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Harnessing the potential of food-derived extracellular vesicles: A paradigm shift in targeted drug delivery and metabolic disease management

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ABSTRACT: The diverse sources and bioactive properties of food-derived extracellular vesicles（FEVs）render them a promising tool for drug delivery and the management of metabolic disease. Despite the growing interest in FEVs, no systematic review has addressed their dual roles in targeted drug delivery and metabolic disease management. Herein, we summarize the latest research progress of FEVs, focusing on their sources and unique properties, and outline their advantages and challenges in the field of drug delivery, including targeting and therapeutic efficacy. In addition, this review discusses the role of FEVs in metabolic diseases, expounds their limitations in current research, and proposes future research directions. Current research on FEVs has primarily focused on their sources, including milk, plants, and microorganisms, as well as their bioactive components, such as proteins, lipids, and nucleic acids. FEVs exhibit stability in harsh environments and can cross biological barriers, thereby making them suitable for drug delivery and the management of metabolic diseases. However, the molecular mechanism by which FEVs-miRNAs regulate host metabolic signaling pathways remains unclear, which limits our understanding of their therapeutic potential. In summary, this review enhances our understanding of FEVs applications, identifies the limitations of the current research, and provides a foundation for advancing their targeted modification and clinical translation.

Keywords: Extracellular vesicles; Drug delivery; Metabolic diseases; Bioactive components; Innovative approaches

1. Introduction

Extracellular vesicles (EVs) are a heterogeneous population of small, lipid-bound nanoparticles that serve as key mediators in numerous physiological and pathological processes.[1] As illustrated in Fig. 1, the formation process of EVs primarily proceeds through three stages. In the first stage, the cell membrane undergoes initial invagination, leading to the formation of endocytic vesicles. Subsequently, multiple endocytic vesicles fuse, resulting in the production of early endosomes. In the second stage, early endosomes undergo invagination, encapsulate cellular materials, and form multiple intraluminal vesicles. These endosomes then mature into late endosomes, also referred to as multivesicular bodies (MVBs). In the third stage, MVBs fuse with the cell membrane, releasing intraluminal vesicles (i.e., EVs) into the extracellular space.[2] EVs contain a variety of biomolecules, including proteins, nucleic acids, lipids, metabolites, and

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amino acids, which dictate their biological activity. The lipid composition of EVs, encompassing phosphatidylserine, cholesterol, sphingolipids, and ceramides, is particularly important for intercellular signalling and structural stability.[3] The formation and release of EVs are tightly regulated processes that involve the participation of numerous proteins, molecules, and signalling pathways. These processes are associated with the endosomal sorting complex required for transport (ESCRT), including ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III, which work together to form MVBs containing related proteins, such as VPS4, VTA1, and Alix.[4] EVs function as key mediators of intercellular communication, transferring information between cells via the carriage and transfer of various biomolecules. EVs can transfer miRNA and proteins between cells, thereby regulating gene expression and function in the recipient cells. These molecules include immune-regulating proteins that modulate the activity of immune cells, such as macrophages and dendritic cells (DCs), thereby influencing inflammation and immune responses. The biocompatibility and targeting capability of EVs render them ideal drug delivery vehicles for transporting therapeutic molecules such as siRNA, mRNA, proteins, and chemotherapy drugs directly to diseased cells, reducing damage to normal cells. Research[5] have demonstrated that specific miRNAs can regulate metabolic activity across cells and species via the EVs pathway, thereby providing critical evidence for their roles in the management of metabolic diseases. EVs can act as biomarkers for the diagnosis and prognosis of metabolic diseases, as changes in the levels of specific EVs miRNAs or proteins in the blood or other body fluids can serve as disease indicators. Furthermore, EVs can be utilized to deliver anti-tumour drugs, by interacting with surface molecules, they target specific tumour cells, thereby improving the targeting efficiency and therapeutic efficiency of the drugs. Additionally, EVs can enhance anti-tumour immune responses via the delivery of immune-stimulatory molecules.[6]

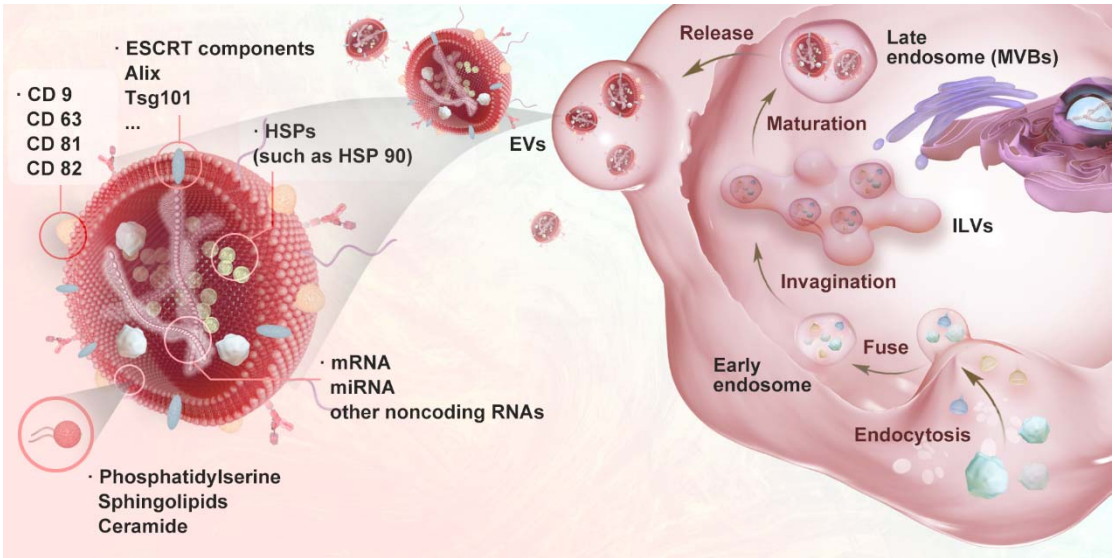

Fig. 1. Schematic representation of EVs structure and composition. The biogenesis of EVs consists of three stages: membrane invagination forming endocytic vesicles, fusion into early then late endosomes, and release from late endosomes via membrane fusion. The structure of EVs consists of a phospholipid bilayer and contains various biomolecules, such as proteins, nucleic acids, and lipids.

Recently, researchers have discovered and isolated EVs from food. Notably, these food-derived extracellular vesicles (FEVs) possess remarkable bioactivity—an attribute that distinguishes them from many

synthetic nanocarriers and even some mammalian cell-derived EVs, laying a critical foundation for their applications in nutrition and medicine. FEVs carry the unique bioactive components of their parental cells，it exert distinct functional properties, and possess dual nutritional and therapeutic properties. Furthermore, FEVs exhibit excellent stability against the harsh digestive environment. FEVs can withstand the harsh conditions of the digestive system, be absorbed by intestinal epithelial cells, and translocate to distant organs via the bloodstream.[7] FEVs are found in various foods, including fruits and vegetables, such as grapes, lemons, ginger, carrots, broccoli, and soybeans. FEVs share similar characteristics with mammalian cells-derived EVs, including a lipid bilayer membrane structure, membrane-associated proteins, and cytoplasmic components such as mRNA, microRNAs, and proteins.[8] However, EVs isolated from different food sources exhibit biological functions specific to their respective origins. For example, ginger-derived EVs exert anti-inflammatory effects, while lemon-derived EVs exhibit anti-cancer activities. These FEVs can also interact with host cells and modulate their functions. Yeo[9] have shown that FEVs possess the potential to regulate the intestinal microbiota, alleviate diseases (e.g., inflammatory bowel disease and cancer), and modulate immune responses. Furthermore, FEVs protect biomolecules from degradation by digestive enzymes, thereby enhancing their stability and bioavailability. FEVs have gained increasing attention due to their extensive source availability, non-toxicity, cost-effectiveness, and environmental friendliness. This is consistent with the concept of "homology of medicine and food". As FEVs, derived from edible sources, represent typical "natural substances with both pharmacological and nutritional properties", they not only supply bioactive components to support physiological metabolism but also exert targeted regulatory effects on pathological processes, thereby bridging the gap between nutritional supplementation and therapeutic intervention.[10] For example, Mori fructus-derived EVs can inhibit the expression of lipid synthesis-related genes via the delivery of miRNAs, thereby suppressing hepatic lipid accumulation, regulating blood lipid levels, and further preventing the formation of atherosclerotic plaques.[11] *Atractylodes macrocephala*-derived EVs can inherit the anti-inflammatory properties of the traditional Chinese herb *Atractylodes macrocephala*, specifically target damaged colonic regions, and significantly promote the recovery of ulcerative colitis.[12] Compared with EVs derived from mammalian cells, FEVs exhibit higher absorption efficiency in the gastrointestinal tract, thereby conferring advantageous for clinical applications. However, it is noteworthy that FEVs and mammalian cell-derived EVs differ at multiple levels, and these differences present both advantages and challenges for their clinical application. The unique composition and functions of EVs from different biological sources render them suitable for specific applications. FEVs contain food source-specific biomolecules, which may contribute to their medicinal potential. Within the digestive tract, FEVs must resist degradation by digestive enzymes to preserve their biological activities.[13]

With the advancement of research on FEVs, it has become increasingly evident that FEVs hold significant potential for applications in the biomedical field and have garnered growing research interest. Due to their edible plant or animal sources, FEVs are regarded as biocompatible, non-toxic, and non-immunogenic, thereby rendering them suitable as functional food ingredients for enhancing the body's natural defence

mechanisms.[10] Furthermore, FEVs exhibit considerable potential and unique advantages in biomedicine field. Their natural origins endow FEVs with unique biological properties, including specific compositional and functional characteristics, which make them sustainable and environmentally friendly biomaterials. Moreover, FEVs can be isolated from mass-produced crops and farmed animals, which confers high scalability to FEVs for meeting clinical and commercial requirements. FEVs can act as drug delivery systems, enabling the direct delivery of therapeutic molecules, such as small-molecule drugs, siRNAs, DNA expression vectors, and proteins, to target tissues. For example, Wang et al.[14] demonstrated that grapefruit-derived EVs enhanced the targeting ability to inflammatory tumour tissues via the activation of leukocyte membrane coatings enriched in inflammatory receptors; Corvigno et al.[15] revealed the substantial potential of edible plant-derived extracellular vesicles (PDEVs) to cross the blood-brain barrier and improve drug delivery efficiency within the tumour microenvironment; and Zempleni et al.[16] illustrated that milk-derived extracellular vesicles (mEVs) participate in the management of metabolic diseases through intercellular communication, regulate gene expression, and interact with the gut microbiome. Therefore, future research on FEVs should focus on the development of more efficient FEVs separation and purification techniques, the engineering of FEVs to enhance therapeutic efficacy, the integration of FEVs with other emerging technologies, such as CRISPR-Cas9 and AI, and addressing challenges and opportunities in clinical translation.

2. Sources of FEVs

2.1. Animals-derived EVs

2.1.1. Milk

2.1.1.1. Bovine milk

Milk contains numerous extracellular vesicles known as mEVs, and these mEVs have emerged as a research focus due to their potential applications in intercellular communication, drug delivery, and disease therapy. mEVs contain lipids, miRNAs, proteins, mRNAs, and DNA with common membrane proteins, including tetraspanin family members (CD9, CD63, and CD81), Alix, Flotillin 1, integrins, and cell adhesion molecules. This composition underscores the complexity of mEVs in mediating cellular communication.[17] mEVs express acid-resistant glycoproteins and surface proteins, which confer resistance to gastric proteases and stability under harsh conditions such as stomach acid (low pH) and high temperatures, thereby rendering mEVs suitable as oral drug delivery carriers.[18] Common isolation methods for mEVs include ultracentrifugation, size-exclusion chromatography, ultrafiltration, and polymer precipitation. In 2024, Wu et al.[19] successfully achieved mEVs large-scale preparation of mEVs using tangential flow filtration technology. They further observed that mEVs exerted significant skin barrier-protective effects, along with anti-inflammatory and antioxidant activities. Chen et al.[20] developed an mEVs-based nanodrug delivery system, which exhibited high biocompatibility and anti-tumour efficacy.

