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Food-derived extracellular vesicles: isolation, characteristics, bioactivities, applications as targeted delivery carriers and adverse consequences

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ABSTRACT

Delivery carriers serve as a highly efficient approach for precision nutrition and medicine; however, artificial delivery carriers are prone to triggering the immune response and have the disadvantages of poor stability and low bioavailability. Extracellular vesicles (EVs), nucleus-free biological particles composed of phospholipid bilayers secreted by living cells, are a new generation of targeted delivery carriers. In recent years, an increasing number of species have been reported to contain EVs. Among them, food-derived extracellular vesicles (FDEVs) show outstanding comprehensive properties. FDEVs are considered to have great application potential due to their wide range of sources, high yields, absence of human pathogenic pathogens, and ethical concerns. In this review, the preparation, nomenclature, physicochemical characteristics, and preservation methods of FDEVs are discussed, as well as their potential protein markers, bioactivities, and applications as novel targeted delivery carriers of FDEVs from animals, plants, and microorganisms. We also summarized the adverse consequences of FDEVs in current studies, and put forward the problems and challenges in the process of FDEVs research and commercialization. In short, the importance of FDEVs has been highlighted, and FDEVs have good application prospects as a new class of targeted delivery carriers. The current problems should be paid attention to and actively solved.

1. Introduction

Precision nutrition aims to provide scientific, efficient, accurate, safe and timely nutritional interventions according to individual genetic background, living habits, physical conditions and other factors to achieve disease prevention and control and promote human health[1-2]. Precision nutrition is essentially to achieve the balance of individual metabolic function through the nutrient absorption of functional factors in food and the regulation of intestinal microorganisms. Therefore, food functional factors are the key to achieving the goal of precision nutrition. However, most food functional factors, including proteins, polysaccharides, lipids, phenols, terpenoids, and vitamins, have disadvantages such as poor stability, poor solubility, and low bioavailability[3]. In order to solve these problems, artificial carriers are often used to load food functional factors to increase their stability and achieve targeted delivery[4], including liposomes, polymer nanoparticles, emulsions, etc. However, artificial delivery carriers are prone to trigger immune responses in humans, along with short circulation *in vivo*, low bioavailability, and potential toxicity[5-6]. Therefore, the scientific community has been working hard to find stable, efficient, green and safe targeted delivery carriers.

Extracellular vesicles (EVs) are biological particles released from cells that are composed of phospholipid bilayers and contain proteins,

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lipids, nucleic acids, polysaccharides, and bioactive molecules. Moreover, it is widely accepted that EVs do not have a nucleus and cannot replicate. Typical EVs can be divided into three categories (Fig. 1A). The first category, known as exosomes, is released by the fusion of multivesicular bodies (MVBs) with the cell membrane, with diameters ranging from 30 to 150 nm[7]. The second category, known as microvesicles or ectosomes, are directly released from the membrane carrying some of the contents, with diameters ranging from 100 to 1 000 nm[8]. The third category, known as apoptotic bodies, is the irregular particles formed by the invagination of the cell membrane during programmed cell death, with diameters ranging from 1 to 5 μm[9]. With the deepening of research and the improvement of isolation methods, more categories of EVs have been reported successively. For example, migrasomes, which are vesicle structures released by breakage of contractile filaments during cell migration[10], and non-membranous extracellular granule subtypes—exomeres and supermeres[11].

When EVs were first discovered, researchers described them as "Platelet dust"[19], which was considered to be only cellular metabolic waste without biological functions. However, people gradually realized that EVs are not only important communication media between cells, but also have great application value in the diagnosis and treatment of various diseases as biomarkers with the deeper understanding of EVs[20]. More surprisingly, EVs have the characteristics of enhanced stability of cargoes in the gastrointestinal environment, targeting ability to specific tissues, high safety and good biocompatibility[21-22], which make them regarded as the next generation of targeted delivery carriers. Existing studies have also proved that some EVs themselves have certain disease prevention and/or treatment effects[23-24]. The general consensus of EVs mentioned above is mainly based on studies in mammalian cells, particularly humans, but there is growing evidence that EVs derived from plants and microorganisms also have similar characteristics and functions. Among them, plant-derived EVs have been sought after by researchers as nano-carriers with a wide range of sources and more promising application prospects[25].

In this review, particles with EVs-like characteristics derived from edible raw materials and accessories are named food-derived extracellular vesicles (FDEVs), and for the first time, their sources are expanded to three categories: animals, plants (including drug-food homologous species) and beneficial food microorganisms (Fig. 1B). FDEVs are abundant in resources, inexpensive, safe, sustainable, and have the potential for large-scale production[26]. Furthermore, FDEVs are free of human pathogenic pathogens and do not pose any ethical issues. Additionally, some FDEVs possess the ability to assimilate bioactive substances present in their source species[27]. FDEVs exhibit remarkable stability within the gastrointestinal tract and possess the ability to efficiently penetrate the intestinal epithelium, facilitating targeted delivery and precise release at the cellular level[28-29]. These attributes confer significant implications for enhancing drug or functional factor delivery. In brief, FDEVs are gradually being recognized as excellent carriers for targeted steady-state delivery.

This review provides a comprehensive overview of EVs derived from animals, plants and microorganisms in food. We systematically introduce the preparation, nomenclature, morphology, physicochemical properties and storage methods of FDEVs. Furthermore, we discuss the potential protein markers, bioactivities and advancements in targeted delivery of food functional factors or drugs within FDEVs. Notably, FDEVs exhibit significant therapeutic effects on various diseases including the nervous system, respiratory system, intestinal system, as well as cancer. FDEVs hold immense application potential in precision nutrition and human health enhancement. Finally, we summarize the reported adverse consequences of FDEVs while highlighting current challenges in their development.

2. Approaching the FDEVs

2.1 Nomenclature and isolation methods

Minimal information for studies of EVs has been published by the International Society of Extracellular Vesicles (ISEV) in 2023 (MISEV2023). The guidelines emphasize the necessity of providing sufficient evidence for biogenesis when using specific terms such as exosome, ectosome and microvesicle[30]. The secretion of milk is an autonomous process carried out by mammary cells. Moreover, these milk-derived EVs encompass a diverse range of protein markers commonly found in typical exosomes, which has led to many studies referring to these isolated nanoparticles as exosomes (Table 1). During the process of juicing or homogenization, plant-derived FDEVs induce cellular damage, resulting in the release of organelle efflux, unnatural EVs and cell debris[25]. Consequently, researchers commonly refer to these vesicles as exosome-like nanoparticles or EVs-like nanovesicles. In fact, there is considerable variability in the nomenclature used for these isolated particles (Table 2). As shown in Table 3, microorganism-derived FDEVs are generally extracted directly from the supernatant and referred to as EVs in most studies. Fungus-derived FDEVs are often termed as exosome-like nanoparticles due to the homogenization treatment. Clear criteria for the nomenclature of FDEVs are still lacking, and the standardization of nomenclature for FDEVs derived from plants and microorganisms can be achieved in accordance with the MISEV2023 guidelines.

In principle, FDEVs can be separated and purified based on their size, density, specific protein composition, charge characteristics, etc. The existing extraction methods include differential centrifugation, ultracentrifugation, density gradient centrifugation, size exclusion chromatography, polyethylene glycol (PEG) precipitation, affinity chromatography, asymmetric flow field, microfluidic chips and commercially available kits[31]. Through the summary of FDEVs, it is evident that differential centrifugation emerges as the most prevailing method for isolating FDEVs, which is also known as the "gold standard"[32]. However, owing to the inherent distinct characteristics of animal, plant and microorganism sources, there are still some differences in the isolation methods of FDEVs from each category. The milk-derived FDEVs have been extensively investigated in the field of animal-derived FDEVs research, with a higher content of protein and fat. Consequently, the extraction process generally requires the elimination of casein and lipids through chemical sedimentation or enzymatic hydrolysis, followed by subsequent filtration and/or centrifugation (Table 1). The isolation of FDEVs can be achieved from juice-rich plants, such as pear, orange, blueberry and grapefruit. However, for melons or herbs, it is necessary to obtain original samples by adding PBS for mixing and homogenization before isolating FDEVs (Table 2). The plant-derived FDEVs were mostly isolated by differential centrifugation

