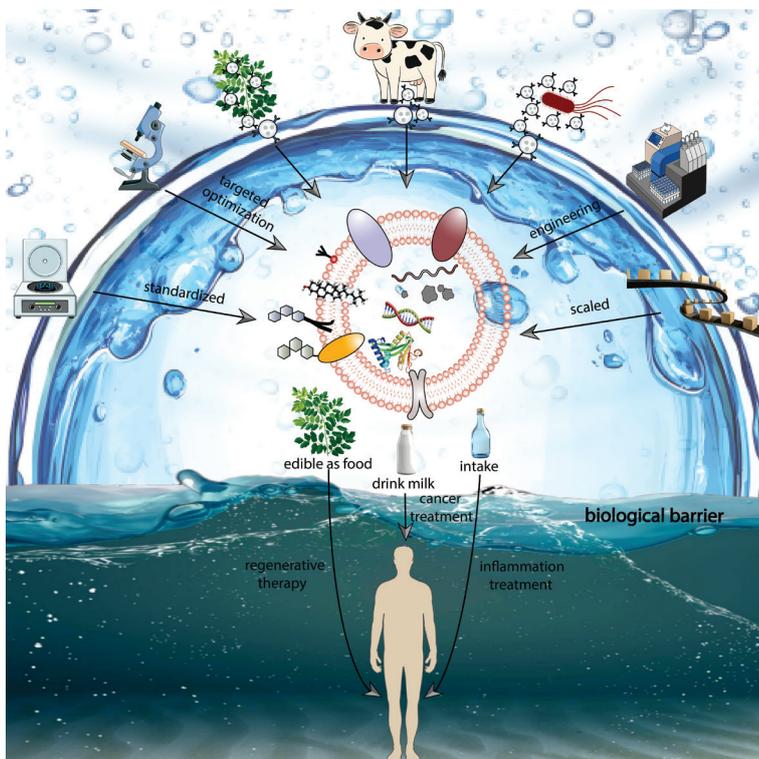# BioIntegration

Exogenous Extracellular Vesicles as Emerging Platforms in Translational Medicine

Graphical abstract

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In brief

This review focuses on exogenous EVs as translational medicine platforms. It explores their sources, biological properties, and roles in disease modulation, highlights applications in oncology, regeneration, and inflammation, and addresses clinical translation challenges, envisioning AI-driven personalized EV therapies.

Highlights

- This review summarizes exogenous extracellular vesicles (EVs) from milk, plants, and microbes, covering their structure, uptake, and roles in intercellular communication.
- Their unique advantages (low immunogenicity, biocompatibility) and challenges (standardization, loading efficiency) for clinical use are analyzed.
- It also details applications in cancer therapy, regenerative medicine, and inflammatory diseases with preclinical/clinical evidence.
- Future directions via AI and synthetic biology for personalized EV-based precision therapy are discussed.

Exogenous Extracellular Vesicles as Emerging Platforms in Translational Medicine

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Abstract

Extracellular vesicles (EVs) are nanoscale, membrane-bound carriers that are naturally secreted by cells and capable of transporting proteins, lipids, and nucleic acids across biological barriers. As key mediators of intercellular communication, EVs participate in immune regulation, tissue repair, and disease progression. The structural integrity of EVs, which is conferred by a lipid bilayer, protects cargo from degradation, while surface molecules facilitate targeted cell interactions. With increasing interest in their therapeutic utility, EVs have emerged as promising candidates in diagnostics, drug delivery, and regenerative medicine. EVs derived from milk, plants, and microbes exhibit distinct bioactivities, broadening the applicability in translational research. The intrinsic biocompatibility and low immunogenicity of EVs further enhance their clinical relevance. This review highlights recent advances in the understanding and biomedical utilization of exogenous EVs. The structural features, mechanisms of cellular uptake, and functional roles in modulating disease pathways are discussed. Furthermore, the unique advantages and challenges of leveraging exogenous EVs for clinical translation are explored, including standardization, loading efficiency, and targeting specificity. With continuous innovation at the interface of nanotechnology, synthetic biology, and biomedicine, exogenous EVs are poised to become next-generation platforms for precision therapy and regenerative strategies.

Keywords

Extracellular vesicles, Immunogenicity, Translational medicine, Plant-derived extracellular vesicles, Outer membrane vesicles.

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Introduction

Cell-based therapies have emerged as transformative tools in regenerative medicine, immuno-oncology, and tissue engineering [1]. However, despite the significant therapeutic potential of cell-based therapies, these approaches face critical limitations, including immune rejection, tumorigenicity, limited engraftment, and complex manufacturing and regulatory hurdles [2]. These challenges have spurred growing interest in cell-free therapeutic strategies that aim to replicate the benefits of living cells, while addressing safety and scalability concerns [3, 4].

Among such strategies, extracellular vesicles (EVs), which are nano-sized, membrane-enclosed vesicles secreted by virtually all cell types, have garnered significant interest as next-generation therapeutic vectors [5]. As natural mediators of intercellular communication, EVs are enriched with diverse bioactive cargos, including proteins, nucleic acids, lipids, and metabolites, which are capable of modulating immune responses, promoting tissue repair, and influencing disease progression [6]. Compared to whole-cell therapies, EV-based approaches offer the following distinct advantages: lower immunogenicity; reduced toxicity; enhanced stability under physiologic conditions; and the intrinsic ability to traverse biological barriers, such as the blood-brain barrier, thereby broadening their clinical utility [7].

Of particular translational interest are exogenous EVs, which are derived from non-autologous sources, such as bovine milk, edible plants, and engineered microorganisms [8]. These vesicles offer notable practical and clinical advantages. Specifically, EVs can be produced at scale through cost-effective pipelines, are amenable to non-invasive delivery routes (e.g., oral or intravenous), and can be engineered to improve targeting or carry customized therapeutic cargos [9]. Additionally, the unique biomolecular signatures of EVs enable innovative

applications in diagnostics and prognostics, particularly in cancer, neurodegenerative diseases, and inflammatory conditions [10].

This review explores the emerging landscape of exogenous EVs in translational medicine (**Scheme 1**) with a focus on their roles in intercellular communication, immune modulation, and therapeutic innovation. The recent advances in the application of EVs in cancer therapy, regenerative medicine, and inflammatory disease management are critically examined, while highlighting key challenges requiring resolution to unlock the full clinical potential (standardization, bioengineering, and regulatory oversight) [11]. Exogenous EVs represent a promising, safe, and scalable alternative to traditional cell-based therapies and are poised to drive the next generation of precision medicine [5].