2.1.1.2. Human milk

The primary source of human breast milk-derived extracellular vesicles (hBM-EVs) is mammary epithelial cells, which play a crucial role in regulating infant immunity and promoting intestinal development in infants. They contain tissue factors that initiate blood coagulation, thereby facilitating wound healing and prevents infection.[21] Compared with hBM-EVs, bovine milk-derived extracellular vesicles(b-EVs) contain proteins with larger molecular weights, which may be less digestible by infants.[22] During the isolation of hBM-EVs, careful removal of potential contaminants (e.g., casein micelles) is essential. Different methods of removal can have varying effects on the particle characteristics and protein labelling enrichment of hBM-EVs. Therefore, an appropriate purification strategy must be selected to ensure high purity of EVs samples.

2.1.1.3. Other mammalian sources

The yield of mEVs is relatively high, studies have shown that via differential centrifugation and filtration, the yield of mEVs can reach 200 mg per liter of milk.[23] In contrast, the yield of EVs from goats and other mammals' species is relatively low. In a comparative study investigating the drug delivery capabilities of FEVs isolated cow, buffalo, and goat milk, goat milk-derived EVs were found to exhibit highest drug-loading capacity. Additionally, Ahmed et al.[24] reported that the doxorubicin encapsulated in goat milk-derived EVs exerted more potent inhibition effects on cell viability, with a lower half-inhibitory concentration (IC50).

2.1.2. Other animal sources

Compared with mEVs, meat-derived EVs exhibit significant size differences from PDEVs in size, and the miRNA composition of meat-derived EVs is different.[25] Shen et al.[26] demonstrated that miRNA in cooked pork-derived EVs, following their entry into mice, may modulate metabolic processes by regulating hepatic gene expression. Zhang et al.[27] demonstrated that albumen-derived EVs can mitigate the inflammatory response of intestinal epithelial cells by inhibiting inflammatory activation and apoptosis via the miR-22/ATM/p53/NF-κB axis, thereby providing a potential natural strategy for improving intestinal health. Currently, research on egg-derived EVs primarily focuses on cell experiments and animal experiments, and the extraction process of egg-derived EVs may be affected by protein impurities, so it is essential to optimize the extraction protocols in subsequent studies.

2.2. Plant-derived extracellular vesicles

2.2.1. Fruits

In recent years, researchers have discovered and isolated FEVs from plants, particularly from edible fruits and vegetables, such as blueberries, grapefruits, lemons, apples, grapes, cantaloupes, kiwis, ginger, tea leaves, carrots, broccoli, and soybeans. PDEVs share structural characteristics with mammalian cell-derived EVs, which include lipid bilayer membranes and membrane-associated proteins. PDEVs also encapsulate plant-specific homologous lipids, proteins, and RNA, which enable intercellular and interspecific communication.[28] However, PDEVs are almost completely devoid of cholesterol, given that they are primarily isolated from fruits, vegetables, and grains. Fruit-derived EVs often exhibit antioxidant activities due to the

presence of bioactive components such as ascorbic acid, catalase, anthocyanins, and glutathione. Perut et al.[29] isolated EVs from strawberry juice with a high content of vitamin C (0.416 nM/μg), which could enhance the survival rate of adipose-derived mesenchymal stem cells under oxidative stress in a dose-dependent manner. Notably, the physiological state and pre-treatment protocols of fruits may affect the extraction efficiency and purity of FEVs, and stability issues may arise during the isolation and storage processes, including temperature fluctuations, pH variations, or physical stress, which can induce FEVs rupture or aggregation. Future research should focus on optimising the storage conditions of fruit-derived EVs, such as using cryoprotectants and proper temperature control to maintain their stability, and developing standardized protocols for FEVs isolation and purification to ensure the comparability of results across.

2.2.2. Vegetables

Recent studies have demonstrated that vegetable-derived EVs exhibit therapeutic potential for various diseases and can modulate the immune system, thereby rendering them promising drug carriers. Deng et al.[30] found that broccoli-derived EVs were internalized up by specific intestinal immune cells, such as DCs and macrophages, and could prevent and alleviate colitis symptoms in mice via the activation of the AMP-activated protein kinase (AMPK) signalling pathway. Some vegetable-derived EVs also modulate the composition of the gut microbiota, thereby promoting the proliferation of beneficial bacteria and enhancing intestinal health. However, the mechanisms underlying they interact with intestinal immune cells and the molecular mechanisms by which they maintain immune homeostasis remain elusive. In contrast to fruit-derived EVs, Zhang et al.[8] compared the protein profiles of FEVs isolated from grapefruit and grapes with those from ginger and found that ginger-derived EVs exhibited lower protein content, with a predominance of cytoplasmic proteins and a relative paucity of membrane proteins. When comparing the RNA yield isolated from FEVs derived from 100 g of different plants (grapes, grapefruits, ginger, and carrots), the total RNA content in root vegetables-derived EVs was higher than that in fruit-derived EVs. Furthermore, these RNAs may play a regulatory role in intestinal homeostasis and immune regulation.[31]

2.2.3. Grains and legumes

Grains and legumes are key sources of plant proteins in daily diets, and the effects of their FEVs on human health have garnered considerable research attention. Xiao et al.[32] demonstrated that via sequencing-based analysis of the miRNAs of 11 PDEVs, such as blueberries, kiwis, soybeans, and tomatoes, soybean-derived EVs exhibited the highest number of miRNA species, with 127 species, and the top 20 most abundant miRNAs accounting for over 92% of total miRNA expression. He et al.[33] discovered that mung bean sprout-derived EVs can alleviate the progression of type 2 diabetes in mouse models via activating of the PI3K/Akt/GLUT4/GSK-3β signalling pathway, reduction of blood sugar levels, and improvement of insulin resistance. Pang et al.[34] found that adzuki bean-derived EVs could ameliorate high-fat diet-induced obesity by enhancing gut abundance and regulating its composition. Grain- and legume-derived EVs hold significant potential for clinical applications and industrialisation.

2.3. Microbial-derived extracellular vesicles

Microbial-derived EVs are distinctive in their pre-isolation pretreatment procedures and protein composition. Microbial fermentation broth is collected and subjected to separation via a combined centrifugation-filtration method; during the separation process, contamination by heterotrophic bacteria must be avoided to preserve the purity of EVs. *Leuconostoc mesenteroides* DB-21(isolated from Camellia japonica)-derived EVs completely retain camellia-derived antibacterial polyphenols and exhibit anti-inflammatory activity. Similar to PDEVs, microbial-derived EVs have a nanoscale phospholipid bilayer structure. This structure confers stability, enables protection of internal bioactive molecules (e.g., nucleic acids, proteins, and lipids), and mediates specific interactions with target cells. Their protein composition differs significantly from that of cell-derived EVs.[35] Specifically, microbial-derived EVs are typically enriched in proteins associated with cell wall synthesis, metabolism, and stress responses (e.g., Hsp70, Enolase), which is linked to the unique biological processes of microbial-derived EVs.[13] *Lactobacillus plantarum* Q7-derived EVs can bind to specific receptors on host cells and modulate the host immune response. They can activate or inhibit immune cells such as DCs, macrophages, T cells, and B cells, thereby affecting the immune response.[36] They can also ameliorate gut microbiota, promote the proliferation of beneficial bacteria, and inhibit the growth of pathogenic bacteria, thus, maintaining the gut microbiota homeostasis. Some microbial-derived EVs can serve as carriers of drugs or bioactive substances. For example, *Akkermansia muciniphila*-derived EVs have been utilized as mucosal delivery carriers to ameliorate obesity-related symptoms in mice, demonstrating their potential for drug delivery.[37] PDEVs are typically isolated from specific plants, whereas microbial-derived EVs can be obtained in large quantities through cultivation. Compared with PDEVs, microbial-derived EVs have lower costs, higher production efficiency, and greater feasibility for large-scale production. In disease therapy, microbial-derived EVs target specific pathogens.

2.4 Isolation and Purification Technologies

2.4.1 Ultracentrifugation

The isolation and purification of EVs represent a critical challenge in EVs-related research and clinical applications, as different isolation methods can induce variations in yield, purity, and downstream applications (Table 1). Ultracentrifugation is the most widely employed method for EVs isolation and purification. The most common protocol involves differential centrifugation, in which cells and cellular debris are first removed via low-speed centrifugation, followed by ultracentrifugation to pellet EVs.[38] This method offers advantages including low reagent costs and a low risk of contamination. However, despite its recognition as the "gold standard" for EVs isolation, preparations obtained via this method are often contaminated with high levels of proteins and lipoproteins. Additionally, the protocol is complex and challenging to scale up for large sample volumes or clinical testing.[39] Notably, for certain PDEVs with thick cell walls, it is necessary to first digest the plant cell walls using cellulase, terminate the enzymatic reaction, and subsequently isolate and purify PDEVs via ultracentrifugation or other methods (e.g., density gradient centrifugation, tangential flow filtration) as described in PDEVs purification protocols.[40] Furthermore,

high-viscosity food samples may require longer ultracentrifugation steps and higher centrifugation speeds, which can adversely impact the purity and recovery efficiency of FEVs.

Table 1: Comparison of isolation techniques for FEVs

Methods	Principle of isolation	Advantages	Disadvantages	Reference
Ultracentrifugation	Density, Size, Shape.	Low cost, wide application, low pollution risk.	High cost of the equipment, the experiment takes a long time. High-speed centrifugation may damage the integrity and biological activity of EVs.	38,39,136
Size-Exclusion Chromatography	Size, molecular weight.	Quick and simple, the obtained EVs have high purity, to ensure the integrity and biological activity of the EVs, the yield is high.	Requires specialized equipment and packing, multiple samples cannot be manipulated at the same time.	41, 138,139
Immunoaffinity capture	Immunomagnetic bead technology, specific binding between antigen and antibody.	It is suitable for the isolation of different subtypes of EVs with high purity.	Reagents are expensive, Low yield, High specificity is required, and antibodies may affect the original biological function and activity of EVs surface proteins.	45,48,140
Microfluidics	Separation using microfluidic devices, immunoaffinity, Size, Density.	The processing speed is fast, the cost is low, the operation is simple and easy to automate.	Both the separation efficiency and specificity depend on the design of the device and are not suitable for the extraction of large numbers of samples.	46,47,48,49

2.4.2 Size exclusion chromatography

Size-exclusion chromatography (SEC) is a chromatographic technique used to separate molecules in a solution based on their size or, in some cases, their molecular weight.[41] SEC columns are typically packed with porous gel particles, such as sephadex, sepharose, and seohacryl. These particles have a porous structure that allows smaller molecules to enter the pores while larger molecules are unable to, resulting in separation between the particles. This technique can separate both large and small molecules, as well as molecules within a specific molecular weight range.[42] Optimizing the column packing material and pore size distribution can enhance the resolution and separation efficiency of SEC.[43] Additionally, SEC can be coupled with highly sensitive detection techniques, such as mass spectrometry, for in-depth proteomic analysis.[44] Due to its ability to obtain highly pure and intact FEVs, SEC is suitable for isolating FEVs for further functional or drug delivery studies.

2.4.3 Immunoaffinity capture

Immunoaffinity capture typically employs techniques such as immunomagnetic beads to capture FEVs expressing specific membrane proteins.[45] This method exhibits high specificity and can enrich FEVs expressing one or more specific surface proteins, while preserving FEVs integrity throughout the experiment—an attribute that facilitates subsequent functional studies. However, the compositional complexity of FEVs necessitates the development of highly specific antibodies for different FEVs sources to accurately identify and capture target FEVs, thereby avoiding non-specific interference and weak binding

events. This method is typically combined with ultracentrifugation or other isolation techniques to enhance purity and yield.

