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PLANT-DERIVED EXOSOMES AND EXTRACELLULAR VESICLES FOR DRUG DELIVERY: EMERGING THERAPEUTIC APPLICATIONS

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ABSTRACT

Vesicular nanoparticles recovered from edible and medicinal plants have moved in little more than a decade from a curiosity of plant pathology to a proposed drug delivery platform. Their appeal is straightforward: grapefruit and ginger yield lipid material at the gram-per-kilogram scale, the starting biomass is agricultural rather than a bioreactor, and a carrier built from a food crop carries no risk of transmitting a human pathogen. Preclinical performance has matched the promise in places. Oral ginger and grape vesicles reach the inflamed colon and the intestinal crypt, folate-decorated grapefruit lipid particles have shown tumour accumulation more than a thousand-fold above unmodified controls, and ginseng vesicles have extended median survival in an orthotopic glioma model. This review synthesises the evidence across biogenesis, composition, isolation, cargo loading, surface engineering and therapeutic application, and argues that the field's principal obstacle is neither delivery efficiency nor toxicity but definitional. The entity most papers call a plant exosome is, in the majority of published work, a mechanically produced mixture of secreted vesicles, intracellular membranes and reassembled lipid particles, and the International Society for Extracellular Vesicles now advises against the term. Reported yields for a single species vary several-fold across laboratories, encapsulation efficiencies span 1% to 96% depending on the cargo, and dose has been reported in at least five mutually inconvertible units. Three registered clinical trials, the earliest opened in 2011, have between them enrolled fewer than one hundred participants and posted no results. The carrier is ahead of the analytics that would allow it to be manufactured, dosed and compared, and closing that gap is the precondition for translation.

Keywords: extracellular vesicles; plant-derived nanovesicles; exosomes; drug delivery systems; nanoparticles; oral administration; pharmaceutical manufacturing.

1. Introduction

The lipid bilayer is the oldest drug delivery motif in pharmaceutics, and every generation of nanomedicine has returned to it. Liposomes established the principle, PEGylation solved circulation time, and ionisable lipid nanoparticles carried the first mRNA vaccines into hundreds of millions of people. Each of these carriers is synthetic, and each inherits the costs of being synthetic: purified lipid feedstock, controlled process chemistry, and an immunological profile that has to be engineered rather than inherited. Mammalian cell-derived extracellular vesicles were proposed as the biological alternative, naturally assembled and naturally tolerated, but a clinical dose needs litres of conditioned medium from a characterised cell bank and the product carries the regulatory weight of a cell-derived biologic, including the obligation to exclude adventitious viral agents.

Plants offer a third route. The lipid material recoverable from grapefruit amounts to about 2.2 g per kilogram of fruit[1], a kilogram of ginger rhizome yields roughly 4g by ultracentrifugation[13], and the feedstock is a commodity crop processed from a blender rather than a bioreactor. No plant virus or plant transposon has a mammalian host. Interest has grown accordingly, from a handful of publications on plant-derived vesicle theranostics in 2009 to several hundred a year now.

That growth has outpaced the field's methodological discipline. The material recovered from a blended rhizome is not obviously the same entity as the vesicle a leaf secretes into its apoplast during fungal attack, yet both appear in the literature under the same names. Sizes reported for a single ginger preparation range widely depending on whether the measurement is nanoparticle tracking analysis or dynamic light scattering[17]. Encapsulation efficiency for a protein cargo has been measured near 1% in one grapefruit preparation and near 90% for curcumin in a ginger preparation[17,18]. Doses appear as milligrams of protein per kilogram of animal, particles per animal, nanomoles of phospholipid, and grams of lyophilised powder per kilogram of herb, and a comparison of two published studies in similar disease models shows an apparent thousand-fold dose separation attributable to the choice of unit rather than to potency[5].

This review takes those inconsistencies as its subject rather than as background noise. It covers what plant-derived extracellular vesicles are and how they are made, what is inside them, how the isolation route determines what is measured, how drugs and nucleic acids are loaded, what the preclinical record actually shows, and where translation stands. The argument developed across these sections is that plant vesicles have already demonstrated the delivery behaviour a carrier needs, and that the barrier to clinical use is the absence of an agreed identity, an agreed dose metric and an agreed set of release specifications. Analytical standardisation, not further demonstration of efficacy in mouse models, is the work the field now owes itself.

2. What is a plant-derived extracellular vesicle?

2.1 Three routes of production, and only one of them is secretion

Plants release membrane-bound particles into the apoplast by more than one mechanism, and the mechanisms are genetically separable. Fusion of multivesicular bodies with the plasma membrane releases intraluminal vesicles in the manner of the canonical animal exosome pathway, and the tetraspanin TET8 marks vesicles on this route [6]. A second route runs through the exocyst-positive organelle, a compartment that fuses with the plasma membrane to deposit a single membrane-bounded vesicle into the cell wall, and autophagy contributes a third population that is subpopulation-specific, since the genetic requirements for TET8-positive and PEN1-positive vesicles differ[6]. These subpopulations are physically resolvable at distinct buoyant densities[8]. Isolating plant vesicles at a single centrifugal force therefore samples an operationally defined slice of a heterogeneous population, and the slice differs between laboratories.