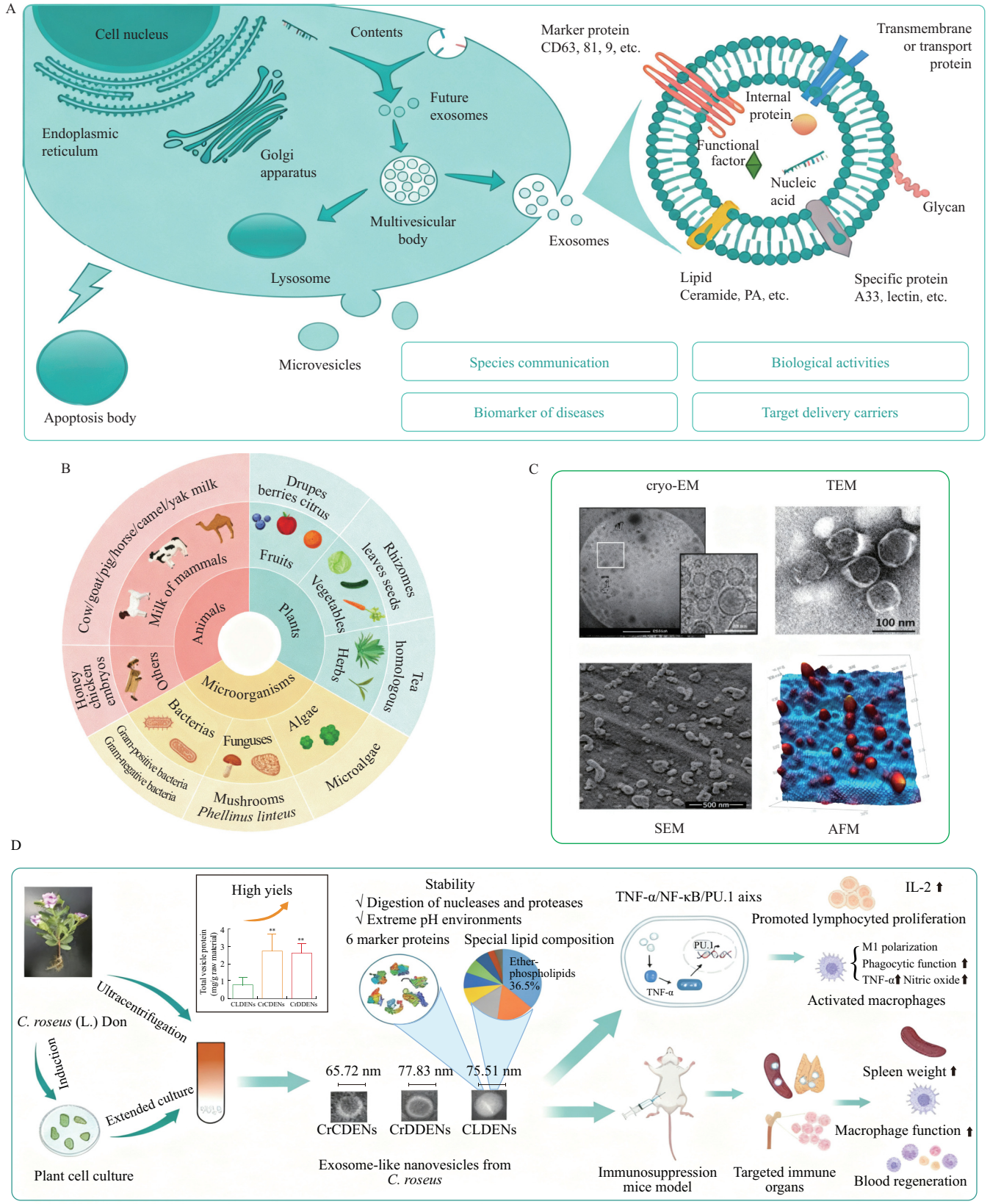

Fig. 1 Biogenesis of EVs, and the sources, morphology, bioactivities, applications and adverse consequence of FDEVs. (A) Typical classification of EVs, and classical exosome structure. (B) The main species of FDEVs. (C) The morphology of milk-derived EVs: cryo-EM[12], TEM[13], SEM[14], AFM[15]. (D) The immunostimulation effect of *C. roseus* derived FDEVs through the TNF-α/NF-κB/PU-1 pathway[16]. CrCDENs, *C. roseus* cambial meristematic cells-derived exosome-like nanovesicles; CrDDEN, *C. roseus* dedifferentiated cells-derived exosome-like nanovesicles. (E) Schematic representation of the CD44 targeted formation of the hyaluronic acid (HA)-modified milk exosome AST delivery system (HA-mExo-AST) and its intervention effect on LPS-induced oxidative damage[17]. (F) Oral administration of milk-derived FDEVs induced primary tumor senescence, but accelerated tumor metastasis in an environmentally dependent manner[18]. Pictures sourced from BioRender.com in Fig. 1B.

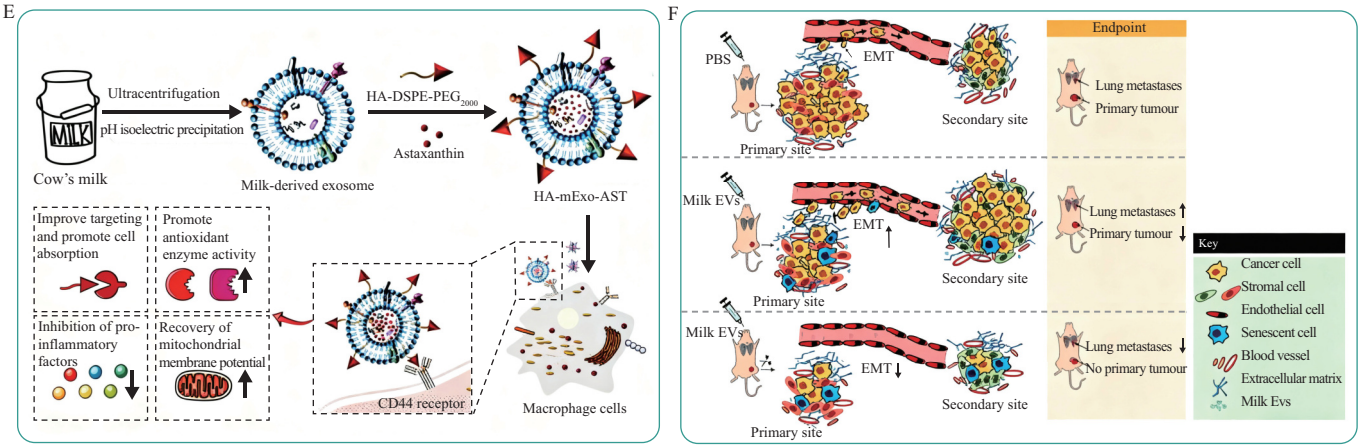

Fig. 1 (*Continued*)

or in combination with density gradient centrifugation. In particular, Regente et al.[33] successfully isolated vesicles from sunflower seeds using the vacuum tissue extraction method, which minimized cellular damage to a significant extent. However, it is important to note that this technique may still result in disruption of cell metabolism and has not gained widespread adoption. The microorganism-derived FDEVs are commonly isolated by differential centrifugation, which does not differ significantly from the centrifugation speeds of animal- or plant-derived FDEVs. The use of differential centrifugation in combination with other separation methods is increasing, but each separation method possesses its own advantages and disadvantages. It is necessary to carefully select the isolation procedure based on the research investigation and subsequent application[34].

2.2 General characteristics

2.2.1 Morphology, size and Zeta potential

To assess the successful isolation of FDEVs, it is essential to determine the morphology, size, and protein markers of the extracted particles. While typical EVs are characterized by saucer-like or spherical stereoscopic biological particles with a membrane structure, EVs secreted by identical cells exhibit heterogeneity, resulting in diverse morphologies[83]. Moreover, differences in measurement methods lead to significant disparities in the outcomes of identical resources. Pre-treatment procedures, such as transmission electron microscope (TEM), scanning electron microscope (SEM), atomic force microscope (AFM), can potentially induce dehydration of FDEVs, leading to detrimental alterations in their original morphology. The ultra-low temperature sample preparation technology of cryo-electron microscope (cryo-EM) enables the preservation of the original morphology of FDEVs to a significant extent, facilitating more accurate imaging and providing a novel approach for the observation of FDEVs. Fig. 1C intuitively demonstrates that milk-derived FDEVs exist as regular round spheres with a membrane structure in the cryo-EM analysis, while other 3 methods display poorer regularity and increased heterogeneity.

The sizes of FDEVs derived from animals and microorganisms showed a unimodal distribution, with most falling within the range of 150 nm, which is consistent with the size of typical EVs. In contrast, the size distribution of plant-derived FDEVs ranged from 50 to 400 nm, covering a wide range with the majority falling above 150 nm. Some plant-derived FDEVs, such as blueberry[48] and yam[55], even exhibited multiple size peaks in their distribution patterns. Similar to the morphological determination of FDEVs, the size determination results can be influenced by variations in sample preparation techniques employed across different methods. The use of TEM, SEM and AFM lead to potential underestimation of the size of FDEVs. Dynamic light scattering (DLS) and nanoparticle tracking analyzer (NTA) may show bias in the results due to the swelling effect on FDEVs during measurement. The cryo-EM offers the best preservation of FDEVs' size, but faces challenges regarding equipment availability. Furthermore, the size of FDEVs can vary significantly depending on the solution, with reduced heterogeneity observed for grapefruit-derived FDEVs in acidic solutions compared to aqueous or alkaline solutions[25]. Therefore, in order to comprehensively comprehend the fundamental characteristics of FDEVs, it is imperative to provide results from multiple determination methods for their size characteristics.

All FDEVs have a negative Zeta potential, which remains unaffected even when loaded with food functional factors or drugs. The electrostatic repulsion between similarly charged particles can prevent the agglomeration of FDEVs and enhance system stability[16]. At the same time, the negative potential confers an advantage to FDEVs by evading clearance from the body' s immune system, enabling prolonged circulation within the organism and thereby enhancing bioavailability and application efficacy[84]. The magnitudes of the various potentials exhibited by FDEVs showed significant variation, with plant-derived FDEVs generally displaying larger values. Interestingly, the potential in the range of 10 to 30 mV demonstrated enhanced physical stability[85]. Therefore, it is crucial to consider the potential characteristics of FDEVs from different sources, as these characteristics may influence their respective application scenarios or engineering approaches.

2.2.2 Components and potential protein markers

The fundamental structure of FDEVs consists of a phospholipid bilayer, with an array of proteins and polysaccharides present on the outer surface, while nucleic acids are confined within the enclosed compartment. The bioactive substances derived from FDEVs exhibit significant diversity depending on their source[26,29,86]. The proteins contained in FDEVs can be classified into skeleton proteins, marker

Table 1
Isolation methods and properties of FDEVs from animals.