2.4.4 Microfluidic techniques

Microfluidics is a fluid manipulation technology that utilizes microfluidic devices at a microscopic scale, based on the physical and biochemical properties of EVs for processing and analysis.[46] These devices comprise numerous microchannels, which are designed and fabricated based on the distinct physicochemical properties of EVs, with channel parameters including length, diameter, shape, and material.[47] This technique offers rapid operation and ease of use, enabling the design of customized microfluidic chips to address diverse separation requirements. It further exhibits potential for automation and high-throughput processing in EVs isolation.[48] However, the design and fabrication technologies for microfluidic devices remain in the developmental stage. The adoption of microfluidic chip-based separation techniques imposes high material and technical demands, frequently leading to elevated costs and a lack of standardized separation criteria. This further hinders the processing of large sample volumes.[49]

3. Unique features of FEVs

3.1. Structural characteristics

The structure of FEVs is analogous to that of cell-derived EVs, with both possessing a lipid bilayer membrane structure. Notably, FEVs exhibit a slightly larger particle size compared to cell-derived EVs. Dynamic light scattering analysis demonstrated that the average particle size of ginseng root-derived EVs (GrEVs) was 92.04±4.85 nm, and these vesicles exhibited a closed spherical structure. The lipid composition of GrEVs differs from that of ginseng root extracts, with unique vesicular characteristics, including diacylglycerol, phosphatidylcholine (PC), phosphatidylethanolamine (PE), and sphingomyelin. Under simulated gastrointestinal acidic conditions, the structure of GrEVs ultrasonic treatment disrupted, preventing the entry of carried miRNAs into cultured cells, indicating the crucial role of physical structure of GrEVs in their function.[50] Grapefruit-derived EVs exhibited a particle size range of 105.7–396.1 nm, with an average size of 210.8±48.62 nm and a negative zeta potential ranging from –49.2 to –1.52 mV. These nanovesicles were rich in PE and PC, with PE and PC accounting for 45.52% and 28.53%, respectively. They remained stable at physiological temperature (37 ℃) and showed reduced size heterogeneity in acidic solutions, but no change in alkaline solution.[51] Ginger-derived nanovesicles (GDNs) had a particle size range of 102.3–998.3 nm, presenting as spherical or elliptical nanoparticles with a negative zeta potential ranging from –24.6 to –29.7 mV. They exhibited stability in simulated gastric (pH 2.0) and intestinal (pH 6.5) environments, with an increase in particle size in gastric fluid and a further enlargement in intestinal fluid.[52] Compared with mammalian cell-derived EVs, FEVs show greater tolerance to harsh environments, rendering them potentially valuable as oral drug delivery carriers.

3.2. Bioactive components

3.2.1. Nucleic acids

EVs are important mediators of intercellular communication and encapsulate abundant small RNA (smRNAs) such as rRNA, snoRNA, tRNA, piRNA, and microRNA (miRNA).[53] Ju et al.[54] demonstrated that RNA could be detected in grape-derived EV-like nanoparticles (GELNs) via agarose gel electrophoresis and that RNase treatment can induce RNA degradation in GELNs. The miRNAs identified in GELNs are primarily from the miR169 family, which play crucial roles in regulating plant growth and development, as well as in responding to environmental stress, and share similarity with two human miRNAs (has-miR-4480 and has-miR-4662a-5p).[31] Xiao et al.[32] employed sequencing-based approaches to analyse the miRNAs in EVs from 11 food sources, including blueberry, ginger, and grapefruit, and found that these plant-derived EVs contained many 20-22nt miRNAs. The miRNA from ginseng-derived EVs can promote skin cell proliferation, regulate the cell cycle, activate the TGF-β signaling pathway, and enhance cellular cytoskeleton structure.[55] The target genes of miRNAs in edible PDEVs are associated with immune cells and are significantly enriched in cancer-related signalling pathways, indicating that miRNAs have immune-regulatory or anti-cancer capabilities. Additionally, edible plant-derived EVs miRNAs specifically interact with mammalian miRNAs and modulate biological processes.[32] The miRNAs and mRNAs in EVs can be internalized by recipient cells and directly modulate gene expression. Specifically, miRNAs can inhibit translation or promote the degradation of target mRNAs via binding to them, thereby regulating the expression of specific proteins and controlling gene expression in recipient cells (Fig. 2b).

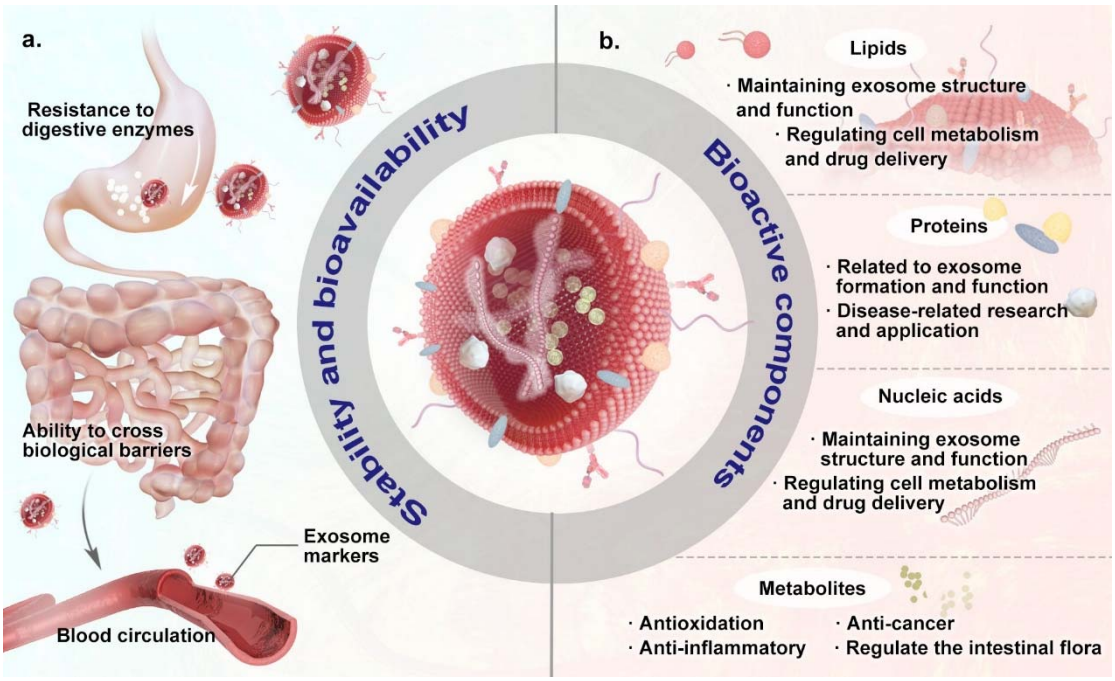

Fig. 2. Unique features of FEVs. In terms of stability and bioavailability, FEVs can resist the degradation of digestive enzymes due to their lipid bilayer structure and can enter the bloodstream through multiple pathways across biological barriers. Their bioactive components include lipids, proteins, and nucleic acids. Lipids maintain the structure and function of EVs and regulate cell metabolism and drug delivery. Proteins are related to EVs formation, function, and disease - related research and applications. Nucleic acids can regulate cell metabolism and drug delivery.

3.2.2. Proteins

EVs are small vesicles that encapsulate proteins derived from various cellular compartments including the cell membrane, cytoplasmic lysosomes, and mitochondria.[56] Owing to their distinct biogenesis and

functional properties, the protein composition of EVs differs from that of cells or other subcellular compartments. Proteins in EVs can be divided into non-specific and specific proteins. Non-specific proteins are typically derived from the cytoplasmic and membrane proteins of EV-producing parent cells. These proteins may contribute to the biogenesis of EVs and include adhesion proteins and transmembrane proteins such as the tetraspanin family, polymeric immunoglobulin receptors, cytoplasmic proteins (actins, tubulin, and moesin), and metabolic enzymes (glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase M2, and heat shock proteins [HSP70 and HSP90]).[57] Specific proteins are associated with the cell type of origin and serve as the focus of protein composition analysis, as they can be used to study the role of FEVs in specific physiological processes and disease pathogenesis. EVs isolated from meat, milk, and soy products exhibit various surface proteins, including αS1-casein, αS2-casein, β-casein, κ-casein, and β-lactoglobulin.[58] Proteomic analysis of EVs isolated from cow milk identified 2107 proteins, among which some are involved in immune functions.[59] In particular, the proteins of mEVs are involved in immunomodulatory processes such as chemokine signaling, and can regulate the function of the infant immune system.[60] hBM-EVs also contain immune-related proteins and miRNAs, suggesting a potential role in early infant development.[61] Characterisation of proteins in FEVs is crucial for understanding FEVs secretion mechanisms, identifying surface markers for various diseases, and developing drug delivery carriers (Fig. 2b).

3.2.3. Lipids

FEVs carry various bioactive factors that participate in intercellular communication and multiple biological processes, such as regulating inflammation, the microenvironment, and the immune system.[62] Lipids and their related factors are key types of bioactive molecules that not only participate in intercellular communication, but also are closely associated with the synthesis and release of FEVs. The lipid composition of FEVs can affect their ability to fuse with cell membranes, thereby influencing their uptake efficiency. Lipids including cholesterol, sphingolipids, and phosphatidylserine play pivotal roles in the generation and functional application of FEVs.[63] These lipid components are essential for maintaining the structural stability and functional integrity of FEVs. For instance, cholesterol-enriched FEVs can mediate direct siRNA cytoplasmic delivery via cell membrane fusion, suggesting that cholesterol regulates the fluidity and structural integrity of FEVs.[64] The lipid components of EVs can serve as biomarkers for disease diagnosis and therapeutic intervention.[65] Additionally, FEVs can regulate the lipid metabolism of recipient cells by delivering specific lipids and proteins such as lipid transport proteins and nuclear transcription factors. In the context of drug delivery, the lipid properties of FEVs render them promising drug carriers.[66] Their lipid membranes encapsulate drug molecules, protect them from entering the bloodstream, and promote intracellular delivery. Therefore, the fusion of FEVs and liposomes is being investigated as a novel strategy to overcome the inefficiencies of traditional FEVs drug carriers (Fig. 2b).

3.2.4. Metabolites

The metabolites of FEVs include proteins, lipids, nucleic acids, and secondary metabolites, which serve as the basis for exerting a variety of biological functions.[67] Primary metabolites account for 38.20% of the

total metabolites, with lipids as the main component. They not only act as the structural basis of vesicles but also possess basic functions such as anti-inflammatory and antioxidant activities. Secondary metabolites constitute 39.35% of the total metabolites, with terpenoids, flavonoids, organic acids, and their derivatives as the main categories. They are crucial for the specific pharmacological functions of *Pinellia ternata*-derived EVs, such as anti-inflammatory and anti-cancer effects.[40] The metabolite components of FEVs exert their core functions in intercellular communication, disease diagnosis, and treatment by regulating signaling pathways or remodeling the microenvironment. Actins, proteases, and transport proteins such as aquaporins and chloride channels may be associated with the endocytic function of FEVs, ensuring their interaction with target cells. Furthermore, they are predicted to possess antioxidant capacity and can alleviate disease symptoms through antioxidant effects.[8] Sphingolipids, phospholipids, and glycerolipids maintain the structural integrity of FEVs membranes and may serve as drug delivery platforms. For example, grapefruit-derived EVs loaded with Stat3 inhibitors can efficiently target brain tumours and inhibit tumour growth.[68] Nucleic acid-based metabolites directly support the core functions in signal transduction and pathological microenvironment regulation by providing energy or regulating gene expression. miR-396e in garlic-derived EVs regulates the expression of PFKFB3, affects metabolic reprogramming of macrophages, and alleviates inflammation in obese adipose tissue.[69] Secondary metabolites encapsulated in FEVs can assist in drug delivery and participate in the regulation of biological functions. For instance, β-glucans in oat-derived EVs promote the uptake of oat-derived EVs by microglia, facilitating their functional exertion in brain-related diseases (Fig. 2b).[70]

3.3. Stability and bioavailability

3.3.1. Resistance to digestive enzymes

Digestive enzymes break down food components in the gastrointestinal tract, and FEVs have been demonstrated to resist this degradation to a certain extent. This is primarily attributed to the lipid bilayer membrane structure of FEVs, which provides a relatively stable microenvironment for encapsulated bioactive molecules, enabling them to resist the hydrolytic activity of digestive enzymes for a specific duration.[71] The gastrointestinal mucosa has barrier structures, including the mucus layer, and FEVs can interact with or adsorb onto components within the mucus layer. This interaction inhibits FEVs from rapidly penetrating the deeper regions of the intestine, thereby extending their residence time in the gastrointestinal tract. Following oral administration, ginger-derived EVs are transported to the liver via the circulatory system rather than the lymphatic pathway. These FEVs are rich in phosphatidic acid (PA), which extends their residence time in the intestine. In a mouse model, the fluorescent signal of ginger-derived EVs was enriched in the middle and distal segments of the small intestine, caecum, and colon, and these FEVs were readily taken up by intestinal stem cells and intestinal macrophages.[72] Endocytosis, as a key process for FEVs to enter target cells or interact with them, modulates relevant signaling pathways and cellular functions, thereby influencing the metabolic processes of FEVs. However, there is currently no well-established theory regarding the metabolic pathways of FEVs in vivo. Nevertheless, it is generally agreed that six distinct endocytic pathways can be differentiated

by adding specific inhibitors.[73] Furthermore, FEVs can recognise and bind to specific receptors on the surface of gastrointestinal mucosal epithelial cells, further enhancing their stability and mitigating their degradation to a certain extent.[74] Processing parameters, including temperature, pH, and physical treatments (such as homogenisation and spray drying), can also influence the stability of FEVs. Elevated temperatures disrupt the lipid bilayer structure of FEVs, whereas variations in pH modulate their surface charge and interactions with digestive enzymes. Freeze-thaw cycles can induce the rupture of FEVs and the degradation of proteins. Therefore, when evaluating the bioavailability of FEVs, their stability in the gastrointestinal tract, ability to resist digestive enzymes, and uptake efficiency by target cells should be considered. By optimising food processing and formulation strategies, the stability and bioavailability of FEVs can be enhanced, thereby augmenting their potential as drug delivery systems or carriers for bioactive molecules (Fig. 2a).

