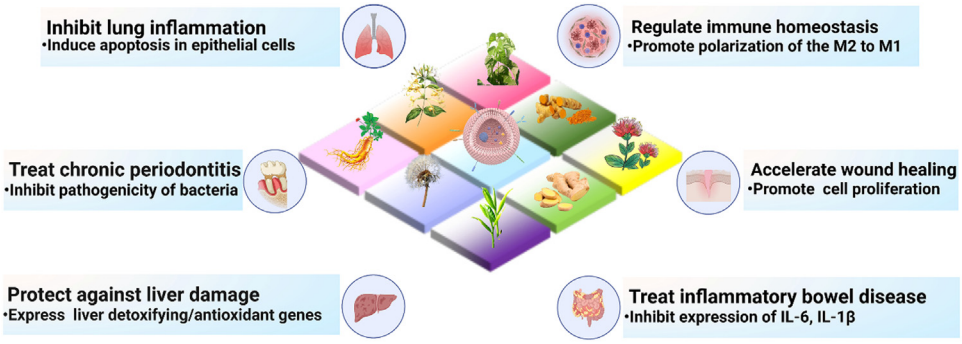

Fig. 2. Applications of PDNVs in the treatment of various disorders, including inhibiting lung inflammation, treating chronic periodontitis, protecting against liver damage, regulating immune homeostasis, accelerating wound healing and treating IBD. This figure is created with biorender.com.

noma (OSCC) and trigger apoptosis via ROS generation [85]. In addition, MCELNs can be internalized by 4T1 breast cancer cells to induce apoptosis and inhibit tumor growth by stimulating ROS production and disrupting mitochondria [86]. Cao et al. used cation-free kiwi-derived EVs (KEVs) to deliver the gene drug Signal Transducer and Activator of Transcription 3 interfering RNA (siSTAT3), exhibiting EGFR-targeting ability [87]. Tail vein injection of this vesicle inhibited EGFR overexpression in nude mice while suppressing tumor growth through STAT3-mediated apoptosis.

4.2. PDNVs derived from herbal medicine sources

As a valuable asset of China, TCM has been used in treating diseases for thousands of years. In recent years, PDNVs from TCM have also been extensively developed. Scholars have embarked on studying PDNVs from traditional Chinese herbal medicine and have now successfully extracted them from ginseng [9], PGY [10], HJT [11], *Lonicera japonica* Thunb. [76], *Andrographis paniculata* (Burm. f.) Nees [11], tea leaves [79], ginger [32], turmeric [80], and so on (Fig. 2). The average particle size distribution and average potential of vesicles are similar to the properties of traditional liposomes and demonstrate superior stability [9,32,40,79].

PDNVs positively influence physiological and pathological processes and hold considerable potential as therapeutic agents [88]. Very distinctive in immunotherapy is the GSDNs, which promote the macrophages' polarization from M2 to M1 and generate ROS to promote mouse melanoma cell death [9]. In addition, the combination of GSDNs and programmed cell death protein-1 monoclonal antibodies altered the cold tumor environment and triggered sustained systemic tumor immunity in multiple mouse tumor models [89]. After presenting the carried mitochondrial DNA to tumor-associated macrophages (TAMs), artemisia-derived nanovesicles (ADNVs) activate the cGAS-STING pathway, induce the polarization of TAMs, and enhance the efficacy when combined with PD-L1 inhibitors [90]. Tea leave-derived ELNs [79] and GDNs can treat inflammatory bowel disease (IBD) [64]. In addition, scholars found that shogaol in GDNs activate a number of liver detoxifying/antioxidant genes and restrain the formation of ROS, providing some protection against alcohol-induced liver damage to some extent [32]. DM-EVs, PD-EVs, TO-EVs, and CO-EVs were isolated from the sap of *Dendropanax morbifera, Pinus densiflora, Thuja occidentalis*, and *Chamaecyparis obtusa*, respectively. It was found that DM-EVs and PD-EVs were harmful to malignant skin tumor cells, while TO-EVs and CO-EVs had no toxic effects on most tumor cells [34].

Interestingly, honeysuckle encodes an atypical microRNA-MIR2911 that exhibits the ability to target the Influenza A virus [76]. Remarkably, MIR2911 conspicuously inhibited viral replication and rescued the weight loss induced by viral infection in mice. Besides, the authors have demonstrated the specific binding of MIR2911 mimetic to SARS-CoV-2 target and the inhibitory effect of exosomes coated with MIR2911 mimetic on SARS-CoV-2 viral multiplication *in vitro*. Other TCMs have also been found to harbor miRNAs capable of targeting SARS-CoV-2, such as GDNs which exhibit expression of miRNAs targeting SARS-CoV-2 through a quantitative reverse transcription-polymerase chain reaction. In a mouse pneumonia model, GDNs microRNAs mitigate lung inflammation induced by exosome^{Nsp12Nsp13}. Nsp12 and spike genes expression is inhibited by GDNs microRNAs, thereby inhibiting the cytopathic consequences of SARS-CoV-2 [91].

5. PDNVs as nanocarriers

Despite the inherent distribution properties of PDNVs, their ability to target the specific lesion site is still limited [103]. Ununiform particle size and inadequate targeting capacity of PDNVs call for further engineering strategies to meet medical requirements. Various bioengineering techniques have been applied to enhance their therapeutic capacity, including standardized loading methods, membrane fusion, sur-

face modification, and responsive material conjunction. In this part, we thoroughly discuss the existing bioengineering strategies for PDNVs to exploit their clinical potential.

5.1. Current bioengineering strategies for PDNVs as nanocarriers

5.1.1. Drug loading methods

Due to the PDNVs' structural similarities with liposomes, hydrophobic and hydrophilic medicines can be effectively loaded into these particles' lipid bilayer or aqueous core. Through passive drug diffusion or rupturing lipid bilayers of PDNVs, small molecules like doxorubicin (DOX) [84], methotrexate (MTX) [31], paclitaxel [24], as well as RNA like siRNA [104] and miRNA [14] have been loaded into PDNVs (Fig. 3a). Co-incubation is the simplest ways to load drugs, but relying solely on simple diffusion may result in poor drug encapsulation rates. For instance, loading DOX into LELNs by co-incubation has reached a loading efficiency of 18.84 ± 0.56% [12]. To achieve higher loading efficiency of DOX, sonication and co-extrusion could be employed to encapsulate DOX into GDNs [84]. However, sonication and co-extrusion may rupture lipid bilayers of PDNVs, resulting in the loss of surface proteins and active ingredients.

In addition, drugs could be chemically coupled to the amino groups on the surface of PDNVs. The reagents EDC and NHS are often applied to activate the reactive group carboxyl. Wang's group [31] employed amino groups on GPDNs to achieve surface coupling of MTX by simple incubation. Through centrifugation after co-incubation, free MTX was isolated, and the coupling of MTX was demonstrated by ultraviolet spectroscopy. Modified GPDNs retained the anti-inflammatory ability of MTX and natural macrophage-targeting properties. Likewise, DOX-loaded heparin-based nanoparticles (DNs) were conjugated to grapefruit vesicles through carboxyl and amino bonding [16]. The conjugates offered a four-fold increase in loading efficiency over encapsulating drugs into PDNVs. Surface chemical coupling could achieve high loading efficiency but requires a specific drug structure and a lengthy incubation period.

5.1.2. Targeting modification strategies

Several studies have shown that different species of ELNs have inherent tissue distributions. DiR labeling is often used to trace the tissue distribution of PDNVs *in vivo*. After oral administration of DiR-labelled GDNs, Zhuang et al. identified fluorescent signals in the liver and mesenteric lymph nodes but not in the spleen and lungs [32]. Confocal microscopic imaging for albumin further confirmed the existence of DiR-labelled GDNs in the liver, indicating that hepatocytes were a crucial target cell for GDNs. However, another research showed that ginger and turmeric-derived PDNVs could remain in the intestine for a long time due to the high level of PA, which was absorbed by LGG in the intestine [64]. The tissue distribution properties of PDNVs originating from the same ginger source exhibit variations, which may be attributed to differences in pathological conditions.