Source	Method	Process	Nomenclature	Filter membrane	Properties	Storage condition
Milk	UC	(1) 13 000 × *g* for 30 min (2) Supernatant: EDTA (3:1, *V*/*V*) for 15 min (3) 100 000 × *g* for 1 h (4) 135 000 × *g* for 1.5 h	Milk-derived exosome	/	DLS: 72.9 nm NTA: 83.2 nm PDI: 0.225 Zeta potentials: –9.8 mV Markers: CD63, CD81, TSG101	PBS –80 °C
Goat milk	UC + SEC	(1) 5 000 × *g* for 10 min (2) Added microbial rennet (3) 5 000 × *g* for 10 min (4) 13 000 × *g* for 35 min (5) 35 000 × *g* for 15 min (6) 10 000 × *g* for 70 min (7) Washed thrice with PBS and purified with PD-10 columns (8) 100 000 × *g* for 1.5 h	Goat milk exosomes	/	DLS: (125.7 ± 3.25) nm NTA: (124.44 ± 8.54) nm PDI: 0.14 Markers: CD9, CD63, CD81, TSG101, HSP90AA1, HSP90AB1, Annexins (Proteomic data)	PBS –20 °C
Horse milk	UC + gel-filtration + affinity chromatography	(1) 100 000 × *g* for 40 min (2) 12 000 r/min for 40 min (twice) (3) Thrice at 30 000 r/min (4) Filtered through a 0.1 μm filter and purified with gel-filtration (5) Further purified on anti-CD81-Sepharose (6) Eluted with the buffer	Horse milk exosomes	0.1 μm	Markers: CD9, CD81, CD63	/
Porcine milk	UC	(1) 2 000 × *g* for 30 min (2) 15 000 × *g* for 30 min (3) Transferred at –80 °C (4) Filtered through a 0.45 μm filter (5) 160 000 × *g* for 1.5 h (6) Filtered through a 0.22 μm filter	Porcine milk exosomes	0.45 μm 0.22 μm	DLS: 155.7 nm Markers: CD9, CD63	PBS –80 °C
Bactrian camel	UC	(1) 8 000 × *g* for 30 min (2) 13 000 × *g* for 30 min (3) 100 000 × *g* for 2 h (4) Filtered through a 0.22 μm filter	Bactrian camel milk-derived exosomes	0.22 μm	TEM: 70–100 nm	PBS –80 °C
Yak milk	(A) UC (B) UC with rennet precipitation	(1) 8 000 × *g* for 30 min (2) 13 200 × *g* for 1 h (A) (3) 120 000 × *g* for 1.5 h (4) Filtered through 0.45 μm and 0.22 μm filters (B) (3) Added rennet (0.025 g/L) for 6 h at 37 °C 4) Filtered through a 0.45 μm filter and 120 000 × *g* for 1.5 h (5) Washed in PBS at 120 000 × *g* for 1.5 h (6) Filtered through 0.45 and 0.22 μm filters	Yak-milk-derived exosomes	0.45 μm 0.22 μm	nano-ZS: (A) (109.2 ± 57.1) nm PDI: 0.256 (B) (112.4 ± 48.6) nm PDI: 0.167 Markers: CD63, TSG101, HSP70, *β*-actin	PBS –80 °C
Honey	UC or density gradient centrifugation	(1) 500 × *g* for 10 min (2) 2 000 × *g* for 20 min (3) 10 000 × *g* for 30 min (4) 100 000 × *g* for 2 h (5) Filtered through a 200 nm filters (6) Alternatively, sucrose gradient (8%/30%/60%) 150 000 × *g* for 16 h and washed in PBS at 100 000 × *g* for 2 h	Honey-derived vesicle-like nanoparticles (H-VLNs)	200 nm	NTA: 142–156 nm H-VLNs contains 142 plant-derived proteins and 82 honey bee-derived proteins.	PBS
Chicken embryo	(A) UC (B) Density gradient centrifugation (C) Membrane affinity (exoEasy kit) (D) PEG precipitation (exoQuick kit)	(1) Type III collagenase (0.5%) enzyme digestion for 30 min at 37 °C (2) 300 × *g* for 15 min (3) 2 000 × *g* for 15 min (4) 20 000 × *g* for 30 min (A) Filtered through a 0.22 μm filter and 100 000 × *g* for 2 h + 1.5 h (B) (5) Filtered through a 100 μm filter and 100 000 × *g* for 2 h (6) 174 000 × *g* for 3 h in sucrose gradient and collected samples at 30% (7) 100 000 × *g* for 1.5 h (C) (5) Filtered through a 0.8 μm filter (6) Added buffer XBP with an equal volume and placed in exoEasy spin (7) 500 × *g* for 1 min and added 10 mL buffer XBP (8) 5 000 × *g* for 5 min and collected by buffer XE (D) 5) Filtered through a 100 μm filter and added exoQuick TC for 12 h (6) 3 000 × *g* for 10 min (7) The precipitate was dissolved in Buffer B + A and placed in a purification column (8) 1 000 × *g* for 1 min and collected by Buffer B + A	Chicken embryo tissue-derived exosomes	(A) 0.22 μm	nanoFCM: average diameter (A) (71.8 ± 11.0) nm (B) (73.4 ± 12.9) nm (C) (79.4 ± 25.9) nm (D) (77.0 ± 24.5) nm Markers: CD63, CD81	PBS –80 °C

Note: / means not reported; Breast milk with potential ethical issues and non-edible animal-derived EVs (chicken allantois) were excluded from the statistics. UC, ultracentrifugation; NTA, nanoparticle tracking analysis; PDI, polydispersity index; SEC, size exclusion chromatography; nano-ZS, Zeta-sizer Nano-series instrument; nano-FCM, nano-flow cytometry.

Table 2
Isolation methods and properties of FDEVs from plants.

Type	Source	Method	Process	Nomenclature	Filter membrane	Properties	Storage condition	Reference
Fruit	Apple	UC	Crushed with the apple skin (1) 2 000 × *g* for 20 min (2) 13 000 × *g* for 70 min (3) 120 000 × *g* for 130 min	Apple-derived nanoparticles	/	qNano: 140 nm	PBS	[42]
	(i) Pear (ii) Kiwifruit	UC	Peeled, wrapped in gauze, and squeezed by hand (1) 1 200 × *g* for 20 min (2) 3 000 × *g* for 20 min (3) 10 000 × *g* for 1 h (4) Filtered through a 1 μm filter (5) 150 000 × *g* for 1.5 h	Edible plant-derived exosome-like nanoparticles (EPDELNs)	1 μm	DLS: (i) about 100–400 nm (ii) about 10–80 nm and 100–500 nm	PBS	[43]
	Coconut	UC	Directly extracted (1) 3 000 × *g* for 1 h (2) 20 000 × *g* for 1 h (3) 120 000 × *g* for 1.5 h (4) Filtered through a 0.22 μm filter	Coconut nanoparticle	0.22 μm	SEM: 26.8 nm TEM: 44.21 nm DLS: 138.1 nm Markers: SNAP25, CD9, CD63	PBS –20 °C	[44]
	Acerola	(A) UC (B) exoEasy maxi kit (C) Exoquick reagent	(A) Not specified (B)(1) Acerola juice filtered through a 0.45 μm polyvinylidene fluoride filter (2) Added buffer XBP with an equal volume and placed in exoEasy spin column (3) Added 800 mL buffer XE and 5 000 × *g* for 5 min (4) 100 000 × *g* for 70 min and collected by PBS (C) (1) Filtered through a 0.45 μm polyvinylidene fluoride filter (2) Added 2 mL ExoQuick reagent (3) Refrigeration for 12 h and 1 500 × *g* for 30 min	Acerola exosome-like nanovesicles	0.45 μm	NTA: (A) (352 ± 180) nm (B) (245 ± 132) nm (C) (340 ± 172) nm	PBS	[45]
	Pomegranate	SEC	(1) Stacked up to 13 mL Sepharose-CL2B and washed thrice with PBS (2) Loaded 1 mL concentrated extract onto the column and collected 0.5 mL of each sample (3) Collected fractions 6–10	Extracellular vesicles from pomegranate juice	/	NTA: 148.7 nm TEM: 122.6 nm	PBS	[46]
	Grape	Density gradient centrifugation	Collected the juice from the peeled fruit (1) 500 × *g* for 10 min (2) 1 200 × *g* for 30 min (3) 10 000 × *g* for 1 h (4) Sucrose gradient (8%/30%/45%/60%) 36 000 r/min for 1.5 h	Grape exosome-like nanoparticles	/	DLS: (380.5 ± 37.47) nm Zeta potentials: (–26.3 ± 8.14) mV	/	[47]
	Blueberry	UC	(1) Added 5 times PBS and passed through a metallic strainer (2) 1 000 × *g* for 10 min (3) 5 000 × *g* for 20 min (4) 11 000 × *g* for 50 min (5) Filtered through a 0.45 μm filter (6) Ultra-filtered in 10 000 MWCO at 125 g/min (7) Filtered through a 0.22 μm filter (8) 120 000 × *g* for 1.5 h	Blueberry-derived exosome-like nanoparticles	0.45 μm 0.22 μm	DLS: (198 ± 112) nm LTS: (108 ± 14) nm and (286 ± 6) nm SEM: (114 ± 36) nm	–80 °C	[48]
	Strawberry	UC	Strained the juice with a colander (1) 3 000 × *g* for 30 min (2) 10 000 for 1 h (3) Filtered through a 100 mesh cell strainer and 16 500 × *g* for 1 h thrice (4) Filtered through a 0.45 μm filter and 110 000 × *g* for 1 h (5) Washed with PBS and 110 000 × *g* for 1 h PBS	*Fragaria*-derived EPDELNs	100 mesh cell strainer 0.45 μm	TEM: 30–191 nm	PBS –80 °C	[49]
	Hami melon	UC	(1) Ground with PBS and 1 200 × *g* for 20 min (2) 3 000 × *g* for 20 min (3) 10 000 × *g* for 1 h (4) Filtered through a 1 μm filter (5) 150 000 × *g* for 1.5 h	EPDELNs	1 μm	DLS: about 100–550 nm	PBS	[43]
	Lemon	UC + Density gradient centrifugation	(1) 4 000 × *g* for 1 h (2) 10 000 × *g* for 1 h (3) 120 000 × *g* for 2 h PBS (4) Sucrose gradient (8%/30%/45%/60%) 120 000 × *g* for 1 h (5) Washed with PBS and 120 000 × *g* for 1 h	Lemon-derived extracellular vesicle-like nanoparticles	/	Band 8/30% NTA: about165 nm Zeta potentials: –22.4 mV	PBS	[50]

Table 2 (*Continued*)