Overview of EVs

Definition and structure of EVs

As research on EVs continues to advance, the essential roles of EVs in intercellular communication, immune modulation, and various physiologic and pathologic processes have become increasingly recognized. EVs are broadly defined as lipid bilayer-enclosed vesicles secreted by cells into the extracellular space [1]. While extensively studied in mammalian systems, EVs are also released by plants and bacteria, highlighting the evolutionary conservation and universal role in cross-kingdom communication. EVs encompass a diverse range of vesicles in multicellular organisms that are secreted by nearly all cell types [12]. EVs are commonly
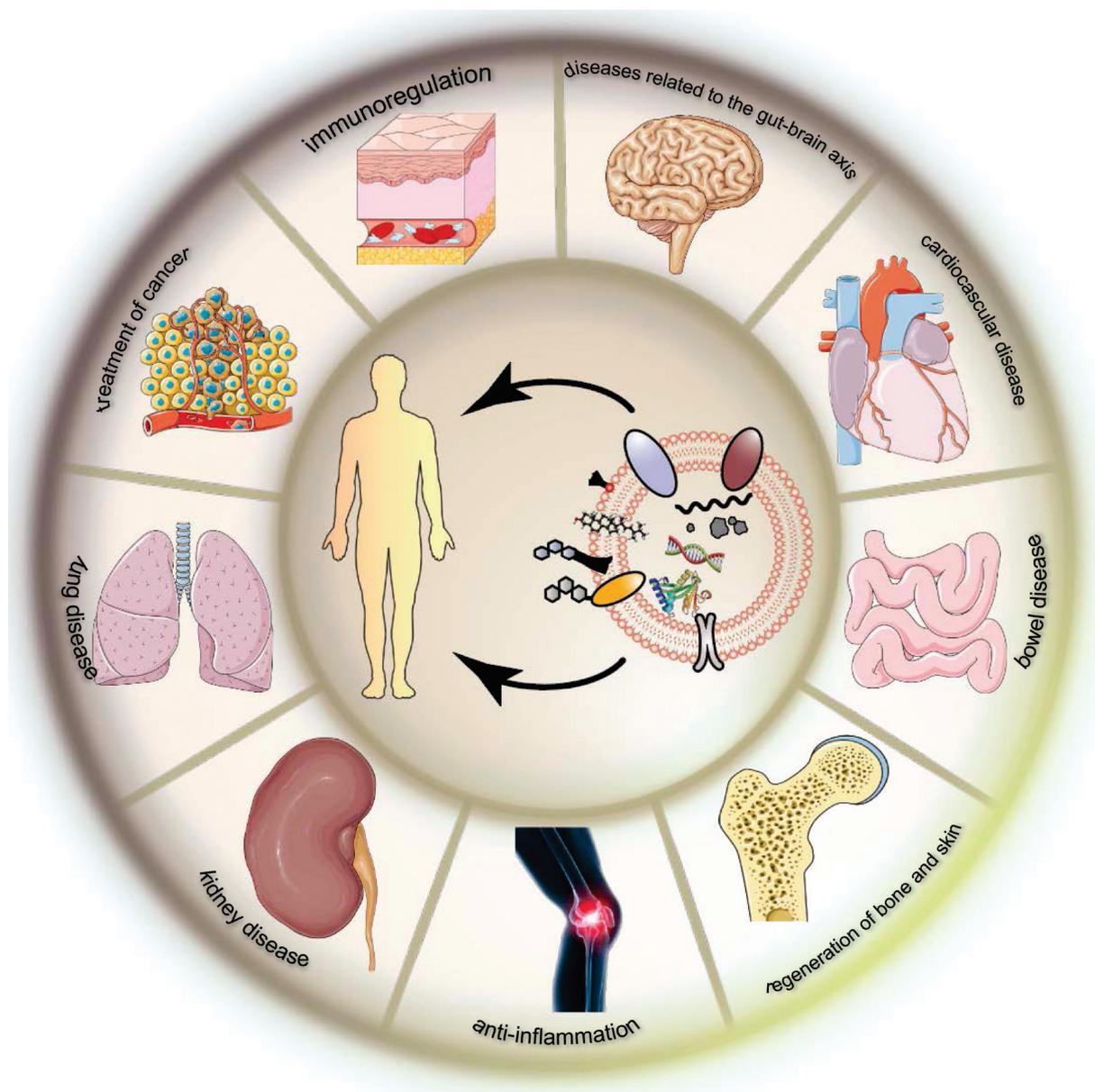
Scheme 1 Exogenous extracellular vesicles as emerging platforms in translational medicine. Created with the Adobe Illustrator.
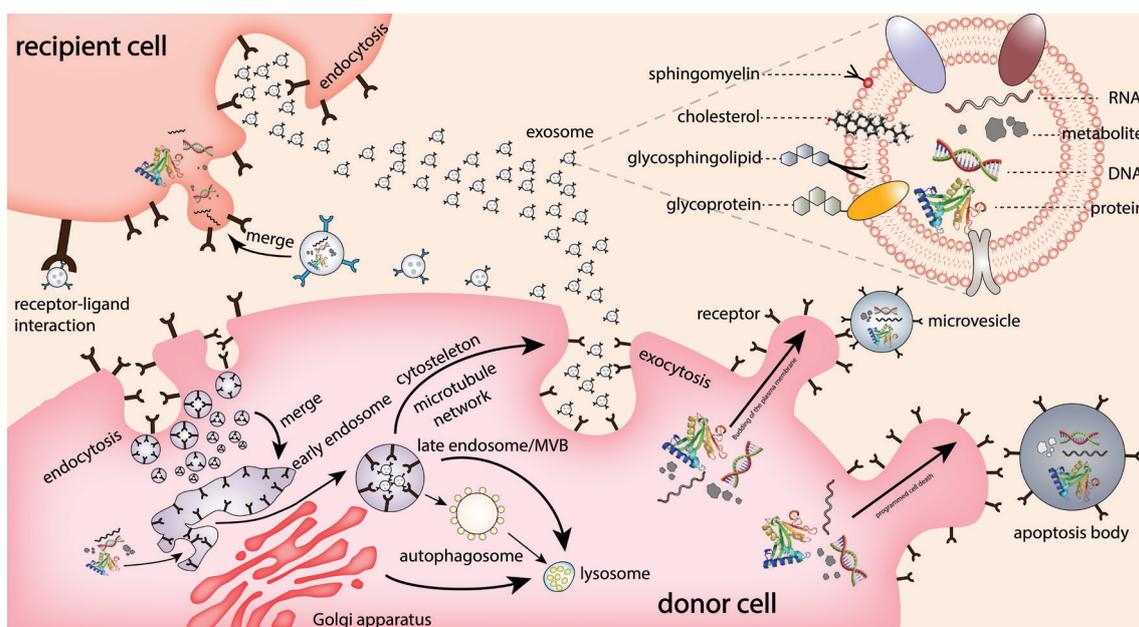
Figure 1 The origin, structure, and function of extracellular vesicles. Created with the Adobe Illustrator.

categorized into exosomes (30–150 nm), microvesicles (100 nm–1 μm), and apoptotic bodies (50–5000 nm) based on size, origin, secretion mechanisms, surface markers, and function (**Figure 1**) [1, 11, 13]. Even larger vesicles (i.e., large oncosomes, 1–10 μm in size) can bud directly from the plasma membrane in cancer [14]. Due to overlapping features and dynamic release patterns, precise classification of EVs remains a challenge [15].

EVs are enclosed by a lipid bilayer that recapitulates the asymmetric architecture of the parent cell membrane, which are enriched in cholesterol, sphingolipids, and glycosphingolipids. This specialized composition preserves membrane integrity, maintains vesicle morphology, and safeguards the encapsulated cargo from extracellular enzymatic degradation [16]. Key membrane proteins, such as tetraspanins (CD9, CD63, and CD81), serve as markers for EV subtypes and mediate interactions with recipient cells [17]. The presence of ceramides and specific phospholipids also implicates lipid rafts in cargo sorting during vesicle formation [18]. EVs carry a variety of bioactive molecules, including mRNA, microRNAs, DNA, proteins, and metabolites, that reflect the physiologic state of the source cells. These cargos are selectively packaged via endosomal sorting complex required for transport (ESCRT)-dependent and -independent mechanisms, enabling EVs to modulate gene expression and signaling pathways in recipient cells [6, 19, 20]. Similarly, plant-derived EVs have been shown to transport small RNAs that regulate microbial gene expression, while bacterial outer membrane vesicles (OMVs), which are equivalent to EVs, carry virulence factors, signaling molecules, and immunomodulatory compounds involved in host-pathogen and microbe–microbe interactions.

Importantly, the composition of EVs is dynamically influenced by the physiologic or pathologic state of the producing cells. Stress or transformation can alter the lipid or protein profiles of EVs, making EVs promising biomarkers for cancer, neurodegeneration, and inflammatory diseases [21]. This structural and functional plasticity also supports the growing interest in engineered EVs for therapeutic delivery with the potential for high targeting specificity and low immunogenicity [22]. In particular, plant- and bacteria-derived EVs offer unique advantages, such as oral bioavailability and immune compatibility, expanding the toolkit for drug and gene delivery. In summary, EVs are structurally organized, membrane-bound carriers that mediate intercellular communication across diverse biological kingdoms. Despite the heterogeneity in size, origin, and cargo, EVs share a defining phospholipid bilayer enriched with functional biomolecules, endowing EVs with stability, targeting capacity, and the ability to deliver bioactive contents to recipient cells [17, 19].

Discovery process and research history of EVs

The study of EVs can be traced back to the mid-20th century, when researchers first identified particulate matter in plasma with biological activity. In the 1940s, Chargaff and West observed that these sedimentable particles could accelerate blood coagulation, implying a functional role [13]. However, due to the limited resolution of early electron microscopy, these vesicles were initially mistaken for cellular debris. Indeed, these structures were initially dismissed as metabolic waste. A pivotal turning point came in the early 1980s, when Pan et al. reported that maturing reticulocytes actively secrete transferrin receptors via small vesicles [23]. This finding provided strong evidence that vesicle release is a regulated process, not merely a byproduct of cell damage [13, 24]. Around the same time, the term, "exosome," was introduced by Trams et al. [25] to describe vesicles shed from the plasma membrane. Later, exosomes

as vesicles were found to form within multivesicular bodies (MVBs) and release through fusion with the plasma membrane [24, 26]. In contrast, microvesicles are generated by direct outward budding from the plasma membrane. Although this biogenetic distinction is widely accepted, significant overlap in vesicle size and content has continued to challenge clear classification [2]. In the late 1980s and early 1990s, research expanded beyond erythroid cells. Immune cells, such as B lymphocytes and dendritic cells, were shown to release EVs capable of antigen presentation and intercellular molecular transfer [6]. These discoveries highlighted the role of EVs in immune regulation and laid the groundwork for future exploration in therapeutic and diagnostic contexts [27].

Despite growing interest in EVs, researchers struggled with the inconsistent terminology. Terms, like "platelet dust," "microparticles," and "exosome-like vesicles," were used interchangeably, reflecting the lack of a unified classification system [13]. To resolve this dilemma, the International Society for Extracellular Vesicles (ISEV) recommended the umbrella term, "extracellular vesicles," to encompass all membrane-bound vesicles with subclassification based on size and molecular markers (e.g., small EVs vs. large EVs) [9, 28]. A major step forward came with the launch of EVpedia in 2012, a public database aggregating EV composition data, homologous molecule identification, and bioinformatics tools [29]. This initiative, along with the release of the minimal information for studies of extracellular vesicles (MISEV) guidelines by the ISEV, helped standardize EV isolation, characterization, and experimental reporting, all of which were key to improving reproducibility and comparability across studies [13, 28]. Taken together, these milestones illustrate the evolving understanding of EVs from overlooked cellular byproducts-to-critical mediators of intercellular communication and potential tools for clinical application. The historical trajectory of EV research reflects a broader shift in cell biology, from static structural views-to-dynamic, communicative systems with therapeutic potential.

Role and mechanism underlying exogenous EVs in biological communication

EVs derived from allogeneic sources, such as different individuals of the same species or from distinct animal species, exhibit highly conserved mechanisms of biogenesis and functional characteristics. This evolutionary conservation suggests that EVs have developed a relatively universal regulatory system across diverse organisms [1]. The formation of EVs involves a series of conserved molecular pathways, including ESCRT-dependent membrane budding, the organization of membrane microdomains by tetraspanin family proteins, and the regulation of vesicle trafficking and secretion by Ras-like proteins from rat brain (Rab) GTPases. These shared mechanisms result in notable similarities among EVs from different sources in terms of structural features, membrane protein expression profiles, and cargo composition,

including RNAs and proteins [24]. Moreover, ligand–receptor interactions on the EV surface are not strictly specific. Some surface molecules, such as integrins and tetraspanins, exhibit broad binding affinities, enabling EVs to recognize and be internalized by heterologous recipient cells. This cross-species targeting capability provides a strong theoretical basis for the application of EVs in interspecies communication and therapeutic interventions [1, 12].