After oral administration, the natural distribution of ELNs is limited to organs such as the liver and intestine. Therefore, it is indispensable to perform engineering modifications to enhance the targeting capability of PDNVs towards the specific lesion site. High-affinity folic acid (FA) receptors are abundantly expressed in tumor cells while being almost negligible in non-tumor cells. Hence, lipophilic FA can be incorporated into the lipid layer of ELNs by mixing with lipid components to enhance the ability to target tumor cells. *In vivo* imaging showed that FA-modified GPDNs were more clustered at brain tumor sites [14]. In addition to FA, cRGD has also been employed for targeted delivery to tumor sites. Integrin $\alpha_V\beta_3$ is highly expressed on both tumor cells and tumor neovascular endothelial cells, and the specific ligand RGD peptide can bind to integrin $\alpha_V\beta_3$ for achieving selective enrichment at the tumor site. Unlike direct surface modification with FA on PDNVs, cRGD can be coupled onto carboxyl groups present on heparin-based nanoparticles and indirectly modified onto ELNs through heparin-based nanoparticles [16]. PEG-ACNVs were obtained by integrating DSPE-PEG into the lipid membrane of *Asparagus cochinchinensis* (Lour.) Merr.-derived ELNs (ACNVs) using PEG engineering technology. PEG-modified PEG-ACNVs cause the escape of ACNVs from the mononuclear phagocyte system, prominently prolong blood retention time and tumor targeting ability, and exert a better therapeutic effect, inhibiting the proliferation of Hep G2 cells and thus inhibiting cell growth [105]. Apart from modifying ligands on the surface of ELNs, leukocyte-derived cell membranes were coated on the GPDNs aiming at ensuring effective targeting capability towards inflammation sites (Fig. 3b, I) [103].

5.1.3. Membrane fusion

In addition to the engineered strategies described above, membrane fusion also imparts a new avenue to enhancing PDNVs' functions. For instance, bacterial outer membrane vesicles (OMVs) derived from E. coli MG1655 are fused with the thylakoid nanovesicles of spinach, resulting in the formation of bacterial-plant hybrid nanovesicles (Fig. 3b, II). On the one hand, thylakoid nanovesicles contain all kinds of enzymes and photosystems that can induce efficient photodynamic effects to release antigens. On the other hand, OMVs serve as an adjuvant to induce dendritic cell maturation. Thus, combining photodynamic therapy and immunotherapy enables bacterial-plant hybrid nanovesicles to achieve better therapeutic effects [106]. With membrane fusion technology, the advantages of membranes from different sources combined in a fusion membrane can yield better results.

5.1.4. Responsive material conjunction

Regarding the transport and release of the drug, achieving precise drug release at the disease site is considered ideal. Responsive release drug delivery platforms can be designed to meet this requirement. Responsive release mechanisms include exogenous stimuli such as light, magnetic fields, ultrasound, as well as endogenous pathological features like pH, enzymes, redox agents, and the like [107] (Fig. 3b, III). However, there are limited examples of responsive release designs for PDNVs. To achieve pH-responsive DOX release, researchers have linked adipic dihydrazide and DOX through a hydrazone bond, which can promote the release of DOX in the tumor microenvironment [16]. Although the designs of responsive drug release in PDNVs are few, references from other drug carriers' responsive-release designs can provide valuable insights [108].

5.2. ELNs inspired nanovesicles

The nanoparticles extracted from ELNs lipids are referred to as ELNs inspired nanovesicles in this study. These ELNs inspired nanovesicles resemble the structure of liposomes without containing water-soluble agents. Compared to ELNs, the ELNs inspired nanovesicles have a more homogeneous particle size. The frequently-used method for extracting ELNs inspired nanovesicles is Bligh-Dyer, followed by a liposome extruder to achieve enhanced homogeneity and uniformity in particle size. For example, during the preparation of GDNs, water and chloroform are sequentially added to native ELNs, and then separated into organic and aqueous phases via centrifugation after layering. Organic phases are then collected, washed, dried, and resuspended in PBS. Ultimately, ELNs inspired nanovesicles pass through a liposome's extruder with a 200 nm polycarbonate membrane [109] (Fig. 3c).

In gene-drug delivery, the cytotoxicity of cationic liposomes makes them challenging to use in the clinical setting [110]. ELNs inspired nanovesicles with improved safety and stability have been developed as carriers for gene drugs such as siRNA [109] and miRNA [14]. Wang et al. conducted a comprehensive comparison between ELN-inspired nanovesicles (GDNs) and liposomes in terms of safety. The MTT experiment demonstrated that GDNs exhibited lower cytotoxicity towards two cell lines, RAW 264.7 macrophage-like cells, and colonic cancer cells, compared to liposomes. Moreover, GDNs maintained the integrity

of the intestinal barrier, while liposomes were toxic to intestinal epithelial cells [109]. In the treatment of colon cancer, GDNs were able to load DOX efficiently and displayed superior pH-dependent drug release profile than commercially available liposomes [84]. Moreover, liposome-like GDNs demonstrated excellent stability, which is associated with the glycolipids-rich GDNs. Glycolipids play a crucial role as stabilizers during freeze-thawing/freeze-drying processes or when encapsulating drugs or siRNA within liposomes [111]. Overall, ELNs inspired nanovesicles exhibit enhanced biocompatibility, greater stability, wider availability, and superior carrier properties compared to traditional liposomes. These hint that ELNs inspired nanovesicles can be used as an alternative to the traditional liposomes.

As for their applications, ELNs inspired nanovesicles retain the lipids of the native ELNs, but inevitably cause the loss of protein, RNA, and other active ingredients, resulting in the absence of certain functions. Thereby ELNs inspired nanovesicles are the most commonly used drug carriers, while native PDNVs are usually used as therapeutic agents [14,84,103]. Furthermore, their small size makes it easy for them to cross biological barriers and enhances the accumulation of nanoparticles at the disease site [112]. A study showed that the particle size of ELNs inspired nanovesicles is smaller and more uniform than native ELNs [24]. On the other hand, Unlike ELNs inspired nanovesicles, engineered PDNVs ensure drug loading while retaining their therapeutic activity. Wang et al. demonstrated that intestinal macrophages selectively ingested GPDNs, which ameliorated dextran sodium sulfate (DSS)-induced colitis in mice by upregulating HO-1 expression and inhibiting IL-1β and TNF-α production. Subsequently, the anti-inflammatory drug was conjugated to the surface of GPDNs in a co-incubation approach, and the conjugates possessed a higher anti-inflammatory effect [31]. However, there are potential difficulties in terms of engineering native PDNVs. Cao's group found that GSDNs significantly reduced their ability to induce macrophage polarization under ultrasound [9]. The causation of this phenomenon may be the loss of active ingredients by membrane deformation of PDNVs. Consequently, the way to avoid losing active ingredients during the engineering process is an issue that needs to be addressed.

6. Engineered PDNVs for treating brain diseases

In recent years, brain disorders, such as brain tumors, psychiatric disorders, and migraines, have seriously damaged human health. The pathogenesis of brain diseases is complex and poses significant challenging. TCM has been extensively employed for treating brain disorders due to its multi-target and efficacy. For instance, *Rheum palmatum* L. has been used to maintain the integrity of the BBB [113], Ziziphi Spinosae Semen regulates the levels of monoamine and amino acid neurotransmitters in the brain [114], and *Ginkgo biloba* L. and *Centella asiatica* (L.) Urba [115] are utilized for treating brain disorders. Nowadays, in response to the call for the modernization of Chinese medicine, an increasing number of technologies are being applied to develop modern therapies for TCM. Nanodrug delivery holds immense therapeutic potential in the treatment of glioma [116]. Encouraged by the advantages demonstrated by TCM-derived PDNVs and edible plant-derived PDNVs mentioned earlier, we also contemplate employing PDNVs for the treatment of brain diseases. However, limited research exists on this front, making it a formidable challenge.

6.1. Diagnosis and treatment of brain diseases

Imaging examinations play a crucial role in the diagnosis of brain diseases, and brain imaging data have garnered significant attention in recent years for studying both brain diseases and functions. Several conventional imaging techniques, including magnetic resonance imaging (MRI), computed tomography (CT), and positron emission tomography (PET), are commonly used in the clinical diagnosis of brain diseases [117,118]. CT images of cranium are preferred for determining whether a stroke is hemorrhagic or ischemic [119]. Unlike CT and X-rays, MRI does not employ potentially harmful ionizing radiation, thereby minimizing its impact on the human body [120]. However, hidden lesions in the brain, such as brain tumor areas with unclear boundaries, can not be displayed through conventional magnetic resonance imaging. The addition of a contrast agent can enhance image observation efficacy. For example, nanovesicles loaded with Gd^{3+} contrast agent exhibit gradually enhanced MRI signals with increasing Gd^{3+} concentration [121].

