
**EXTRACELLULAR VESICLES AS NUTRITIONAL MESSENGERS:
CROSSTALK BETWEEN DIET, GUT MICROBIOME AND HOST
PHYSIOLOGY**

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ABSTRACT

Extracellular vesicles (EVs) are nano-sized, lipid bilayer-enclosed structures secreted by plant, microbial, and mammalian cells. Recent scientific advances have revealed that EVs function as nutritional messengers, mediating dynamic communication between dietary components, gut microbiota, and host physiological processes. Unlike traditional nutrients, EVs carry highly stable bioactive cargos—including microRNAs, lipids, peptides, metabolites, and functional proteins—which can withstand gastrointestinal digestion and be absorbed into systemic circulation. Dietary EVs derived from milk, fruits, and vegetables regulate intestinal immunity, epithelial barrier integrity, and gene expression pathways involved in metabolism. Concurrently, gut microbiome-derived EVs act as potent biological signals modulating host inflammation, cellular signaling, and metabolic homeostasis. A growing body of evidence suggests that diet alters the microbiome's EV secretion profile, while microbial EVs shape host metabolic and immune responses, thus forming a bidirectional communication axis. This review synthesizes current knowledge on EV biogenesis, dietary uptake mechanisms, microbiome-derived EVs, and diet-microbiome-host interactions, with emphasis on technological advances in EV isolation and characterization. We also highlight the therapeutic potential of EVs in functional foods, nutraceuticals, and targeted drug delivery. Understanding EV-mediated crosstalk provides a new paradigm in nutrition science and opens avenues for personalized diet-based interventions.

KEYWORDS: Extracellular vesicles; Gut microbiome; Inter-kingdom communication; Nutritional signaling; Functional foods; Host physiology.

1. INTRODUCTION

Extracellular vesicles (EVs) are membrane-bound nanostructures released by nearly all living systems. They play vital roles in transferring regulatory molecules such as proteins, lipids, DNA, mRNAs, and non-coding RNAs. EVs encompass multiple subclasses, primarily exosomes and microvesicles, which differ in origin and size but share a common function as mediators of intercellular communication [1].

The concept of EVs as nutritional messengers has recently emerged, indicating that dietary EVs and gut microbial EVs influence host physiology beyond classical nutrient pathways. This is based on growing evidence that plant-, milk-, and microbiota-derived EVs retain structural stability during digestion, are taken up by intestinal cells, and act on systemic organs.

With global demand for functional foods, microbiome-based therapies, and nutraceuticals, understanding diet–EV–host crosstalk has become essential in pharmaceutical sciences and human nutrition [2].

The aim of this review is to present an in-depth, evidence-based exploration of:

- EV types and origins
- Dietary EV absorption and stability
- Gut microbial EVs and their systemic effects
- Tripartite communication between diet, microbiota, and host physiology
- Applications in health and disease, analytical tools, and future research directions [3].

2. Types and Origin of Extracellular Vesicles

2.1 Classification of EVs

EVs are broadly classified into:

2.1.1 Exosomes (30–150 nm)

- Formed via inward budding of endosomal membranes
- Released when multivesicular bodies fuse with the plasma membrane
- Enriched in CD63, CD81, CD9, TSG101, Alix [4].

2.1.2 Microvesicles (100–1000 nm)

- Bud directly from the plasma membrane
- Contain cytosolic proteins, lipids, receptors

2.1.3 Apoptotic Bodies (500–2000 nm)

- Generated during programmed cell death
- Contain fragmented DNA, organelles [5].

2.2 Biological Sources of EVs Plant-derived EVs

- Found in ginger, grapes, citrus fruits, broccoli, carrot
- Rich in small RNAs, lipids, antioxidants
- Highly stable in the gut

Animal-derived EVs

- Present in human and bovine milk
- Contain growth-promoting and immune-regulating microRNAs

Microbial EVs

Bacteria, fungi, archaea release EVs:

- Gram-negative bacteria release outer membrane vesicles (OMVs)
- Gram-positive bacteria release cytoplasmic membrane vesicles [6]

2.3 Structural Composition of EVs

Extracellular vesicles (EVs) possess a highly organized structural architecture that enables them to function as efficient carriers of biologically active molecules. Their outer boundary is formed by a lipid bilayer, enriched with lipids such as phosphatidylserine, cholesterol, sphingomyelin, and ceramides, which not only maintain membrane stability but also influence vesicle curvature, fusion, and cellular uptake. Embedded within this lipid membrane are numerous proteins, including tetraspanins (CD9, CD63, CD81) that serve as EV markers, and heat shock proteins (HSP70, HSP90) that aid in protein folding and stress responses. Additionally, membrane-bound receptors, adhesion molecules, integrins, and enzymes contribute to EV-mediated signaling and selective cell targeting [7].