In recent years, growing attention has been directed toward EVs derived from animals, plants, and even microorganisms for applications in disease treatment, drug delivery, and inflammation modulation. Owing to the low toxicity, minimal immunogenicity, and effective cross-species functionality of EVs, these naturally sourced EVs are emerging as promising candidates for next-generation biotherapeutic delivery systems.

Milk-derived EVs (milk EVs)

Milk EVs are primarily secreted by mammary epithelial cells with contributions from immune cells, stem cells, and other specialized cells [7]. A defining feature of these EVs is a robust lipid bilayer, which preserves the bioactive cargo under harsh conditions, such as digestive enzymes, low pH, high temperatures, and freezing. This stability ensures functional delivery during milk processing, storage, and gastrointestinal transit [30, 31]. In addition to nutritional roles, milk EVs serve as intercellular messengers, modulating immune development, gut homeostasis, and systemic responses [32, 33]. Notably, EV functions extend beyond infancy, potentially mediating interspecies communication and affecting health in adult dairy consumers [31].

Milk EVs are enriched with functional proteins, including classic exosomal markers (CD63, CD9, and Alix) that identify EVs and mediate cell signaling [34, 35]. Milk EVs also transport growth factors, cytokines, and enzymes involved in metabolism, inflammation, and tissue repair [30, 36]. The lipid composition of EVs, rich in phospholipids, cholesterol, and sphingolipids, facilitates membrane fusion and intracellular delivery. Some lipids further activate signaling pathways, such as ERK/MAPK, which promotes cell survival and proliferation [35, 37]. A key component of milk EVs is the RNA cargo, including thousands of mRNAs and regulatory non-coding RNAs (miRNAs, lncRNAs, and circRNAs). Dominant miRNAs, such as miR-148a, miR-21, and miR-146a, regulate immune responses, cell proliferation, and metabolism by modulating post-transcription gene expression [30, 34]. These RNAs act as epigenetic regulators, influencing chromatin structure and transcriptional activity, thereby affecting processes, like differentiation, development, and stress adaptation [33, 36].

Milk EVs interact with target cells through receptor binding or endocytic uptake [31, 32]. Once internalized, milk EVs release cargo into the cytoplasm, modulating signaling pathways, such as NF-κB, Wnt/β-catenin, PI3K/AKT, and mTORC1. These pathways govern inflammation, tissue repair, and epithelial integrity [31, 36]. Uptake efficiency is affected by membrane composition and environmental cues, like calcium, which also stabilize EVs during digestion [31, 38].

Review

Milk EVs carry TGF-β and immune-regulatory miRNAs that promote regulatory T cell differentiation and modulate innate and adaptive immune cells [31, 34]. These effects contribute to neonatal immune maturation and tolerance [32, 33]. Moreover, milk EVs may exert long-term immunomodulatory effects, with implications in autoimmune diseases, allergies, and even cancer [35, 39].

Milk EVs deliver mRNAs and proteins that stimulate proliferation, enhance stem cell activity, and repair damaged tissues in tissue regeneration [30, 36]. Milk EVs support epithelial integrity and regeneration in the gut via activation of PI3K/AKT and mTORC1 signaling, which is aided by EV cargo (growth factors and cytokines) [7, 31, 40, 41]. Milk EVs also influence epigenetic programming, especially in early life. EV miRNAs can downregulate DNA methyltransferases, which alters gene methylation patterns that shape immune tolerance, metabolism, and neurodevelopmental changes with lasting health consequences [31, 33, 34, 42]. Milk EVs uniquely facilitate cross-species communication. The structural resilience of milk EVs allows milk EVs to survive digestion and potentially affect gene expression in non-native species, including humans consuming bovine milk [31, 33]. Milk EVs have attracted interest as natural drug delivery vehicles based on milk EV stability, biocompatibility, and low immunogenicity. Milk EVs can encapsulate and protect therapeutic agents, including chemotherapeutics and siRNAs, which enhances bioavailability while minimizing toxicity [31, 35]. For example, paclitaxel-loaded milk EVs demonstrate improved delivery and reduced side effects [38, 43]. Furthermore, milk EV composition reflects the health status of the lactating organism, making milk EVs promising non-invasive biomarkers for conditions, like metabolic disorders and cancer [30, 39].

In conclusion, milk EVs represent a powerful natural system for intercellular and interspecies communication with roles in immune regulation, tissue repair, developmental programming, and therapeutic delivery [7, 34, 42]. As research progresses, milk EVs hold great potential for clinical translation in diagnostics and targeted therapies [30, 31, 36].

Plant-derived EVs

The cross-kingdom communication capacity of plant-derived EVs holds profound implications for agriculture and medicine [44]. These EVs can penetrate rigid plant cell walls and be taken up not only by neighboring plant cells but also by microbial pathogens, symbionts, and even animal cells. EVs transport small RNAs (sRNAs) and defense proteins to pathogenic fungi, such as *Botrytis cinerea* and *Verticillium dahliae*, effectively silencing key virulence genes. This cross-kingdom RNA interference (RNAi) mechanism illustrates how plants utilize EV-mediated RNA transfer as an adaptive defense strategy to inhibit pathogen proliferation [45, 46].

In addition to pathogen defense, plant EVs mediate broader interspecies communication. During mutualistic symbiosis with arbuscular mycorrhizal (AM) fungi, EVs likely facilitate molecular signaling at the symbiotic interface, promoting nutrient exchange and modulating fungal metabolism and colonization to benefit both partners [47]. Similarly, pollen-derived EVs have critical roles in plant reproductive biology by facilitating molecular dialogues with the pistil that is essential for fertilization [46].

The antifungal function of plant EVs is well-supported by experimental evidence. For example, EVs isolated from sunflower seedling apoplastic fluid significantly inhibit fungal spore germination and induce morphologic abnormalities, confirming the presence of antifungal molecules that disrupt fungal cell integrity. Proteomic analyses further reveal that these EVs deliver enzymes involved in cell wall remodeling and defense, underscoring a multilayered antifungal strategy [48].

In parallel, plant-derived EVs are emerging as highly promising platforms for nanomedicine, owing to the biocompatibility, ability to cross biological barriers, and therapeutic versatility. EVs isolated from fruits and vegetables, such as grapes, grapefruit, bitter melon, and ginger, have demonstrated anti-inflammatory, anticancer, and gut-modulatory properties [49, 50]. Notably, folate-modified ginger-derived EVs have shown efficacy in remodeling the immune microenvironment, offering targeted therapeutic benefits for rheumatoid arthritis [51].

Plant-derived EVs also contribute to the modernization of traditional Chinese medicine. For example, exosome-like nanoparticles from *Platycodon grandiflorus* ("Jiegeng") are rich in bioactive proteins and RNAs, exhibit low toxicity and excellent biocompatibility, and can significantly alleviate lung inflammation in animal models by modulating macrophage metabolism and polarization to suppress inflammatory cascades [52]. Similarly, ginseng-derived EV-like nanoparticles (GENs) alleviate colitis by modulating the gut microbiota and immune responses, helping to maintain intestinal homeostasis and prevent bacterial dysbiosis, which highlights the promise of GENs as novel therapeutics for inflammatory bowel disease (IBD) [53].

Furthermore, yam-derived nanovesicles (YNVs) enhance osteoblast differentiation and mineralization, showing significant therapeutic effects in an ovariectomy (OVX)-induced mouse model of osteoporosis [53]. Nanovesicles derived from goji berries (GqDNVs) promote MC3T3-E1 cell proliferation and differentiation, while accelerating bone fracture healing in mouse models, effects that are closely associated with activation of the PI3K/Akt/mTOR/p70S6K/4EBP1 signaling pathway [54]. The inherent robustness and ability of nanovesicles to traverse biological barriers, such as the gastrointestinal tract, position plant-derived EVs as ideal carriers for the oral delivery of therapeutics [49].

The uptake of plant EVs by recipient cells occurs through multiple mechanisms, including receptor-mediated endocytosis and direct membrane fusion. Intestinal epithelial cells in mammals internalize plant EVs via endocytosis, enabling plant EV cargo to modulate gene expression and immune responses [49]. Clathrin-mediated endocytosis appears to have a role in fungal EV uptake, although further investigation is needed to fully elucidate these pathways. Lipidomic profiling has revealed that plant EV membranes are enriched with bioactive lipids, such as phosphatidic acid, which may facilitate targeted membrane fusion [55].

In conclusion, plant-derived EVs represent sophisticated, dynamic carriers that reflect the complexity of cellular

regulation and enable intra- and cross-kingdom communication. The ability of plant-derived EVs to transport functional RNAs and proteins across cellular barriers allows plants to coordinate defense responses, adapt to environmental challenges, and interact within diverse ecosystems [46, 48]. Continued advances in plant-derived EV science promise to unlock the potential of plant-derived EVs for enhancing crop resistance, developing innovative agricultural solutions, modernizing traditional medicine, and pioneering new nanomedicine applications [52, 56, 57].

Microbial-derived EVs

Microbial-derived EVs serve as fundamental communication tools bridging the microbial world and their hosts. One of the core functions is modulation of the host immune system. EVs from symbiotic and probiotic microbes have a crucial role in maintaining immune homeostasis and promoting tolerance, thereby preventing excessive inflammatory responses in the gut [58, 59]. These vesicles carry effector molecules capable of stimulating pro- or anti-inflammatory cytokine production. For example, EVs from probiotic strains, such as *Escherichia coli* Nissle 1917, promote the secretion of regulatory cytokines and induce T regulatory (Treg) cells, which are essential for balancing protective and tolerant immune responses [60, 61]. In contrast, EVs from pathogenic bacteria may carry virulence factors that trigger strong pro-inflammatory reactions by activating immune pathways, such as inflammasomes, contributing to disease pathogenesis, including IBD [62, 63]. Thus, microbial EVs have a dual function in immune regulation: maintaining intestinal barrier integrity and immune tolerance; and potentially exacerbating inflammation and tissue damage when derived from pathogens [58].