PDNVs inherit certain advantages of liposomes, MDEs, and polymer-based systems while avoiding some of their shortcomings. The most notable advantage of PDNVs is the ability to cross the BBB, which is recognized as the primary biological obstacle for delivering drugs to the brain. BBB mainly consists of brain microvascular endothelial cells, astrocytes, pericytes, and basement membrane [122]. On the one hand, BBB protects the brain parenchyma from invading pathogens and toxins in the circulatory system. On the other hand, it restricts the action of therapeutic drugs [122,123]. Over 98% of small molecule drugs and almost 100% of large molecule drugs are blocked from the brain by the BBB [61]. The enhanced BBB penetration exhibited by PDNVs improves drug bioavailability [13]. Nanoparticles can cross the BBB through various mechanisms including physical barrier disruption [61], receptor-mediated transcytosis [124], and carrier-mediated transcytosis [125]. In terms of PDNVs specifically, biomimetic GPDNs-DNs penetrate glioma tissues via receptor-mediated translocation and membrane fusion. This significantly enhances cell internalization capability as well as anti-proliferation efficacy while prolonging cycle time [16]. Oat ELNs (OatN) cross BBB via both free diffusion and active transport mechanisms [13]. Meanwhile, PDNVs with high yield, comprehensive sources, reduced immunogenicity [83], and high safety without carrying zoonotic pathogens [37] meet the requirements of green TCM for safety and quality. Additionally, due to their structural similarity with MDEs and liposomes, PDNVs can be easily modified to enhance their bioactivity.

6.2. Administration of PDNVs

The route of administration is particularly essential for the distribution of PDNVs *in vivo*. Wang and colleagues investigated the impact of different injection methods on the distribution of DiR-labeled GPDNs. At 72 h post tail vein or intraperitoneal injection, GPDNs were mainly distributed in liver, kidney, lung, and spleen tissues. In contrast, intramuscular injection resulted in confinement of GPDNs mainly to muscle tissue. Intranasal administration led to the majority of GPDNs being located in the lung and brain [24]. It is interesting to note that nasal administration is a non-invasive route that bypasses the BBB by directly entering the brain through olfactory bulb and trigeminal nerve pathways. Moreover, nasal administration avoids the high risk of infection caused by intraventricular and interstitial injections [126]. Consequently, nasal administration has emerged as a promising strategy for treating brain diseases due to its high targeting efficiency towards the brain, convenient drug delivery method, and rapid onset. Temozolomide (TMZ), which can bypass BBB but suffers from poor brain localization and drug resistance issues when used alone. By coupling TMZ to the surface of gold nanoparticles, the accumulation of nanoparticles in the brain was improved after intranasal administration [127]. Small interfering RNA efficiently silences target gene expression with specificity. Various nano-delivery systems like micelles [128] have been extensively employed for delivering small interfering RNA to the brain. Combined with nasal administration, it has achieved remarkable inhibitory effects against glioma.

Another study demonstrated that tea flower-derived ELNs mainly accumulated in the liver and lungs after intravenous administration [43]. Intravenous administration allows PDNVs to concentrate more effectively at the tumor site, but it may also induce liver toxicity. Oral or intragastric administration leads to preferential accumulation of PDNVs in gastrointestinal organs and the liver for a limited duration, which is particularly advantageous for treating gastrointestinal-related diseases

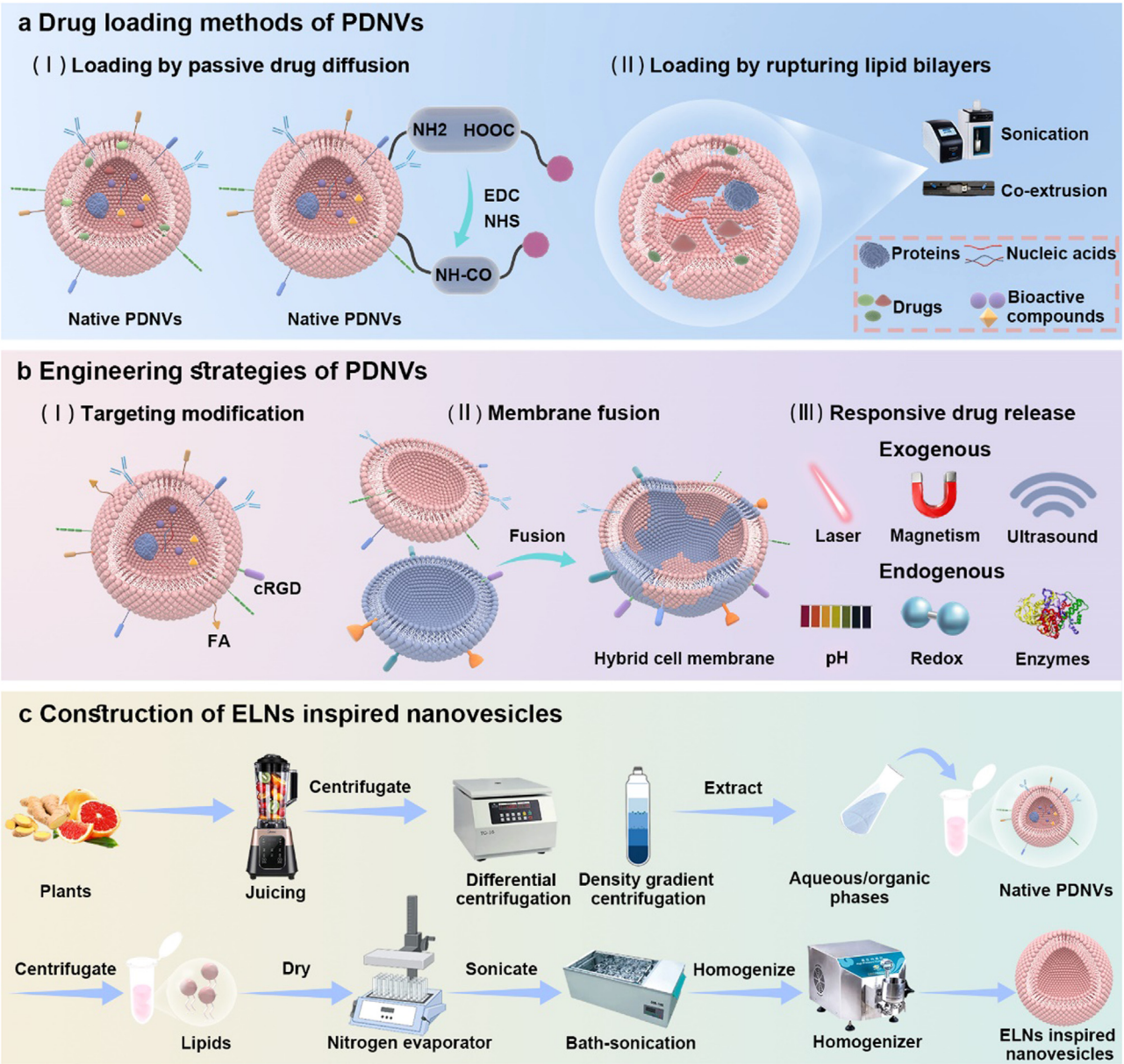

Fig. 3. PDNVs perform as drug delivery nanocarriers. (a) Drug loading methods of PDNVs. (I) Loading by passive drug diffusion. (II) Loading by rupturing lipid bilayers. (b) Engineering strategies of PDNVs. (I) Targeting modification. (II) Membrane fusion. (III) Responsive drug release. (c) Construction of ELNs inspired nanovesicles.

[129,130]. Transdermal administration bypasses the hepatic first-pass effect, offers ease of application, and is commonly employed for dermatologic drug delivery. The diameter of broccoli-derived PDNVs allows passage through the stratum corneum and induces permeability of the plasma membrane of keratinocytes after transdermal administration [131]. Moreover, the presence of Aquaporin dramatically improves stability and holds the potential to be used as a vehicle for transdermal drug delivery. Applying cross-linked gel dressings loaded with CXCL12 and GSDNs at the site of total skin excision would promote skin healing and recruit neural differentiation of BMSCs, which possesses excellent potential in neural regenerative medicine [75].

The main modes of administration in existing strategies for the treatment of brain diseases with PDNVs are intranasal administration, intravenous administration, and oral administration (Fig. 4). Intranasal administration is considered to be a non-invasive administration that bypasses the BBB, allowing high drug accumulation in the brain [24]. Intravenous administration has also been employed for drug delivery. Oral administration has the benefits of convenience, noninvasiveness, and low toxicity [132]. The gut-brain axis serves as a unique mechanism underlying the therapeutic effect of oral administration, which is a bidirectional communication channel connecting the CNS and intestinal nervous system. In addition to intestinal flora, the gut contains a diverse array of hosting abundant nerve cells and immune cells. The gut microbiome produces a range of metabolites, such as dopamine, that indirectly or directly send signals to the nervous system that influence human behavior and mood [133]. Recent studies have linked gut microbiota to many diseases, including NDs, obesity, and depression. Therefore, oral drug delivery via the gut-brain axis has potential in the treatment of brain diseases.