Inside the vesicle lumen, EVs encapsulate a diverse cargo of nucleic acids, such as microRNAs (miRNAs), messenger RNAs (mRNAs), long non-coding RNAs, circular RNAs,

and even fragments of double-stranded or mitochondrial DNA. These genetic materials remain protected from extracellular degradation due to the lipid bilayer, allowing them to modulate gene expression in recipient cells. EVs also contain metabolites, including amino acids, bioactive lipids, and precursors of short-chain fatty acids (SCFAs) that participate in metabolic communication between tissues and the gut microbiome.

The coordinated arrangement of these components makes EVs highly specialized nanocarriers, allowing them to transport stable molecular signals across cells, tissues, and even organ systems. Because of this, EVs act as vehicles for protected molecular delivery, playing a crucial role in intercellular communication, nutrient signaling, immune modulation, and disease progression [8].

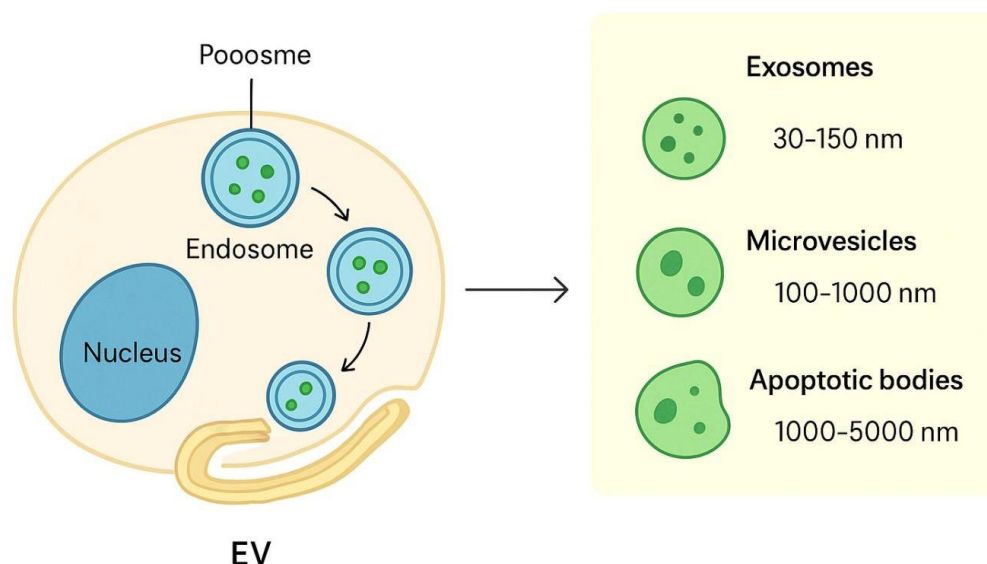


Figure 1: Overview of EV Biogenesis and Classification.

3. Dietary Extracellular Vesicles: Uptake and Bioavailability

Dietary extracellular vesicles (EVs), obtained from foods such as milk, fruits, and vegetables, exhibit remarkable stability and functional bioavailability within the human gastrointestinal tract. Their ability to remain intact during digestion and subsequently enter host circulation enables them to significantly influence gut health, immune regulation, and metabolic processes [9].

3.1 Stability of EVs During Digestion

One of the key properties that enable dietary EVs to exert biological effects is their high stability in the harsh gastrointestinal environment. The lipid bilayer surrounding EVs is

composed of cholesterol, phospholipids, and sphingolipids that provide strong resistance to gastric enzymes such as pepsin and lipases, preventing vesicle degradation. This membrane also shields the internal cargo—particularly microRNAs, mRNAs, and proteins—from RNases and acidic hydrolysis. Additionally, many dietary EVs, especially those derived from plants (e.g., ginger, grapes) and mammalian milk, exhibit significant pH tolerance, enabling them to withstand the highly acidic pH of the stomach and the alkaline conditions in the small intestine. These properties ensure that EVs reach the intestinal lumen intact, where they interact with mucosal cells and gut immune structures.

3.2 Absorption Mechanisms

Once dietary EVs reach the intestine, they are absorbed through multiple cellular pathways, allowing their bioactive cargo to enter systemic circulation [10].

3.2.1 Endocytosis by Enterocytes

A major route for EV absorption is endocytosis by intestinal epithelial cells (enterocytes). This process can occur via clathrin-dependent or caveolin-dependent pathways, where the EV membrane interacts with cell-surface receptors and is internalized into vesicular compartments [11]. Following internalization, some EVs release their cargo into the cytoplasm to regulate gene expression, while others translocate across the epithelial layer into the bloodstream (transcytosis). This mechanism explains how dietary EVs can influence distant tissues and organs [12].