In addition to immune modulation, microbial EVs are vital for maintaining gut barrier function and preventing systemic dissemination of pathogens and toxins. Microbial EVs influence the expression of tight junction proteins, such as occludin, claudins, and zonula occludens, thereby enhancing barrier integrity [60, 64]. This regulation of tight junctions is not only critical for local gut health but also impacts systemic metabolism. For example, EVs from *Akkermansia muciniphila* improve intestinal permeability, which in turn regulates glucose metabolism and insulin sensitivity, possibly underlying the protective effects against obesity and metabolic disorders [63, 65]. Additionally, these vesicles often carry enzymes that degrade dietary polysaccharides, which aid in the production of beneficial metabolites, like short-chain fatty acids (SCFAs) that serve as energy substrates for colonocytes and have significant roles in host metabolic regulation [60, 66].

Moreover, recent studies have shown that the concentration of specific types of EVs is closely correlated with the progression of some diseases. For example, patients with IBD exhibit a significant reduction in *Lactobacillus plantarum* within the gut microbiota. Supplementation with *L. plantarum* or *L. plantarum*-derived EVs can alleviate IBD symptoms in mouse models [61]. Further investigations

demonstrated that colonization by *L. plantarum* and *L. plantarum*-derived EVs exerts therapeutic effects on IBD severity by inhibiting intestinal epithelial cell apoptosis through the mPTP–CytC–caspase-9–caspase-3 signaling pathway [67].

Microbial EVs are key factors in bacterial communication in addition to mediating microbe-host interactions. By packaging and transferring genetic materials, such as DNA and antibiotic resistance genes, microbial EVs facilitate horizontal gene transfer, which enhances bacterial adaptability and enabling the spread of traits, like drug resistance and virulence within microbial communities [68]. EV-mediated communication also involves the transfer of quorum-sensing molecules and other signaling compounds, and coordinating collective behaviors, such as biofilm formation and phage defense mechanisms [63, 69].

Emerging research indicates that microbial EVs participate in the gut-brain axis, a novel field linking gastrointestinal microbiota composition with central nervous system function. EVs can cross the intestinal barrier and potentially the blood-brain barrier, delivering neuroactive compounds, including neurotransmitters, neuromodulators, and RNAs, that influence neuronal activity and behavior [69, 70]. For example, EVs derived from probiotic lactobacilli have been shown to regulate brain-derived neurotrophic factor (BDNF) and serotonin levels, producing antidepressant-like effects and affecting cognitive functions. These effects may result from direct neuronal uptake of EVs or indirect modulation through immune signaling and endocrine responses [45, 71]. Furthermore, the ability of EVs to deliver specific RNAs and regulatory molecules suggests a bidirectional communication pathway, whereby gut microbes may influence brain gene expression, neuroinflammation, and neurodegenerative disease processes [63, 72].

Microbial EVs primarily affect host cell function through RNA cargo. Bacterial- and host-derived RNAs, including mRNAs, small non-coding RNAs, and miRNA-like molecules, are delivered to target cells, where the RNAs regulate gene expression by modulating transcriptional and post-transcriptional processes [69, 72]. These regulatory RNAs can interact with host innate immune receptors, such as RIG-I and NOD1, triggering interferon production and other inflammatory responses [59, 73]. Conversely, host miRNAs can enter bacteria via EVs, potentially affecting bacterial gene expression related to biofilm formation and antibiotic resistance. This bidirectional RNA-mediated communication underscores the complexity of microbe-host interactions [63, 73].

In conclusion, microbial EVs are vital biological messengers that influence a broad spectrum of host functions through precise cargo delivery and receptor-mediated signaling. The roles of microbial EVs in immune regulation, barrier maintenance, metabolic homeostasis, and even brain function highlight the indispensability of microbial EVs in the complex ecosystem of host-microbiota interactions. With ongoing advances in research and technology, these vesicles are poised to further illuminate the molecular dialogues underpinning health and disease, ushering in a new era of microbiome-based clinical applications [64, 69].

Comparison of EVs from different sources

Milk EVs, particularly milk-derived EVs from bovine sources, offer the highest natural yield (up to 10^{12} particles/mL) and benefit from mature industrial supply chains, making them attractive for large-scale and GMP-compliant production [74]. Milk EV membranes are enriched with tetraspanins (CD9, CD63, and CD81) and ligands, such as MFG-E8 and integrins, which confer selective cellular targeting [75, 76]. These vesicles efficiently carry miRNAs, proteins, and other bioactive molecules, which have strong stability, efficient uptake, and low toxicity, although downstream purification from complex milk matrices still poses a cost challenge [77, 78].

In contrast, plant-derived EVs have intrinsically lower yields and face heterogeneity in raw materials and isolation protocols, resulting in limited scalability [79, 80]. Despite these limitations, plant EVs exhibit distinct molecular characteristics, including plant-specific tetraspanins (TET8/9) and unique lipid components, such as glycosyl inositol phosphoryl ceramides, which influence receptor recognition and vesicle stability [45, 77, 77]. With low immunogenicity and oral bioavailability, plant EVs hold promise for nutraceutical and therapeutic applications once standardization and large-scale manufacturing are established.

Microorganism-derived EVs present a cost-effective and scalable alternative due to the ease of bioreactor cultivation, theoretically producing 10^{17}–10^{18} vesicles per batch. However, purification requires stringent removal of endotoxins and microbial contaminants. Unlike milk or plant EVs, microbial EVs rely on broad host-pathogen recognition mechanisms through pattern-recognition molecules and receptors rather than precise ligand–receptor interactions. These properties enable strong immunomodulatory effects but also necessitate thorough safety evaluation before clinical use [81, 82].

Immunogenicity of exogenous EVs

The immunogenicity of exogenous EVs largely depends on the parental cell origin and molecular composition. Compared to autologous EVs, allogeneic or xenogeneic EVs are more prone to recognition and clearance by innate immune cells, such as mononuclear phagocytes, and adaptive immunity, especially with repeated administration. This property is partly due to the major histocompatibility complex (MHC) molecules on EV surfaces, which are significantly enriched relative to the source cells and can activate T cells via antigen presentation, thus triggering adaptive immune responses [83].

When utilized as therapeutic delivery vehicles, EV immunogenicity is influenced by the parental cell type and the nucleic acid or protein cargo that is carried. Despite concerns that EVs from human immortalized cell lines (e.g., HEK293T) might harbor oncogenic or toxic elements, studies reveal that wild-type and engineered HEK293T-derived EVs, administered to mice over 3 weeks, induce no significant toxicity or immune activation [84]. Assessments

covering blood biochemistry, immune cell phenotyping, and histopathology demonstrated no inflammatory infiltration, tissue damage, or functional abnormalities. Minor fluctuations in immune markers, such as IP-10 and VCAM-1, only occur in engineered EV groups and remain within physiologic limits, indicating that engineered EVs with exogenous RNAs or protein tags maintain low immunogenicity, supporting clinical translation safety [85].

Mononuclear phagocytes discriminate self from non-self EVs through polymorphic ligand-receptor interactions. Therefore, xenogeneic and allogeneic EVs may be rapidly cleared even without adaptive immune involvement [86, 87]. For example, SIRPα receptor polymorphisms in mice increase macrophage phagocytosis of xenogeneic EVs, resembling xenograft clearance. Despite this finding, clinical trials report no evident immunotoxicity from autologous or allogeneic exosomes and plasma transfusions containing native EVs do not provoke significant adverse reactions, which underscores the high biocompatibility [88].

A central challenge in cross-individual EV immunogenicity arises from immune memory responses triggered by repeated dosing. Donor-recipient genetic differences can elevate the immunogenic risk of allogeneic or xenogeneic EVs. Nevertheless, studies show minimal immune responses in mice exposed to human-derived HEK293T EVs, with no significant changes in splenic T, B, or NK cell populations or systemic inflammatory cytokines (e.g., IL-6 and TNF-α) [85]. In non-human primates, repeated intravenous allogeneic EV injections cause accelerated blood clearance (ABC), which is marked by a shortened EV circulation half-life and increased EV-specific IgG antibodies, indicating antibody-mediated immune memory that is likely driven by EV surface MHC molecules [89]. In contrast, engineered EV surface tags, such as FLAG or PC94 peptide, do not elicit anti-drug antibodies, suggesting inherent immune "silencing" properties of EVs. Future studies should confirm if such immune evasion applies broadly across diverse EV types and animal models [85].

EVs often acquire biomolecular coronas (adsorbed plasma proteins, including complement factors and immunoglobulins) *in vivo* that can exacerbate immune recognition by activating complement cascades or Fc receptor-mediated phagocytosis. Gene-editing techniques knocking out B2M in EV-producing cells effectively lower MHC-I expression to reduce cross-individual immunogenicity, which significantly diminishes allogeneic EV immunogenicity [89]. Additionally, mesenchymal stem cell (MSC)-derived EVs naturally exhibit low immunogenicity and immunosuppressive properties. For example, MSC-EVs modulate ischemia-reperfusion injury by downregulating pro-inflammatory cytokines and promoting anti-inflammatory macrophage polarization, which favors allogeneic application [1]. Genetic engineering to overexpress immune checkpoint molecules, like PD-L1 on EV surfaces, further enhances the ability to suppress T cell activation and evade adaptive immunity [90].

The cross-species immunogenicity of EVs is primarily dictated by cellular origin and immune recognition. Compared to autologous EVs, xenogeneic and allogeneic EVs display greater susceptibility to innate and adaptive immune clearance, especially under repeated administration.