6.3. Applications of plant-derived nanocarriers in various types of brain diseases

6.3.1. Brain tumors

Glioma account for approximately 51.4% of primary brain tumors [134]. In adults, glioblastoma (GBM) accounts for more than half of all

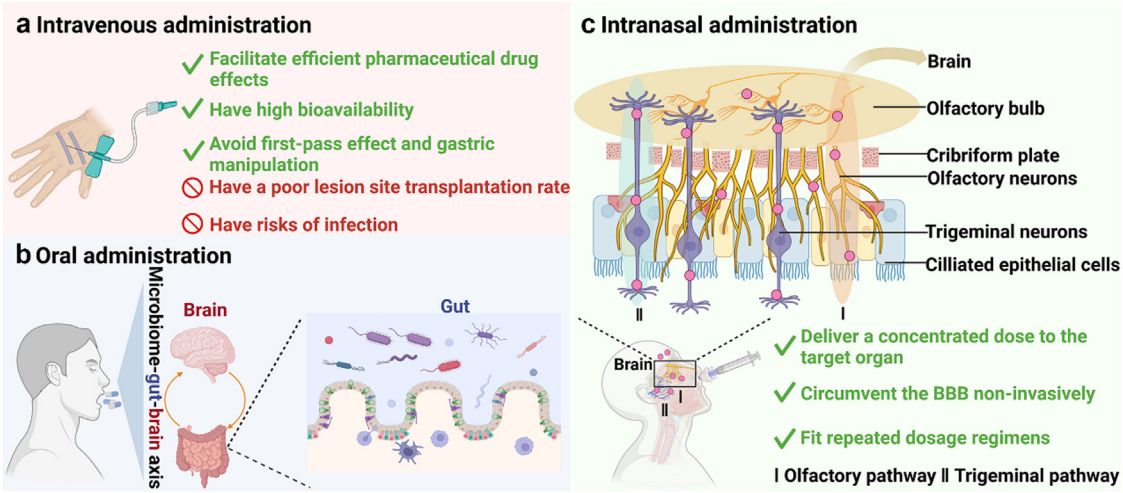

Fig. 4. PDNVs are administered through different routes and cross the BBB into the brain to exert different therapeutic effects. (a) Intravenous administration. (b) Oral administration. (c) Intranasal administration. This figure is created with biorender.com.

gliomas and represents the most prevalent malignant primary glioma [135]. Glial cells, such as astrocytes or oligodendrocytes, are often thought to be the origin of gliomas [136]. A proportion of patients with gliomas (about 5%) are associated with specific genetic syndromes [137], while the remaining cases are sporadic. An essential aspect of glioma pathogenesis is malignant transformation [138]. Malignant transformation is caused by the sequential accumulation of genetic alterations and aberrant regulation of growth factor signaling pathways. Histologically diagnosed GBM is characterized by increased cell density, atypical cell abnormalities, areas of necrosis, and robust angiogenesis [139]. The Cancer Genome Atlas project has improved the characterization of GBM through whole genome sequencing and identified vital oncogenic signaling pathways. Molecular abnormalities required for gliomagenesis include mutations in P53, retinoblastoma (RB) and the receptor tyrosine kinase/ Ras/ phosphatidylinositol 3-kinase (PI3K) / protein kinase B (AKT) signaling pathway [140]. In addition, epithelial growth factor (EGFR) expression is amplified in some GBM, leading to increased cell proliferation via the receptor tyrosine kinase/ Ras/ PI3K/ AKT signaling pathway [141].

Due to the aggressive tumor growth, high heterogeneity, complex oncogenic pathways, and inherent resistance to cell death, low overall survival in patients with malignant glioma and tumor recurrence are inevitable [142–144]. Moreover, the presence of BBB poses significant challenges for drug delivery to the brain. The current standard of care for newly diagnosed GBM involves surgical resection followed by radiotherapy and subsequent temozolomide chemotherapy [142]. It is noteworthy that advancements in nano-drug delivery technologies have enabled more precise targeting and modification, leading to enhanced efficacy [145]. The commonly used nanocarriers for glioma treatment include liposomes, micelles, nanogel systems, and cell membranes. The main nano-strategies for enhancing anti-glioma efficacy are gene therapy [146], immunotherapy [147], photodynamic therapy [148], and tumor therapy fields [143].

The lipid bilayer of PDNVs forms a protective barrier around the encapsulated drugs, enabling drug delivery to target lesions through the BBB. By conjugating adipic dihydrazid to DOX with a hydrazone bond, a pH-sensitive DNs was prepared that promotes the release of DOX in an acidic tumor microenvironment. Wang et al. [16] patched DNs onto the surface of grapefruit EVs to obtain biomimetic EV-DNs, which significantly bypassed the BBB and enhanced drug enrichment at brain tumor sites, resulting in tumor growth inhibition (Fig. 5a-c). In order to reinforce the targeting ability of EV-DNs, cRGD, which has the ability to connect with the integrin v3 receptor in glioma cells, was also conjugated to the heparin's carboxyl group (Fig. 5d, e). Intranasal delivery is often considered a practical, non-invasive approach for delivering therapeutic drugs to the brain. Zhuang et al. [14] developed an FA-coated grapefruit-based nanovectors (GNVs) mixed with polyethyleneimine (FA-pGNVs) for effective intranasal delivery of miR17 to suppress brain tumor growth (Fig. 5f). MiR17 mediates the downregulation of MHC-1 expressed on tumor cells leading to activation of NK cells and inhibition of tumor growth [149]. Odyssey imaging showed that FA-pGNVs/ miR17 had enhanced efficiency in targeting brain tumor cells in a mouse model (Fig. 5g). PDNVs can also exert anti-tumor effects directly as therapeutic agents. *Momordica charantia* has shown therapeutic potential in glioma [150]. Epithelial-mesenchymal transition, a reversible process where epithelial cells transform into mesenchymal cells, plays a crucial role in cancer invasion and metastasis. MC-ELNs could suppress U251 glioma cell migration and invasion by undergoing the epithelial-mesenchymal transition process and down-regulating matrix metalloproteinase 9 (MMP-9). For PDNVs derived from other sources, it is worth investigating whether their anti-tumor properties can also be applied in the treatment of brain tumors. GDNPs, for instance, have demonstrated the ability to modulate M2 polarization both *in vivo* and *in vitro*, thereby facilitating their anticancer effects [9]. Moreover, citrus nanovesicles are internalized by human cancer cells by specifically reaching tumor sites, and trigger cell death by activating the TNF-related apoptosis-inducing ligand/DR5 pathway and preventing the secretion of cytokines implicated in angiogenesis [67].

Unlike PDNVs, researchers have discovered that active ingredients in TCM easily self-assemble into nanoparticles due to their unique structures and modification sites, thereby enhancing therapeutic efficacy, improving biodistribution *in vivo*, and reducing ingredients toxicity [151]. TCM self-assembled nanoparticles also hold potential for treating brain illnesses. Using natural macromolecules to construct drug carriers can effectively avoid the need for chemical reagents, which is advantageous for applications in the biopharmaceutical industry and the promotion of safety [152]. Hollow casein (CA) nanospheres possess tremendous capacity to penetrate cell membranes without causing cellular damage and cytotoxicity [153,154]. The 10-Hydroxycamptothecin (HCPT) was loaded onto a self-assembled nanoparticles system called HCPT-M-CA-NPs using CA as a carrier and menthol as a brain-targeting ligand. It was reported that the ability of drugs to penetrate the BBB and disperse in the brain is enhanced by menthol [155]. Consequently, this system provided efficient and safe brain tumor targeting ability while improving the treatment effect of HCPT [156]. Tanshinone IIA and glycyrrhetinic acid self-assembled into TanIIA-GL nanomicelles (TGM), which were then enveloped by serum exosomes and modified with CpG oligonucleotides to obtain CpG-EXO/TGM nanoparticles [157]. CpG-EXO/TGM