3.2.2 M Cell-Mediated Transport Across Peyer's Patches

Another important uptake route involves M cells (microfold cells) located in Peyer's patches of the small intestine. These specialized cells transport particles and antigens from the gut lumen to underlying immune tissues. EVs interacting with M cells are rapidly translocated across the epithelial barrier and delivered to immune cells such as dendritic cells, B cells, and T cells. This mechanism highlights how dietary EVs can modulate immune responses and promote mucosal immunity [13].

3.2.3 Phagocytosis by Intestinal Macrophages

Dietary EVs can also be taken up by intestinal macrophages through phagocytosis. Once engulfed, the vesicles release their lipid, protein, and RNA components, influencing macrophage activity. This includes modulation of inflammatory cytokine secretion, enhancement of gut barrier maintenance, and regulation of microbiota-immune interactions.

Through this pathway, dietary EVs play a direct role in shaping intestinal immune homeostasis [14].

3.3 Case Studies of Dietary EVs Milk-Derived EVs

Milk-derived EVs are rich in immune-regulatory microRNAs, such as miR-21 and miR-148a, which remain intact even after pasteurization and digestion. These EVs enhance gut epithelial development, strengthen intestinal tight junctions, and promote immune maturation in infants. Their miRNA cargo can regulate genes involved in inflammation, metabolism, and intestinal growth, contributing to the well-documented health benefits of breast milk and dairy intake [15].

Ginger-Derived EVs

Ginger-derived EVs are widely studied for their anti-inflammatory and gut-protective properties [16]. They contain bioactive lipids and RNAs capable of suppressing pro-inflammatory pathways such as NF- κ B and promoting the production of anti-inflammatory cytokines. Additionally, these EVs play a significant role in supporting gut barrier integrity by enhancing epithelial cell proliferation and reducing oxidative stress [17]. Their plant origin makes them highly stable and effective in delivering therapeutic molecules to intestinal immune cells [18].

Grape-Derived EVs

EVs derived from grapes contain antioxidants, polyphenols, and specific RNAs that help modulate gut microbiota composition, increasing beneficial bacterial populations [19]. They also support cellular antioxidant defense by upregulating detoxification enzymes and reducing oxidative stress markers. As a result, grape EVs contribute to improved gut health, enhanced immune function, and protection against inflammatory disorders [20].

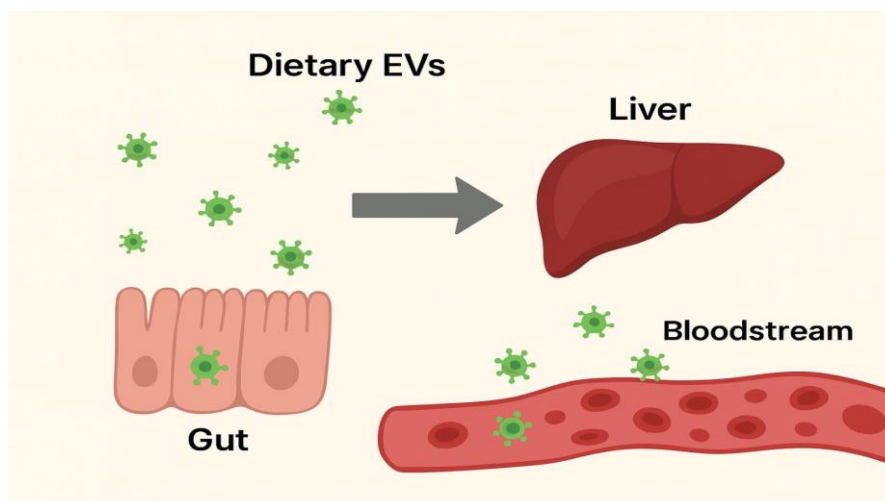


Figure 2. Uptake and Systemic Circulation of Dietary Extracellular Vesicles (EVs)

4. Gut Microbiome-Derived EVs and Host Interactions

Gut microbial communities continuously release extracellular vesicles (EVs) that act as potent mediators of communication between microbes and the host. These nano-sized vesicles contain lipids, proteins, nucleic acids, metabolites, and cell-wall components that enable them to cross the intestinal mucus layer and interact directly with epithelial and immune cells. Through these interactions, microbial EVs influence immune homeostasis, intestinal barrier integrity, and metabolic signaling, ultimately shaping the host's physiological responses [21].