None of this describes how most vesicles used in drug delivery research are obtained. Those come from homogenised fruit, juice or rhizome, a process that ruptures every intracellular compartment and mixes their membranes with whatever was genuinely secreted. Microfiltration through 0.22 or 0.45 µm membranes then selects, and arguably generates, particles in the 30 to 200 nm range that the field expects to see. The resulting preparation contains secreted vesicles, intracellular vesicles and reassembled membrane fragments in unknown proportion, and no published method distinguishes them[4].

2.2 The nomenclature problem is a scientific problem

The 2018 ISEV position statement established extracellular vesicle as the generic term and recommended operational descriptors over biogenesis-implying names, noting that small EV should be understood as under 100 nm or under 200 nm depending on the separation used [2]. The 2023 revision went further, instructing authors to avoid exosome-like vesicle and related constructions precisely because they imply a biogenesis that has not been demonstrated, and introducing non-vesicular extracellular particle for cell-derived material that is not membrane-delimited[3]. Both documents are written from mammalian data and neither carries a substantive plant section, resting instead on the assertion that the principles should transfer[3].

The plant field has not adopted the advice, and the terms plant-derived extracellular vesicle, plant-derived exosome and plant-derived exosome-like nanoparticle are used in comparable proportions. An intermediate proposal, first articulated in a 2021 call for rigour, reserves plant extracellular vesicle for material recovered without tissue disruption and assigns plant-derived nanovesicle or nanoparticle to everything obtained by grinding, juicing or blending[4]. Since the two categories differ measurably, with mechanically derived preparations tending to be larger, more lipid-rich and more RNA-laden, the distinction carries

information rather than merely tidying its origin.

vocabulary. Figure 1 sets out the distinction and

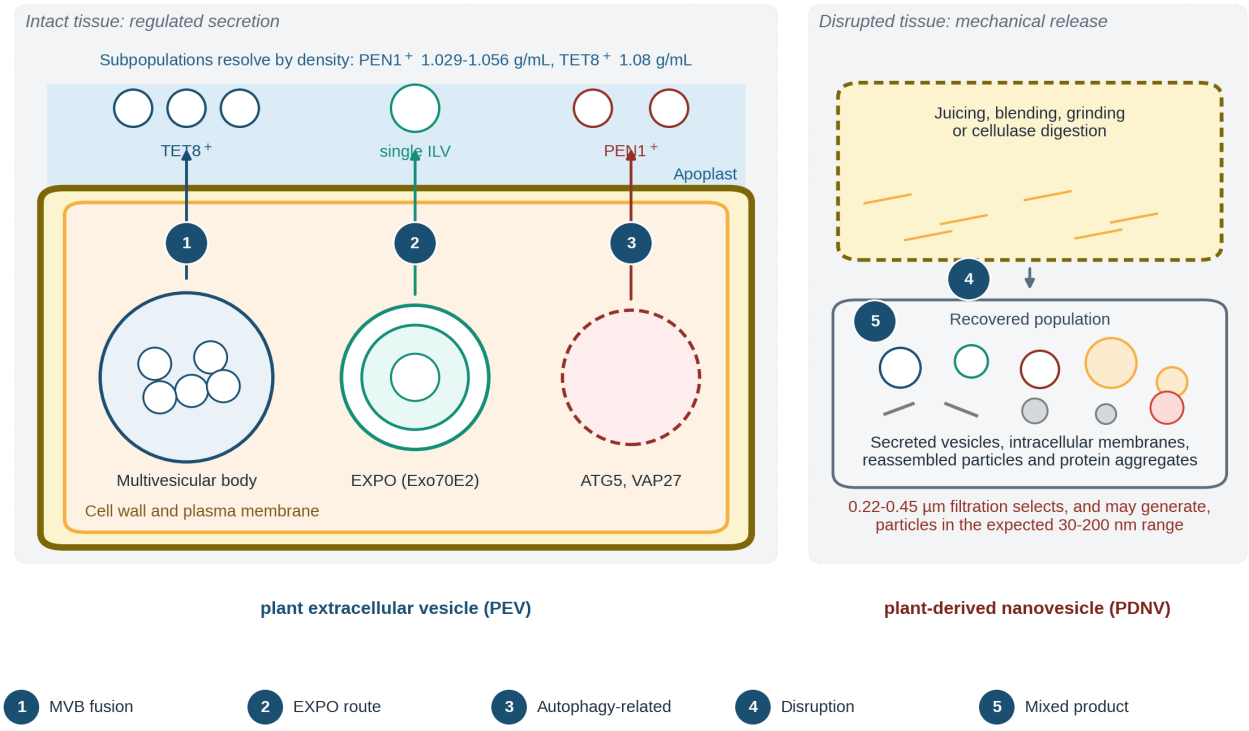

Figure 1. Vesicles reaching a preparation by different routes are not the same entity, and the isolation route determines which term applies. Left: in intact tissue, vesicles are secreted into the apoplast by at least three genetically separable mechanisms. Fusion of multivesicular bodies with the plasma membrane releases intraluminal vesicles carrying the tetraspanin TET8 (step 1); the exocyst-positive organelle deposits a single intraluminal vesicle into the cell wall (step 2); an autophagy-related route contributes a further population, while PEN1-marked vesicles depend on a distinct pathway (step 3). The resulting subpopulations resolve at distinct buoyant densities [4,6,8].