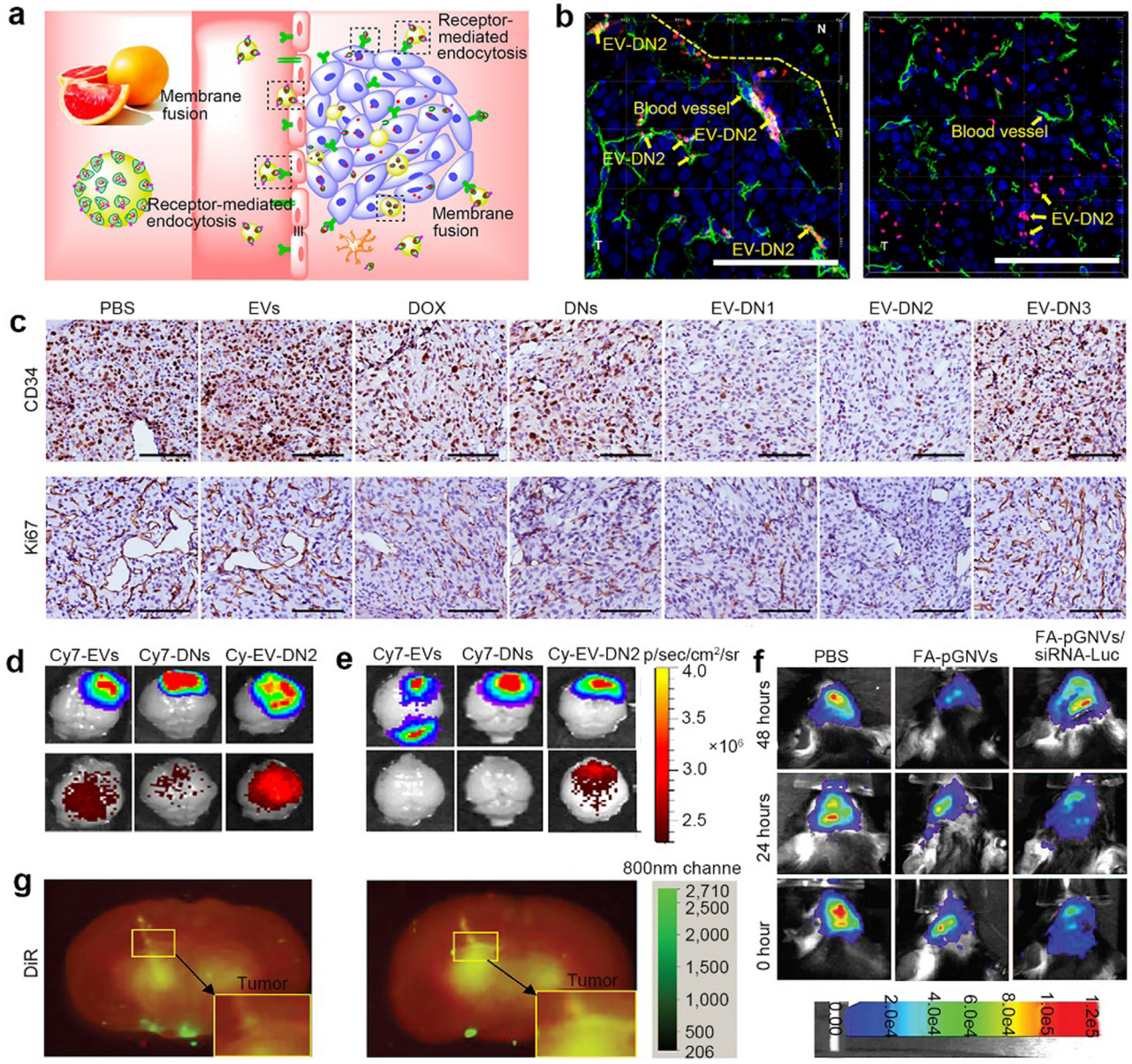

Fig. 5. PDNVs are utilized in the treatment of glioma. (a) Grapefruit EVs-DOX loaded nanoparticles for glioma treatment. (b) The glioma-bearing brain tissues' 3D confocal pictures following intravenous injection of EV-DN2. N and T stand for healthy and tumor tissues, respectively. Scale bar: 100 μm. (c) Various treatment groups' glioma tissues were stained with CD34 to identify endothelial vessels and Ki67 for cell proliferation. Scale bar=100 μm. At 96 h after administration of Cy7-EVs, Cy7-DNs, and Cy7-EV-DN2 (d) without and with (e) perfusion, Cy7 and Luc signals were measured in LN229-luc glioma mice *in vitro*, reproduced from Wang et al. (2021) [16] with permission from American Chemical Society. (f) 6-week-old wild-type B6 mice received intracranial injections of 2×10^4 GL26-luc cells each. Afterward, mice with tumors were given either FA-pGNVs/siRNA-luc or FA-pGNVs/siRNA scramble control intravenously every day. The mice were photographed during the times listed in (f). (g) Imaging data in brain tumor associated photons in DiR^+ FA-pGNVs/miR17-DY547 or pGNVs/miR17-DY54-treated mice, reproduced from Zhang et al. (2016) [14] with permission from Elsevier.

was able to cross BBB through transferrin-mediated endocytosis. Therapeutically, CpG-EXO/TGM stimulated dendritic cell maturation and induced polarization of TAMs, demonstrating promising efficacy against glioma while inhibiting tumor recurrence.

6.3.2. *Neurodegenerative diseases*

NDs, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis, have increased in prevalence as the ageing population. However, lacking accurate biomarkers poses a significant challenge in diagnosing NDs. In addition, effective drug delivery to lesion regions is impeded by the BBB and blood-brain tumor barrier. Reports suggested that the aggregation of mutant NDs proteins such as amyloid-β (Aβ), α-synuclein, and huntingtin in brains plays a pathogenic role in NDs such as AD, PD, and HD, respectively. Notably, levels of tau protein are found to be three times higher in AD patients compared to healthy older adults. Tau protein phosphorylation is thought to be one of the main neuropathological mechanisms in AD [158], disrupting the homeostasis of the nervous system and normal neuronal function, ultimately leading to the development of AD [159].

Even though clinical trials are limited, exosomes have been evidenced to have a natural property to cross the BBB [160]. Three natural compounds, hederagenin, baicalin, and neferine, are encapsulated by neuron-2a cell-derived exosomes to obtain Exo-compounds. These Exo-

compounds exhibited a greater capacity to attenuate the protein levels of P301L tau, huntingtin74, or A53 α-synuclein compared to the individual compounds alone [161]. Apart from the hyperphosphorylation of Tau protein, Aβ deposition remains one of the causations of AD [162]. APP/PS1 double transgenic mice were selected as a classic AD model for studying Aβ deposition in the brain. Exo-neferine demonstrated superior efficacy in reducing Aβ deposition levels in mouse brains when compared with neferine alone. Component-based Chinese medicine (CCM) has also been investigated for its potential role in AD treatment. Xingnaoyizhi CCM, composed of Onjisaponin, icariin, Panax notoginsenosides, and borneol significantly improves the spatial learning ability of the AD rat compound animal model after gavage.

NDs have been found to be associated with oxidative stress. Previous studies indicate that PDNVs can infiltrate mammalian cells and function as cross-kingdom messengers [163,164]. PD is the most prevalent neurodegenerative motor disorder, affecting approximately 1% of the global population aged 65 and above [165]. The motor dysfunction in PD arises from the selective loss of dopamine neurons and their axonal terminals in the dense part of the substantia nigra in the midbrain [166]. The elevation of oxidative stress response in dopaminergic neuron degeneration leads to PD, making it crucial to enhance oxidative stress resistance and reduce apoptosis for effective PD treatment. Carrot-derived nanovesicles (Carex) were characterized by low cytotoxicity and notably restricted neuroblastoma cell apoptosis originating from oxidative stress in cells [94] (Fig. 6a). TEM revealed the spherical morphology of Carex, while NTA was employed to study the size distribution (Fig. 6b and c). Moreover, carex apparently suppressed the decline in Nrf-2, HO-1, and NADPH quinone oxidoreductase-1 (NQO-1) expression, thereby protecting cells from oxidative stress (Fig. 6d-f). In addition, ginseng has a neuroprotective effect with its main active ingredients including ginsenoside Rg1, Rb1, Rd, Rg5, and Rc that enhance brain function, and alleviate various neurological disorders such as neuroinflammation and oxidative stress [167–170]. However, ginsenosides have limited transport capacity through BBB and low bioavailability in the brain, so it is necessary to develop safe and effective drug delivery strategies that guide nanomedicine in diagnosing and treating brain diseases such as PD and AD. In particular, GSDNs successfully promote the neural differentiation of BMSCs via upregulating phosphatidylinositol 3 kinase signaling pathway leading to increased expression of neurotrophic factors and affecting the Ras/Erk pathway. Therefore, GSDNs hold great potential for treating neurological disorders [75].

6.3.3. Other brain diseases

In addition to the two types of brain diseases, PDNVs are also employed in other brain diseases. Brain inflammation is closely associated with the pathogenesis of CNS disorders. Microglia facilitate the shaping of neural circuits via promoting neurogenesis, patterning synaptic connections, and removing apoptotic cells [171,172]. Targeted delivery of therapeutics to microglia poses significant challenges but is essential for effective treatment. Inflammatory cytokines such as IL-1β, TNF-α, and interferon (IFN)-γ are released in response to high-fat diet (HFD) eating, which encourages the death of neuronal cells and brain inflammation. Using a mouse obesity model via the gut-brain axis, GaELNs were transported to the brain for preferential uptake by microglia. This transport inhibited brain inflammation through the IDO1-mediated AHR route and c-Myc-mediated c-GAS/STING inflammatory route, as well as significantly improving memory function [95] (Fig. 7a-d). As a result, in mice given the HFD, brain inflammation and neuron cell death were repressed. Moreover, through a natural oral administration route, OatN could cross the BBB and be internalized by microglia, effectively reducing alcohol-induced brain inflammation and restoring brain memory functions [13] (Fig. 7e-g). In a mouse alcohol use disorder model, OatN prevented alcohol-induced brain inflammation through the β-glucan (BG) mediated hippocalcin pathway (Fig. 7h). It was found that OatN-derived BG directly binds hippocalcin rather than dectin-1 on the microglia membrane, avoiding the activation of inflammatory pathways.