4.1 Immune Modulation

Microbiome-derived EVs play a crucial role in modulating the host immune system by regulating both innate and adaptive immune responses. These vesicles can activate macrophages, leading to changes in inflammatory cytokine production such as IL-6, IL-10, and TNF- α , which determine whether the immune response becomes pro-inflammatory or anti-inflammatory. EVs also influence T-cell differentiation, promoting the development of specific T-cell subsets that help maintain immune balance. A widely studied example is *Bacteroides fragilis*-derived EVs, which contain polysaccharide A (PSA). These EVs facilitate the induction of regulatory T cells (Tregs), thereby enhancing anti-inflammatory pathways and protecting against intestinal inflammatory disorders. Through these mechanisms, microbial EVs help maintain immune tolerance while preventing excessive inflammation [22].

4.2 Maintenance of Intestinal Barrier Integrity

The gut barrier functions as a selective interface that prevents harmful microbes and toxins from entering the bloodstream. Microbial EVs significantly contribute to the maintenance of this barrier by enhancing the expression of tight junction proteins such as ZO-1, claudin, and occludin. These proteins strengthen the epithelial layer and reduce intestinal permeability, effectively preventing —leaky-gut conditions that can trigger chronic inflammation. An important example is *Lactobacillus rhamnosus*-derived EVs, which have been shown to stimulate epithelial repair, reinforce tight junction organization, and protect the gut lining from oxidative and inflammatory damage. By supporting barrier integrity, microbial EVs safeguard against pathogen invasion and systemic inflammatory diseases.

4.3 Microbial EVs as Metabolic Signals

Beyond immune and barrier functions, gut microbial EVs act as metabolic messengers that influence host metabolism. These vesicles carry metabolites, small molecules, lipopolysaccharide (LPS) fragments, bacterial enzymes, and short-chain fatty acid precursors that interact with metabolic pathways in host tissues. Microbial EVs can modulate insulin signaling by affecting inflammatory responses in adipose tissue and influencing glucose uptake pathways. They may also contribute to adipocyte inflammation, a key event in obesity and insulin resistance, by delivering LPS and pro-inflammatory molecules that trigger cytokine release. Through these metabolic interactions, microbial EVs link gut microbial composition with systemic metabolic health, contributing to conditions such as metabolic syndrome, type 2 diabetes, and obesity [23].

5. Diet–Microbiome–Host Crosstalk via Extracellular Vesicles

Dietary patterns profoundly influence the composition of the gut microbiome and, consequently, the nature and function of extracellular vesicles (EVs) produced within the gut environment. These EVs serve as molecular messengers that connect dietary components, microbial activity, and host physiology. Through the cargo they carry—such as lipids, proteins, RNAs, and metabolites—EVs mediate dynamic and bidirectional communication, orchestrating changes in inflammation, metabolism, immunity, and intestinal homeostasis [24].

5.1 Diet Alters EV Composition

The types of foods consumed significantly modify the biochemical composition and functional properties of both dietary EVs and microbiome-derived EVs. Fiber-rich diets enhance the growth of beneficial bacteria such as Bifidobacteria and Lactobacilli, leading to the production of anti-inflammatory EVs enriched in short-chain fatty acid precursors, regulatory RNAs, and protective proteins. These EVs help reduce inflammation, strengthen the gut barrier, and support metabolic balance. In contrast, high-fat diets increase the abundance of pathogenic or pro-inflammatory bacterial species that release EVs containing lipopolysaccharides (LPS), virulence factors, and inflammatory RNAs [25]. Such pro-inflammatory EVs contribute to gut permeability, systemic inflammation, and metabolic disorders. Additionally, polyphenol-rich foods—such as berries,

grapes, and green tea—can modify microbial EV secretion by promoting antioxidant-producing microbes and altering EV-associated enzymes and signaling molecules. Thus, diet-driven changes in EVs directly influence host health outcomes [26].

5.2 Microbial EVs Affect Host Gene Expression

Microbial EVs interact with host cells and modulate gene expression through several well-defined intracellular signaling pathways. A key mechanism involves the NF- κ B pathway, which regulates inflammatory responses. EVs carrying LPS or inflammatory RNAs can activate NF- κ B, leading to the production of cytokines such as IL-6 and TNF- α , whereas EVs derived from beneficial microbes suppress NF- κ B activity and promote anti-inflammatory gene expression. Another critical pathway influenced by EVs is the MAPK (Mitogen-Activated Protein Kinase) pathway, which controls cell survival, stress responses, and epithelial repair. By altering MAPK activity, microbial EVs help regulate cell proliferation and mucosal healing [27]. Moreover, EVs interact with Toll-like receptors (TLRs) on immune and epithelial cells. These interactions initiate immune surveillance, maintaining a balance between tolerance and defense. Through these pathways, microbial EVs exert profound effects on host transcriptional activity and physiological responses [28].