For a drug delivery audience the practical consequence is specific. Almost all pharmacologically interesting material in this literature is plant-derived nanovesicle by that definition, and several of the most-cited delivery vehicles are further removed still. Grapefruit-derived nanovectors are built by extracting total fruit lipid, drying it to a film with the drug, and sonicating it into particles [1]. These are lipid nanoparticles formulated from a botanical lipid source, and their advantages, which are real, belong to the lipid composition rather than to any vesicular provenance. The term used throughout this review is plant-derived extracellular vesicle (PDEV) for the general class, with reconstructed vesicle reserved for material that has been disassembled and reformulated.

2.3 Markers, and the absence of a plant tetraspanin

Mammalian EV work rests on the tetraspanins CD9, CD63 and CD81. Plants have no orthologues of these, which removes both the identity assay and the immunocapture handle [5].

Candidate replacements have been assembled from proteomic consensus, including aquaporins, V-type ATPase subunits, syntaxins and two plant-specific families, and PEN1 and the patellins PATL1 and PATL2 are the best-established markers in Arabidopsis apoplastic preparations, with a substantial proportion of vesicle proteins conserved between Arabidopsis and Sorghum[7,8]. Antibodies against most candidate markers are not commercially available[7], so in practice identity is asserted from size, morphology and a particle count rather than from a marker. Negative markers matter as much: preparations from green tissue should be shown to be depleted of RuBisCO, and any plant preparation should exclude oleosins, which flag co-isolated oil bodies that behave as lipidic nanoparticles of similar size[7].

3. Composition: the cargo is also the confounder

Plant vesicles are lipid-dominated and the lipid classes differ sharply from the phosphatidylcholine-rich mammalian profile. Ginger preparations are enriched in phosphatidic acid and the galactolipids digalactosyldiacylglycerol and monogalactosyldiacylglycerol, with phosphatidylcholine and phosphatidylserine present at low levels[11]. The precise proportions are unstable across reports, and the discrepancy is traceable to which sucrose gradient band was analysed rather than to disagreement about the chemistry, since the denser and lighter ginger bands rank the lipid classes differently [11]. Post-harvest handling alone reshapes the carrier: drying a preparation can halve the number of detectable lipid species and invert the ratio of phospholipids to neutral lipids[17].

The protein cargo is smaller and more metabolic than its mammalian equivalent, dominated by patellins, HSP70 chaperones, 14-3-3 proteins and glycolytic enzymes, and a typical plant vesicle carries far fewer proteins and microRNAs than a mammalian exosome[7]. RNA is the most contested compartment. Small RNA profiling of Arabidopsis vesicles found that conventional 18 to 34 nucleotide trimming retained only about a fifth of reads, and widening the window revealed a population of tiny RNAs peaking at 10 to 15 nucleotides that standard pipelines discard[9]. Reported microRNA counts for ginger alone range across an order of magnitude between studies, a spread reflecting library preparation and annotation more than biology[17].

Whether these RNAs are inside the vesicle at all is now openly disputed. The case that they are rests on the demonstration that Arabidopsis delivers host small RNAs into *Botrytis cinerea* to silence fungal virulence genes, with the transferred species detected in vesicle libraries and *tet8 tet9* double mutants showing enhanced susceptibility[6]. The case against is that most apoplastic small RNA is degraded by RNase only after protease treatment, indicating protection by protein rather than by membrane, and that the small RNAs and circular RNAs most cargo claims depend on do not co-fractionate with TET8-labelled vesicles[10]. Both positions come from established laboratories, and the honest summary is that tiny RNAs of 10 to 17

nucleotides are genuinely vesicle-enriched while the longer species are substantially extravesicular.

Secondary metabolites travel with the lipid. Ginger vesicles concentrate 6-gingerol and 6-shogaol at values that are band-dependent within a single gradient and enriched roughly ten- to thirty-fold over ginger slices[11]. This dual character, in which the carrier is itself pharmacologically active, is the source of the field's most persistent interpretive difficulty. When a loaded vesicle outperforms free drug, the excess may belong to the delivery or to the vesicle's own anti-inflammatory lipids and phytochemicals, and few studies include the vehicle-only arm that would separate them.