Stroke is the third leading cause of mortality worldwide, yet it remains a major contributor to disability [173]. Oxidative stress has been identified as a significant factor in brain damage following ischemic stroke [174]. Excessive production of ROS disrupts protein synthesis and damages DNA structure, impairs mitochondrial function and energy production, peroxidizes cellular lipids and degrades phosphate, and compromises endothelial cells while increasing blood-brain barrier permeability, ultimately resulting in neuronal cell death [175]. Therefore, strategies aimed at reducing ROS generation or scavenging existing ROS and enhancing antioxidant defense mechanisms represent the primary approaches for antioxidant therapy in stroke. Furthermore, emerging evidence suggests that inhibiting post-stroke inflammatory responses by promoting microglial polarization towards the M2 phenotype can effectively prevent brain damage [176]. A major pathological characteristic of acute ischemic stroke is the disruption of BBB integrity [177]. By destroying tight junction proteins, activated MMPs increase BBB permeability and cause brain injury in stroke patients [178]. MC-ELNs exert neuroprotective effects on ischemic brain injury by means of restraining MMP-9 and activating the AKT/GSK-3β pathway [179] (Fig. 6G). Dil-labeled MC-ELNs were found in the infarcted region; treatment with MC-ELNs prominently improved the destruction of the BBB, diminished the size of the infarct, enhanced the quantity of viable neurons in the CA1 area (Fig. 6H and I), and decreased apoptosis in the MCAO ischemic brains' striatum and hippocampus (Fig. 6J and K). Besides, Qingkailing, a representative drug in CCM, consists of baicalin, geniposide, cholic acid, and Margaritifera Concha at 5:25:7:50. This formulation provides protection to brain tissue after ischemia from three aspects: preservation of BBB integrity, anti-inflammatory activity, and neuronal cell protection.

Depression is a common psychiatric disorder, yet its biological basis of depression remains largely unclear. Its pathogenesis mainly involves synaptic plasticity, oxidative stress, gut flora, hypothalamic-pituitary-adrenal (HPA) axis dysregulation and alterations in neurotransmitter metabolism and neuroinflammation [180,181]. Major depressive disorder (MDD) is also characterized by regional brain volume changes, particularly in the hippocampus, as well as functional modifications in brain circuits [182]. Evidence for a genetic component is provided by studies of twins, with an estimated heritability of 38% for MDD [183,184]. In addition, environmental factors such as sexual abuse, physical abuse or emotional abuse during childhood are thought to influence disease risk. In particular, stressful life events throughout the lifespan play an essential role in the etiology of the disease [185]. A combination of multiple factors is involved in the pathogenesis of depression.

Synaptic plasticity, oxidative stress, gut flora, dysregulation of the hypothalamic-pituitary-adrenal axis, changes in neurotransmitter metabolism, and neuroinflammation are the primary factors in the etiology of depression [180,186]. Ginsenoside Rg3 has been shown to prevent lipopolysaccharide-induced depressive behavior and inflammation-related depressive behavior [187]. Treatment with ginsenoside Rg1 significantly ameliorates depressive-like behavior in mice of depression caused by chronic unpredictable mild stress, and this inhibitory induced via a mechanism that inhibits oxidative stress and neuroinflammation [188]. Previous studies have demonstrated the presence of Ginsenoside Rg3 in GSDNs [9]; thereby, GSDNs have the potential to treat depression. Furthermore, several findings have also provided evidence that depressed patients are often accompanied by gastrointestinal diseases, such as nausea, vomiting, and constipation [189,190]. Since PDNVs contain biologically active molecules such as proteins, lipids, miRNAs, and active ingredients of TCM, and the active ingredients in TCM have antidepressant effects, such as Trans-cinnamaldehyde in *Cinnamomum cassia* [191], Perillaldehyde in *Perilla frutescens* (L.) Britt [192]. Oral administration of PDNVs can improve the intestinal microenvironment, which inspired us to explore whether PDNVs contain active ingredients that can exert antidepressant behaviors and whether PDNVs can be used to treat depression through the gut-brain axis. Additionally, albiflorin has various pharmacological activities, including antioxidant stress [193],

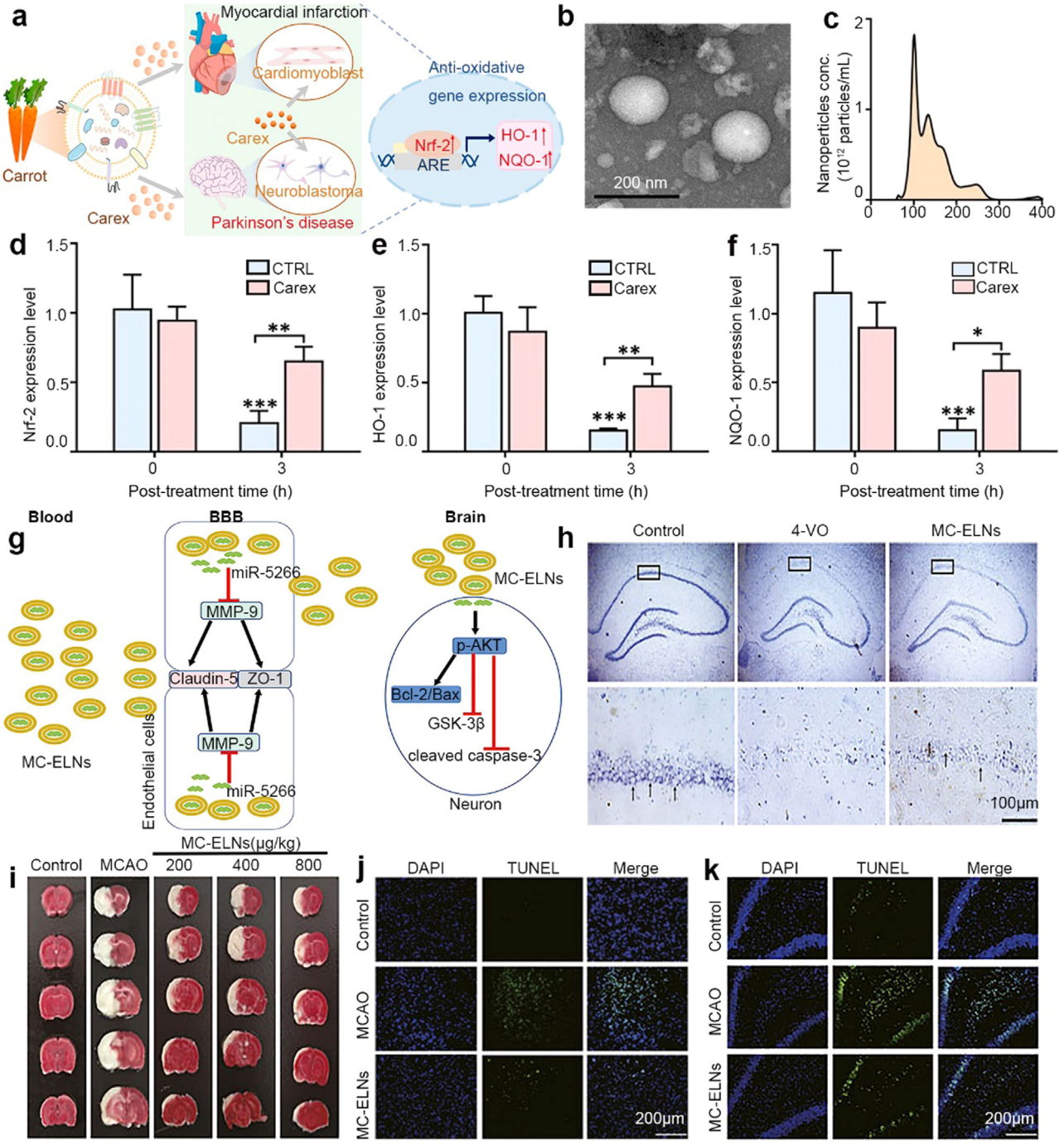

Fig. 6. Applications of Carex and MC-ELNs in the treatment of brain diseases. (a) Illustration of the separation of Carex from carrots and how antioxidative properties and molecular changes are studied in cardiomyoblast and neuroblastoma cells. (b) TEM was used to examine the morphology of Carex. (c) The representative size distribution of Carex was analyzed using NTA. (d-f) Analysis of the levels of Nrf-2 (d), HO-1 (e), and NQO-1 (f) mRNA expression in SH-SY5Y cells after 6-OHDA treatment using RT-PCR. All values are expressed as mean ± SD ($n = 3$); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, reproduced from Rhee et al. (2021) [94] with permission from Multidisciplinary Digital Publishing Institute. (g) MC-ELNs prevent BBB damage from ischemia-reperfusion and prevent neuronal apoptosis, most likely by increasing the activity of the AKT/GSK3β signaling pathway. (h) Representative photos demonstrate the CA1 neurons density in the hippocampal region. The live neurons are denoted by black arrows. (i) The cerebral infarct area in middle cerebral artery occlusion (MCAO) rats treated with MC-ELNs at various concentrations is visible on representative TTC staining images. (j) Images of the ipsilateral striatum stained with TUNEL for each group. (k) Images of the hippocampus DG region stained with TUNEL for each study group, reproduced from Qi et al. (2022) [179] with permission from Frontiers Media SA.