3.3.2. Ability to cross biological barriers

FEVs are capable of crossing biological barriers, and their intestinal absorption mechanisms involve multiple pathways, including transcellular and paracellular transport, which collectively to facilitate the entry of FEVs into the systemic circulation.[75] Tian et al.[18] demonstrated that b-EVs exhibit good stability and could resist degradation by gastric acid, low pH conditions, and gastric proteases. This stability is primarily attributed to the resistance of glycoproteins and membrane surface proteins, such as XDH, BTN, and MUC1. Membrane proteins and the lipid bilayer structure facilitate their fusion with the intestinal cell membrane and the deliver their cargo to intestinal cells.[18] Studies have shown that b-EVs can be internalised by intestinal epithelial cells via endocytosis, with their miRNAs and proteins being released within the cells.[76] Stable FEVs are able to traverse the intestinal epithelial and vascular endothelial cell barriers and enter the systemic circulation. Multiple studies have confirmed the systemic distribution of FEVs through the detection of FEVs markers in bloodstream. FEVs possess specific markers, including characteristic membrane proteins (CD63 and CD81), lipid components, and internally encapsulated nucleic acids (miRNA). By detecting ELISA or rt-PCR to using the presence and changes in the levels of these FEVs markers in the bloodstream, it is feasible to infer whether FEVs have entered the bloodstream and undergone dynamic changes in the blood. Zempleni et al.[16] demonstrated that DiR-labelled mEVs can accumulate in the liver, spleen, pancreas, and kidneys of nude mice following oral gavage and can also be detected in the lungs, colon, and brain. However, subsequent studies have indicated that at low concentrations, the majority of FEVs accumulate in the intestinal mucosa, liver, and colonic contents, with concentrations in other tissues close to background levels. The final clearance of FEVs primarily occurs via lysosomal degradation in recipient cells and excretory pathways. Within lysosomes, FEVs are degraded into small molecules such as amino acids, nucleotides, and lipids, and these products can be reutilised by recipient cells to maintain metabolic homeostasis. In contrast, a small subset of FEVs can release their cargo into the cytoplasm via membrane fusion or endosomal rupture, although this efficiency is relatively low. Current studies have demonstrated that cytoplasmic delivery efficiency can be enhanced through the overexpression of fusion proteins.[77] In a mice model of myocardial infarction, red blood

cell-derived EVs are internalized and degraded by cardiomyocytes. The released hemoglobin-derived iron ions can promote iron metabolism in cardiomyocytes and alleviate iron deficiency following ischemia.[78]

4. Potential applications in drug delivery

4.1. Advantages over synthetic nanocarriers

EVs exist naturally in all bodily fluids and exhibit biocompatibility and biodegradability. Compared with synthetic nanocarriers, EVs exhibit lower toxicity, making them a more advantageous option. Owing to EVs their secretion by endogenous cells, they are less prone to inducing immune responses upon in vivo administration, thereby minimizing their immunogenicity. This characteristic is particularly critical for drug delivery systems. EVs also possess inherent targeting capabilities, enabling them to specifically target specific cell types and tissues and deliver RNA, proteins, or cytokines to target cells. For example, LAMP-28 is a commonly used EVs surface protein. Fusion of its N-terminus with a targeting sequence enables the generation of EVs with specific targeting capabilities.[3] EVs can also be conjugated with antibodies or affinity proteins, which can specifically recognise antigens or receptors on the surface of target cells, thereby facilitating targeted delivery. For instance, the fusion of the HER2-binding affinity protein zHER with the EVs surface protein LAMP-2 enables specific binding to HER2-positive tumour cells.[79] Additionally, EVs can carry natural ligands, such as folate, transferrin, and insulin, which can bind to the corresponding receptors on target cells, enhancing the interaction between EVs and target cells and facilitating EV internalization. Studies have demonstrated that folate-modified EVs can be internalized by target cells via folate receptor-mediated endocytosis for targeted delivery.[80] The phospholipid bilayer of EVs can interact with lipids on the surface of target cells, such as cholesterol, thereby promoting EVs adhesion and internalization. Thus, the lipid components of FEVs can function as ligands during targeting. The phospholipid bilayer of FEVs can spontaneously fuse with the cell membrane of target cells, releasing the FEVs contents directly into the target cells. This mechanism can enhance the FEVs delivery efficiency under certain situations. In the context of drug delivery, FEVs hold significant potential as oral biopharmaceuticals owing to their advantages of intestinal stability, targeting capability, and biocompatibility. mEVs encapsulate various proteins and miRNAs with immunomodulatory and nutritional support properties, which confer diverse functionalities for drug delivery. The abundance of food sources facilitates the relatively easy and sustainable isolation of FEVs. Different food sources can yield FEVs with distinct characteristics, offering multiple options to address diverse therapeutic needs, and robust support for FEVs as oral biopharmaceuticals. Numerous studies have confirmed that FEVs can be utilized for the treatment of various diseases, including tumours, alcoholic liver disease, and enteritis[72]. Mu et al.[31] discovered that EVs from ginger, carrots, grapes, and grapefruit can inhibit inflammatory responses and enhance intestinal barrier function by inducing the nuclear translocation of Nrf2 and activating the Wnt/TCF4 pathway in macrophages. Furthermore, these FEVs can be utilized for immune modulation and serve as carriers for delivering chemotherapy drugs, siRNAs, DNA expression vectors, and protein transport to various cell types. Targeting modification of FEVs, the specificity of the delivery system can enhance, further improving the therapeutic effects of drugs. Compared with artificially synthesised

nanocarriers, FEVs exhibit substantial advantages. Wang et al.[68] demonstrated that grapefruit-derived EVs cannot cross the placental barrier in pregnant mice when injected through the tail vein, indicating their potential for treating diseases in pregnant women.

4.2. Targeted delivery to specific tissues or organs

Using the natural tropism of FEVs, they can exert diverse biological functions. For example, ginger-derived EVs are internalised by human and mouse intestinal epithelial cells, demonstrating their capacity to withstand harsh conditions in the gastrointestinal tract.[8] These FEVs can also traverse the endothelial cell barrier and enter the bloodstream, accumulating in multiple organs, including the liver, spleen, brain, intestines, stomach, and lungs.[7] This natural tropism is dictated by multiple factors, including the surface molecular characteristics, size, and charge of FEVs. The proteins and lipids on the surface of FEVs can recognise and bind to corresponding receptors on target cells, directing FEVs to specific tissues or organs. Studies have shown that the targeting capability of FEVs can be enhanced via surface modification, including antibody conjugation, genetic engineering, and physicochemical modification. Antibody conjugation involves immobilizing specific antibodies to the surface of FEVs, enabling the targeted recognition of cells or tissues expressing a specific antigen. Genetic engineering involves fusing specific protein or peptide gene sequences with gene sequences of EVs lipid membrane proteins to enhance targeting capability. Physical modification employs lipophilic molecules to anchor to the EVs membrane, enabling surface modification similar to that of other carriers, such as liposomes.[81] Chemical modification involves chemical reactions to modify the EVs surface, such as using click chemistry to conjugate azide-fluor 545 to EVs. Characterisation assays have shown that this modification does not impact the size of EVs or their adhesion to receptor cells.[82] However, research on the surface modification of FEVs remains limited, necessitating additional in-depth studies. In the context of organ-specific delivery, EVs have been extensively investigated for drug delivery to organs such as the brain and liver. Hemp-derived EVs exert a hepatoprotective effect, significantly increasing liver regenerative capacity in a mouse model of colitis.[83] This finding demonstrated the considerable potential of FEVs as natural nanocarriers (Fig. 3).

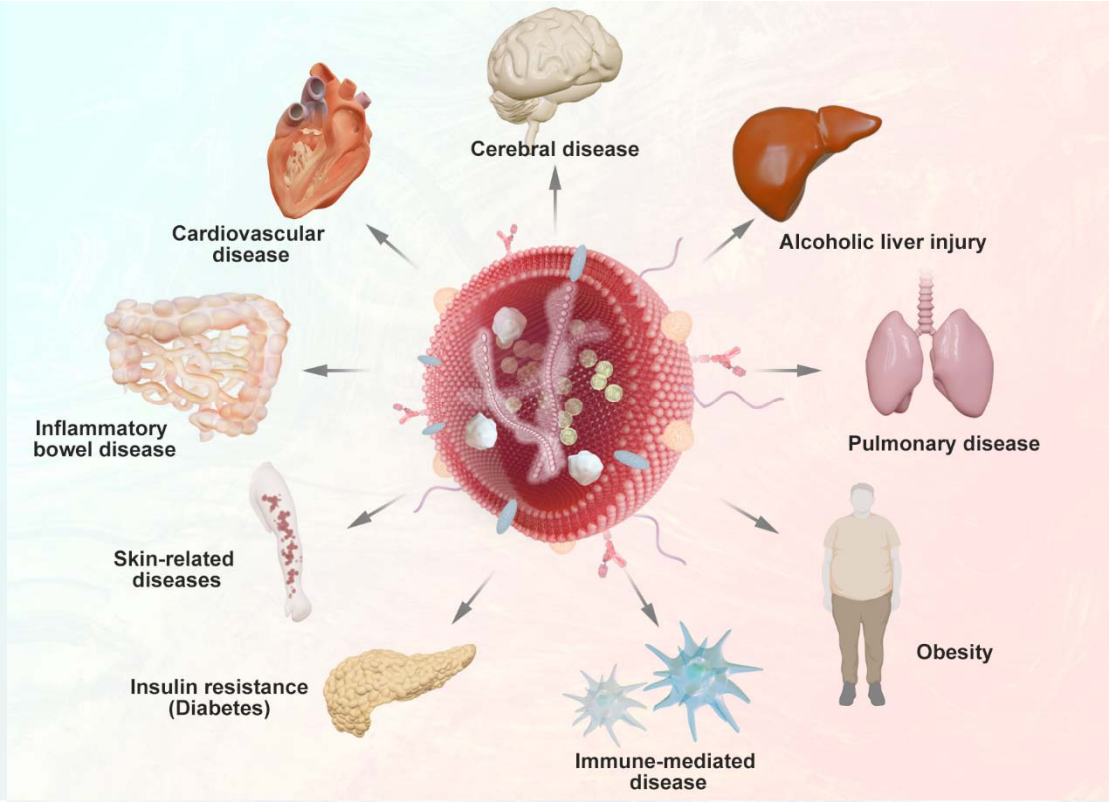

Fig. 3. FEVs can be used as natural nanocarriers to intervene in a variety of diseases. FEVs exhibit therapeutic potential in a wide range of diseases, including cerebral disorders, cardiovascular diseases, alcoholic liver injury, inflammatory bowel disease, pulmonary conditions, obesity, insulin resistance (diabetes), immune-mediated disorders, and skin-related pathologies. Their efficacy is attributed to their natural targeting ability, biocompatibility, and capacity to modulate immune responses and metabolic pathways.