4. Isolation determines what is measured

4.1 Method sets yield, purity and function independently

The clearest evidence comes from head-to-head comparisons on a single batch of material. Ultracentrifugation, sucrose gradient ultracentrifugation, polyethylene glycol precipitation and membrane filtration applied to the same ginger gave protein yields spanning more than an order of magnitude, with all four giving a mean particle size near 120 nm[12]. Yield and purity moved in opposite directions: the sucrose gradient placed a higher fraction of particles within the 30 to 150 nm window than simple ultracentrifugation, at the cost of yield. Zeta potential tracked the method rather than the material, running from about −26 mV for ultracentrifugation to about −9 mV for polyethylene glycol, consistent with polymer adsorption[12]. Most consequentially, the polyethylene glycol product was inactive against two lung cancer lines at a concentration where the others were active, attributed to residual polymer[12]. Independent work quantifies that residue at tens of milligrams per gram of isolate and shows the same method recovering only about a third of the polyphenol content obtained by ultracentrifugation while delivering most of its mass yield[13].

Chromatographic polishing achieves what centrifugation cannot. Anion-exchange chromatography applied to *Brassica oleracea* vesicles after tangential flow filtration raised the purity index by roughly an order of magnitude, removing about 90% of contaminating protein with essentially no particle loss, and tightened the size distribution from a mean near 222 nm to 138 nm[14]. Tangential flow filtration is the route most likely to survive scale-up: a microalgal process using a filtration cascade concentrated more than a litre of culture several hundred-fold in a single batch and was explicitly presented as suitable for GMP-compliant production[15]. Comparable data for higher plants at pilot scale have not been published. Table 1 sets the principal routes side by side.

Table 1. Isolation routes for plant-derived extracellular vesicles, with reported yield, purity and functional consequences. Values are as stated in the cited source and are not interconvertible between rows because the source materials and units differ.

Method	Source material	Yield reported	Purity or size outcome	Functional or practical note	Ref.
Differential ultracentrifugation	Ginger rhizome	Highest of four methods on one batch	64% of particles in 30-150 nm; ζ ≈ −26 mV	Reference yield; moderate purity	[12]
Sucrose gradient ultracentrifugation	Ginger rhizome	Lower than dUC	78% of particles in 30-150 nm; ζ ≈ −17 mV	Purity gained at the cost of yield	[12]
PEG 6000 precipitation	Ginger rhizome	Comparable mass to dUC	ζ ≈ −9 mV; residual PEG tens of mg/g	Biologically inactive; residual polymer	[12,13]
Membrane filtration	Ginger rhizome	Very low mass yield	Highest particle count	High count, low mass	[12]
TFF + anion-exchange chromatography	Brassica oleracea	Recovery essentially quantitative	Purity index up ~10-fold; mean 222 → 138 nm	~90% of contaminating protein removed	[14]
Tangential flow filtration cascade	Microalgal culture	~2 mg vesicles per 5 L culture	85 ± 5 nm	>1 L concentrated several hundred-fold; GMP-ready	[15]

dUC, differential ultracentrifugation; PEG, polyethylene glycol; TFF, tangential flow filtration; ζ, zeta potential.

4.2 Characterisation, and the numbers it produces

Sizing technique changes the answer. The same Sorghum preparation gave a nanoparticle tracking mean near 170 nm, a dynamic light scattering mean near 182 nm, and a cryo-electron tomography median near 104 nm for single-layered vesicles, with the tomography census showing most vesicles single-layered[8]. Tracking analysis is number-weighted, light scattering is intensity-weighted and hydrodynamic, and each interrogates a different observable. Even within one technique the agreement is imperfect: a controlled study across two nanoparticle tracking instruments operated by a single analyst found day-to-day variation of up to a quarter and significantly different results between the two instruments under identical software settings, resolvable only by instrument-specific optimisation[16]. A pragmatic minimum follows. Report the separation in full including rotor and g-force, give size by at least two orthogonal methods, state particle concentration and protein content so a purity ratio can be computed, and demonstrate depletion of at least one compartment marker. Figure 2 places the three principal sources of dispersion side by side.