anti-inflammatory effects [194], and neuroprotective effects [195]. An albiflorin nanogel-loaded self-assembled thermosensitive hydrogel system (albiflorin-NGSTH) was constructed, and it had antidepressant effects [196]. Intranasal administration of low doses of albiflorin-NGSTH alleviated depressive behavior, reduced proinflammatory cytokines levels, and repaired neuronal damage in rats subjected to chronic unpredictable mild stress, suggesting a promising depression therapeutic potential.

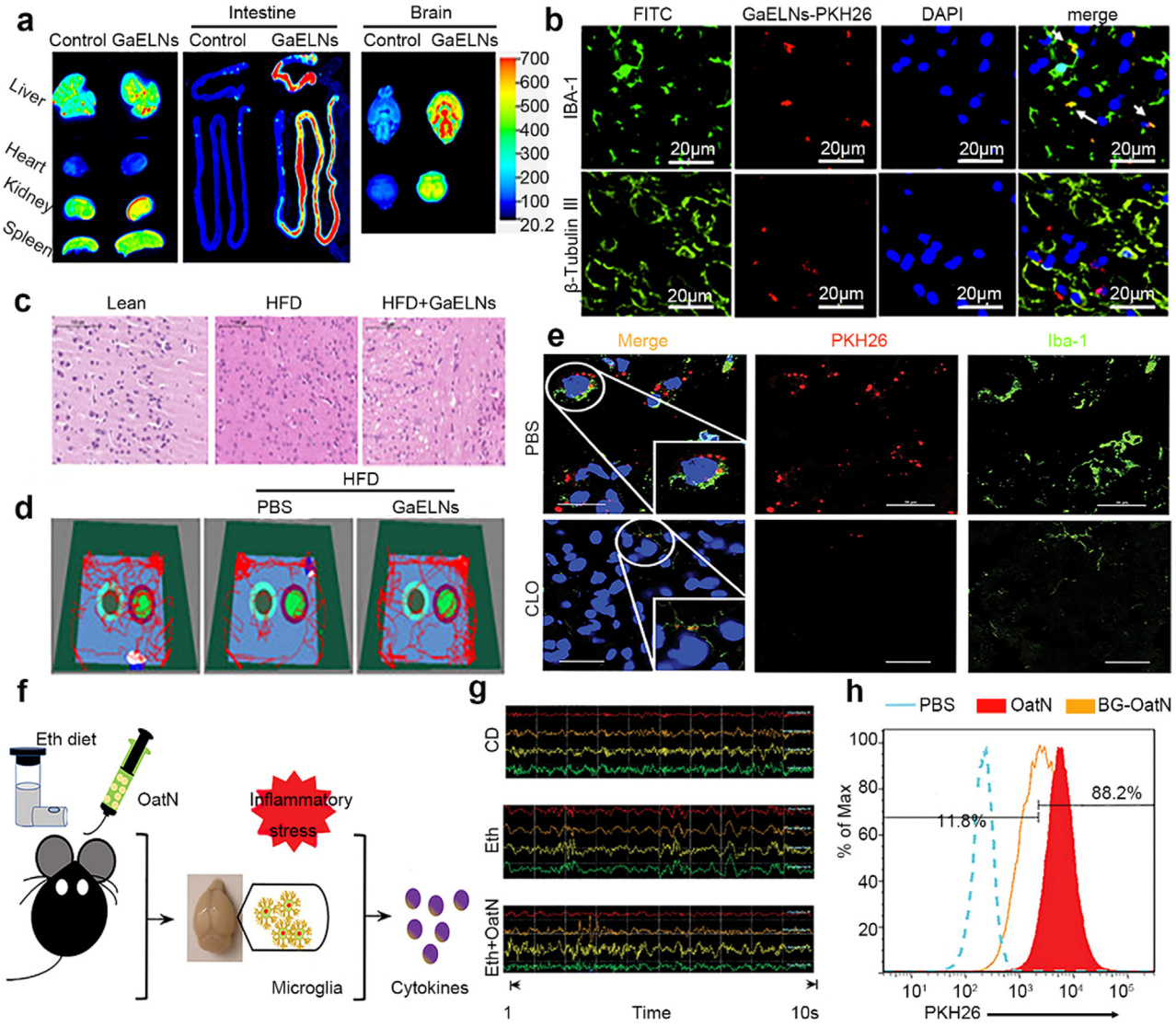

Fig. 7. GaELNs and OatN reduce inflammation in the brain and enhance mouse brain memory. (a) GaELNs (1×10^{10}) that were PKH26 and DiR dye-labeled were orally administered to HFD-fed mice for 24 h, and imaging analysis was used to show the *in vivo* distribution of the GaELNs in several organs. (b) Using confocal imaging, the uptake of GaELNs by brain microglial and neuronal cells was assessed. IBA-1 and β-tubulin III were specific markers for labeling microglial and neuronal cells, respectively. (c) The brain's inflammatory status is shown through brain histological sections. (d) A NORT analysis of movement traces from lean, HFD, and GaELNs treated HFD mice. Illustrations of movement traces from lean, HFD, and HFD mice treated with GaELNs determined by NORT, reproduced from Zhang et al. (2022) [95] with permission from Ivyspring International. (e) Mice were administered clodronate (CLO) through intracisternal injection before receiving PKH26-labeled OatN via gavage. After brain tissue had been sectioned and stained with anti-Iba-1, confocal imaging assays were performed. Scale bars, 20 mm. (f) A diagram depicts OatN biological effects on microglia cells during alcohol-induced chronic brain inflammation in mice. (g) Mice were gavage-fed OatN three times per week for 28 days while fed either a control liquid diet or a control liquid diet containing 5% ethanol (Eth). EEG data were represented by four spots in the mice's brains. (h) Commercially available BG was used to inhibit BV2 cells before PKH26 labeled OatN was introduced to the cell media, reproduced from Zhang et al. (2022). [13] with permission from Wiley.

7. Safety assessment and clinical trials

When PDNVs are applied to disease treatment and before clinical trials, their safety assessments must be addressed. When conducting safety assessments, tumor-bearing mice with corresponding diseases are often used. The commonly used administration methods include oral, intravenous, and intraperitoneal injections. To evaluate the safety of PDNVs *in vivo*, experimental mice are usually given therapeutic doses of PDNVs in different administration methods, and the control group is set. H&E staining is performed after sacrifice and then observed whether there are apparent signs of tissue or cell damage in the organs of various groups [16,80,84]. Blood biochemical analysis can also be performed on mice to observe changes in blood cells, hemoglobin, and platelets [9]; serum alanine aminotransferase and aspartate aminotransferase can be measured to assess the degree of liver damage.

Current preclinical and clinical trials are underway to determine the efficacy and safety of PDNVs. Susan and her colleagues evaluated the safety of exosomes with and without curcumin in patients with IBD and estimated the effects of curcumin on ginger exosomes (NCT04879810). A completed clinical trial investigated grape exosomes' ability to prevent oral mucositis related to chemotherapy and radiation treatment for head and neck cancer. Grape exosomes will be tested for their impact on cytokines, as well as immunological responses to exosomal tumor antigens, metabolism, and molecular indicators (NCT01668849). In addition, the effects of vesicles from *Citrus Limon* (L.) Juice on several cardiovascular factors was studied (NCT04698447), and the therapeutic effects of ex-

osomes from ginger and aloe vera on polycystic ovary syndrome were also investigated (NCT03493984).