5.3 Bidirectional Communication

Extracellular vesicles form the basis of a complex bidirectional communication system among diet, microbiota, and host tissues. Dietary components influence the composition and activity of gut microbes, which in turn shape the profile and bioactivity of microbial EVs—thereby completing the first part of the communication loop: Diet \rightarrow Microbiota modulation. The second part of the loop occurs when these microbial EVs interact with host immune cells, epithelial cells, and metabolic organs, affecting processes such as cytokine production, insulin sensitivity, oxidative stress responses, and overall immune balance. This represents Microbiota \rightarrow Host immune and metabolic signaling. Through this continuous exchange of vesicle-mediated signals, the diet–microbiome–host axis remains dynamically interconnected, enabling the body to adapt to nutritional changes and maintain homeostasis [29].

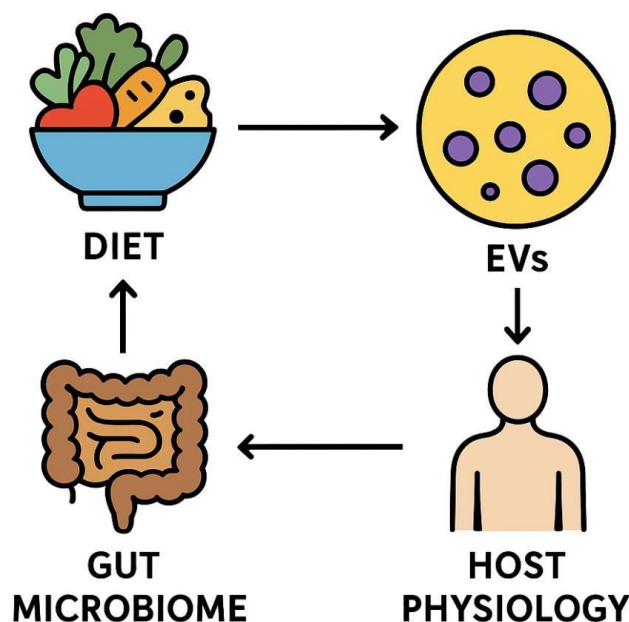


Figure 3. Conceptual Diagram of Diet–EV–Gut Microbiome–Host Physiology Crosstalk

6. Implications for Health and Disease

Extracellular vesicles (EVs) derived from diet and the gut microbiome play a central role in shaping host physiology, influencing gut integrity, metabolism, immune function, and even neurological health. Their ability to transport stable bioactive molecules across cellular and tissue barriers makes them critical mediators in the diet–microbiome–host axis. Understanding their functions provides new opportunities for disease prevention, therapeutic intervention, and nutraceutical development [30].

6.1 Gut Integrity

Dietary EVs, especially those derived from milk, fruits, and vegetables, have demonstrated significant benefits in maintaining and restoring gut integrity. These EVs help reduce intestinal inflammation by suppressing pro-inflammatory pathways such as NF- κ B and enhancing anti-inflammatory cytokines [31]. They also stimulate mucus production, increasing the thickness and protective function of the mucosal barrier, which is essential for preventing pathogen penetration and toxin absorption. Additionally, dietary EVs support epithelial renewal by promoting enterocyte proliferation, enhancing tight junction formation, and accelerating tissue repair after injury. Through these combined effects, dietary EVs contribute to stronger gut barrier function and protection against gastrointestinal disorders such as colitis and leaky-gut syndrome [32].

6.2 Metabolic Disorders

Abnormal EV-mediated communication between the gut microbiome and host tissues has been strongly linked to the development of metabolic diseases. Pro-inflammatory microbial EVs— especially those enriched in lipopolysaccharides (LPS) or inflammatory microRNAs—can promote adipose tissue inflammation, a key driver of obesity and insulin resistance. These EVs impair insulin signaling pathways, elevate systemic inflammation, and disrupt glucose metabolism. Moreover, dysfunctional EV signaling can alter lipid metabolism, contributing to dyslipidemia, including elevated triglycerides and abnormal cholesterol profile [34]. In contrast, EVs derived from beneficial microbes or plant-based foods can counteract these processes by improving insulin sensitivity, reducing oxidative stress, and regulating energy homeostasis. Thus, EVs represent both biomarkers and potential therapeutic targets in metabolic syndromes [35].