This characteristic arises from foreign antigenic molecules on EV surfaces, especially elevated MHC-I levels in xenogeneic EVs, that interact with T cell receptors to activate adaptive immunity. Innate immune cells also recognize polymorphic differences in molecules, like CD47 through SIRPα receptors, which triggers phagocytosis [86]. Nevertheless, preclinical studies reveal low immunogenicity of human EVs in mouse and non-human primate models. For example, repeated injections of human Expi293F EVs do not cause significant toxicity or systemic inflammation, although circulation times decrease due to ABC, which correlates with EV-specific IgG production [89]. These findings suggest that EV immunogenicity is context-dependent and influenced by origin, dosage, and host immune status.

Exogenous EVs are promising therapeutic carriers given the low immunogenicity. Multiple studies have confirmed the safety of human-derived EVs. Zhu et al. demonstrated that wild-type and engineered HEK293T EVs did not induce toxicity in mice after 3 weeks with only minor immune marker changes in the engineered EV groups [85]. Similarly, Saleh et al. reported that Expi293F-derived EVs did not impair HepG2 cell viability *in vitro* or provoke systemic immune responses in BALB/c mice, further supporting human EV safety [89].

Engineering approaches can enhance EV immune compatibility and expand clinical utility. Genetic modifications or chemical conjugations embedding chimeric proteins or pre-miR-199a-3p reduce immune marker fluctuations, improving biosafety. Zhu et al. reported minimal VCAM-1 changes from engineered EVs without significant toxicity differences versus controls, demonstrating a balance of functionality and safety [85]. Species differences in EV surface proteins, like MHC, may influence immune responses in cross-species applications. However, murine tolerance to human EVs is encouraging for preclinical research.

Clinical interest focuses on EVs from regenerative cells. Cardiovascular progenitor cell-derived EVs for non-ischemic dilated cardiomyopathy (trial NCT05774509) are administered repeatedly to elicit reparative immunomodulation without harmful immune activation. Early data showed low immunogenicity and safety in repeated dosing. MSC-derived EVs, leveraging the parent cell intrinsic immunosuppressive properties, are under clinical evaluation (trial NCT05787288). These EVs shift immune phenotypes from pro- to anti-inflammatory and induce regulatory T cell expansion. The low immunogenicity is due to minimal MHC-II expression and immunoregulatory cargo (microRNAs and proteins) that reprogram recipient immunity. Preclinical studies have confirmed that MSC-EVs suppress allogeneic peripheral blood mononuclear cell activation without cytokine storm or adverse events.

Intravenous infusion of bone marrow MSC-derived EVs (ExoFlo) in COVID-19-associated ARDS patients (trial NCT04493242) showed no treatment-related immunogenic adverse events despite extensive immune monitoring. Other trials (NCT06002841, NCT06242379, and NCT06825572) evaluating MSC-EVs in liver failure post-transplant patients revealed no notable immune rejection or pathologic inflammation when combined with standard immunosuppression.

In contrast, pathogen-derived EVs (pEVs) possess marked immunogenicity due to cargo, including lipopolysaccharides, peptidoglycans, and microbial nucleic acids (pathogen-associated molecular patterns [PAMPs] [91]. These PAMPs are recognized by host pattern recognition receptors (PRRs), such as toll-like receptors (TLRs), NOD-like receptors, and STING, all of which activate potent innate immune responses. pEVs also carry toxins and virulence factors that can amplify inflammation or suppress immunity to favor pathogen survival [92]. This finding illustrates the inherent capacity of exogenous EVs to carry antigenic material triggering innate and adaptive immunity [93]. In addition to pathogen recognition, EVs regulate allogeneic immune responses, especially in transplantation. Donor EVs can transfer intact MHC molecules to recipient cells ("cross-dressing"), enabling recipient antigen-presenting cells to activate allogeneic T cells. Some EV subtypes also deliver immunosuppressive factors, such as Fas ligand, TGF-β, and microRNAs, that induce T cell anergy or regulatory T cell expansion, potentially fostering graft tolerance over rejection [94].

Placenta-derived EVs promote immune tolerance, which is crucial in pregnancy. By modulating maternal immunity, placenta-derived EVs prevent rejection of the semi-allogeneic fetus, balancing pathogen defense with fetal tolerance [95]. This mechanism offers valuable paradigms for the safe clinical use of EVs in transplantation and regenerative medicine, highlighting the therapeutic potential in immune regulation without provoking harmful immunogenicity [96].

In conclusion, the immunogenicity of exogenous EVs depends strongly on cellular origin, cargo, and surface molecules. Regenerative cell-derived EVs have low immunogenicity and potent immunomodulatory effects, supporting the therapeutic potential (e.g., clinical trials NCT05774509 and NCT05787288). Conversely, pathogen- or tumor-derived EVs exhibit high immunogenicity, triggering robust innate and adaptive immune responses [91, 93]. This dual nature positions EVs as versatile immunotherapeutics, for which rational engineering and dosing strategies are essential to optimize safety and efficacy [96, 97].

Application of exogenous EVs in biomedicine

Building upon the intrinsic properties and source-specific advantages of exogenous EVs, the exploitation in biomedicine has gained significant momentum (**Table 1**), particularly in areas with high unmet clinical needs and where traditional therapies face limitations [98]. The ability of EVs to navigate biological barriers, deliver bioactive cargo with precision, and modulate cellular functions, often with inherent low immunogenicity and favorable safety profiles, makes EVs uniquely suited for therapeutic intervention [22]. This section delves into the burgeoning applications of exogenous EVs across three key translational domains: cancer therapy (leveraging targeting, drug delivery, and immunomodulation); regenerative medicine (capitalizing on

Table 1 Application of Exogenous Extracellular Vesicles in Translational Medicine

	Source	Vesicle Type and Size (diameter/nm)	Active Molecules	Isolation Method	Engineering Strategy	Disease Model	Therapeutic Effect	Advantages & Disadvantages	Ref
Milk-derived EVs	Human	Breast milk exosomes (30–150)	Lipids	Ultracentrifugation	None	Necrotizing enterocolitis (NEC)	Administration of full-term and preterm milk exosomes significantly enhanced *in vitro* epithelial proliferation and migration, and improved the severity of NEC *in vivo*	Disadvantage: Low lipid content extracted	[8]
	Human	Breast milk exosomes (30–150)	CD36	Ultracentrifugation	None	Non-alcoholic fatty liver disease (NAFLD)	Inhibited lipogenesis and increased lipolysis, effectively alleviating high-fat diet-induced hepatic steatosis and insulin resistance in NAFLD mice	Limitation: The composition of bioactive compounds in breast milk, including EV concentration, varies across lactation stages; conclusions only apply to EVs from human milk administered intraperitoneally, not orally	[51]
	Human	Human colostrum and derived extracellular vesicles (50–200)	MFG-E8	ExoQuick purification	Co-incubation	Resistance to rotavirus and respiratory syncytial virus	Intrinsic antiviral activity of human colostrum against rotavirus and respiratory syncytial virus	Contributes to revealing new mechanisms of the functional role of breast milk as a protective and therapeutic agent for preterm infants; ExoQuick purification is a method with "high recovery rate and low specificity," cannot exclude the presence of protein contaminants or antiviral molecules	[132]
	Human	Human milk exosomes (30–150)	Growth Factors	Ultracentrifugation	None	Murine Necrotizing Enterocolitis (NEC)	Reduced hypoxia, inflammation formation, and intestinal damage	Not mentioned	[133]
	Pig	Porcine milk exosomes (40–100)	miR-4334, miR-219, and miR-338	Ultracentrifugation	None	LPS-induced intestinal inflammation and apoptosis	Inhibited cellular inflammation and prevented apoptosis, thereby protecting intestinal epithelial cells from LPS-induced damage	Porcine milk exosomes contain a large amount of miR-NAs, whose target genes are rich in immunity, and miRNAs encapsulated in exosomes can survive gastrointestinal conditions	[134]
	Pig	Porcine milk exosomes (40–100)	miRNAs, mRNAs, and proteins	Centrifugation	None	Murine intestinal proliferation	Promoted intestinal cell proliferation and intestinal development	Not mentioned	[135]

Table 1 Continued

Source	Vesicle Type and Size (diameter/nm)	Active Molecules	Isolation Method	Engineering Strategy	Disease Model	Therapeutic Effect	Advantages & Disadvantages	Ref
Cow	Bovine milk exosomes (30–200)	Active proteins	Ultracentrifugation	None	Hair regeneration	Milk-exo promoted the proliferation of hair dermal papilla cells and rescued dihydrotestosterone (DHT, androgen)-induced hair follicle developmental arrest	Compared to animal cell-derived exosomes, milk-derived exosomes have very high yields, which have economic advantages in drug commercialization	[136]
Cow	Bovine milk exosomes (30–201)	miRNA-2478	Centrifugation	Co-incubation	Melanin formation	Bovine milk exosomes reduced melanin content, tyrosinase activity, and expression of melanogenesis-related genes in melanoma cells and melanocytes, and bovine-specific miR-2478 inhibited melanin production	Contributes to the development of functional whitening cosmetics with low cytotoxicity	[137]
Cow	Bovine milk-derived EVs [BMEVs] (30–200)	Not specifically mentioned	Centrifugation	None	Murine osteogenesis	Increased the number of osteoblasts and woven bone formation, contributing to altered osteoblast differentiation and bone matrix formation	BMEV seems to contribute to the development and dominance of osteoblasts in the immature stage, with faster differentiation into osteoblasts in the later stage	[138]
Plant-derived EVs	Grape	Grape exosome-like nanoparticles (EP-DEN) (380.5 ± 37.47)	Lipids	Centrifugation	Sucrose step gradient	Murine colitis	Not only regulates intestinal tissue renewal processes but also participates in the remodeling in response to pathological triggers	1. No cytotoxic effects; 2. Higher uptake efficiency for the majority of investigated cells (even B and T cells), compared toliposomal formulation; 3. No immune reaction detectable; 4. No observable adverse effects; 5. Intranasal nanovector application delivered miR-17 to brain tumor cells within a short time in mice, whereas liposomes did not reach the brain; 6. Intravenous injection of nanovectors delivered miR-18a to liver macrophages and, consequently promoted anti-tumor M1 macrophage induction; 7. Highly efficient cell internalization and cancer suppression of aptamer-doxorubicin loaded nanovectors [55]	[138]