Type	Source	Method	Process	Nomenclature	Filter membrane	Properties	Storage condition	Reference
	Orange	UC	(1) Filtered through a strainer and 4 000 × *g* for 30 min (2) 10 000 × *g* for 1 h (3) 100 000 × *g* for 2 h (4) Re-suspended in 0.9% NaCl + 1% DMSO and filtered through a 0.22 μm filter	EVs extracted from orange juice	0.22 μm	NTA: (167 ± 10) nm	0.9% NaCl + 1% DMSO –80 °C	[51]
	Grapefruit	UC	(1) Initial juice 1 200 × *g* for 20 min (2) 3 000 × *g* for 20 min thrice (3) 10 000 × *g* for 1 h (4) 15 000 × *g* for 1 h (5) 10 000 × *g* overnight and further at 150 000 × *g* for 2 h (6) Swaying overnight in PBS and 150 000 × *g* for 2 h (7) Shaking in PBS for 1 h and frozen in liquid nitrogen	Grapefruit-derived EVs	/	NTA: (52 ± 8) nm DLS: about 30 nm Cryo-EM: (41 ± 13) nm	PBS –80 °C	[52]
	(i) Commercially 100% natural pineapple juice (ii) Commercially 100% natural grapefruit juice	UC	(1) 2 000 × *g* for 5 min (2) Filtered through 0.45 and 0.2 μm pore filters (3) 100 000 × *g* for 4 h	Exosome-like nanovesicles from fruit juice	0.45 μm 0.22 μm	NTA: (i) (169 ± 70) nm (ii) (258 ± 104) nm	/	[53]
Vegetable	Carrot	SEC	(1) 8 000 × *g* 1 h (2) 20 000 × *g* for 1 h (3) Concentrated with a Amicon Ultra-15 filter unit and eluted with PBS	Carrot-derived extracellular vesicles (Carex)	Amicon Ultra-15	NTA: 143.9 nm PDI: 0.43 Zeta potentials: –10.2 mV	PBS	[54]
	Yam	UC	Blended with ice-cold PBS (1) 500 × *g* for 10 min (2) 2 000 × *g* for 20 min (3) 10 000 × *g* for 30 min twice (4) 100 000 × *g* for 1 h and shaking overnight in PBS (5) 100 000 × *g* for 1 h (6) Shaking in 0.5 mL of PBS for 1 h at 4 ℃ and frozen in liquid nitrogen	Yam-derived exosome-like nanovesicles	/	NTA: 168 and 328 nm	PBS –80 °C	[55]
	Ginger	UC + Density gradient centrifugation	Homogenized in a high-speed blender for 1 min from the peeled fruit (1) 1 000 × *g* for 10 min (2) 2 000 × *g* for 20 min (3) 4 000 × *g* for 30 min (4) 10 000 × *g* for 1 h (5) 100 000 × *g* for 1.5 h (6) Sucrose gradient (8%/30%/45%/60% in 20 mmol/L Tris-HCl) 100 000 × *g* for 1.5 h	Ginger-derived exosome-like nanoparticles	/	NS: (206.8 ± 81.1) nm	/	[56]
	Turmeric	UC + Density gradient centrifugation	Ground to obtain juice in an extractor (1) 3 000 × *g* for 20 min (2) 10 000 × *g* for 40 min (3) 150 000 × *g* for 2 h (4) Sucrose gradient (8%/30%/45%, *m*/*V*) 150 000 × *g* for 2 h	Turmeric-derived exosome-like nanoparticles	/	Band 30%/45%: DLS: 177.9 nm Zeta potentials: –21.7 mV	PBS –80 °C	[57]
	Garlic	Aqueous two-phase systems	(1) Garlic juice 300 × *g* for 10 min (2) Mixed with ATPS-EV solution (1:1) and 1 000 × *g* for 10 min (3) Added PEG rich washing solution and 1 000 × *g* for 10 min (twice) (4) Filtered through a 0.22 μm filter	Garlic derived small EVs	0.22 μm	DLS: 50–150 nm Markers: HSP70, CD9, CD63	/	[58]
	Onion	UC	(1) Onion juice 1 000 × *g* for 5 min (2) 17 000 × *g* for 15 min (3) 200 000 × *g* for 1 h	Onion-derived nanoparticles	/	DLS: 185.3 nm	PBS –80 °C	[59]
	Celery	UC + Density gradient centrifugation	(1) Celery juice 2 000 × *g* for 20 min (2) 5 000 × *g* for 30 min (3) 10 000 × *g* for 1 h (4) 100 000 × *g* for 2 h in PBS (5) Sucrose gradient (30%/45%/60%) 100 000 × *g* for 1 h (6) Collected Band 30%/45% in PBS 100 000 × *g* for 1 h	Celery exosome-like nanovesicles	/	Band 30%/45% NTA: 111.8 nm DLS: 115.7 nm Zeta potentials: –34.24 mV	PBS	[60]

Table 2 (*Continued*)

Type	Source	Method	Process	Nomenclature	Filter membrane	Properties	Storage condition	Reference
	Cucumber	UC	(1) Juiced from the peeled cucumbers and added the protease inhibitor cocktail (2) 400/800/2 000/15 000 × *g* for 30 min (3) Filtered through a 0.45 μm filter (4) 120 000 × *g* for 1 h and added fresh samples until all the juice was processed (5) Resuspended in PBS and vortexed for 20 min	Cucumber-derived plant exosome-like vesicles	0.45 μm	NTA: 123 nm DLS: (167 ± 3) nm PDI: 0.2 ± 0.02 Zeta potentials: (–32 ± 0.13) mV	PBS –80 °C	[61]
	Momordica charantia	UC + Density gradient centrifugation	(1) MC juice 1 000 × *g* for 10 min (2) 3 000 × *g* for 20 min (3) 10 000 × *g* for 40 min (4) 150 000 × *g* for 1.5 h (5) Sucrose gradient (8%/30%/45%/60%) 150 000 × *g* for 1.5 h (6) Collected Band 30%/45% and 150 000 × *g* for 1.5 h (7) Filtered through a 0.22 μm filter	*M. charantia*-derived extracellular vesicles-like nanovesicles	0.22 μm	Band 30/45% NTA: 120 nm Markers: CD54, CD63, TSG101	PBS	[62]
	(i) Cabbage (ii) Red cabbage	(A) PEG-based precipitation (B) UC (C) SEC	1) 8 000 × *g* for 1 h 2) 20 000 × *g* for 1 h (A) Mixed PEG with juice at 1:5 (*m*/*V*) overnight at 4 °C and 1 500 × *g* for 30 min (B) 120 000 × *g* for 2 h (C) Concentrated with a Amicon Ultra-15 filter unit and eluted with PBS	Exosome-like nanovesicles from cabbage and red cabbage (Cabex and Rabex)	Amicon Ultra-15	(i) Cabex: Zeta potentials: –14.8 mV NTA: (A) 148.2 nm (B) 134.2 nm (C) 98.8 nm (ii) Rabex: Zeta potentials: –15.2 mV (A) 185 nm (B) 128 nm (C) 103 nm	PBS –80 °C	[63]
	Tomato	UC	(1) 1 500 × *g* for 30 min (2) 3 500 × *g* for 20 min (3) 10 000 × *g* for 1 h (4) 16 000 × *g* for 1 h (5) 10 000 × *g* overnight (5) 150 000 × *g* for 2 h and swaying in 2 mL of PBS overnight (6) Added 8 mL of PBS and 150 000 × *g* for 2 h (7) Resuspended in PBS for 1 h at 4 °C and frozen in liquid nitrogen	Tomato exosome-like vesicles	/	NTA/DLS: 140–170 nm Zeta potentials: –11 to –25 mV	PBS –80 °C	[64]
	Broccoli	UC/+SEC	Broccoli flower heads placed in 1:1.5 (*m*/*V*) milli-Q water and homogenized for 2 min (1) 5 000 × *g* for 30 min (2) 10 000 × *g* for 30 min (3) 25 000 × *g* for 30 min (4) 35 000 × *g* for 1 h (5) 135 000 × *g* for 105 min (6) Resuspended in PBS and filtered through a 0.45 μm filter (7) Loaded 0.6 mL concentrated extract onto the column and eluted with PBS	Broccoli-derived EVs	0.45 μm	UC NTA: (146.7 ± 7.2) nm SEC NTA: (174.3 ± 5.5) nm	PBS –80 °C	[22]
	Petasites japonicu	UC	Ground PJ leaves to obtain juice (1) 200 × *g* for 10 min (2) 2 000 × *g* for 20 min (3) 10 000 × *g* for 30 min (4) Filtered through a 0.22 μm filter (5) 100 000 × *g* for 1 h (6) Filtered through a 0.22 μm filter	*P. japonicus*-derived EVs	0.22 μm	DLS: 122.6 nm TEM: 100–140 nm	PBS –80 °C	[65]
	Paprika	UC	(1) 2 000 × *g* for 5 min (2) Filtered through filter paper (3) Filtered through 0.45 and 0.2 μm pore filters (4) 100 000 × *g* for 4 h	Exosome-like nanovesicles EVs	0.45 μm 0.2 μm	NTA: (232 ± 114) nm	/	[53]
	Sunflower seed	Vacuum pulses + UC	(1) Soaked in water for 2 h and peeled (2) Immersed in 50 mmol/L Tris-HCl (pH 7.5), 0.6% NaCl, 0.1% 2-mercaptoethanol and subjected to three vacuum pulses (10 s on and 30 s off) (3) 400 × *g* for 20 min (4) Filtered through a 0.5 μm filter (5) 10 000 × *g* for 30 min (6) 40 000 × *g* for 1 h (7) 100 000 × *g* for 1 h	Vesicular or exosome-like vesicles	0.5 μm	TEM: 50–200 nm	20 mmol/L Tris-HCl pH 7.5	[37]

Table 2 (*Continued*)