4.3. Enhancement of drug efficacy and reduced side effects

In addition to functioning as drug delivery carriers, FEVs can also enhance drug efficacy and mitigate drug-related side effects. FEVs are nanoscale vesicles enclosed by a phospholipid bilayer whichcan encapsulate drugs within their internal lumen, creating a relatively stable microenvironment. This structure feature can protect drugs from external perturbations including enzymatic degradation, pH fluctuations, and redox reactions, thereby enhancing drug stability. Studies have demonstrated that U937 cells encapsulate doxorubicin into EVs and release it into the extracellular space, demonstrating that EVs can act as potential therapeutic targets by facilitating drug efflux from progenitor cells and reducing intracellular drug accumulation.[84] FEVs regulate drug release rates by modulating their membrane structure and cell interactions. For example, in an in vivo setting, FEVs can fuse with target cells or be phagocytosed by cells, thereby releasing encapsulated drugs into the cytoplasm and enabling targeted delivery.[85] Furthermore, lipids in the FEVs membrane can interact with drug molecules, which in turn influences drug solubility. The phospholipid bilayer of mEVs can improve drug aqueous solubility and protect the encapsulated drug cargo from degradation, thereby enhancing drug stability and bioavailability. In the context of controlled-release properties, FEVs hold significant potential as they can protect drugs from in vivo degradation, enhance drug stability and bioavailability, and enable site-specific targeted release. Modification with ginger-derived EVs reduces the immunotoxicity of nanoparticles; during the 50h detection period, the drug concentration remains

consistently high. This indicates that ginger-derived EVs modification significantly reduces the recognition and clearance of nanoparticles by the immune system, providing theoretical support for their potential long-term applications.[86] Beyond lipids, proteins on the FEVs membrane also contribute to the regulation of membrane structure and drug release. Membrane proteins can function as drug receptors or carriers, bind to drug molecules, and transport them to specific sites. Alterations in the activity and conformation of membrane proteins may also impact membrane permeability and regulate drug release.[87] Studies have demonstrated that following treatment of cells with different concentrations of staurosporine, the expression levels of FEVs release-related proteins (flotillin-1 and CD63) increase with increasing staurosporine concentration, indicating that FEVs release and properties may be influenced by cellular stress.[88] Drug loading methods is also critical factors influencing the controlled-release properties of FEVs, as different loading approaches can affect the distribution and binding of drugs within FEVs, thereby influencing their drug release profiles. Teng et al.[89] proposed an innovative controlled-release system integrating EVs and hydrogels, which could effectively promote skin wound repair via photothermal-controlled EVs release, offering novel insights for EVs drug-loading methods.

4.4. Examples of successful drug delivery using FEVs

FEVs possess inherent targeting capabilities and the potential to enhance drug efficacy. Zhuang et al.[90] investigated the application of grapefruit-derived nanocarriers (GNVs) for delivering miR17 to treat brain tumours in mice. This directly supports our view that FEVs possess inherent targeting and biological barrier-crossing capabilities for efficient drug delivery. Via nasal administration, miR17 can be rapidly delivered to the brain and selectively internalized by GL-26 tumour cells, thereby inhibiting tumour growth. Notably, folate-modified GNVs (FA-GNVs) can target folate receptor-positive GL-26 brain tumour cells, thereby enhancing the carrier's targeting capability toward tumour cells. In the context of siRNA and miRNA delivery, Janos et al. found that mEVs contain various miRNAs, including miR-148a.[16] Specifically, mEVs are internalized by intestinal cells via endocytosis and delivered to various tissues and organs through the bloodstream, demonstrating that miRNAs can regulate gene expression by binding to complementary sequences of target mRNAs, inhibiting tumour cell proliferation, migration, and invasion, as well as regulating immune cell function. Numerous FEVs including broccoli, grapefruit, and ginger carry and deliver siRNAs and miRNAs. Owing to their excellent biocompatibility and stability, they can encapsulate siRNAs and miRNAs, protect these nucleic acids from nuclease-mediated degradation, and target specific cells and tissues via surface modification and ligand binding, thereby improving their delivery efficiency and therapeutic efficacy. Furthermore, the application of FEVs as carriers in drug delivery systems for loading proteins or peptides has been validated.[91] In summary, FEVs have emerged as a promising strategy in the field of drug delivery systems as they integrate natural origins, excellent biocompatibility, and inherent targeting abilities, thus laying the foundation for ongoing clinical trials.

4.5. Mechanisms of FEVs-mediated drug delivery by different source

FEVs may exhibit intrinsic pharmacodynamic characteristics, which play a critical role in surface signaling communication with target cells. Specifically, when specific proteins on the exosome surface interact with receptors on target cells, they trigger a series of biochemical cascades, thereby activating specific signaling pathways within target cells. As natural drug delivery carriers, FEVs exhibit differences in their drug delivery mechanisms due to variations in their sources. The biogenesis pathways of PDEVs differ from those of animals-derived EVs. PDEVs primarily rely on plant-specific endosome-organelle interaction pathways for their biogenesis, which are mainly classified into the MVBs pathway, vacuolar pathway, and EXPO pathway. These pathways directly influence the structural stability and cargo-loading capacity of PDEVs.[22] PDEVs carry plant-specific marker proteins on their surface, which can mediate cell adhesion. Some PDEVs contain transport proteins (e.g., aquaporins, chloride channels), which can enhance their uptake by intestinal epithelial cells. The lipid composition of PDEVs is dominated by phospholipids and sphingolipids.[92] The inherent targeting capability of PDEVs enables their preferential accumulation in the gastrointestinal tract following oral administration. Specifically, ginger-derived PDEVs target intestinal inflammatory regions by binding to the HBP35 protein on intestinal epithelial cells via surface phosphatidic acid.[93] Furthermore, the engineering of PDEVs is associated with lower costs and less complexity compared to animals-derived EVs. It is easier to enhance their targeting to specific tissues via surface modification; for example, grapefruit-derived EVs, which are fused with the membrane of mesenchymal stem cells overexpressing CCR6, target inflammatory regions in autoimmune skin diseases.[67] According to current studies, PDEVs from different plant sources are suitable for different drug delivery scenarios due to differences in their compositions. Specifically, fruit-derived EVs are rich in antioxidant components (e.g., ascorbic acid, flavonoids) and are suitable for delivering chemotherapeutic drugs.[94] Vegetable-derived EVs are more suitable for delivering nucleic acid-based drugs; for instance, osa-miR-530-5p from ginger-derived EVs targets the ORF1ab gene of SARS-CoV-2.[95] Chinese herbal medicines-derived EVs from contain regeneration-related components and are suitable for delivering pro-repair drugs. For example, ginseng-derived EVs deliver miRNAs to endothelial cells in a high-glucose environment, regulating cellular glucose metabolism and promoting angiogenesis.[96] Animal-derived EVs contain cholesterol on their surface, and their cellular uptake is primarily mediated by clathrin-dependent endocytosis, making them prone to lysosomal degradation. In contrast, PDEVs can avoid degradation via the caveolin-dependent pathway.[97] Microbial-derived EVs contain LPS on their surface, which are more likely to activate host immune responses. In drug delivery applications, they are more suitable for delivering bacteriostatic peptides, but their ability to protect nucleic acids is weaker than that of PDEVs.[98]

5. Role in metabolic diseases

5.1. Current understanding of FEVs in metabolic disorders

FEVs play a crucial role in intercellular communication and are involved in the pathogenesis of metabolic diseases. FEVs can transfer bioactive molecules to recipient cells, thereby facilitating the exchange of genetic information and the reprogramming of recipient cells. Accumulating evidence indicates that FEVs mediate intercellular communication in metabolic tissues and play a pivotal role in metabolic diseases,

including diabetes mellitus, obesity, and cardiovascular diseases (Table 2). FEVs can regulate gene expression and signalling pathways in target cells via the miRNAs and proteins they carry, thereby influencing cellular function and metabolism. For example, Zhang et al.[8] found that ginger-derived nanoparticles encapsulated 125 miRNAs with lengths ranging from 15 to 27 nucleotides. Among these 125 miRNAs, 124 may potentially target and regulate human genes by binding to the 3'-untranslated regions. These miRNAs are also involved in regulating the expression of inflammatory factors, decreasing the levels of pro-inflammatory cytokines TNF-α, IL-6, and IL-1β, and modulating the expression of genes such as cyclin D1, thereby inhibiting cellular proliferation. Zhuang et al.[52] demonstrated that GDNs can inhibit ROS production in the liver during alcohol metabolism. GDNs are primarily internalized by hepatocytes, indicating their liver-targeting and hepatoprotective capabilities. A high-fat diet may impair renal metabolism and function. However, research on the targeting effects of FEVs on kidney tissues, including the regulation of electrolyte balance, acid-base balance, and urine concentration is limited. Thus, additional in-depth research is warranted in this regard. Different types of FEVs exert distinct effects on metabolic diseases. For example, in the context of intestinal function regulation, broccoli-derived EVs can be taken up by DCs in the mesenteric lymph nodes and colon of mice, activating the AMPK signalling pathway and inducing tolerance in DCs, thereby regulating the intestinal immune microenvironment.[30] In contrast, grapefruit-derived EVs are rich in PE and PC, which act on intestinal macrophages to induce the expression of the antioxidant gene HO-1 and anti-inflammatory cytokine IL-10, and inhibit the production of pro-inflammatory cytokines IL-1β and TNF-α in intestinal macrophages, thereby enhancing the anti-inflammatory ability of host cells and regulating intestinal function.[51] The size and surface charge of FEVs also contribute to their regulatory effects on metabolic diseases. For example, smaller EVs (30 to 100 nm) are more likely to cross biological barriers and be taken up by endocytosis, whereas larger FEVs may be taken up by phagocytosis.[99] However, challenges persist in controlling the release of FEVs content and their targeted delivery to specific sites remain.

Table 2: Summary of food-derived extracellular vesicles' effects on metabolic diseases

Source of FEVs	Main components	Target organ/tissue	Types of metabolic diseases	Mechanism	Findings	Potential applications	References
Edible ginger	Lipids, few proteins, 125 miRNAs, lots of bioactive ingredients (6-gingerol and 6-shogaol)	Colon	Inflammatory bowel disease	Modulating the immune response by increasing anti-inflammatory cytokines and decreasing pro-inflammatory cytokines; It promotes the survival and proliferation of intestinal epithelial cells	It reduces symptoms of acute colitis； Enhanced intestinal repair and prevention of chronic colitis and colitis-related cancers	Therapeutic agents for inflammatory bowel disease that enhance mucosal healing	[8]
Ginger	Protein, lipids, RNAs	Liver	alcohol-induced liver damage	A group of hepatic detoxification/antioxidant genes is expressed and inhibits reactive oxygen species production	Protecting mice from alcohol-induced liver injury; Reduced liver triglyceride level and liver weight in mice	Agents that protect the liver from injury	[52]
Ginger	Lipids	intestinal epithelial cells（IECs）； liver and adipose tissue	Insulin resistance and obesity	Restoring intestinal epithelial FoxA2-mediated signaling to prevent insulin resistance; Increases Foxa2 expression and protects Foxa2 from Akt-1-mediated phosphorylation and inactivation	Improved glucose tolerance and insulin response in mice; It prolongs the lifespan of mice and inhibits skin inflammation	Transport therapeutic agents for the prevention and treatment of metabolic diseases	[101]
Turmeric	Curcumin, Bioactive compounds	Adipose tissue (particularly white adipose tissue); liver; gut microbiome	Obesity and related metabolic abnormalities	Promote lipolysis and inhibit lipogenesis; Promote the Browning of adipocytes; Induced apoptosis of adipocytes; Altering the composition of the gut microbiota	Reducing obesity	Anti-obesity therapeutic agents	[104]
Broccoli	Lipids, especially sulforaphane	Colon	Colitis	Activation of AMPK in DCs; It reduced the expression of pro-inflammatory factors and increased the expression of anti-inflammatory factors in the colon. Induction of tolerogenic DCs	Reduced mucosal inflammatory cell infiltration; Increased colonic goblet cells; Protection of mice against colitis	Therapeutic agents for dysregulation of intestinal immune homeostasis	[30]
Grapefr	Lipids,	intestinal	Colitis	It can regulate the immune system	Increased resistance to DSS-induced	Novel oral delivery	[51]