6.3 Neuroinflammation and the Gut–Brain Axis

One of the most fascinating discoveries in EV biology is the ability of microbiome-derived EVs to cross the blood–brain barrier (BBB). Once inside the central nervous system, these EVs can modulate neuronal pathways and immune responses within the brain [36]. They may activate microglia, the resident immune cells of the brain, influencing the balance between neuroprotection and neuroinflammation. Additionally, microbial EVs can affect neurotransmitter pathways, altering levels of serotonin, dopamine, and GABA, ultimately impacting mood, cognition, and stress responses [37]. Dysregulated EV signaling has been implicated in conditions such as anxiety, depression, neurodegeneration, and Parkinson’s disease, highlighting the important role of gut-derived EV communication in the gut–brain axis [38].

6.4 Dietary Interventions

Growing evidence underscores the potential of dietary strategies to modulate EV-mediated health benefits [39]. Foods naturally rich in EVs—such as milk, citrus fruits, ginger, and grapes— provide EV-rich nutritional support that enhances gut and immune health. Probiotics, particularly strains like *Lactobacillus* and *Bifidobacterium*, are known to release beneficial EVs carrying anti-inflammatory and barrier-protective molecules [40]. These probiotic-derived EVs represent a promising tool for both preventive and therapeutic interventions. Additionally, emerging research is exploring plant-derived EV-based nutraceutical formulations, where purified EVs from edible plants are used as natural delivery

systems for therapeutic RNAs, antioxidants, and bioactive lipids [41]. Such interventions may offer safe, biocompatible alternatives to synthetic nanoparticles for targeted therapy. Overall, dietary modulation of EV profiles holds significant promise for disease prevention, metabolic balance, and long-term wellness [42].

7. Emerging Technologies and Analytical Methods

The study of extracellular vesicles (EVs) has rapidly advanced due to improvements in isolation techniques, characterization tools, and analytical platforms [43]. These technologies allow researchers to separate EVs from complex biological samples, identify their structural and molecular composition, and understand their functional roles in diet–microbiome–host interactions [44]. Despite these advances, challenges remain regarding standardization, purity, and scalability, making EV research an evolving and technologically demanding field [45].

7.1 Isolation Techniques

Efficient isolation of EVs is essential for high-quality analysis, and several methods are now commonly used. Ultracentrifugation remains the gold standard, where EVs are separated based on density and size through high-speed spinning; however, it can be time-consuming and may damage delicate vesicles. Density gradient centrifugation provides better purity by layering EVs according to buoyant density, reducing contamination from proteins and cell debris [46]. Another widely used method is size exclusion chromatography (SEC), which separates EVs by passing the sample through porous beads that allow smaller molecules to enter and delay, while larger vesicles elute first—resulting in minimal structural alteration and high reproducibility [47]. More recently, microfluidics-based isolation has emerged as a cutting-edge approach, utilizing microscale channels to separate EVs through immunocapture, acoustic forces, or nano-filters.

This method offers rapid processing, minimal sample volume, and the potential for clinical point-of-care diagnostics [48].

7.2 Characterization Tools

Once isolated, EVs are characterized using a range of biophysical and molecular techniques [49]. Nanoparticle tracking analysis (NTA) measures particle size and concentration by tracking the Brownian motion of vesicles in solution, providing quantitative insights into EV populations. Transmission electron microscopy (TEM) offers high-resolution imaging that

reveals EV morphology, confirming their characteristic cup-shaped or spherical structures [50]. Flow cytometry, especially high-sensitivity or nano-flow systems, enables surface marker profiling using antibodies against EV-associated proteins such as CD9, CD63, and CD81 [51]. At the molecular level, techniques like proteomics and RNA sequencing are used to map the protein and nucleic acid cargos of EVs, helping to uncover functional pathways and biomarker signatures. These tools collectively allow for comprehensive characterization of EV identity, purity, and bioactivity [52].

7.3 Challenges

Despite technological progress, EV research faces significant limitations. One major challenge is the lack of standardized protocols for isolation and characterization, leading to variations in EV yield, purity, and reported outcomes across studies. Batch variability further complicates reproducibility, especially when EVs are derived from biological sources such as milk, plants, or microbial cultures. Storage stability is another concern, as EV integrity can be compromised during freeze–thaw cycles or long-term storage, affecting functional assays and molecular profiles [53]. Additionally, EV preparations often suffer from contamination with non-EV particles, including protein aggregates, lipoproteins, and cell debris, making it difficult to distinguish true EV-derived effects from artifacts. Addressing these challenges requires refinement of methodological guidelines, development of EV-specific standards, and greater transparency in reporting experimental parameters [54].

Table 1: Analytical Methods for EV Isolation and Their Advantages and Limitations.