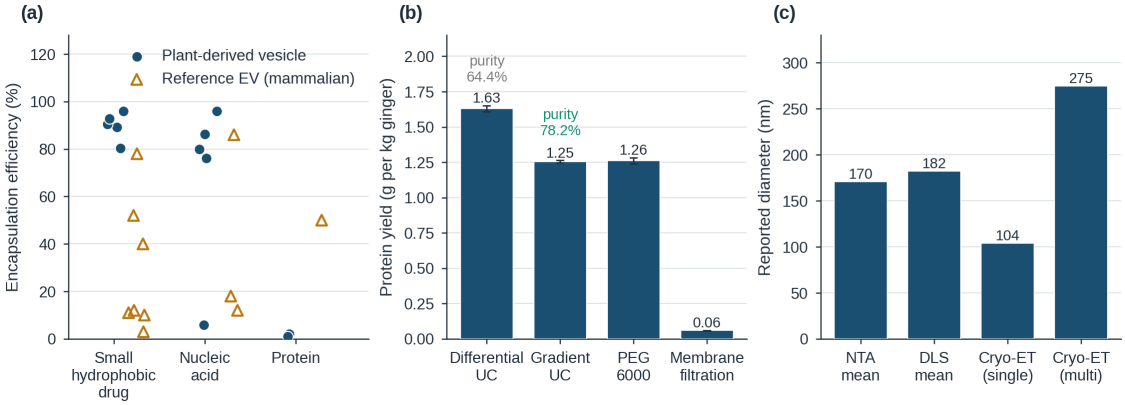

Figure 2. Three sources of dispersion in the plant vesicle literature, plotted from values reported in the cited studies. (a) Encapsulation efficiency separates by cargo class rather than by carrier: small hydrophobic drugs load at roughly 80% to 96% into plant vesicles, nucleic acids from about 6% to 96% depending on complexation, and proteins at about 1% to 2%; each point is a single reported value with no averaging applied [1,17,18,19]. (b) Protein yield from four isolation methods applied to one batch of ginger; gradient centrifugation trades yield for purity, and polyethylene glycol precipitation produced biologically inactive material despite a comparable yield [12]. (c) Diameter of the same Sorghum bicolor preparation measured four ways; the cryo-electron tomography values are medians and the others are means [8].

5. Loading and engineering

5.1 Encapsulation efficiency depends on the cargo, not the vesicle

Reported loading performance for plant vesicles spans nearly two orders of magnitude, and the pattern in the spread is chemical. Small hydrophobic molecules partition into the bilayer and load well: curcumin into ginger vesicles by sonication reaches close to 90% encapsulation, and doxorubicin by extrusion exceeds 95%[17]. Nucleic acids fall in the middle and respond strongly to formulation: microRNA loading into grapefruit vesicles rises from about 6% to more than 85% when the vesicle is coated with a polyethylenimine complex[19]. Proteins load poorly, with sonication of HSP70 into grapefruit vesicles capturing only about 2% of the input protein[18]. Method matters within a cargo class but less than cargo identity, so a single headline encapsulation efficiency for plant vesicles is not defensible, and comparisons between papers using different cargoes carry no information about the carriers.

Integrity after loading is reported far less often than efficiency. The most careful measurement available used cryo-electron microscopy before and after sonication and found grapefruit vesicles essentially unchanged in size with an insignificant fraction of broken vesicles[18]. Against this, repeated freeze-thaw cycles produce structural damage and cargo loss unless a cryoprotectant or preservative system is used[17].

5.2 Surface engineering works, and the effect sizes are large

The foundational demonstration remains folate conjugation to grapefruit lipid nanovectors, which increased tumour distribution more than a thousand-fold in colorectal models and improved siRNA-mediated knockdown several-fold[1]. Aptamers have been coupled by click chemistry to a lipid inserted into grapefruit vesicles, and

folate has been displayed on an anchored RNA three-way junction in ginger vesicles, where the orientation of the construct determined binding[19]. Membrane hybridisation is the more distinctly biological strategy. Fusing lemon vesicles with homotypic tumour cell membrane gave roughly six-fold selective uptake in the matched cell line, with doxorubicin encapsulation above 90% and a blood half-life of about 3 h[21]. Coating strategies of this kind place plant vesicles among the earliest membrane-fusion hybrids rather than within an established literature. Release behaviour has been engineered as well: doxorubicin release from plant nanovesicles reaches roughly 88% at pH 5.0 against 70% at pH 7.5, with the zeta potential shifting from about −25 mV at neutral pH to slightly positive under acidic conditions, a combination well suited to endosomal escape and the tumour interstitium [20]. Figure 3 summarises the loading and engineering options together with the cellular route they are designed to exploit.