uit	Flavonoids	macrophages		by up-regulating the expression of HO-1 and inhibiting the production of IL-1β and TNF-α; drug delivery was used to improve the therapeutic effect	colitis in mice	systems to deliver small-molecule drugs to the gut; Intestinal immunomodulators	
Kidney bean	Protein	Gut microbiota ; liver	Obesity	Increasing the diversity and composition of gut microbiota; Regulating the levels of inflammatory factors; It regulates lipid metabolism	The body weight and liver weight of obese rats were reduced. Improved blood glucose and lipid levels in obese rats	Therapeutic agents to reduce obesity and improve metabolic diseases	[34]
Garlic	Protein, Lipids, Nucleic acid	Liver	Nonalcoholic fatty liver disease	The interaction between macrophages and hepatocytes can inhibit inflammatory response and improve lipid metabolism	Reduce liver inflammation and improve liver function; It regulates lipid metabolism	Beneficial treatments that are anti-inflammatory and improve lipid metabolism	[105]
Cooked pork	Protein, Lipids, Nucleic acid	Liver	Insulin resistance ; lipid metabolism disorders	It affects metabolic pathways associated with insulin resistance and lipid metabolism in mice	Abnormal glucose metabolism and insulin resistance were observed in mice after exosome ingestion. Increased lipid droplets in the liver; Differentially expressed genes enriched in metabolic pathways were increased	Meat product-derived exosomes may not be beneficial for health	[26]
Milk	Protein, Lipids, Nucleic acid	Brain; Liver; Spleen; Pancreas; Kidney; Lung; Colon; Placenta and fetus	Loss of cognitive function; Purine metabolites increased; Decreased fertility	Intercellular communication; Gene regulation; Regulation of immunity	High bioavailability; Gut microbiome interactions	As a drug carrier; Examining the potential of infant formula; As a bioactive ingredient	[16]
Orange Juice	Lipids, carbohydrates, diacylglycerol, and free fatty acids	Small intestine, especially jejunum	Obesity, insulin resistance, non-alcoholic fatty liver disease, dyslipidemia and high plasma triglycerides	Increasing the size of jejunal villi; Reducing triglyceride content; It regulates inflammatory factor levels, barrier permeability, and fat absorption	Reversed diet-induced intestinal changes; It increased villus size, reduced triglyceride content, and regulated the mRNA levels of related genes in the jejunum	Dietary supplements that modulate lipid metabolism	[137]
Momordica charantia L.	RNA, Protein, Metabolites, lipids	Heart; Liver; Spleen	Cardiotoxicity	Reducing reactive oxygen species and protecting mitochondrial integrity in H9c2 cells; Reduced myocardial cell apoptosis	Improved cardiac function and myocardial structure in rats. Promoting H9c2 cell viability, reducing apoptosis and restoring mitochondrial function	Cytoprotective agents	[107]

5.2. Therapeutic potential

5.2.1. Diabetes

Strategies for β-cell protection and regeneration based on FEVs represent emerging research directions in diabetes mellitus treatment. Studies have demonstrated that EVs secreted by pancreatic β-cells contain various miRNAs, which can exert β-cells by regulating gene expression. For example, miR-26a in EVs can protect β-cells by enhancing insulin sensitivity and reducing β-cell apoptosis.[100] Research has further demonstrated that miR-15 plays a crucial regulatory role in insulin biosynthesis by β-cells, and EVs secreted by β-cells are an important source of circulating miR-15a. Elevated plasma levels of miR-15a in patients with diabetes mellitus with diabetes patients are associated with disease severity. Kumar et al.[101] demonstrated that treatment with ginger nanoparticles (GDNPs) can induce miR-375 expression, inhibit AhR expression, prevent high-fat diet-induced weight gain, reduce high-fat diet-induced increases in liver weight and white adipose tissue mass, reduce the insulin resistance index, improve glucose tolerance and insulin resistance, and enhance insulin secretion. Concurrently, GDNPs can regulate the Foxa2 signalling pathway in intestinal epithelial cells, protect Foxa2 from Akt-1-mediated phosphorylation, preserve its activity, and improve its metabolic function. Zhao et al.[102] demonstrated that a purple ginseng-derived nanovesicle formulation improved insulin sensitivity, alleviated metabolic disorders and liver oxidative stress, and reduced blood glucose levels in rats. Ginseng-derived EVs have been shown to enhance angiogenesis and neovascular network reconstruction in full-thickness diabetic skin ulcer wounds in mice. They have been also proven to be a promising nanotherapeutic agent for stimulating glycolytic reprogramming-mediated angiogenesis in diabetic ulcers, exhibiting high biosafety.[96] In terms of drug delivery, tartary buckwheat-derived EVs not only function as delivery carriers but also possess intrinsic glucose metabolism-regulating activity. They exert a synergistic anti-inflammatory effect with chlorogenic acid, further enhancing glucose-lowering efficacy.[103] Research on the regulation of insulin sensitivity by FEVs is still in its early stages, although some potential mechanisms and pathways have been identified; additional research is needed to elucidate the distinct effects of FEVs from different sources on β-cells and the differences in their regulation of insulin sensitivity. Furthermore, additional research is warranted to investigate the interactions between FEVs, other nutrients, and drugs; for example, do FEVs affect the efficacy of specific hypoglycaemic agents or do they exert a synergistic effect when combined with other nutrients to better regulate insulin sensitivity?

5.2.2. Obesity

FEVs, which encapsulate containing miRNAs and proteins, can regulate inflammatory responses and modulate the recruitment and function of immune cells in adipose tissue. FEVs carry chemokines or their antagonists, which modulate the migration of immune cells to adipose tissue. FEVs can also influence energy metabolism in adipose tissue, and miRNAs in EVs can regulate lipid metabolism-related genes in adipocytes. Wang et al.[104] developed a reconstructed turmeric-derived nanovesicle for obesity treatment, which is

internalized by adipocytes via clathrin- and caveolae-mediated endocytosis. This nanovesicle promotes lipolysis, inhibits adipogenesis, and exerts lipid-lowering effects through multiple pathways. Pang et al.[34] demonstrated that orally administered kidney bean-derived exosome-like nanovesicles can enhance gut microbiota diversity and ameliorate obesity by modulating gut microbiota composition, increasing the abundance of beneficial bacteria, and elevating the levels of short-chain fatty acid metabolites. This study definitively links gut microbiota modulation of FEVs to improvements in obesity. Liu et al.[105] demonstrated that garlic-derived EVs can regulate the expression of fructose-2,6-bisphosphatase 3, a key enzyme in macrophage glycolysis, through miR-396e, thereby affecting metabolic reprogramming and polarisation. This, in turn, alleviates hepatic inflammation and metabolic function through macrophage-adipocyte crosstalk and regulates liver dysfunction in high-fat diet-induced obese mice. Furthermore, oral administration of honey-derived vesicular nanoparticles to naturally ageing mice has been shown to protect the liver against the development of non-alcoholic steatohepatitis. These nanoparticles are primarily internalized by hepatic Kupffer cells, where they inhibited the progression of chronic liver inflammation, fibrosis, and nodule formation in aged mice. These findings further demonstrate the considerable potential of FEVs in the treatment of metabolic diseases and provide novel insights into strategies for managing related diseases.[106]

5.2.3. Cardiovascular diseases

FEVs play crucial roles in signalling, inflammation, and coagulation, among other biological functions, in the pathogenesis of cardiovascular diseases. Several have studies confirmed the cardioprotective effects of FEVs. Ye et al.[107] demonstrated that bitter melon (*Momordica charantia* L) exosome-like nanoparticles stabilise p62 protein levels by inhibiting DOX-induced p62 ubiquitination and degradation, thereby promoting p62 binding to Keap1, facilitating Nrf2 nuclear translocation, and induces the expression of HO-1 downstream genes, ultimately promoting cardiomyocyte survival. FEVs can also carry growth factors and signalling molecules, such as insulin-like growth factor-1 and vascular endothelial growth factor, which promote cardiomyocyte proliferation and differentiation. FEVs can also transport enzymes and metabolism-regulating signaling molecules, such as AMPK-related regulators, which activate glucose uptake and utilisation in cardiomyocytes and enhance energy production. Additionally, FEVs can modulate cardiomyocyte metabolism to ensure a adequate energy supply under diverse physiological and pathological conditions. These studies have yielded promising results, highlighting the potential value of FEVs for cardio protection. Atherosclerosis is a chronic inflammatory disease characterized by endothelial dysfunction, inflammation, and dyslipidemia.[108] Intercellular communication is essential for nearly all physiological and pathological processes, including atherosclerosis. As key participants in intercellular communication, FEVs contribute to atherosclerotic calcification, may function as actors, bystanders, biomarkers, and therapeutic mediators.[109] Lipid oxidation is a critical step in atherosclerosis. Pretreatment with blueberry-derived exosome-like nanoparticles can counteract TNF-α-induced ROS production and reduced cell viability and modulate the differential expression of 29 genes induced by TNF-α, to prevent vascular system damage caused by various stressors.[110] Despite the valuable findings from these studies, several issues remain unresolved. FEVs from

diverse sources exhibit complex and heterogeneous compositions, and their regulatory effects on cardiovascular diseases may vary depending on factors, including food type and FEVs isolation methods. Additional in-depth research is warranted to identify the specific targets of FEVs and evaluate their long-term safety.

6. Innovative approaches and future directions

6.1. Engineering FEVs for enhanced therapeutic effects

6.1.1. Genetic modification of source cells

Although we have outlined the intrinsic targeting capability and biocompatibility of natural FEVs, their therapeutic efficacy is frequently constrained by inadequate cell specificity and suboptimal drug-loading efficiency. To fully validate the proposed hypothesis—that FEVs represent a transformative tool for targeted therapy—it is imperative to employ engineering strategies to enhance their intrinsic properties. Notably, the intrinsic nanoscale structure and excellent biocompatibility of FEVs provide a versatile platform for such engineering approaches, which facilitates their customization for improved encapsulation and delivery of therapeutic agents. Genetic modification of source cells is a promising strategy for introducing specific genes into source cells via genetic engineering techniques, resulting in overexpression.[111] Overexpression of genes associated with FEVs biogenesis, stability, or targeting may enhance the production of FEVs, improve stability, or confer specific targeting capabilities.[112] Additionally, for therapeutic applications, FEVs can be overexpressed via gene carriers, allowing them to transport therapeutic molecules such as drugs, nucleic acids, or proteins (Fig. 4a).