EV Isolation Method	Principle Description	Advantages	Limitations	Reference
Differential Ultracentrifugation (UC)	Sequential high-speed centrifugation separates EVs based on size and density.	Widely used; suitable for large sample volumes; cost-effective on large scale equipment available.	Time-consuming; requires expensive ultracentrifuge; may co-isolate protein aggregates; high force.	[55]
Density Gradient Ultracentrifugation	EVs separated based on flotation density using sucrose	Higher purity than differential UC; reduces protein/lipoprotein	Complex protocol; low throughput; longer processing time.	[56]

	gradients.	contamination.		
Size Exclusion Chromatography (SEC)	Physical separation based on size using porous beads.	High EV purity; gentle on EV structure; suitable for downstream omics analyses.	Lower concentration yield; requires post-concentration step; batch variability.	[57]
Ultrafiltration (Membrane Filtration)	Size-based retention of EVs through molecular weight cut-off membranes.	Fast; scalable; no need for expensive equipment.	Membrane clogging; may deform EVs; loss of small EV subpopulations.	[58]
Precipitation-based Kits (PEG Precipitation)	EVs precipitated using polymer-based reagents (e.g., PEG).	Simple; specialized instruments; good yield.	Low purity; polymer contamination; cannot be used for proteomics/functional assays without cleanup.	[59]
Immunoaffinity Capture	Antibodies targeting EV surface proteins (CD9, CD63, CD81) capture EV subtypes.	High specificity; useful for biomarker studies; isolates defined EV populations.	Expensive; low yield; biased toward known EV surface markers.	[60]
Microfluidics-based Isolation	EVs captured using microchannels, acoustic waves, or nano-immuno chips.	Fast; small sample volume; high sensitivity; integrates isolation + analysis.	Limited scalability; specialized chips required; high cost.	[61]
Tangential Flow Filtration (TFF)	Continuous filtration and EV concentration using tangential flow.	Scalable for industrial production; preserves EV integrity; suitable for nutraceutical applications.	Initial setup cost high; risk of membrane fouling.	[62]

Field-Flow Fractionation (FFF)	Separation based on hydrodynamic properties under external fields.	High resolution; can distinguish EV subpopulations.	Requires technical expertise; expensive instrumentation.	[63]
Chromatographic Affinity Methods	Affinity ligands or aptamers capture EVs; based on surface properties.	High specificity; gentle process; suitable for clinical applications.	Low yield; requires well-characterized EV markers.	[64]
Acoustic Nanofiltration	Ultrasonic standing waves separate EVs by size and density.	Label-free; rapid; preserves EV morphology.	Still emerging; low availability; may require calibration for different EV types.	[65]
Magnetic Bead-Based Isolation	EVs bound to magnetic beads coated with antibodies/peptides.	Easy and fast; suitable for diagnostic workflows.	Limited throughput; costly; captures	[66]
Polymer-free Aqueous Two-Phase Systems	Partition of EVs into two immiscible aqueous phases based on surface properties.	Good yield; mild isolation conditions.	Lower purity; optimization required for each sample type.	[67]

Table 2: Summary of Analytical Methods for EV Characterization.

Characterization Method	Principle / What It Measures	Key Outputs	Advantages	Limitations	Reference
Nanoparticle Tracking Analysis (NTA)	Tracks Brownian motion of particles using	Size distribution, particle concentration.	High sensitivity; quantifies size and number; real-time	Cannot distinguish EVs from non-EV particles;	[68]

	laser illumination.		measurement.	requires dilution optimization.	
Dynamic Light Scattering (DLS)	Measures fluctuations in scattered light intensity due to particle movement.	Average particle size (hydrodynamic radius), polydispersity.	Fast; simple; non-destructive.	Low resolution for polydisperse EV samples; dominated by larger particles.	[69]
Transmission Electron Microscopy (TEM)	Electron beam imaging of EV morphology at high resolution.	Shape, membrane structure, EV integrity.	High-resolution visualization; gold standard for morphology.	Labor-intensive sample prep; not quantitative; potential for artifacts.	[70]
Cryo-Electron Microscopy (Cryo-EM)	Imaging vitrified EVs without staining or fixation.	Near-native structure, membrane topology.	Most accurate morphology; preserves hydration.	Expensive; technically demanding; low throughput.	[71]
Atomic Force Microscopy (AFM)	Scanning probe maps EV surface topography.	3D surface structure, stiffness, mechanical properties.	Provides biomechanical data; minimal sample prep.	Small field of view; slower scanning; requires expertise.	[72]
Flow Cytometry	Uses fluorescent antibodies to detect EV surface markers.	Marker expression, EV subpopulation analysis.	High throughput; detects specific EV subsets.	Struggles with EVs <200 nm; potential for antibody background noise.	[73]
High-Resolution / Nano-Flow Cytometry	Modified cytometry optimized for nanoscale particles.	EV count, phenotype, size distribution.	Enables single-EV analysis; detects EVs down to ~40–70 nm.	Very expensive; requires protocol standardization.	[74]
Western Blotting	Protein detection using antibodies.	Presence of EV markers (CD9, CD63, HSP70).	Confirms EV protein identity; good for purity checks.	Not quantitative; requires large sample; cannot detect heterogeneity.	[76]
Proteomics (LC-MS/MS)	Mass spectrometry profiling of EV proteins.	Full proteome mapping, biomarker discovery.	Highly sensitive; deep characterization.	Costly; requires skilled handling; time-consuming.	[77]
RNA Sequencing (miRNA/mRNA profiling)	Sequencing EV-associated nucleic acids.	miRNA cargo, mRNA, lncRNA profiles.	High-resolution transcriptomics; detects functional	Needs high-quality RNA; susceptible to contamination.	[78]