Type	Source	Method	Process	Nomenclature	Filter membrane	Properties	Storage condition	Reference
	(i) *Juglans regia* (ii) *Juglans californica* (iii) *Corylus avellana* (edible dried nuts)	UC	(1) Homogenized and mixed with 1:10 (*m*/*V*) PBS (2) 20 000 × *g* for 15 min (3) 10 000 × *g* for 1 h (4) Filtered through a 0.45 μm filter (5) 16 500 × *g* for 1 h (6) 110 000 × *g* for 70 min (7) Filtered through a 0.45 μm filter (8) 16 500 × *g* for 10 min	Nanovesicles	0.45 μm	DLS: (i) 90 and 230 nm (ii) 230 nm (iii) 90 and 560 nm Zeta potentials: –21 to –23 mV	/	[66]
	(i) Soybean (ii) Pea	UC	Ground with PBS (1) 1 200 × *g* for 20 min (2) 3 000 × *g* for 20 min (3) 10 000 × *g* for 1 h (4) Filtered through a 1 μm filter (5) 150 000 × *g* for 1.5 h	EPDELNs	1 μm	DLS: (i) about 100–400 nm (ii) about 100–550 nm	PBS	[43]
	Oat	UC + density gradient centrifugation	Oat bran meal was dissolved and incubated in PBS for 30 min at 37 °C (1) 1 000 × *g* for 10 min (2) 2 000 × *g* for 20 min (3) 4 000 × *g* for 30 min (4) 10 000 × *g* for 1 h (5) 100 000 × *g* for 2 h (6) OptiPrep gradients (10%/20%/40% in Tris-HCl) 100 000 × *g* for 2 h	Oat exosome like nanoparticles	/	NS: 135 nm	/	[67]
	Corn	UC + density centrifugation	(1) Homogenized the edible portion for 2 min and 2 000 × *g* for 20 min (2) 5 000 × *g* for 30 min (3) 10 000 × *g* for 1 h (4) Filtered through a 0.45 μm filter (5) Added 2 mL of 60% sucrose to 38 mL filtered sample and 100 000 × *g* for 2 h	Corn-derived nanoparticles (cNPs)	0.45 μm	DLS: (83.3 ± 22.2) nm Zeta potentials: (–17.4 ± 1.8) mV (3.56 ± 0.19) × 10^{12} particles/mL	/	[68]
Herb	Ginseng	UC + density gradient centrifugation	(1) 3 000 × *g* for 30 min (2) 10 000 × *g* for 1 h (3) 150 000 × *g* for 2 h PBS (4) Sucrose gradient (8%/30%/45%/60%, *m*/*V*) 150 000 × *g* for 2 h	Ginseng-derived exosomes	/	Band 30%/45% (144.1 ± 2.8) nm Zeta potentials: (–27.4 ± 0.45) mV	PBS –80 °C	[69]
	Pueraria lobata (Gegen)	Centrifugation	(1) Ground the fresh leaves and stems (2) The juice through a filter paper (2) 10 000 × *g* for 10 min (3) Filtered through a 0.22 μm filter (4) 5 000 × *g* for 10 min (5) Filtered through an Amicon Ultra-4 PL 100 KDa centrifugal filter	*P. lobata*-derived exosomes	0.22 μm 100 kDa filter	DLS: 40–150 nm NS: 1 × 10^{11} particles/mL	PBS	[70]
	Salvia dominica hairy root	UC	(1) Conditioned medium 300 × *g* for 10 min (2) 2 000 × *g* for 20 min (3) Filtered through a 0.45 μm filter 15 000 × *g* for 20 min (4) 100 000 × *g* for 3 h	*S. dominica* hairy root-derived extracellular vesicles	0.45 μm	DLS: 140–210 nm NTA: (153 ± 1.8) nm (1.89 × 10^{9} ± 8.6 × 10^{7}) particles/mL Markers: actin, HSP70/80/90, enolases, tetraspanin-7, ATPase8, etc. (143 proteins data)	MilliQ water –80 °C	[71]
	Catharanthus roseus (L.) Don	UC + Density centrifugation	(1) The leaves were digested with cellulase (3 g/100 mL) and pectinase (0.2 g/100 mL) for 12 h (2) 3 000 r/min for 20 min (3) 1 000 r/min for 40 min (4) Concentrated by hollow fiber module (5) Added 7 mL of 0.971 mol/L sucrose and 150 000 × *g* for 1.5 h (6) Removed the sucrose using an ultrafiltration tube	*C. roseus*-derived exosome-like nanovesicles (CLDENs)	Hollow fiber module	DLS: 141.7 nm nano-flowmeter: (75.51 ± 10.19) nm Zeta potentials: –21.8 mV Potential marker: 6 proteins: ABC transporter C family member 10, DnaJ homolog subfamily C GRV2-like, etc. (Proteomic data)	PBS –80 °C	[16]
	Aloe	UC	(1) The gel and rind were ground, and through a filter (2) 1 000 × *g* for 10 min (3) 3 000 × *g* for 20 min (4) 10 000 × *g* for 45 min (5) 100 000 × *g* for 20 min (6) Filtered through a G-25 desalting column	Nanovesicles derived from aloe	Desalting column	Gel: NTA: 138.7 nm Zeta potentials: –7.4 mV Rind: NTA: 220 nm Zeta potentials: –8.9 mV Marker: HSP70, GAPDH, Annexin, Adenosyl-homocysteinase, Glutathione S transferase, etc. (Proteomic data)	PBS –20 °C	[72]

Table 2 (*Continued*)

Type	Source	Method	Process	Nomenclature	Filter membrane	Properties	Storage condition	Reference
	Tea leaf	UC + Density gradient centrifugation	(1) Homogenized with cold PBS for 10 min (2) 1 000 × *g* for 20 min (3) 5 000 × *g* for 40 min (4) Sucrose gradient (8%/30%/45%/60% in 20 mmol/L Tris-HCl, pH 7.2) 120 000 × *g* for 2 h	Tea leaf exosome-like nanotherapeutics	/	Band 30%/45% DLS: 166.9 nm PDI: 0.1 TEM/AFM: 70 nm Zeta potentials: −28.8 mV	−80 °C	[73]
	Tea flower	UC + Density gradient centrifugation	(1) Mixed with 1:2 (*m/V*) PBS and homogenized for 5 min (2) 3 000 × *g* for 20 min (3) 10 000 × *g* for 1 h (4) 150 000 × *g* for 2 h (5) Sucrose gradient (8%/15%/30%/45%/60%, *m/V*) 150 000 × *g* for 2 h	Tea flower exosome-like nanoparticles	/	Band 15%/30% DLS: 131 nm PDI: 0.155 TEM: 20 nm	−80 °C	[74]

Note: / means not reported; LTS, laser transmission spectroscopy, NS: nano-sight NS300 instrument; MWCO, molecular weight cut off.

Table 3
Isolation methods and properties of FDEVs from microorganisms.

Type	Source	Method	Process	Nomenclature	Filter membrane	Properties	Storage condition	Reference
Gram-positive bacteria (G+)	*Lactobacillus plantarum*	UC	(1) 3 000 × *g* for 15 min (2) 35 000 × *g* for 1 h (3) 200 000 × *g* for 1 h (4) Filtered through a 0.22 μm filter	*L. plantarum*-derived extracellular vesicle (*L. plantarum*-derived EVs)	0.22 μm	DLS: (124.1 ± 6.9) nm ($3.15 \times 10^8 \pm 8.04 \times 10^7$) particles/mL (24 h) (129.0 ± 6.8) nm ($3.80 \times 10^8 \pm 6.92 \times 10^7$) particles/mL (48 h)	PBS	[75]
G+	*Lactobacillus rhamnosus* GG	UC	(1) 2 000 × *g* for 10 min (2) 10 000 × *g* for 30 min (3) Filtered through a 0.22 μm filter (4) 100 000 × *g* for 1 h	*L. rhamnosus* GG-derived extracellular vesicles (LDEVs)	0.22 μm	TEM: 30–100 nm (48 h)	PBS –80 °C	[76]
G+	*Lactobacillus casei* BL23	UC	(1) 4 000 × *g* for 25 min (2) Filtered through 0.8, 0.65 and 0.45 μm filters (3) Concentrated with 100 kDa cut-off filter and further filtered through a 0.45 μm filter (4) 110 000 × *g* for 2 h	Microvesicles from *L. casei* BL23 (*L. casei* BL23 MVs)	0.8 μm 0.6 μm 0.45 μm	DLS: (47 ± 3) nm PDI: 0.38 ± 0.04 AFM: (33 ± 3) nm TEM: (48 ± 3) nm Zeta potentials: (–8.7 ± 1.9) mV (48 h)	PBS or QuickZol reagent –80 °C	[77]
G+	*Clostridium butyricum* MIYAIRI II 588	UC	(1) 8 000 × *g* for 30 min (2) 20 000 × *g* for 45 min (3) Filtered through a 0.22 μm filter (4) 120 000 × *g* for 2 h (5) Resuspended in PBS and 120 000 × *g* for 2 h	*C. butyricum*-derived extracellular vesicles (*C. butyricum*-derived EVs)	0.22 μm	NTA: (149.3 ± 50.9) nm	PBS	[78]
Gram-negative bacteria (G–)	*Akkermansia muciniphila*	UC	(1) 10 000 × *g* for 20 min (2) Filtered through a 0.45 μm vacuum filter and enriched using QuixStand (3) Filtered through a 0.22 μm filter (4) 150 000 × *g* for 2 h	*A. muciniphila*-derived EVs	0.22 μm	DLS: 40–60 nm	PBS –80 °C	[79]
Fungus	*Phellinus linteus*	UC	Homogenized in a blender and obtain the PL juice (1) 3 000 × *g* for 30 min (2) 10 000 × *g* for 1 h (3) 150 000 × *g* for 1.5 h	Fungi exosome-like nanovesicles derived from *P. linteus*	/	Nano-ZS: 100–260 nm	PBS	[80]
Fungus	Mushrooms*	UC	Grounded for 15 s in PBS (1) 500 × *g* for 10 min (2) 2 000 × *g* for 20 min (3) 10 000 × *g* for 30 min (4) 100 000 × *g* for 2 h	Exosome-like nanoparticles	200 nm	NS: 100–140 nm	PBS	[81]
Algae	Microalgae	(A) UC (B) Tangential flow filtration (C) Density gradient centrifugation	(A) Large EVs: 10 000 × *g* for 30 min Small EVs: 118 000 × *g* for 70 min (B) (1) Concentrated a 450 nm hollow fibre membrane (> 450 nm sized particles) (2) Concentrated a 200 nm hollow fibre membrane (< 450 nm sized particles) (3) Concentrated a 50 kDa MWCO hollow fibre membrane (< 200 nm sized particles) (C) (1) 110 000 × *g* for 2 h and vigorously mixed for 20 min (2) OptiPrep gradient (10%/30%/50%) 110 000 × *g* for 24 h	Nanoalgosome	(B) Healthcare polysulfone hollow fibre membrane (450 nm, 200 nm and 50 kDa MWCO)	DLS: 120–135 nm Zeta potentials: (–13 ± 12) mV Markers: Alix, Enolase *β*-actin, H+/ATPase	PBS	[82]

Note: / means not reported; *Mushrooms were white beech (*Hypsizygus tessellatus*), brown beech (*H. tessellatus*), white button (*Agaricus bisporus*), Swiss brown (*A. bisporus*), king oyster (*Pleurotus eryngii*), shiitake (*Lentinula edodes*), oyster (*Pleurotus ostreatus*). Harmful bacteria or pathogenic bacteria were not included in the statistics.

proteins, channel proteins, specific proteins, and others. These have many homologous sequences with the proteins identified in mammal-derived EVs[25]. Lipids found in FDEVs include glycerides and phospholipids, which play a pivotal role in the mechanisms of disease prevention and control[87]. For instance, lemon-derived FDEVs were enriched in phosphatidylcholine (42.12%), diacylglycerol (17.05%), and sphingomyelin (10.63%)[50]. The nucleic acids of FDEVs primarily comprise microRNA and mRNA, potentially accompanied by circular RNA and DNA fragments. These nucleic acids exhibit robust biological activities; however, the precise mechanisms of disease prevention and treatment remain elusive. FDEVs can incorporate bioactive substances derived from their sources, thus exerting their preventive and therapeutic potential of FDEVs against associated diseases[73-74]. Currently, enzyme-linked immunosorbent assay (ELISA), sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), Western blot, liquid chromatograph-mass spectrometry (LC-MS) and other technologies are used for the comprehensive analysis of FDEV components.