Table 1 Continued

Source	Vesicle Type and Size (diameter/nm)	Active Molecules	Isolation Method	Engineering Strategy	Disease Model	Therapeutic Effect	Advantages & Disadvantages	Ref
Ginger	Ginger EPDENs (0–250)	Heme oxygenase-1 (HO-1) and anti-inflammatory cytokine IL-10	Centrifugation	Sucrose step gradient	Murine *in vitro* experiments	Ginger EPDENs preferentially induced the expression of the antioxidant gene, heme oxygenase-1 (HO-1), and the anti-inflammatory cytokine, IL-10.		[139]
Ginger	Ginger EPDENs (0–250)	Nuclear factor erythroid 2-related factor 2 (Nrf2)	Centrifugation	Sucrose step gradient	Murine liver injury	Mediated activation of Nrf2 leading to the expression of a set of liver detoxification/antioxidant genes and inhibition of reactive oxygen species production.		[140]
Grapefruit	Grapefruit-derived nanovesicles (0–250)	Heme oxygenase-1 (HO-1)	Centrifugation	Sucrose step gradient	Murine colitis	Vesicles were selectively taken up by intestinal macrophages and improved murine colitis		[141]
Lemon	Citrus limon-derived nanovesicles (50–70)	TRAIL	Ultracentrifugation	Sucrose step gradient	Murine *in vitro* model	Inhibited cancer cell proliferation in different tumor cell lines by activating TRAIL-mediated apoptotic cell death		[142]
Carrot	Carrot EPDEN	Nuclear factor (erythroid-derived 2)-like 2 (Nrf2)	Centrifugation	Sucrose step gradient	Murine inflammation model	Carrot EPDEN increased nuclear translocation of Nrf2		[139]
Grapefruit	Grapefruit-derived nanovesicles (0–250)	Not mentioned	Gradient centrifugation	Vortex mixing and assembly	In vitro cell culture model and murine tumor model	Excellent candidates for delivering various types of therapeutic agents		[143]
Arabidopsis	Exosome-like extracellular vesicles	Small RNAs (sRNAs)	None	None	*Arabidopsis* fungus	Silenced genes related to fungal pathogenicity		[126]
Ginger	Folic acid-modified ginger-derived extracellular vesicles (268)	TNF-α 和 IL-1β	Combined electrophoretic technique with 300 kDa cut-off dialysis bag (named ELD)	Conjugating GDEVs with folic acid (FA)	Mouse arthritis model	Demonstrated significant ability to alleviate RA symptoms in mouse models		[51]
Microbial-derived EVs	*Pediococcus pentosaceus*	Extracellular membrane vesicles (235–245)	IL-10, arginase-1, PD-L1	Ultracentrifugation	None	Mouse models of liver fibrosis, peritonitis, and colitis	Reduced pathologic processes with potent immunoregulatory effects	Not mentioned	[144]

Table 1 Continued

Source	Vesicle Type and Size (diameter/nm)	Active Molecules	Isolation Method	Engineering Strategy	Disease Model	Therapeutic Effect	Advantages & Disadvantages	Ref
Propionibacterium freudenreichii CIRM-BIA 129	Extracellular Vesicles (84.80 ± 2.34)	SlpB	Centrifugation	None	HT-29/kbseap-25 cell inflammation model	Attenuated OMV-driven neuroinflammation by inhibiting LPS-induced NF-κB signaling pathway	Whether *P. freudenreichii* EVs exert immunoregulatory effects *in vivo* is unknown	[145]
Lactobacillus paracasei	*L. paracasei*-derived extracellular vesicles (34.22 ± 6.876)	ER stress-related proteins, IL-10, and TGFβ	Ultracentrifugation	None	LPS-induced inflammation in HT29 human colorectal cancer cells and DSS-induced colitis in C57BL/6 mice	Alleviated LPS-induced intestinal inflammation through ER stress activation	Not mentioned	[146]
E. coli Nissle 1917	Outer membrane vesicles (OMVs) [size not mentioned]	tight junction proteins ZO-1 and claudin-14, and IL-22 activation	Ultracentrifugation	Genetic engineering	Human intestinal epithelial barrier model	OMVs contributed to enhancing epithelial barrier integrity directly through transcriptional regulation of TJ proteins and indirectly through modulation of IL-22	Not mentioned	[147]
Akkermansia muciniphila	*A. muciniphila*-derived extracellular vesicles (AmEVs) [40–60]	occludin	Ultracentrifugation	None	Diabetic mouse model	AmEVs may function as a component controlling intestinal permeability, and the regulation of intestinal barrier integrity can improve metabolic functions in HFD-fed mice	The results do not verify whether EVs remain intact after systemic travel	[148]
L. plantarum AP-sulloc 331261	*L. plantarum*-derived EVs (LEVs) [104 ± 42.4]	IL-10, IL-1B, and GM-CSF	Ultracentrifugation	Density gradient	Human inflammatory skin disease cell model	LEVs can trigger M2 macrophage polarization *in vitro* and induce anti-inflammatory phenomena in human skin	Low-cost production; specific EV factors responsible for effects are unknown	[149]

Table 1 Continued

Source	Vesicle Type and Size (diameter/nm)	Active Molecules	Isolation Method	Engineering Strategy	Disease Model	Therapeutic Effect	Advantages & Disadvantages	Ref
L. plantarum	L-EVs (particle size not mentioned)	Neurotrophic factors (Bdnf1/4 and Nt4/5)	Ultracentrifugation	None	HT22 cell model treated with the stress hormone glucocorticoid (GC; corticosterone), depressive-like mouse model	Can alter the expression of neurotrophic factors in the hippocampus and provide antidepressant-like effects in mice with stress-induced depression	Not mentioned	[150]
B. thetaiotaomicron	Bt OMV (a mean size of 237)	KGF-2	Centrifugation	Genetic engineering	DSS colitis model	Reduced disease severity and promoted intestinal epithelial repair	Safe, relatively inexpensive and easy to produce, highly suitable for use in vaccines, can also overcome limitations associated with traditional OMV-related toxins, reducing the risk of side effects	[151]

pro-regenerative signaling and biomaterial integration); and inflammatory diseases (exploiting anti-inflammatory and immunoregulatory actions).

Tumor therapy

In recent years heterologous EVs have attracted significant attention in the field of cancer therapy. Among the heterologous EVs, animal- and plant-derived EVs have emerged as promising candidates for next-generation drug delivery vehicles due to unique biological properties and therapeutic potential. Specifically, milk EVs have gained widespread interest owing to excellent biocompatibility, intrinsic targeting ability, and scalability for large-scale production.

Samuel et al. [38] demonstrated that bovine milk EVs effectively induce cellular senescence and apoptosis in primary colorectal cancer cell lines (LIM1215 and SW620) through a series of *in vitro* and *in vivo* experiments ($P < 0.01$). Notably, milk EVs also uncovered a bidirectional regulatory effect of these EVs on tumor metastasis. While promoting the metastatic potential of pancreatic and breast cancer cells in the presence of a primary tumor, the EVs significantly suppress metastasis after surgical removal of the primary lesion. This discovery highlights the context-dependent functionality of milk EVs within the tumor microenvironment and offers new insights for designing postoperative strategies for metastatic cancer patients.

Wang et al. [99] developed an innovative milk EV-based carrier platform (i.e., the KME system) that enables the co-delivery of celastrol (CEL) and PD-L1 siRNA, aiming to synergize chemotherapy and immunotherapy. In a colorectal cancer mouse model, this system led to a 68.3% ± 5.7% reduction in tumor volume, demonstrating remarkable therapeutic efficacy and laying a solid foundation for the application of milk EVs in combined chemo-immunotherapy.

In addition to animal-derived vesicles, plant-derived EVs have also gained momentum as promising delivery vectors due to the abundant sources, oral bioavailability, and low immunogenicity. A recent study [100] reported that aloe vera–derived exosome-like vesicles treated with indocyanine green (ICG)-induced hypoosmotic stress exhibited an approximately 40% increase in mechanical strength, as measured by atomic force microscopy, significantly improving drug stability and bioactivity retention during transport. Furthermore, these vesicles showed high tumor-targeting efficiency *in vivo*, reaching 85.2% ± 3.1%, highlighting the potential of natural plant EVs for tumor-specific drug delivery.