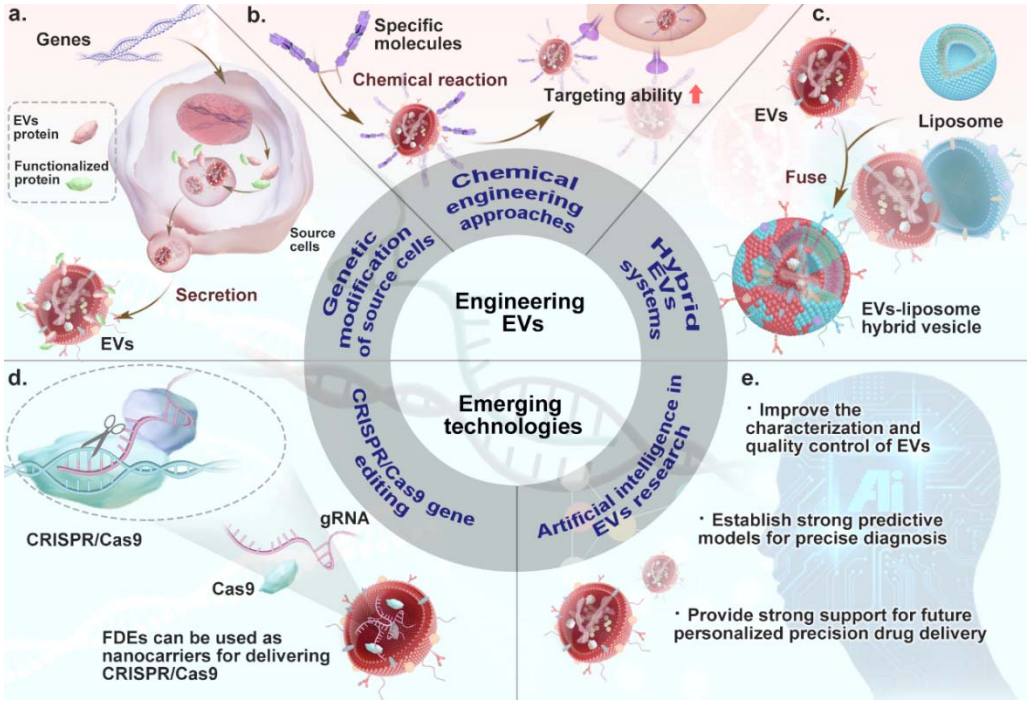

Fig. 4. Conceptual framework for integrating FEVs with emerging technologies. Gene editing of source cells can regulate EVs production, stability, and targeting. Chemical engineering methods can modify the surface of EVs to enhance their targeting ability. The construction of hybrid EVs systems combines the advantages of EVs and materials like liposomes to improve drug-carrying and targeting performance. In addition, FEVs can be used as nanocarriers to deliver CRISPR/Cas9 for gene editing, and AI is applied to the analysis, diagnosis, and treatment optimization in EVs research, providing new directions for the application of FEVs.

6.1.2. Chemical engineering approaches

Chemical engineering techniques can be employed to modify their surfaces (Fig. 4b). By using chemical reactions to attach specific molecules such as targeting ligands, antibodies, and drugs to the surface of FEVs, this method can enhances their targeting capability, allowing them to reach specific tissues or cells more accurately.[113] Via copper-catalyzed azide-alkyne cycloaddition "click" reaction, the amino groups of EVs proteins can be covalently conjugated to molecules containing alkynes or azides, enabling the binding of targeting ligands to the surface proteins of EVs. For example, the glioma-targeting RGE peptide can be conjugated to the surface of EVs via a cycloaddition reaction with sulfonyl azide, which can be utilised for targeted therapy of glioma.[114]

6.1.3. Hybrid exosome systems (HEs)

HEs refer to a combination of EVs with other biological materials that enhance the drug-loading capacity of EVs and targeting capability, thereby improving therapeutic efficacy (Fig. 4c).[115] HEs exhibit characteristics similar to those of natural EVs, including a size range of 30–150 nm, which makes them suitable carriers of small therapeutic agents. Fusing EVs with liposomes, integrates the biocompatibility of EVs and the flexibility of liposomes, significantly enhancing the drug- loading capacity and targeting ability. Xiao et al.[116] modified the surface properties of b-EVs-liposome hybrid vesicles (mExos@DSPE-Hyd-PMPC) by introducing a pH-sensitive hydrazone bond between highly hydrophilic zwitterionic polymers and phospholipids. This modification leverages the pH microenvironment on the small intestinal surface, allowing the hybrid vesicles to adapt to diverse physiological conditions. Compared with natural b-EVs, the drug encapsulation efficiency of hybrid EVs increased by 2.4 times, demonstrating the capacity of hybrid EVs to improve drug-loading efficiency. Additionally, the hydrophilicity and neutral charge of the hybrid EVs in the intestinal lumen contribute to enhanced protection of membrane proteins and effective penetration through the mucus barrier. Upon reaching the surface of intestinal epithelial cells, the positively charged and well-preserved membrane proteins of the hybrid EVs effectively overcome the apical, intracellular transport, and basal efflux barriers, resulting in an increased oral bioavailability of 8.7%, significantly enhanced pharmacological effects, and improved mucus penetration. This successful application of HEs not only validates the FEVs engineering concept but also directly addresses the key challenges of drug stability and absorption in the gastrointestinal tract that were discussed in Section 3.3. Through the synergistic combination of the advantages of natural FEVs and synthetic carrier systems, HEs provide a promising platform that supports our central thesis regarding the immense potential of engineered FEVs in the revolutionization of oral drug delivery. Cellular uptake studies of hybrid EVs have shown that the delivery functionality of EVs can be modulated by altering their lipid composition or properties. For example, PEG-modified EVs significantly increase cellular uptake efficiency, likely due to the fact that PEG reduces electrostatic repulsion between EVs and target cell membranes, thereby increasing the interaction between EVs and cells.[115] Additionally, the inherent targeting capability of EVs and the stability of liposomes can be utilised to fuse synthetic liposomes with EVs, enabling the simultaneous delivery of multiple drugs and therapeutic molecules, thereby achieving

more precise drug delivery.[117] In conclusion, HEs represents an innovative strategy for producing EVs with broad application potential. Future research should investigate the preparation methodologies, functional mechanisms, and safety of hybrid EVs to provide robust support for their application in nutritional supplementation, drug delivery, and disease diagnosis.

6.2. Integration with other emerging technologies

6.2.1. CRISPR/Cas9 gene editing

The CRISPR/Cas9 system is a gene-editing technology that utilises the Cas9 nuclease and a guide RNA complex to recognise and cleave target gene sequences, enabling gene deletion, insertion, and mutation (Fig. 4d).[118] Using RNA interference or gene editing tools, such as CRISPR/Cas9, specific genes can be silenced in source cells, which can be used to eliminate or reduce unwanted components in EVs or inhibit the expression of disease-associated genes. However, the CRISPR/Cas9 system currently exhibits limitations in delivery applications as its vectors have high immunogenicity and low cellular tolerance.[119] Thus, FEVs can serve as nanocarriers to deliver CRISPR/Cas9. Cai et al.[120] demonstrated that *Arabidopsis*-derived EVs can deliver sRNAs to *Botrytis cinerea*, achieving specific knockout and silencing of pathogenic genes through CRISPR/Cas9 and RNA interference techniques, thus, elucidating the mechanism by which *Arabidopsis* inhibits fungal pathogenicity through EVs-mediated sRNA delivery. FEVs have inherent tissue-targeting capabilities, high cellular uptake, excellent biocompatibility, and strong stability. Compared with artificial nanoparticles, they exhibit lower toxicity and do not require additional modifications to enhance their performance. They can serve as carriers to encapsulate siRNAs, miRNAs, chemotherapeutic agents, and hydrophobic compounds. In addition, they have a long circulation time in the bloodstream, enable accurate tissue targeting, reduce systemic effects, optimise therapeutic outcomes, and minimise adverse effects. However, they still have limitations such as inconsistent sizes and physical properties, unclear mechanisms of action, and the potential to cause unknown immune reactions. In the future, the isolation process of FEVs should be optimised, and their morphology, quantity, and chemical composition should be characterised to clarify their functions and properties. Although PENs still face challenges in clinical applications, such as incomplete standardisation of the production process, they have been evaluated in preclinical and clinical trials. For example, FEVs derived from grapes, ginger, and aloe vera have been registered in clinical trials, and several studies have combined FEVs with the CRISPR/Cas9 technology for the treatment of diseases such as colon cancer. However, further studies are required to confirm their safety and efficacy.[121] Nevertheless, FEVs may still represent a promising strategy to deliver CRISPR/Cas9 in the future, and additional research is warranted to overcome these challenges and achieve more effective targeted gene-editing therapy for specific tissues.

6.2.2. Artificial intelligence (AI) in EVs research

EVs are key mediators of intercellular communication and function as drug delivery carriers. AI exhibits robust predictive capabilities in drug development, allowing the analysis of large datasets to optimise drug design and delivery strategies. The integration of FEVs and AI has multiple potential advantages and

prospects.[122] Incorporating AI technology, more efficient and accurate analyses enables in the isolation, characterisation, and functional investigation of FEVs. The application of machine learning strategies to spectral data processing can effectively handle complex EVs datasets and establish strong predictive models for precise diagnosis. Using machine learning algorithms, the spectral and imaging signals of EVs can be analysed to enhance the accuracy and reliability of EVs detection.[123] Shin et al. [124] proposed a method using AI to analyse surface-enhanced Raman spectroscopy profiles of EVs and identify six types of early-stage cancers. Multiple machine learning models, including convolutional neural networks, support vector machines, and multilayer perceptron networks, have been utilised to enhance the accuracy of cancer detection. The advantages of this method include rapid detection (EVs isolation and detection are completed in approximately 1 h, with a final diagnosis made in seconds), reduced resource requirements for diagnosis (no additional reagents required), and the potential to expand its application to diagnose. However, this method exhibits limitations, including the need for expanded training samples, external testing, prospective clinical trials for validation, consideration of potential confounding factors, correlation analysis of major spectral features with pathological factors, introduction of a standardised and mass-producible SERS detection chip in clinical settings, and establishment of a comprehensive diagnostic workflow. As drug delivery carriers, FEVs hold significant potential for tracking disease progression. The utilisation of AI and deep learning technologies can optimise EVs delivery and personalise EVs drug delivery as part of precision medicine. Deep learning, more accurate standards can be provided for the industrial production of EVs, particularly regarding EVs cell sources, purification processes, and drug formulations, which can accelerate the establishment of more unified and advanced standards for EVs medicinal research. In conclusion, the application of AI in EVs research has significant potential, particularly for FEVs research. The utilisation of AI technology can significantly enhance the characterisation and quality control of EVs, optimise predictive models for EVs therapy, and provide robust support for personalised precision drug delivery (Fig. 4e).

6.3. Challenges and opportunities in clinical translation

6.3.1. Scalability and manufacturing

FEVs encounter substantial challenges in clinical translation but possess considerable potential. Currently, the production scale of FEVs is limited and cannot meet the high demands of clinical applications. The identification of efficient and reproducible large-scale production methods remains a key challenge. This necessitates the development of advanced technologies and processes to ensure stable production and quality of FEVs. The Temporary Immersion Bioreactor System mitigates the issue of quality fluctuations in the production of PDEVs.[125] However, the isolation of EVs poses numerous challenges. Although various isolation techniques have been developed based on physicochemical and biochemical properties, such as ultracentrifugation, density gradient centrifugation, ultrafiltration, size exclusion chromatography, polymer precipitation, immunoaffinity capture, and microfluidics, an effective method that can simultaneously ensure the yield, purity, and biological activity of EVs has not yet been identified. Ultracentrifugation is widely utilised due of its simplicity and cost-effectiveness; however, it exhibits low reproducibility and may

compromise the integrity of EVs. Density gradient centrifugation yields high purity but is complex to operate. Size exclusion chromatography preserves the integrity of EVs but has low throughput and high equipment costs. Ultrafiltration is straightforward to perform but results in low yield and purity. Polymer precipitation provides high yield but low purity and is susceptible to polymer contamination. High molecular weight components in plant juice can impede centrifugation, and the combined application of different isolation methods or emerging technologies may improve separation efficiency and purity. Kalarikkal et al.[57] used a combination of PEG-6000 and ultracentrifugation to isolate EVs from fresh ginger roots, which exhibited cost-effectiveness and high purity. The combination of ultracentrifugation with ExoQuick can enhance the isolation purity and colloidal stability of ginseng-derived EVs.[126] In the future, further exploration and optimisation of these techniques are required to achieve efficient and reproducible large-scale production. It is also worthwhile to investigate the biosynthetic mechanism of EVs to identify strategies for enhancing their production. Furthermore, due to the wide range of sources (including milk, plants, and microorganisms) and diverse preparation methods of FEVs, significant variations exist in their quality. This is not only reflected in the size and morphology of FEVs but, more importantly, in the inconsistency of their bioactive components and functions. For example, FEVs from different sources or batches may differ in the type and quantity of bioactive substances they carry.[127] The high-yield and low batch-to-batch variation characteristics of TIBS can address the issue of quality fluctuations in FEVs products. Through a non-destructive culture mode, TIBS increases FEVs yield by 3.2-fold compared to traditional methods; furthermore, it enables precise programmable control of culture parameters, resulting in a batch-to-batch variation of < 8% for FEVs and a coefficient of variation of < 5% for key bioactive components—providing a foundation for establishing quality standards for FEVs.[125] Standardisation is necessary to ensure that FEVs produced in different batches have consistent therapeutic efficacy, thereby improving the safety and efficacy of clinical applications. This necessitates the establishment of detailed operating procedures and quality standards at every step of the production process, strict adherence to these protocols, and the implementation of a comprehensive quality monitoring system—all to conduct thorough and rigorous testing of the production process and final products.