The animal-derived FDEVs contain some protein markers, such as CD63, CD81, TSG101 and HSP70, which are similar to those found in typical EVs. Therefore, these FDEVs can be identified according to the MISEV2023 guidelines. Unfortunately, there is currently no recognized protein marker available for plant-derived FDEVs, but a few studies have suggested potential protein markers that could be applicable to plant-derived FDEVs. Pinedo et al.[88] identified 3 common protein markers in plants, namely HSP70, *S*-adenosyl-homocysteinase (SAHH) and glyceraldehyde 3 phosphate dehydrogenase (GAPD), throug a comparative analysis of protein data. Interestingly, these 3 protein markers are also found in typical EVs markers. Zeng et al.[72] reported the consistent identification of 5 proteins in plant-derived FDEVs, including HSP70, SAHH, GAPDH, glutathione S transferase, and Annexin, with the first three markers matching the conclusions of Pinedo et al[88]. In addition, we found that FDEVs derived from coconut[44], garlic[58], bitter gourd[62] and aloe[72] have been reported to contain protein markers such as CD63, TSG101 and HSP70. MISEV2023 states that broad protein markers for Gram-negative and -positive bacteria can be biomarked by lipopolysaccharide (LPS) and lipotechoic acid (LTA), respectively[30]. This applies to microorganism-derived FDEVs, but ongoing attention is required for generalizability. Although the proteomic and Western blot data of FDEVs derived from plants and microorganisms are limited, available data suggest that these FDEVs possess protein markers that are either identical or structurally and functionally similar to those found in typical EVs. It is crucial to urgently identify the markers of FDEVs derived from plants and microorganisms, followed by comprehensive research based on the marker classification outlined in the MISEV2023 guidelines. The collaborative efforts and data sharing among botanists, microbiologists and EVs researchers are anticipated to promote consensus regarding the protein markers of FDEVs from different sources.

2.3 Storage of FDEVs

The combination of PBS resuspension and cryogenic freezing is a widely used method for storing FDEVs (Tables 1–3), which is also frequently utilized in studies of typical EVs. Wu et al.[13] measured the particle size, Zeta potential, and protein content of milk-derived FDEVs resuspended in PBS (pH 7.4) during storage at −80 °C for 7 weeks, and observed no significant changes in milk-derived FDEVs throughout the storage duration. Zeng et al.[72] found that aloe gel-derived FDEVs loaded with the photosensitive material indocyanine green had no significant difference in the therapeutic efficacy against melanoma even after 30 days of storage when compared with freshly prepared FDEVs. However, in contrast to this finding, Gelibter et al.[89] evaluated the effect of eight storage methods and freeze-thaw cycles on plasma and glial cell-derived EVs over a storage period of up to 6 months. The results revealed that the storage at −80 °C did not guarantee optimal preservation of EVs. Both the purity and concentration of EVs decreased with the prolongation of storage time, accompanied by agglomeration and potential changes. Furthermore, the EVs concentration was significantly reduced after the first freeze-thaw cycle, and the EVs particle size increased with the subsequent cycles. Similarly, the study conducted by Maroto et al.[90] demonstrated that storing exosomes at −80 °C resulted in a 25% increase in diameter compared with storage at 4 °C and freshly prepared bronchoalveolar lavage fluid exosomes, which had the least effective outcome. It seems that the storage stability of FDEVs is better than that of typical EVs, but this needs to be confirmed by further studies. Moreover, we still need to pay attention to the potential effects of storage methods on FDEVs.

The storage conditions of FDEVs vary due to the lack of clear standards for research operations and the constraints imposed by experimental conditions. The ubiquitous presence of cryopreservation in practical research is attributed to the extraction procedure and yield of FDEVs. At the same time, large-scale production and future practical applications still cannot avoid the need for FDEVs storage. Therefore, it is necessary to optimize the storage conditions and maintain the optimal state of FDEVs. In a two-year study, Gorgens et al.[91] compared the protective effects of nine different conditions on the stability of MSC and HEK293T:CD63eGFP cell-derived EVs. The findings showed that a PBS solution with human albumin and trehalose exhibited superior preservation capabilities for EVs, resulting in significantly improved the short- and long-term recovery rates of EVs when stored at −80 °C. Although this study focused on human cell-derived EVs, it holds significant implications for the storage of FDEVs. At present, research on FDEVs storage remains limited. Further studies are required to explore an improved buffer system and storage conditions of FDEVs in order to maintain their characteristics and quality to the fullest extent possible. Until then, we still recommend utilizing freshly prepared FDEVs in mechanistic studies and downstream analyses to avoid any potential uncertain effects that may arise from their changes during storage on experiments.

3. Bioactivity of FDEVs

After oral administration, FDEVs exhibit stability in the gastrointestinal environment and accumulate within the intestine, liver and other systemic compartments to achieve internal circulation and cellular uptake[13,28]. This orchestrated distribution enables the maintenance of intestinal homeostasis while offering promising prospects for disease prevention and treatment. Ma et al.[78] showed that administration of 50 μg/day of *Clostridium butyricum*-derived FDEVs protected the intestinal barrier, effectively alleviated dextran sodium sulfate (DSS)-induced colitis, and elevated the abundance of

Butyricoccus and butyrate-producing *Clostridium sensu stricto*-1 in mice. Certainly, FDEVs can also exert their biological activities at specific sites through localized administration. Wang et al.[92] found that grapefruit-derived FDEVs did not penetrate the placental barrier after tail vein injection in mice. Meanwhile, bitter gourd[62] and garlic[93] studies demonstrated the ability of FDEVs to penetrate the blood-brain barrier and selectively target nerve cells or brain tumors. Thereby, FDEVs present an excellent vehicle for drug development in special populations such as pregnant women. It should be noted that the route of administration can significantly affect the distribution *in vivo* of FDEVs[16,60]; therefore, further investigate into the targeting properties of different FDEVs is necessary. It can be determined that FDEVs can eventually be recognized or taken up by cells at the targeted site to realize interspecies signal transmission and material exchange. We summarized the bioactivities and mechanisms of FDEVs in maintaining intestinal homeostasis, exerting anti-inflammatory effects, exhibiting anticancer activity, and other relevant functions (Table 4).

Zhou et al.[94] reported that the plant microRNA MIR2911, enriched in Jinyinhua decoction, exerts a direct antiviral effect against the influenza A virus (H1N1, H5N1, and H7N9 subtypes) by binding to viral mRNA and blocking their protein translation. Oral administration of honeysuckle decoction effectively prevented infection and reduced mortality in H5N1-induced mice. Subsequent studies revealed that MIR2911 also had an inhibitory potential on SARS-CoV-2 replication, and administration of a decoction prepared from 30 g of dried honeysuckle (MIR2911 level, 10.5 pmol) to subjects accelerated negative transformation in infected patients. Unfortunately, the authors did not directly confirm the origin of the enriched microRNAs in Jinyinhua soup originated from EVs, therefore, this study is excluded from Table 4.

Following entry into the body *via* distinct pathways, FDEVs can effectively navigate to the target site through internal circulation and interact with specific target cells, modulating the expression of relevant genes or interfering with specific signaling pathways. Fig. 1D illustrates that vincristine-derived FDEVs inhibited leukopenia and bone marrow cell cycle arrest and alleviated cyclophosphamide-induced immunosuppression *via* the TNF-α/NF-κB/PU-1 pathway[16]. Additionally, the bioactive substances contained in FDEVs mayserve as the foundation for their biological activities. Chen et al.[73-74] reported that tea leaves and edible tea flowers-derived FDEVs contain polyphenols and flavonoids that have demonstrated anti-tumor effects, including epigallocatechin gallate (EGCG), vitexin-2-*O*-rhamnoside, apigenin-8-*C*-glucoside, myricetin-3-*O*-rhamnoside and kaempferol-3-*O*-galactoside. Zeng et al.[72] used high-performance liquid chromatography (HPLC) to identify the constituents in FDEVs derived from aloe rind and gel, revealing the presence of functional factors such as aloe emodin, aloin, and *β*-sitosterol. Man et al.[95] showed that the content of 6-gingerol, 8-gingerol and 10-gingerol in ginger-derived FDEVs was 10.21, 22.69, and 32.36 times higher than those of ginger slices of equivalent mass, respectively. Sundaram et al.[87] found that ginger-derived FDEVs are selectively taken up by *Porphyromonas gingivalis* in a phosphatidic acid (PA)-dependent manner, and the cargo miRNAs of FDEVs synergistically interact with multiple pathogenic factors in recipient bacteria to induce bacterial apoptosis. However, not all studies have shown a significant application effect. Leiferman et al.[96] reported that dietary supplementation of milk exosomes (RNA cargo deficient or abundant) had no significant impact on bone strength, gene expression, and muscle amino acid profile in mice. Therefore, FDEVs may exhibit various biological activities, and we need to study whether they have significant application effects at specific sites according to the requirements.

4. Application of FDEVs in targeted delivery and precision release

Currently, food functional factors and drugs have been successfully loaded into FDEVs using various methods including freeze-thaw cycles, ultrasonication, passive incubation, recombinant packaging, electroporation and others. As vehicles, the engineered FDEVs retained their carrier characteristics and demonstrated significant application effects (Table 5).