In another study Yan et al. [101] (**Figure 2A**) successfully isolated exosome-like nanovesicles (BF-Exos) from the fruit of the traditional Chinese medicinal plant, *Brucea javanica*, for the first time. miRNA sequencing revealed that these vesicles are enriched in multiple anticancer bioactive components. In a triple-negative breast cancer mouse model, BF-Exos achieved a tumor growth inhibition rate of 72.4% ± 6.3% and reduced angiogenesis by 65.8% ± 5.2%, demonstrating potent antitumor and anti-angiogenic properties, and providing robust preclinical evidence for the therapeutic application of herbal EVs.
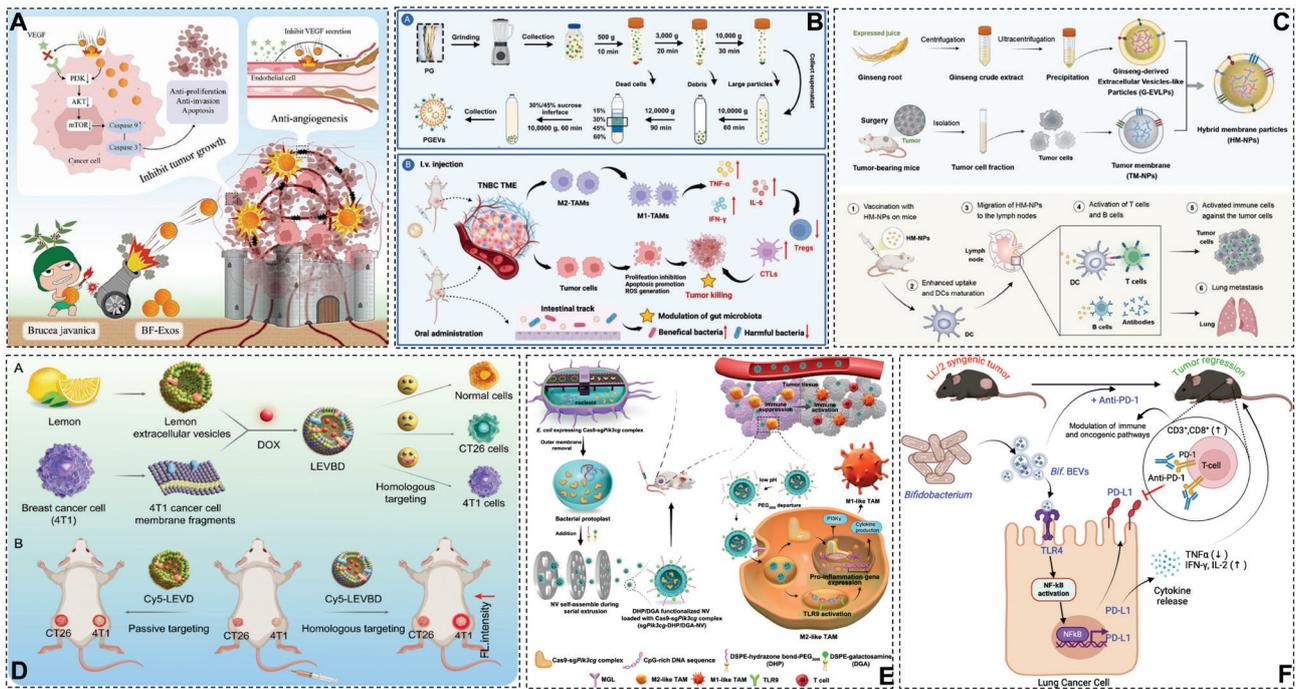
Figure 2 The application of exogenous EVs in tumor therapy. A. *Brucea javanica*-derived exosome-like nanovesicles deliver miRNAs for cancer therapy [101]. Copyright 2024, Elsevier B.V. All rights reserved. B. *Platycodon grandiflorum*-derived EVs suppress triple-negative breast cancer growth by reversing the immunosuppressive tumor microenvironment and modulating the gut microbiota [102]. Copyright 2025, The authors. C. Hybrid Ginseng-derived EVs-Like Particles with Autologous Tumor Cell Membrane for Personalized Vaccination to Inhibit Tumor Recurrence and Metastasis [103]. Copyright 2024, The authors. D. Enhanced tumor self-targeting of lemon-derived EVs by embedding homotypic cancer cell membranes for efficient drug delivery [104]. Copyright 2025, The authors. E. Bacterial protoplast-derived nanovesicles carrying CRISPR-Cas9 tools re-educate tumor-associated macrophages for enhanced cancer immunotherapy [105]. Copyright 2024, The author(s). F. Gut commensal *Bifidobacterium*-derived EVs modulate the therapeutic effects of anti-PD-1 in lung cancer [106]. Copyright 2025, The author(s).

Platycodon grandiflorum-derived EVs (PGEVs) have been shown to promote the polarization of tumor-associated macrophages (TAMs) toward the M1 phenotype, enhance the infiltration of cytotoxic T lymphocytes (CTLs) into tumor tissues, suppress PD-1 expression within the tumor microenvironment, and boost CTL-mediated cytotoxicity against tumor cells, thereby inhibiting tumor growth (**Figure 2B**) [102]. Moreover, PGEVs can improve gut microbiota composition by increasing microbial diversity and abundance in tumor-bearing mice, contributing to therapeutic outcomes by alleviating dysbiosis. Similarly, functional hybrid nanovesicles (HM-NPs), formed by fusing ginseng-derived extracellular vesicle-like particles (G-EVLPs) with membranes extracted from resected autologous tumor tissue, have been reported to enhance antigen-specific immune responses, effectively suppressing tumor recurrence and metastasis in subcutaneous and orthotopic tumor models (**Figure 2C**). [103]. In addition, vaccination with HM-NPs also induces long-term immune protection, leading to prolonged survival, while maintaining excellent biocompatibility. This personalized hybrid tumor vaccine platform based on G-EVLPs offers a promising strategy to prevent postoperative tumor recurrence and metastasis [103].

In another example, a novel nanoplatform (LEVBD) was developed by encapsulating membrane fragments from breast cancer cells within a scaffold of lemon-derived nanovesicles (LEVs). LEVBD demonstrates efficient and selective targeting to homologous tumor sites, even in the

presence of competing heterologous tumors, significantly suppressing tumor growth without any observable systemic toxicity (**Figure 2D**) [104]. Furthermore, researchers have engineered an *in vivo* gene editing system that used bacteria-derived nanovesicles (NVs) to efficiently deliver CRISPR-Cas9 components [105]. This approach enables effective reprogramming of endogenous TAMs, producing notable anticancer effects without triggering systemic immune responses (**Figure 2E**).

Microbial-derived EVs also exhibit distinct advantages in modulating the tumor immune microenvironment. Recent findings showed that EVs from *Bifidobacterium* (Bif.BEVs) [106] can modulate the lung tumor microenvironment via the gut–lung axis (**Figure 2F**), enhancing the response rate to anti–PD-1 therapy from 35% to 62% ($P < 0.001$). This work provides valuable insight into the long-range regulatory mechanisms linking the gut microbiota to tumor immunity and paves the way for integrating microbiome-targeted interventions into cancer immunotherapy. Regulation of the tumor microenvironment has been widely studied and confirmed to be related to the overall condition of patients, such as muscle integrity and inflammation, which profoundly affects the prognosis and quality of life of patients. In addition, these studies have also opened up therapeutic targets for EVs, broadening the perspective for the exploration of EVs [107, 108].

Additionally, Qin et al. [109] engineered a probiotic-based delivery system (ENZC) that used dual nanobody technology

Review

to specifically recognize and bind tumor-derived exosomes (TDEs) via PD-L1 and CD9 antigens. *In vivo* experiments showed that this system could enhance CD8+ T cell infiltration by 3.2-fold and inhibit tumor metastasis with an efficiency of 78.5% ± 4.3%, demonstrating great promise for targeting tumor immune evasion mechanisms.

The above-mentioned examples vividly illustrate the potent utility of exogenous EVs in oncology, primarily leveraging the capabilities for targeted drug co-delivery, modulation of the immunosuppressive tumor microenvironment, and induction of anti-tumor immunity. In addition to the transformative impact in cancer, the inherent ability of EVs to shuttle bioactive molecules, such as growth factors, regulatory RNAs, and immunomodulatory signals, between cells positions EVs as equally compelling candidates for regenerative medicine. In this domain the focus shifts from combating malignant growth to actively promoting tissue repair, functional restoration, and constructive immune remodeling at sites of injury or degeneration.

Regenerative medicine

Exogenous EVs, due to efficient delivery of bioactive molecules, are emerging as promising therapeutic carriers in regenerative medicine. EVs orchestrate cellular proliferation, differentiation, and immune microenvironment remodeling at injury sites acting through the intercellular transfer of functional cargos, such as miRNAs, mRNAs, and cytokines, thereby promoting multidimensional tissue repair and regeneration.

Mesenchymal stem cell (MSC)-derived EVs, with potent osteoinductive properties, represent a highly promising acellular approach for bone defect repair [12]. Wang et al. engineered a biohybrid scaffold by combining decellularized fish scale scaffolds with osteogenically induced bone marrow MSC-EVs, achieving sustained EV release and promoting BMSC differentiation [110]. Similarly, Kang et al. developed an EV-functionalized scaffold composed of human MSC-EVs, magnesium ions, and gallic acid (GA) with a nanoscale interface that significantly enhanced osteogenesis [111]. Moreover, neutrophil-derived EVs have also been shown to accelerate bone regeneration by promoting BMSC osteogenic differentiation. Based on this finding, Wang et al. proposed the integration of these EVs with BMSC-based cell sheets to further enhance bone repair [112].

Another innovative approach involved encapsulating bacterial EVs loaded with BMP-2 and VEGF (BEVspBMP-2-VEGF) with IL-4 into GelMA hydrogels to create a multifunctional composite (IL-4/BEVspBMP-2-VEGF@GelMA) [113]. IL-4 reshaped the immune microenvironment by promoting macrophage polarization from pro-inflammatory M1 to reparative M2 phenotypes, while BEVspBMP-2-VEGF boosted BMP-2 and VEGF expression at fracture sites, thereby accelerating osteogenesis and angiogenesis. *In vitro* studies using RAW264.7 cells, BMSCs, and vascular endothelial cells (VECs) confirmed the composite anti-inflammatory, osteogenic, and pro-angiogenic effects. Furthermore, bone organoid models and *in vivo* studies in osteoporotic fracture (OPF) mouse models demonstrated that the composite significantly enhanced fracture healing by increasing M2 macrophage recruitment, osteogenic protein expression, and type-H vessel formation (**Figure 3A**) [113]. In parallel, an engineered bacterial EV platform was developed by displaying BMP-2 and CXCR4 proteins on the surface of *E. coli*, yielding BEVs-BC vesicles with excellent bone-targeting properties [114]. These engineered vesicles significantly enhanced BMSC osteogenic differentiation and effectively inhibited bone loss in ovariectomized (OVX) mouse models, thereby reversing osteoporosis progression (**Figure 3B**) [114].