6.3.2. Standardization of isolation and characterization methods

The field of FEVs lacks a consistent standard for isolation, basic structure-function relationships, and nomenclature, which hinders the comparability and reproducibility of research results and severely impedes its development.[128] The development of reference materials and protocols can provide researchers with standardised operating guidelines, enhancing the consistency and comparability of experimental results. For example, a unified isolation method can ensure that FEVs obtained from different laboratories exhibit consistent purity and biological activity, thereby improving the reliability of research outcomes. Reference materials should be representative and stable, accurately reflecting the characteristics of the FEVs. They can be used to calibrate and validate the accuracy of isolation and characterisation techniques, assisting researchers in identifying error sources and optimise experimental conditions. For instance, specific EVs markers or components can be established as reference standards to determine the recovery rates and purities

of different isolation techniques.[129] Protocols should cover all aspects of FEVs isolation and characterisation, including sample collection, preprocessing, selection and operating procedures of isolation techniques; application of characterisation techniques; and data analysis and reporting. The implementation of these protocols requires close collaboration and information sharing among researchers as well as the establishment of corresponding training and quality control systems to ensure the proper execution of the protocols. Different laboratories and research teams utilise different methods and standards to report EVs data, complicating cross-study comparisons and data integration. Standardising reporting standards for FEVs can enhance research transparency and reproducibility, enabling other researchers and clinicians to accurately understand and evaluate research findings. Reports should provide detailed information on the sources of FEVs (plant or animal species and growth environment), isolation methods (reagents, instruments, and operating parameters), characterisation results (size, morphology, surface markers, and bioactive components), and quality control measures during the experimental process. Furthermore, promoting international cooperation and establishing a unified reporting standard and data-sharing platform will facilitate global collaboration in FEVs research and data interoperability. Concurrently, it will benefit regulatory authorities in reviewing and regulating related products, thereby fostering the healthy development of the FEVs industry.

6.3.3. Regulatory considerations

The classification of FEVs remains ambiguous, complicating the determination of their locations within regulatory frameworks. Debate exists regarding whether they should be regulated as pharmaceuticals, biologics, or food products. For example, they have therapeutic effects analogous to those of drugs but are derived from food, which sets them apart from traditional drugs and food in terms of their inherent nature and intended use. Regulatory authorities must clarify the classification of FEVs and develop corresponding guidance documents. Thus, in-depth investigations into the pharmacological effects, bioavailability, and safety of FEVs are required. Factors such as the composition, function, and intended use of FEVs can also impact their classification. If FEVs are primarily intended for disease treatment and contain clearly defined therapeutic components, they are more likely to be classified as pharmaceuticals or biological products. If they are primarily used as nutritional supplements or functional food ingredients, they can be categorised as food products. However, many FEVs possess multifunctional properties, which complicates their classification. Clear classification is critical for the formulation of appropriate regulatory policies. Different classifications correspond to distinct approval processes, quality standards, and market supervision requirements. For pharmaceuticals or biological products, rigorous clinical trials are required to validate their safety and efficacy, whereas food products should focus on safety and nutritional composition. Accurate classification facilitates the reasonable use of FEVs in the market, protects consumer rights, and fosters the healthy development of this field. In addition, comprehensive safety assessments, including toxicity, immunogenicity, and biocompatibility, are required for FEVs. Notably, as FEVs are non-human-derived, their safety profile is inherently linked to the route of administration. Animal-derived EVs exhibit species-specific membrane

proteins on their surface; these components share high homology with humans and are readily recognized as "semi-foreign substances" by the host immune system, triggering adaptive immune responses.[130] In contrast, PDEVs contain plant-specific components and lack homologous receptors recognizable by the human immune system, thus rarely inducing adaptive immune responses (e.g., antibody production).[101] γδ T cells have been found to play a critical role in the progression of tumours, infections, and autoimmune diseases.[131] Following oral administration of garlic-derived EVs, the proportion of γδ T cells among $CD3^+$ T cells in the mouse intestine was approximately 2.5-fold higher than that in the untreated group, accounting for 30%-40% of total T cells. Additionally, garlic-derived EVs exerted a certain activating effect on intestinal immune cells such as dendritic cells and macrophages.[132] Notably, oral administration of garlic-derived EVs exerted no significant toxic side effects in mice, whereas intravenous injection of garlic-derived EVs, although not significantly affecting tumour growth, induced obvious side effects in mice. This indicates that the route of EVs administration is crucial for their immune responses and safety, and further investigations into the mechanisms underlying these side effects are warranted in future studies. On the other hand, the low toxicity advantage of edible-medicinal homologous-derived EVs indirectly influences regulatory classification determinations by affecting their safety assessment outcomes; this classification requires comprehensive consideration of factors such as FEVs sources, functional properties, and clinical applications.[10]

While oral administration benefits from inherent gastrointestinal tolerance, non-gastrointestinal routes (e.g. intravenous, intranasal or topical administration) require rigorous validation of bioavailability, tissue distribution and potential adverse reactions. For instance, compared with mice in the control group, mice administered high concentrations of mEVs via oral gavage exhibited no significant difference in immune cell counts; however, researchers observed that mEVs at concentrations of 25 mg/kg and above significantly increased the metastatic burden of pancreatic cancer cells to the liver, which represents a key mechanism underlying accelerated tumour metastasis.[133] When investigating the effects of FEVs on different organs and tissues, it is critical to monitor whether they induce inflammation, allergies, or other adverse events and assess their long-term safety. Currently, researchers are assessing the impact of lemon-derived EVs on cardiometabolic risk factors in subjects with metabolic syndrome, though this research is still in its early stages—this study also highlights that the collection of experimental data on EVs immune tolerance during long-term application is currently ongoing (ClinicalTrials.gov: NCT04698447). The mechanism of action of FEVs is not fully elucidated, which hinders the assessment of their safety and efficacy. Further research is required to determine its distribution, metabolism, and target sites in the body.

6.3.4. Establish immune tolerance and long-term safety

Regulating the phenotypic transition of macrophages can prevent excessive activation or insufficient suppression of immune responses, and represents a core mechanism for modulating immune responses and maintaining immune homeostasis. *Lactobacillus murinu*-derived EVs are internalized by macrophages, which activates the TLR2 signaling pathway and prevents intestinal inflammatory damage caused by excessive accumulation of M1 macrophages.[134]

On the other hand, cytokine balance and inflammatory pathways are core regulatory components for the establishment and maintenance of immune tolerance, and these three elements are closely linked through a logical chain of "suppressing excessive inflammation - maintaining immune homeostasis - promoting tolerance formation. *Roseburia intestinalis*-derived EVs reduce the expression of pro-inflammatory cytokines, increase the expression of anti-inflammatory cytokines, modulate the activity of NF-κB and STAT3 via the PI3K signaling pathway, enhance intestinal barrier function, reduce immune-inflammatory damage, and maintain immune balance.[135]

Direct oral administration of anti-inflammatory agents tends to induce systemic immunosuppression and may exacerbate intestinal inflammation. However, grapefruit-derived EVs can serve as carriers for anti-inflammatory agents. In this study, researchers found that after loading anti-inflammatory agents, FEVs did not induce abnormal changes in immune cells of the intestinal lamina propria in mice, nor did they cause hepatic toxicity. This indicates that while synergistically enhancing anti-inflammatory effects, FEVs can maintain normal immune homeostasis in the host and avoid the risks associated with excessive.[51]

Current studies indicate that FEVs exhibit favorable biocompatibility in short-term use. Combined with existing short-term safety-related data, future research should further evaluate long-term safety-related indicators of FEVs, including assessments of long-term in vivo accumulation and organ toxicity, observation of adaptive immune responses, and evaluations of long-term metabolic and reproductive developmental safety.

7. Conclusion and future perspectives

This review highlights the important role of FEVs in the management of metabolic diseases, while underscoring their unique advantages as drug delivery systems. In recent years, substantial progress has been achieved in research on FEVs, highlighting their transformative potential in the field of biomedicine. FEVs are EVs isolated from foods characterised by unique structural features and bioactive components. An advantage of FEVs is their natural origin, which contributes to their low immunogenicity. Substances isolated from natural foods are generally regarded as non-toxic and environmentally friendly, making them attractive for medical applications. FEVs play a pivotal role in intercellular communication by modulating gene expression and function and carrying and transferring various biomolecules. A distinguishing feature of FEVs is their capacity to withstand the harsh conditions of the digestive system, be internalized by intestinal cells, and reach distant organs via the systemic circulation. Compared with other administration routes such as intravenous injection, oral administration is simple, cost-effective, safe, and while also improving patient-centered care. Additionally, FEVs have shown considerable potential in regulating the gut microbiota, alleviating inflammatory bowel disease and cancer, and modulating immune responses. Compared with mammalian cells-derived EVs, FEVs have a higher absorption efficiency in the gastrointestinal tract, conferring them advantages for clinical applications. The non-toxicity, cost-effectiveness, and environmental benignity of FEVs render them ideal drug delivery carriers. Furthermore, the unique composition and functions of FEVs make them suitable for specific applications; for example, ginger-derived EVs have anti-inflammatory effects

and lemon-derived EVs have anticancer properties. These characteristics render FEVs widely applicable in the field of biomedicine, particularly in drug delivery and the management of metabolic diseases.

Despite the significant progress in FEVs research, numerous challenges and unresolved issues persist. First, isolation and purification techniques for FEVs demand further advancement. Current techniques, such as ultracentrifugation, size exclusion chromatography, and immunoprecipitation exhibit limited efficacy; however, they are associated with issues such as low efficiency, high cost, and complex operational procedures. In addition, the stability of FEVs represents a critical issue that must be addressed, particularly in terms of stability in the gastrointestinal environment and resistance to digestive enzymes. Future research should focus on developing more efficient isolation and purification techniques for FEVs, enhancing drug-loading efficiency, optimising in vivo stability and biodistribution, and evaluating their safety and efficacy in clinical settings. The mechanism of action of FEVs is not fully elucidated, which hinders the assessment of their safety and efficacy. Future studies should focus on gaining a deeper understanding of the distribution, metabolism, and target sites in the body. Various methods can be employed to enhance the targeting ability of EVs, such as antibody conjugation, genetic engineering, and physicochemical modifications. However, current targeting modification strategies for FEVs remain limited. Furthermore, classification and regulatory considerations of FEVs in clinical applications must be addressed. The multifunctionality of FEVs enable their classification as pharmaceuticals, biologics, and food product complexes. Therefore, regulatory authorities must establish the appropriate classification of FEVs and develop the corresponding guidance principles. Thus, further studies on the pharmacological effects, bioavailability, and safety of FEVs are required.

The study of FEVs is a multidisciplinary field that encompassing disciplines, such as food science, molecular biology, pharmaceutical sciences, and nanomaterial science. This interdisciplinary nature necessitates close collaboration among experts from diverse fields to address challenges in FEVs research. For example, food scientists can provide insights into the sources and isolation methods of FEVs; molecular biologists can investigate the bioactive components and mechanisms of action of FEVs; pharmaceutical scientists can explore the potential of FEVs as drug delivery carriers; and nanotechnology experts can advance the isolation and purification techniques of FEVs. To facilitate the clinical translation of FEVs, it is essential to establish a multidisciplinary collaboration network, promote data and resource sharing, and develop unified research and reporting standards. This will facilitate global cooperation, enhance research transparency and reproducibility, and promote the development of the FEVs industry. Interdisciplinary collaboration can also promote innovation in FEVs research, explore new applications of FEVs in drug delivery and metabolic disease management, and ultimately achieve personalised precision medicine.

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Fig. 3 was created with BioRender. com.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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