Sasaki et al.[68] produced cNPs by ultracentrifugation and prepared PC liposomes (PC-Lips) using the conventional membrane hydration method. The results showed that the yield of cNPs was 10 times higher than that of PC-Lips, indicating the potential of cNPs as a feasible material for large-scale production employing simple techniques. However, it is important to continuously optimize the loading conditions for food functional factors or drugs based on experimental requirements, as no universally effective and flawless loading method currently exists[100]. The engineered FDEVs exhibited resilience against the harsh conditions of oral enzymes, gastrointestinal tract or detergents, effectively safeguarding the loaded material as demonstrated by both *in vitro* and *in vivo* experiments[101]. This remarkable resistance can be attributed to the bilayer structure characteristics of FDEVs and their ability to maintain structural integrity even after engineering[82].

The inherent body targeting property of engineered FDEVs serves as the fundamental basis for their functionality. As previously mentioned, the targeted aggregation characteristics of FDEVs vary among different and delivery routes from the same source can significantly affect their aggregation results. In addition to withstanding the extreme conditions, FDEVs must effectively penetrate the intestinal mucosal barrier in order to exert their intended therapeutic effects. Interestingly, Wu et al.[13] revealed that FDEVs had superior penetration ability compared to artificial carriers and did not destroy cell integrity. The delivery of engineered FDEVs, either orally or intraperitoneally, typically necessitates precise targeting *via* the internal circulation. Numerous studies have substantiated the stability of engineered FDEVs within the blood circulation, their efficient targeting capabilities[102], as well as their recognition and internalization by cells[17] (Fig. 1E). Pomatto et al.[51] used orange juice-derived FDEVs as a carrier for the oral and nasal delivery of *SARS-CoV-2* mRNA vaccines; the results indicated the efficient encapsulation of diverse mRNA molecules within FDEVs, safeguarding them against degradation by gastric acid and ribonucleases, while successfully facilitating targeted delivery to recipient cells and subsequent translation into functional proteins.

The FDEVs are derived from edible raw materials and possess a theoretically high safety. Furthermore, experimental results have confirmed the low immunogenicity of FDEVs[103], establishing them as a secure targeted delivery carrier. In addition, Wu et al.[104] compared the efficacy of insulin loaded with PEG polymer nanoparticles and milk-derived FDEVs in the treatment of type I diabetes, and the

Table 4
Bioactivities and mechanisms of FDEVs.

Sources	Subjects	Administration routes	Accumulation site	Bioactivities	Mechanisms	Reference
Goat milk	Mice	Intravenous injection	Peritoneal inflammation region	Visualizing inflammation with BDP or SCy-5 as a natural probe	The complexes were taken up by macrophages and neutrophils.	[35]
C. roseus (L.) Don	Mice	Intraperitoneal injection	Liver and kidney	Alleviated cyclophosphamide-induced immunosuppression as well as leukopenia and bone marrow cell cycle arrest in mice	Stimulated the secretion of TNF-α, activated the NF-κB signaling pathway, and increased the expression of the hematopoietic function related transcription factor PU.1	[16]
Honey	Mice	Intraperitoneal injection	Liver	Alleviated inflammation of experimental acute liver injury in mice	The miR-4057 inhibited NLRP3 inflammasome formation and activation in macrophages	[40]
Lemon	Mice	Oral administration	Kidney	Hindered the formation and development of kidney stones	Transported by microtubules and eventually recruited into lysosomes, CaOx crystallized into unstable subtypes and inhibited endoplasmic reticulum stress in tubule cells	[50]
C. butyricum (G+)	Mice	Orally gavaged	Gut	Alleviated symptoms of ulcerative colitis and improved intestinal microbiome homeostasis	Decreased the proinflammatory gene expression, increased the beneficial bacteria abundance and enhanced gut barrier integrity by regulating TJ proteins Claudin, Zo-1 and JAM-A	[78]
Garlic	Human kidney carcinoma cell line A498 and human lung carcinoma cell line A549	/	/	Leaded to S phase arrest of kidney and lung cancer cells and induced cell apoptosis	ROS levels and anti-apoptosis Bcl-2 protein were increased, VEGF angiogenesis protein and the basal Cas3 activity were decreased	[58]
M. harantia	Human glioma cell lines (U251) and mice	Intraperitoneal injection	Subcutaneous tumors and brain	Penetrated the blood-brain barrier and inhibited glioma cell invasion and tumor growth	Inhibited glioma proliferation, migration and invasion by regulating the PI3K/AKT signaling pathway	[62]
S. dominica hairy root	MIA PaCa-2 and MCF-7 cells	/	/	Significantly decreased the viability of pancreatic cancer cells and breast cancer cells (3×10^8 EVs/mL for 24 h)	Activated procaspase-3, degrade PARP-1 enzyme, and promoted programmed cell death	[71]
L. rhamnosus GG	HepG2 cells	/	/	LDEVs at 100 mg/mL produced significant toxic effects on liver cancer cells	Increased the expression ratio of *bax/bcl-2* genes	[76]
Yam	Mice	Oral administration	Liver and small intestine	Promoted bone formation in postmenopausal mice with osteoporosis	The differentiation, proliferation and mineralization of osteoblasts were activated by the BMP-2/p-p38-dependent Runx2 pathway	[55]
P. linteus	Volunteer skin, mice, HaCaT, HSFs and HEK 293T cells	Cream containing the 2% *P. linteus*	/	Inhibited UV-induced skin aging and had anti-inflammatory effects	The miR-CM1 in fungi exosome-like nanovesicles inhibited the expression of Mical2 and MMP1, reduced the level of reactive oxygen species and SA-*β*-galactosidase, and increased the activity of SOD	[80]
Garlic	Mice	Orally gavaged	Liver, brain and intestine	Inhibited the symptoms of high-fat diet induced encephalitis, increased glucose tolerance and insulin sensitivity, and improved memory function	The combination of PA and BASP1 inhibited c-Myc-mediated expression of STING, reduced the production of inflammatory factors, and inhibited neuronal cell death	[93]
Ginger	*P. gingivalis* and mice	Added to the drinking water	/	Inhibited the growth of *P. gingivalis* (periodontitis related pathogens), reduced the adhesion to oral epithelial cells, and inhibited alveolar bone loss	Increased cell depolarization, induced cytoplasmic protein release by directly acted on HBP35 protein	[87]

Note: / means not reported.

Table 5
Bioactivities and mechanisms of FDEVs.

Source	Loaded material	Load mode	Critical process	Subjects	Administration routes	Results	Mechanisms	Reference
Milk	Anti-lipopolysaccharide factor peptide	Freeze-thaw cycle	(1) Peptide:MEVs = 10:3 (2) Incubated for 2 h at room temperature (RT) (3) Rapidly frozen to −80 °C (4) Thawed at RT (5) Repeated thrice	Human m onocytic cell line (THP-1 cells)	/	Immunoregulation	Effectively delivered the peptide into monocytes, increased the reactive oxygen species and superoxide anion levels, and phagocytosis, activated the secretion of cytokines in THP-1	[14]
Milk	Astaxanthin	Ultrasoni-cation	Astaxanthin:mExo = 1:10 (1) Ultrasonic probe at 200 W for 2 min (15 s on/15 s off) (2) Incubated for 2 min on ice (3) Repeated thrice (4) Incubated for 1 h	Macrophage	/	Increased the cellular uptake of astaxanthin and reduced the inflammatory response	Reduced the production of reactive oxygen species and mitochondrial depolarization, inhibited the expression of IL-1β, IL-6 and other inflammatory factors	[17]
Milk	Curcumin and resveratrol	Passive incubation	(1) Dissolved in EtOH:PBS (1:1) and exposed to milk exosomes (250 μg/mL) (2) 37 °C for 1 h in the dark	Breast cancer cells (MCF-7 and MDA-MB-231)	/	The bioavailability and anticancer activity of both substances were improved, and escaped from cancer cells' ATP-binding cassette transporters-mediated chemoresistance	Increased the cells G0/G1 phase and reduced S phase, induced cell death by the mitochondrial apoptosis, and entered the cells *via* clathrin-mediated endocytosis	[97]
L. plantarum	Fucoxanthin (carotenoids)	Ultrasoni-cation	(1) Dissolved in ethanol and added an equal mass of EVs (2) Ultrasonicated 35 kHz for 30 min with an ice ultrasonic bath	Mice	Oral administration	The stability and therapeutic effect for colitis of fucoxanthin was improved	Promoted the polarization of macrophages to anti-inflammatory M2 type, restored the balance of intestinal flora	[98]
Grapefruit juice	Heat shock protein 70	Passive incubation + Ultrasoni-cation	(1) HSP70 (0.1 mg/mL) added to GEVs (about 2×10^{12} vesicles/mL) and incubated overnight at 4 °C (2) 35 kHz for 15 min at RT (3) Incubated for 1.5 h at 4 °C	Glioma cells	/	Increased the delivery efficiency of HSP70 to cells	There was a strong correlation between the properties of EVs and the delivery efficiency	[64]
Milk	miR-31-5p	Electropo-ration	miR-31-5p:EVs = 1:1 (1) Perforation voltage was 110 V, penetration voltage was 25 V, capacitance was 940 μF, 6 ms on/10 ms off (2) Incubated for 30 min at 37 °C	Mice	Wound treatment	It promoted angiogenesis of endothelial cells and healed the wound on the back of diabetic mice	Mir-31-5p was delivered to cells to inhibit HIF1AN expression	[99]
Milk	Insulin	Ultrasoni-cation	(1) Insulin:EVs = 1:8 (2) 100 W for 3 min, 2 s on/2 s off	Rat	Oral administration and subcutaneous injection	Oral administration of 50 IU/kg was better than that of subcutaneous injection in hypoglycemic effect. Significantly increased the bioavailability of insulin	Activation of ERK1/2 and p38 MAPK pathways increased the internalization of EV@INS by intestinal epithelial cells	[13]
Milk	Paclitaxel	Passive incubation	(1) Dissolved in acetonitrile:ethanol (1:1, *V/V*) (2) Incubated for 15 min at RT	Nude mice	Oral administration	Significantly inhibited lung tumor growth	EVs made more drugs to be delivered to the tumor site, milk exosome reloading drugs was safer than administration alone.	[15]
Celery	Doxorubicin	Passive incubation	(1) DOX:EVs = 1.01×10^{11} particles: 1 mg 2) Incubated for 1 h at 37 °C	Nude mice	Intraperitoneal injection and tail vein injection	The composite carrier inhibited tumor proliferation and reduced cardiotoxicity	The cell proliferation marker Ki67 was decreased and the apoptosis marker Caspase-3 was increased	[60]
Ginseng	GEV and CXCL12 were implanted in crosslinked hydrogels	Hydrogel	(1) Methacryloyl (MA) and gelatin MA were dissolved in PBS containing 0.5% LAP (2) Pipetted into a mold and exposed to UV light (360−480 nm, 6.9 mW/cm^2) for 60 s	Rat	Wound treatment	Repair the nervous system of the damaged skin	The miRNA was transferred to bone marrow mesenchymal stem cells to activate PI3K signaling and transcription, and promoted stem cell differentiation and neural development	[69]
Aloe	Indocyanine green	Passive incubation	(1) Indocyanine green:FDEVs = 3:2 (2) Incubated for 30 min at 37 °C	Mice	Intratumor injection	Inhibition of melanoma growth	The transdermal properties were remarkable, and the temperature increased after near infrared light irradiation, which led to cell death	[72]

Note: / means not reported.

findings demonstrated that insulin loaded with milk-derived FDEVs exhibited a more pronounced hypoglycemic effect. In order to enhance their loading capacity and targeting characteristics, FDEVs can be fused with liposome membranes or coated with receptor-rich membranes. These engineering approaches have successfully achieved precision drug delivery and showed significant therapeutic effects of plant-derived exosomes-like nanoparticles in the amelioration of periodontal diseases[105].