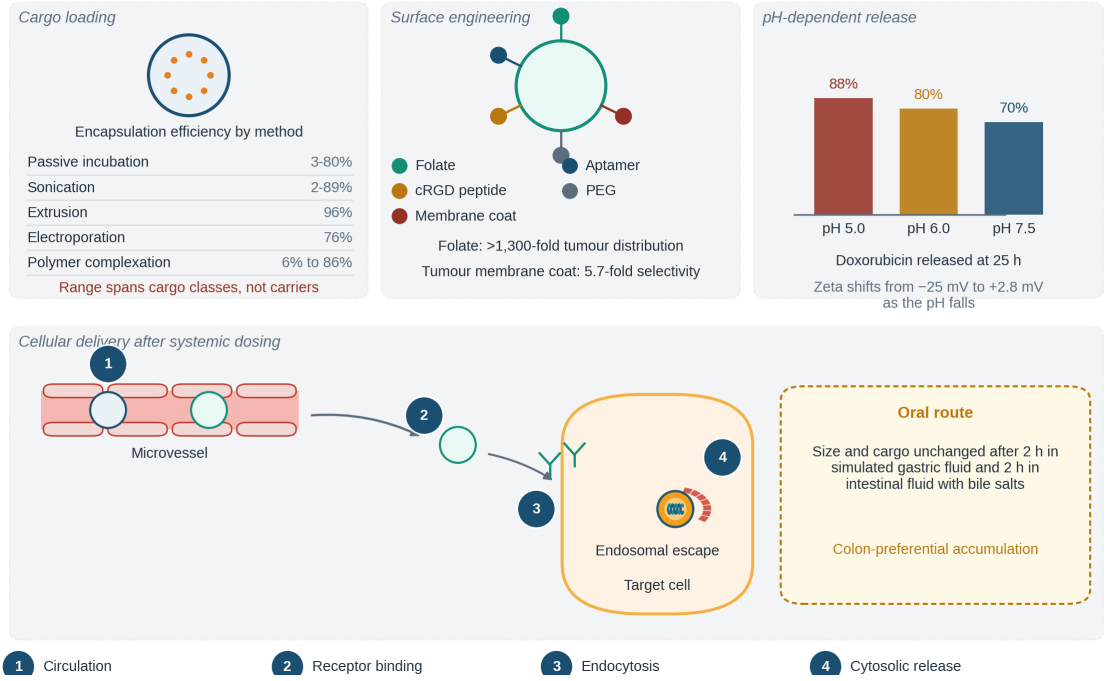

Figure 3. Loading, surface engineering and cellular fate of plant-derived vesicles. (a) Encapsulation efficiency by loading method, given as the range across cargoes reported for plant vesicles; the spread within a method reflects the physicochemistry of the cargo rather than the carrier [1,17,18,19]. (b) Ligands and coatings that have been placed on plant vesicles, with representative reported targeting gains [1,19,21]. (c) pH-dependent doxorubicin release from engineered plant nanovesicles, together with the accompanying shift in zeta potential from about −25 mV at neutral pH to slightly positive under acidic conditions [20]. (d) Fate after systemic dosing: circulation (1), receptor binding through a displayed ligand (2), endocytosis (3), and cytosolic release after endosomal escape (4).

5.3 Stability and the oral route

The most-repeated argument for plant vesicles is oral robustness, and the evidence for it is better than for most claims in this area. Radish seed nanoparticles subjected to a full simulated digestion sequence, from oral fluid through gastric fluid with pepsin at pH 3 to intestinal fluid with pancreatin and bile salts, showed no

significant change in size at any stage[23]. Ginger vesicles held in gastric fluid retained size, concentration and intact cargo proteins and RNAs[24]. Set against this, membrane integrity is compromised below pH 4 in some preparations, with cargo leakage[17]. Storage is less settled. Grapefruit lipid nanovectors remain intact beyond a month at 4 °C [1], while freeze-drying with trehalose or sucrose maintains encapsulation for months where omitting the cryoprotectant produces aggregation and cargo loss[17]. No consensus protocol exists for storage temperature, shelf-life, permitted freeze-thaw cycles or preservation medium.

6. Therapeutic applications and what the preclinical record shows

6.1 The gastrointestinal tract is the strongest case

The gut is where plant vesicles have the clearest mechanistic and pharmacokinetic advantage, since oral delivery places the carrier at its target without requiring systemic absorption. Grape vesicles given orally protected reporter mice against dextran sulfate sodium colitis and increased intestinal stem cell proliferation through Wnt/β-catenin signalling, and the active fraction was lipid, since particles reconstituted from grape vesicle lipids alone reproduced the stem cell targeting[25]. Ginger vesicles are preferentially taken up by lactobacilli in a lipid-dependent manner, and a ginger microRNA silences a *Lactobacillus rhamnosus* monooxygenase, raising indole-3-carboxaldehyde, an aryl hydrocarbon receptor ligand that induces IL-22 and improves barrier function; the anti-colitic effect of ginger vesicle RNA was IL-22-dependent[26]. This is a cross-kingdom RNA effect on a bacterium rather than on a host transcript, and it is considerably better supported than the broader claim discussed below. Delivery of exogenous agents to the same territory has followed, with reconstructed ginger nanolipids carrying siRNA and small-molecule cargoes localising to colon tissue after oral administration [17].

6.2 Liver, metabolic and systemic disease

Oral ginger nanoparticles protected mice against alcohol-induced liver injury, and the biodistribution data from that study remain among the most informative in the field: signal localised predominantly to liver with hepatocytes as the primary target cell, uptake was macropinocytosis-dependent, and Nrf2 nuclear translocation and HO-1 expression rose within hours [27]. Garlic vesicles reversed established high-fat-diet obesity through binding of a garlic phosphatidic acid species to a microglial protein, acting along the gut-brain axis [28]. A series of edible-plant vesicles from other sources converge on the same pattern of gut-microbiome remodelling and reduced hepatic inflammation across independent laboratories and disease models. That convergence is itself an argument: five plant sources and five disease models converging on a shared outcome fits a common lipid and phytochemical mechanism better than it fits cargo-specific pharmacology.