			miRNAs.		
qPCR / Digital PCR	Quantifies specific EV-associated RNAs.	Absolute or relative RNA abundance.	Highly sensitive; ideal for targeted studies.	Requires known targets; influenced by RNA isolation efficiency.	[79]
Zeta Potential Analysis	Measures particle surface charge in suspension.	Surface charge, colloidal stability.	Useful for storage stability and formulation work.	Cannot differentiate EVs from contaminants.	[80]
Fourier-Transform Infrared Spectroscopy (FTIR)	Detects vibrational spectra of EV biochemical components.	Lipid, protein, and nucleic acid signature.	Rapid; label-free; small sample required.	Limited structural detail; requires interpretation skill.	[81]
Raman Spectroscopy	Laser-based molecular fingerprinting.	Chemical composition and biomolecular signatures.	Non-destructive; detects subtle compositional changes.	Low sensitivity; weak signals from nanosized EVs.	[82]

8. Future Perspectives

The field of extracellular vesicle (EV) research is entering a transformative phase, with promising applications in nutrition, disease prevention, diagnostics, and therapeutic delivery. As understanding of diet–microbiome–host communication deepens, EVs are emerging as powerful biological tools capable of reshaping personalized health strategies and modern nutraceutical science.

One of the most exciting future directions is the development of EV-based functional foods designed to deliver targeted health benefits. These foods could be naturally enriched with beneficial plant- or milk-derived EVs or could be fortified with engineered vesicles that carry specific microRNAs, proteins, or metabolites aimed at improving gut health, reducing inflammation, or enhancing metabolic balance. Such innovations could redefine dietary interventions by offering precision-based nutritional support.

Another promising area is personalized nutrition guided by EV biomarker profiling. Because EVs circulate in blood, saliva, urine, and stool, they provide a minimally invasive window into an individual's metabolic state, microbiome activity, and nutritional needs. Profiling EV cargo—such as microRNAs associated with inflammation, metabolic dysfunction, or gut barrier impairment—could allow clinicians and nutritionists to tailor diet plans unique to each person's biological signature.

Advances in AI-driven multi-omics integration are also expected to revolutionize EV research. Integrating proteomics, metabolomics, lipidomics, and transcriptomics with machine learning will enable comprehensive mapping of EV networks and prediction of their functional outcomes. AI tools can help identify EV-derived biomarkers, improve classification accuracy, and optimize therapeutic EV formulations, accelerating both research and clinical translation.

A rapidly emerging therapeutic concept is the use of plant-derived EVs as oral drug delivery systems. Plant EVs are stable in the gastrointestinal tract, naturally biocompatible, and capable of crossing biological barriers. This makes them ideal candidates for delivering therapeutic RNA molecules, antioxidants, anti-inflammatory agents, and even conventional drugs in a safe, targeted manner. Their scalable production from edible plants further enhances their clinical potential.

Finally, as EV applications expand, there is a growing need for robust regulatory frameworks governing EV-based nutraceuticals and functional foods. Guidelines must address safety, purity, batch consistency, labeling standards, and clinical validation before EV-based products can enter the market. Establishing such regulations will ensure that EV technologies are translated into reliable, consumer-safe health solutions.

9. CONCLUSION

Extracellular vesicles represent a transformative concept in nutrition science, acting as molecular messengers that connect dietary components, gut microbiota, and host physiology. Their stability, bioactivity, and inter-kingdom communication potential make them key contributors to metabolic health, immune regulation, and disease prevention. Understanding EV-mediated pathways will accelerate innovations in functional foods, pharmacological therapeutics, and personalized nutrition strategies. Standardized methodologies and clinical research are urgently required to harness EVs for translational applications.

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