Given the current scarcity of clinical trials, it is evident that more clinical trials are needed to advance the application of PDNVs in future clinical treatments. In transforming PDNVs from laboratory to clinical practice, several key issues still need to be addressed: first, the production of PDNVs. Although PDNVs have a wide range of plant sources conducive to large-scale production, there still needs to be standard extraction and separation methods, and purified PDNVs need to meet current good manufacturing practices. Secondly, a standardized naming method must be specified to better define the PDNVs obtained by different extraction methods. Finally, a large number of clinical trials are still needed to reveal the *in vivo* processes related to the absorption, distribution, metabolism, excretion, safety, and adverse reactions of PDNVs in clinical applications.

8. Conclusion

Intensive efforts have been dedicated to developing the applications of PDNVs because of the abovementioned advantages. Due to the variations in original plants, different therapeutic effects are exerted by PDNVs for treating different diseases [14]. Exploring the biogenesis of PDNVs facilitates the study of cross-kingdom regulation. The uptake potential of PDNVs by cells is an essential prerequisite for studying delivery strategies. Improving the isolation and characterization methods is the focus of recent research on upgrading the PDNVs' quality control system. The analysis of lipids, proteins, nucleic acids, and active ingredients in biochemical characterization helps us to study the functions of PDNVs. The diverse content and composition of each component confer rich biological activities on PDNVs as therapeutic agents and nanocarriers. In addition, engineering strategies are essential for PDNVs to perform their function better. This article carefully discusses current modification methods for PDNVs and their corresponding effects.

Nowadays, drug delivery to the brain remains a formidable challenge in the treatment of brain diseases. In this review, we concentrate on the applications of PDNVs and self-assembled nanoparticles of TCM and the mechanisms of their therapeutic effects on brain diseases, such as glioma [16], NDs [94], brain inflammation [13], and stroke [179]. Despite limited examples of PDNV applications in treating brain diseases, we aim to exploit their advantages and explore their potential as an area of interest for further research. Firstly, one of the most significant advantages of PDNVs is their ability to penetrate the BBB [16], which is a critical obstacle hindering the treatment of most brain diseases. Secondly, due to their lipid bilayer membrane structure, PDNVs can serve as carriers for delivering nucleic acids, lipids, proteins, and other small metabolites into the brain. Compared with traditional synthetic nanoparticles such as liposomes, PDNVs have superior biocompatibility and minimal cytotoxicity when used *in vivo* [197]. Thirdly, apart from serving as carriers, PDNVs also possess therapeutic effects themselves. For example, GSDNs promote the polarization of M2 macrophages towards M1 macrophages, the predominant immune cell types in the microenvironment of glioma, indicating the potential application of GSDNs in treating glioma [9]. Oxidative stress and inflammatory response are major contributors of brain injury after ischemic stroke. Recent studies demonstrate that strawberries-derived PDNVs display antioxidant activity without significant toxicity *in vitro* [78], while GPDNs reduce the expression of pro-inflammatory cytokines and chemokines [31]. These findings provide valuable insights for researchers exploring the use of PDNVs in stroke therapy. Furthermore, together with the oral safety and high bioavailability of PDNVs, the drug delivery strategy of absorbing PDNVs orally from the intestine to the brain is worthy of further investigation.

Although research on PDNVs is gradually increasing, the challenges cannot be ignored. Our understanding of the mechanisms of PDNVs' biogenesis, uptake, and function remains limited. The lack of standard separation methods may result in heterogeneity and hinder commercialization. Further investigations are required to assess the stability of PDNVs during preparation and preservation conditions, as well as their potential risk in therapeutic applications. At the same time, the absence of specific markers confines the accurate characterization of PDNVs. Ensuring the activity of engineered PDNVs and modifying them to effectively target distant and challenging tumors without eliciting an immune response necessitate further consideration. While PDNVs are generally considered non-immunogenic and non-toxic, safety concerns arise due to their carrying foreign substances from the human body; thus additional research is needed in this area. Factors such as organic reagents introduced during the extraction and separation process may pose a threat to human health [109]. The metabolism, distribution, absorption processes within the body for PDNVs remain unresolved issues that require investigation. The composition analysis of PDNVs remains incomplete, so it is essential to study their safety. To achieve the clinical application of PDNVs, it is imperative to conduct more comprehensive and long-term safety studies.

In addition, it is essential to acknowledge the dearth of research on the applications of PDNVs in brain diseases, and the translation process from animal experiments to clinical practice poses significant challenges. The intricate pathogenesis of brain diseases presents difficulties in selecting appropriate PDNVs, while limited reports exist regarding their efficacy in traversing the BBB for efficient brain delivery. Nevertheless, they have emerged as promising drug carriers due to their ability to cross the BBB and lower toxicity. PDNVs are structurally similar to liposomes, and their ability to penetrate the skin can also be envisioned as potential delivery nanoplatforms for transdermal vaccination [198]. In conclusion, we have come a long way in understanding the biomedical potential of PDNVs over the past decade, but we have only just begun to scratch the surface of what they can do. Undoubtedly, future research will encounter numerous challenges, but these challenges will eventually be overcome and lead us to new areas.

Abbreviations

Aβ, amyloid-β; AD, Alzheimer's disease; albiflorin-NGSTH, albiflorin nanogel-loaded self-assembled thermosensitive hydrogel system; AFM, atomic force microscopy; AMPK, adenosine monophosphate-activated protein kinase; BBB, blood-brain barrier; BELNs, blueberry-derive ELNs; BG, β-glucan; BMSCs, bone marrow-derived mesenchymal stem cells; CA, casein; Carex, carrot-derived nanovesicles; CCM, component-based Chinese medicine; CDKN1, cyclin-dependent kinase inhibitor 1; Cer, ceramide; CML, chronic myelogenous leukemia; CNS, central nervous system; COVID-19, coronavirus disease 2019; Cryo-EM, cryo-electron microscopy; DGDG, digalactosyldiacylglycerol; DGMG, digalactosyl monoacylglycerol; DLS, dynamic light scattering; DNs, doxorubicin-loaded heparin-based nanoparticles; DOX, doxorubicin; DSS, dextran sodium sulfate; EC, epicatechin; ECG, epicatechin gallate; EGCG, epigallocatechin gallate; ELNs, exosome-like nanovesicles; EM, electron microscopy; Eth, ethanol; EXPO, exocyst-positive organelle; EVs, extracellular vesicles; FA, folic acid; GaELNs, garlic-derived ELNs; GalN, D-galactosamine; GBM, glioblastoma; GNVs, grapefruit-based nanovectors; GDNs, ginger-derived ELNs; GPDNs, grapefruit-derived ELNs; GSDNs, ginseng-derived ELNs; HCPT, hydroxycamptothecin; HD, Huntington's disease; HFD, high-fat diet; HJT, *Rhodiola rosea* L.; HO-1, heme oxygenase-1; IBD, inflammatory bowel disease; IFN, interferon; IL, interleukin; LGG, *Lactobacillus rhamnosus* GG; LELNs, lemon-derived ELNs; LPS, lipopolysaccharide; MCAO, middle cerebral artery occlusion; MC-ELNs, *Momordica charantia*-derived ELNs; MDD, Major depressive disorder; MDEs, mammalian-derived extracellular vesicles; MMP, matrix metalloproteinase; MTX, methotrexate; MVBs, multivesicular bodies; NDs, neurodegenerative diseases; NLRP3, nucleotide-binding domain and leucine-rich repeat related family, pyrin domain containing 3; NQO-1, NADPH quinone oxidoreductase-1; Nrf2, nuclear factor (erythroid-derived 2)-like 2; NTA, nanoparticle tracking analysis; OatN,

oat ELNs; OATP, organic-anion-transporting polypeptide; OMVs, outer membrane vesicles; OSCC, oral squamous cell carcinoma; PA, phosphatidic acid; PC, phosphatidylcholines; PD, Parkinson's disease; PE, phosphatidylethanolamine; PGY, *Taraxacum mongolicum*; PDNVs, plant-derived nanovesicles; ROS, reactive oxygen species; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SEM, scanning electron microscopy; TAMs, tumor-associated macrophages; TCM, traditional Chinese medicine; TEM, transmission electron microscopy; TET8, Tetraspanin-8; TLRs, toll-like receptors; TMZ, Temozolomide; TNF-α, tumor necrosis factor α.

CRediT authorship contribution statement

Ruoning Wang and Liuqing Di conceived and designed this review. Yingjie Zhang, Yumiao Guo and Wei Zeng analyzed the literatures and summarized the results. Jinge Li, Jie Wu, Nengjin Li, Anran Zhu and Jiale Li reviewed and edited this review. Peng Cao, Ruoning Wang and Liuqing Di revised this review. All of the authors have read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no conflicts of interest in this work.

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