Compared with artificial carriers such as liposomes or polymer nanoparticles, FDEVs exhibit pH adaptation in the gastrointestinal tract, targeting capabilities to specific tissues, low immunogenicity, and high bioavailability[13], rendering them gradually prominent in the global scientific community. However, the application of FDEVs remains understudied and their mechanism of action is poorly understood. With advancements in FDEVs engineering methods and further exploration, FDEVs are poised to emerge as the novel generation of targeted delivery carriers with significant potential for precision nutrition and disease prevention and control.

5. Limitations and challenges of FDEVs

5.1 Adverse consequences

Milk-derived FDEVs have demonstrated the ability to modulate intestinal microbiota composition[106], enhance osteoporosis management[107], and also exhibit favorable stability and safety profiles asdelivery carriers. However, Melnik[108] proposed that milk-derived FDEVs microRNAs may excessively activate hepatic mTORC1 signaling and serve as a significant risk factor for hepatocellular carcinoma pathogenesis. Furthermore, they also identified milk-derived FDEVs microRNAs as potential pathogens of B-cell lymphoma that should be eliminated by heat sterilization or fermentation[109]. Tan et al.[110] demonstrated that a high-protein diet promotes the production of succinate by the gut microbiota, leading to intestinal bacterial stress and the generation of reactive oxygen species (ROS), thereby augmenting microbial-derived cellular EVs. These EVs directly activate the TLR4 receptor on intestinal epithelial cells, triggering downstream NF-κB signaling and upregulating the expression of APRIL, CCL28, and PIGR. Consequently, these drive a T cell-independent secretory IgA response. Increased TLR4 signaling during this process exacerbates the proinflammatory response, resulting in more severe colitis. Samuel et al.[18] pointed out that oral administration of milk-derived FDEVs was able to induce the senescence of primary tumors, but accelerated tumor metastasis in an environmentally dependent manner at the same time (Fig. 1F).

Milk EVs encompass the miR-29 family, which exhibits a dual role as an inhibitor of the expression of extracellular matrix gene. The miR-29 family has been demonstrated to mitigate tissue fibrosis[111] and exhibit therapeutic efficacy in cardiovascular diseases; however, it may also compromise tissue structure and impede wound healing processes, particularly in the context of skin[112]. Furthermore, elevated levels of miR-29 expression have been associated with reduced glucose uptake and impaired insulin signaling within the body[113], suggesting a potential link between the milk-derived FDEVs miR-29 and diabetes. However, the undeniable significance and promising application prospects of milk-derived FDEVs cannot be overlooked, as an increasing number of studies have substantiated their favorable characteristics and effects. The research on FDEVs derived from plants and microorganisms is still in its nascent stages, with limited available data on the proteomics, metabolomics, and genetics. Consequently, comparing the results with those obtained from typical EVs poses a significant challenge.

In the future application of FDEVs, it is imperative to meticulously assess their potential hazards and comprehensively evaluate the biological safety in an objective manner. The scientific management of potential adverse consequences associated with FDEVs should be prioritized. By gaining a profound understanding of their mechanisms, efforts should be made to prevent any adverse consequences during drug delivery, disease prevention, and treatment processes, thereby maximizing the benefits and promoting human health through FDEVs utilization.

5.2 Standardization and consensus

Since the turn of this century, the research of EVs has increased exponentially, and ISEV has issued 3 minimum guidelines on EVs research in 2014, 2018 and 2023. However, the majority of these guidelines are based on the studies of mammalian cells. In recent years, these studies and ISEV minimum guidelines have been used for the isolation and investigation of FDEVs. Due to the similarities in characteristics, size, and protein markers between FDEVs and typical EVs, FDEVs from animals are commonly referred to as exosomes in most studies. For plant-derived FDEVs, pretreatment methods such as juicing or homogenisation introduce cellular debris into the extract samples. Moreover, extraction operations further contribute to the heterogeneity of FDEVs, thereby impeding consensus on the nomenclature of FDEVs.

At present, although the existence of FDEVs from plants and microorganisms has been observed by electron microscope, their biogenesis, biomarker, cell wall shuttle mechanism, and communication mechanism in organisms or species are still unclear. Therefore, we advocate for a comprehensive description of experimental details in accordance with the ISEV minimum guidelines and an active exploration of the biological mechanisms of FDEVs. These efforts will facilitate the expedited development of a consensus in the field of FDEVs research and the establishment of industry standards.

5.3 Explore the conditions for mass production

The exosomes diagnosis and treatment projects of companies such as Evelo Biosciences and Mantra Bio have progressed to the clinical trial stage, indicating that the commercial application of exosomes is imminent. Although FDEVs have shown remarkable effects in targeted delivery and disease prevention and/or control, it is crucial to consistently obtain high production of FDEVs in large-scale applications while ensuring their stability and superior quality. The high production volume of FDEVs depends on the utilization of expensive existing equipment and technology. Therefore, the development of isolation methods with a streamlined and efficient process is one of the future research directions.

In order to obtain stable EVs, Ou et al.[16] established a plant cell culture system. Through structural characterization and in-depth functional analysis, it was revealed that the plant cell culture can provide a

stable and high-quality source of EVs, exhibiting striking similarities with directly isolated EVs in terms of morphology, particle size, and biological activity. Wang et al.[114] prepared exosome mimetics (EMs) through continuous extrusion of bone marrow mesenchymal stem cells (BMSCs), and the yield of EMs was 20 times higher than that of EVs obtained by the same number of cells. TEM analysis revealed that EMs had the characteristic morphology of spherical lipid bilayer vesicles ranging 30−200 nm, which were similar to the structure of EVs. Western blot analysis confirmed the presence of typical exosomal markers, including CD63, TSG101, and ALIX within the EMs. *In vitro* experiments demonstrated that engineered EMs loaded with DOX displayed enhanced tumor suppressive activity and reduced side effects compared to free DOX. Although this study was conducted on BMSCs, the method of continuous extrusion of cell membranes is likely to be extended to the application of FDEVs. The aforementioned methods have significantly enhanced the yield of EVs, substantiated the efficacy of the obtained particles through their characteristics and functionalities, and presented opportunities for large-scale production. However, further research and exploration into more refined production conditions are required to ensure consistent high yield and stable quality during industrial manufacturing.

5.4 Application stability and safety

A large number of studies have demonstrated the high safety profile of FDEVs, with large doses and various delivery methods (such as gavage, intravenous injection, intraperitoneal injection) showing no significant impact on organs and physiological states in animal experiments[23,55,60]. The study by Agrawal et al.[15] also demonstrated that FDEVs loaded with paclitaxel exhibited lower immunotoxicity compared to free paclitaxel administered by intravenous injection. However, caution must be exercised regarding the use of FDEVs due to potential risks introduced by solvents or substances used in the isolation, storage, and encapsulation of food functional factors or drugs. *In vitro* biological tests often fail to directly and effectively translate into pharmacological effects *in vivo*, owing to several factors including the route of administration, drug interactions, genetic predisposition, among others[6]. Scientific and rigorous clinical trials should be conducted to ensure the absence of any overt or covert adverse consequences in the body caused by FDEVs. Furthermore, comprehensive investigations are warranted to elucidate the transport characteristics, cellular uptake pathways, carrier release mechanisms, and ultimate biodistribution of FDEVs within the human body. Despite the remarkable efficacy of FDEVs demonstrated, comprehensive studies must be conducted to ensure their stability and safety before practical clinical application and therapeutic utilization due to the relatively narrow focus of current research endeavors in this field.

6. Conclusion

In this study, we proposed the concept of FDEVs from animal, plant and microorganism sources for the first time and comprehensively summarized 67 species of FDEVs with their physicochemical properties, bioactivity and applications as natural targeting carriers. FDEVs have garnered significant attention due to their abundant sources, moderate price, safety, sustainability, and absence of ethical issues. Notably, they have demonstrated remarkable efficacy in mitigating inflammatory responses, maintaining intestinal homeostasis, inhibiting tumor initiation and progression, etc. As novel targeted delivery carriers with excellent stability and non-immunogenicity *in vivo*, they successfully load various food functional factors (e.g., resveratrol, curcumin, fucoxanthin) and drugs to enhance the stability, bioavailability and therapeutic effect of the loaded materials. However, several studies have also reported the adverse consequences of FDEVs. For example, milk-derived FDEVs have been shown to accelerate cancer metastasis and impair insulin signaling while promoting tumor apoptosis. These findings emphasize the need for cautious handling of FDEVs to ensure their safe applications. Although there are still some problems to be addressed, FDEVs have emerged as a promising player in targeted delivery with their remarkable application efficacy and pivotal role. In summary, FDEVs, efficient and sustainable carriers for steady-state targeted delivery, exhibit promising application prospects in precision nutrition, disease prevention and control, as well as the promotion of human health in the future. It is imperative to proactively address the current challenges of FDEVs and optimize their potential contributions to human health.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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