6.3 Oncology and dermal application

Grapefruit lipid nanovectors carrying a STAT3 inhibitor extended survival in a brain tumour model, and folate-targeted particles carrying paclitaxel held tumour volume well below controls [1]. Ginseng vesicles given intravenously produced higher brain fluorescence than control, penetrated three-dimensional spheroids more deeply than liposomes, and extended median survival in glioma from roughly 63 to 105 days [29]. Topical plant vesicles show consistent effects on wound and photoageing endpoints: lemon vesicles delivered in a hydrogel reprogrammed diabetic wound macrophages from a pro-inflammatory to a reparative phenotype and accelerated closure [22]. What is missing is the measurement that would justify the transdermal claim on which much cosmetic product is sold. No published study reports quantitative penetration of plant vesicles across intact stratum corneum, and the absence is conspicuous. Table 2 collects representative preclinical studies and shows the dose-metric incompatibility that prevents quantitative pooling across the field.

Table 2. Representative preclinical studies of plant-derived vesicles as therapeutic agents or carriers, showing the dose-metric incompatibility that prevents quantitative pooling. Every row states the dose in the unit the source used.

Plant source	Cargo	Model	Route and dose	Principal outcome	Ref.
Grape	None (vesicle as agent)	DSS colitis, reporter mice	Oral, 2 mg/mouse daily × 7 d	Protection from injury; stem cell proliferation via Wnt/β-catenin; lipid fraction sufficient	[25]
Ginger	Endogenous miRNA	DSS colitis, mice	Oral, every other day × 3 doses	miRNA silences *L. rhamnosus* gene, raising indole-3-carboxaldehyde; effect IL-22-dependent	[26]
Ginger	None	Alcohol-induced liver injury	Oral gavage, 50 mg/mouse/day	Hepatocyte uptake by macropinocytosis; Nrf2 translocation; HO-1 induction	[27]
Garlic	None	Diet-induced obesity	Oral gavage, 1 × 10^{10} particles/day	Reduced weight and hepatic fat; PA 36:4 binds microglial BASP1	[28]
Grapefruit (lipid)	JSI-124; paclitaxel + folate	Brain tumour; colorectal	Intranasal; intravenous	Extended survival; tumour volume controlled; >1,000-fold tumour distribution with folate	[1]
Ginseng	None	Orthotopic glioma	Intravenous	Deeper spheroid penetration; median survival 63 → 105 d	[29]
Lemon (in hydrogel)	None	Diabetic wound, rat	Topical	Macrophage repolarisation toward reparative phenotype; accelerated closure	[22]

6.4 The cross-kingdom microRNA dispute

The claim that dietary plant microRNAs regulate mammalian genes originates in a 2012 report that rice MIR168a is detectable in serum after ingestion and suppresses a hepatic transcript[30]. A 2013 feeding study found no detectable systemic absorption of plant microRNAs in mice[31], and the dispute has not resolved. The defensible position is narrow: plant vesicle RNA acting on gut bacteria is well evidenced[26], while plant microRNA acting directly on mammalian transcripts after dietary exposure is not established, is confounded by sequencing contamination, and should be described as contested wherever it appears. For the delivery field specifically, the RNA that most cargo claims depend on is substantially extravesicular, which weakens the case that the vesicle is the vehicle.

7. Translation: the clinical record, regulation and quality control

7.1 Three trials, no results

The clinical record is smaller than the preclinical literature implies. A search of the trial registry identifies three plant exosome studies, all sponsored by one institution. The first, a phase 1 study of plant exosomes delivering curcumin to normal and malignant colon tissue with a planned enrolment of 35, opened in 2011 and its status is now listed as unknown[33]. The second, a phase 1 study of grape extract against chemoradiation-induced oral mucositis, enrolled 60 participants and is completed[34]. The third, a pilot study of ginger exosomes with or without curcumin in inflammatory bowel disease, completed with an actual enrolment of four[35]. None has posted results. That record deserves plain statement. Fifteen years after the first plant exosome trial opened, fewer than one hundred participants have been enrolled worldwide, no efficacy signal has been reported, and the intervention in the largest of the three is registered as a dietary supplement rather than a characterised vesicle product. Reviews citing these registrations as clinical validation are citing an intention, not a finding.

7.2 No regulator has a pathway for this

There is no approved exosome product anywhere, and no drug regulatory authority has issued technical evaluation guidance specific to extracellular vesicle medicines. The United States Food and Drug Administration has stated that exosome products intended to treat disease are drugs and biological products requiring premarket approval, and it issued a public safety notification after reports of serious adverse events in patients given unapproved products [32]. A plant vesicle product sits awkwardly within this framework, being a biologic by mechanism, a botanical by starting material and a nanomedicine by physical form, and no existing document addresses the combination.

7.3 The specification problem

Chemistry, manufacturing and controls is where the field is weakest, and the deficiencies are specific rather than general. Identity has no marker: without a plant tetraspanin there is no equivalent to the CD9/CD63/CD81 panel, so identity is asserted from size and morphology[5,7].

Potency has no assay, a failing worse for plants than for mammalian vesicles because the vesicle is simultaneously excipient and active substance. Dose has no unit: published plant vesicle doses appear as body weight, protein mass, particle count, phospholipid quantity and lyophilised mass, and comparison across these is not possible, so two studies in comparable models can differ a thousand-fold on the reported number for reasons of metric and route rather than potency[5]. The batch has no definition either, since vesicle yield, size and cargo vary with cultivar, geographic origin, harvest season, soil and post-harvest processing[5,17], and plant-specific contaminants including agrochemical residues and recognised allergens add a further dimension. The measurement itself is not reproducible between instruments without local optimisation[16].

7.4 What the comparison with mammalian vesicles and liposomes actually supports

Two claims made for plant vesicles are well supported. Biosafety is real: plants host no zoonotic or human pathogens, so the adventitious agent testing burden that dominates cell-derived vesicle manufacture largely disappears. Abundance is real: yield figures of about 2.2 g/kg from grapefruit [1] and about 4 g/kg from ginger [13] have no analogue in mammalian cell culture. Two further claims are asserted more often than they are evidenced. Lower cost is universally stated and, in the accessible literature, never quantified. Scalability is asserted from the abundance of the feedstock, but the only quantitative pilot-scale filtration data come from microalgae[15], and no GMP batch record or batch-to-batch variability statistic has been published for a higher-plant vesicle product. The comparison that would matter most, plant vesicles against clinically validated lipid nanoparticles on the same cargo in the same model, has not been performed. Meanwhile marketed plant vesicle cosmetics are sold as phyto-exosomes or botanical nanocarriers, often without biochemical verification of vesicular identity, and the gap between what is sold and what is demonstrated is the field's most immediate reputational risk.

8. Critical appraisal and priorities

The preclinical literature reviewed here is encouraging on the questions a delivery scientist asks first. Plant vesicles survive simulated digestion[23,24], reach the colon and liver after oral dosing[25,27], accept small hydrophobic drugs at high efficiency[17] and nucleic acids when appropriately complexed[19], take folate, aptamers and whole cell membranes with large gains in targeting[1,21], and have produced no toxicity signal in the rodent studies that report one. It is also weaker than it appears, in four specific ways.

Vehicle controls are frequently absent. Because the carrier is pharmacologically active, a loaded-vesicle arm compared only against free drug cannot attribute the difference to delivery, and every study reporting a therapeutic effect should include an empty vesicle arm at matched particle dose. Effect sizes are often unextractable, with outcomes reported only as figure panels bearing

significance markers rather than numeric values; that, together with incompatible dose metrics and incommensurable endpoints, is why this review presents a structured narrative synthesis rather than a pooled estimate. The decision not to pool is a finding about the literature, not a limitation of the method. Safety data are thin to the point of absence: no acute or repeat-dose rodent toxicology study conducted to regulatory standard was identified, no immunogenicity dataset, and nothing on allergen carryover, despite the sources being foods that include recognised allergens. For a platform proposed for chronic oral use this is the most serious gap in the record. Finally, the identity of the tested article is often unclear, because the isolation is reported in insufficient detail to tell whether the material was a secreted vesicle, a homogenate-derived mixture or a reconstructed lipid particle.

Four priorities follow, in order of leverage. First, a plant-specific reporting standard: until an ISEV Plant Task Force checklist exists, papers should report the full separation protocol with rotor and g-force, source cultivar and harvest conditions, two orthogonal sizing methods, a particle-to-protein purity ratio, and at least one positive and one negative marker [3]. Second, a dose metric that converts: simultaneous reporting of particle count, protein mass, total vesicle mass and lipid quantification, with the yield relating them, would make published doses comparable retrospectively[5], and costs nothing but discipline. Third, reference reagents: the candidate marker panel is now defined[7], and raising and distributing validated antibodies against two or three members would convert plant vesicle identity from an assertion into an assay. Fourth, a regulatory-grade safety package on one lead material: a single well-characterised preparation taken through repeat-dose oral toxicology, immunogenicity and allergen screening under good laboratory practice would do more for the field than another twenty efficacy studies in mice, because it is the study a regulator asks for first and no one has done it.

9. Conclusion

Plant-derived extracellular vesicles have earned their place in the delivery literature. They are abundant where mammalian vesicles are scarce, carry no human pathogen risk, survive the gastrointestinal tract better than most carriers, and respond to surface engineering with targeting gains measured in orders of magnitude. The obstacle to using them clinically is not that they fail to deliver. It is that the field cannot yet say precisely what it is delivering. The material called a plant exosome is usually a mixture of provenances, produced by a method that determines its yield, purity, charge and biological activity, characterised by instruments that disagree with one another, and dosed in units that do not convert. Three registered trials over fifteen years and no posted results are the visible consequence. The work that will move plant vesicles from an attractive preclinical platform to a medicine is analytical rather than pharmacological: a plant-specific reporting standard, marker reagents that make identity testable, a convertible dose metric, and one lead material taken through a regulatory-grade safety

package. Those are tractable problems, and they are the ones that matter now.

Declarations

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