Traditional Chinese medicine (TCM)-derived NVs are gaining attention in bone regeneration. For example [53], yam-derived exosome-like nanovesicles (YNVs) promote osteoblast growth, differentiation, and mineralization, mitigating bone fragility and improving bone regeneration in OVX-induced osteoporotic mice (**Figure 3C**) [53]. Similarly, RDNVs, NVs isolated from *Drynaria fortunei* root juice, exhibit strong bone-targeting activity and anti-osteoporotic effects in OVX mouse models [115]. RDNVs are efficiently internalized by human BMSCs (hBMSCs), which enhances the proliferation, ERα expression, osteogenic differentiation, and bone formation (**Figure 3D**) [115].

To summarize, regenerative medicine integrates biological and engineering strategies to repair or reconstruct damaged tissues and organs [116]. In addition to the differentiation potential, MSCs contribute significantly through the secretion of EVs, which deliver bioactive molecules that regulate tissue regeneration [117]. With continued advances in EV engineering and a deeper understanding of EV biology, tissue engineering and clinical therapy applications are expected to expand, accelerating the development and clinical translation of next-generation acellular regenerative therapies [118].

Inflammatory diseases

Inflammation is a key driver in diseases, like IBD, psoriasis, osteoarthritis (OA), and rheumatoid arthritis (RA). Chronic or excessive inflammation causes tissue damage and immune imbalance, making suppression of inflammation essential for therapy. Recently, EVs from animals, plants, and microbes have gained attention due to the biocompatibility, low immunogenicity, and efficient delivery of bioactive molecules.

IBD, including ulcerative colitis (UC) and Crohn's disease (CD), is marked by impaired intestinal barriers and systemic release of pro-inflammatory cytokines that can trigger distant organ inflammation. Plant- and microbe-derived EVs have strong anti-inflammatory effects and restore gut homeostasis. For example, Lu et al. [109] isolated exosome-like NVs (PLDENs) from *Pueraria lobata*, which significantly reduced TNF-α, IL-6, and IL-1β ($P < 0.01$), restored barrier integrity, and suppressed lung inflammation in DSS-induced colitis. Hwang et al. [53] developed YNVs that inhibited TIMP2 signaling, decreased pro-inflammatory cytokines, and promoted beneficial gut bacteria, which lowered disease severity ($P < 0.05$). Wu et al. [67] demonstrated that *Lactobacillus plantarum* EVs improved epithelial cell
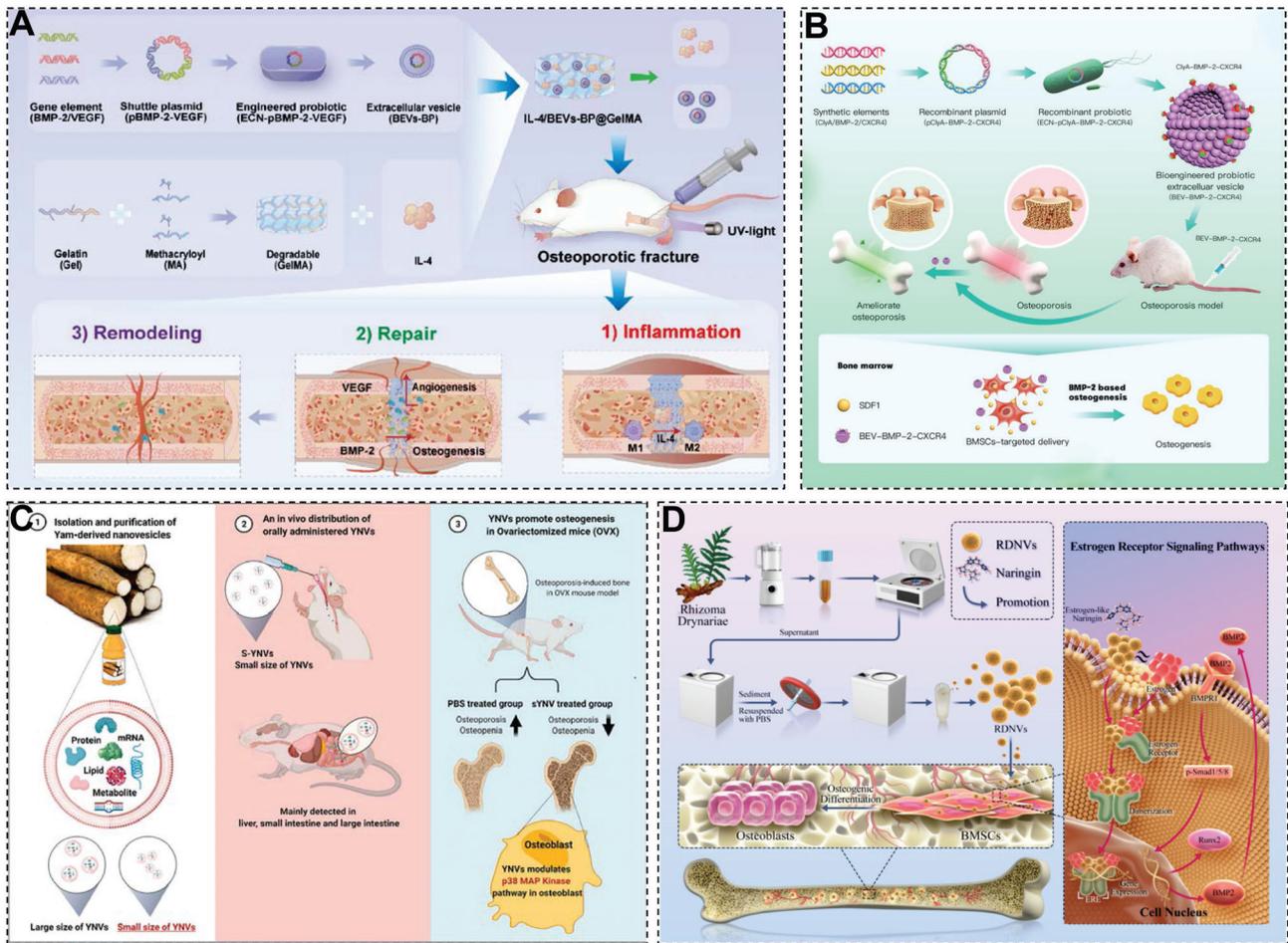
Figure 3 The application of exogenous EVs in regenerative medicine. A. Synthetically engineered bacterial EVs and IL-4-encapsulated hydrogels sequentially promote osteoporotic fractures repair [113]. Copyright 2025, American Chemical Society. B. Synthetic biology-based bacterial EVs displaying BMP-2 and CXCR4 to ameliorate osteoporosis [114]. Copyright 2024 The authors. C. Yam-derived exosome-like nanovesicles stimulate osteoblast formation and prevent osteoporosis in mice [53]. Copyright 2023 Elsevier B.V. All rights reserved. D. *Rhizoma drynariae*-derived nanovesicles reverse osteoporosis by potentiating osteogenic differentiation of human bone marrow mesenchymal stem cells via targeting ERα signaling [115]. Copyright 2024 The authors.

survival, enhanced tight junction proteins, and reduced colonic inflammation by 60%, suppressing cytokine release.

A research team has achieved a stable and efficient engineering method by hybridizing ethanol-mediated EVs with functionalized lipid nanoparticles (LNPs) containing polymeric denatured lipid components. During the hybridization process, the internal bioactive factors and targeted parts maintain the characteristics of LNPs and EVs. An Ab-hybrid was successfully synthesized by hybridization with nicotinamide encapsulated Col2A1 antibody-modified liposomes and EVs overexpressing transforming growth factor (TGF)-β1. The Ab-hybrid was used for surgical instability of the medial meniscus in rats induced by osteoarthritis (OA). Ultimately, the Ab-hybrid demonstrated excellent cartilage protection and anti-inflammatory effects in OA lesions with targeting and persistence (**Figure 4A**) [119]. In another innovative approach, researchers engineered *Bacillus subtilis* as a chassis organism to express the broad-spectrum antimicrobial peptide, cathelicidin-BF (Cathe), which was derived from the banded krait (*Bungarus fasciatus*;

Figure 4B) [120]. Cathe was fused with the spore coat protein, CotC, and subsequently presented within *B. subtilis*-secreted EVs (CEVs) using surface display technology. These engineered CEVs demonstrated significant therapeutic efficacy in alleviating dextran sulfate sodium (DSS)-induced acute colitis. *Folium Artemisiae* Argyi-derived exosome-like NVs (FAELNs) have demonstrated promising therapeutic potential for UC (**Figure 4C**) [121] FAELNs reached peak fluorescence intensity in the mouse intestine within 2 h following oral administration and remained detectable for up to 24 h, predominantly localizing to the gastrointestinal tract and selectively accumulating at inflamed sites. *In vitro* studies revealed that FAELNs are internalized by cells through multiple complex pathways. FAELNs effectively reduce pro-inflammatory cytokine levels, promote macrophage polarization toward the M2 phenotype, scavenge reactive oxygen species (ROS), and modulate apoptosis. FAELNs significantly improved clinical indicators in UC mouse models, such as body weight, colon length, and disease activity index (DAI), while alleviating tissue inflammation and injury. Moreover, FAELNs
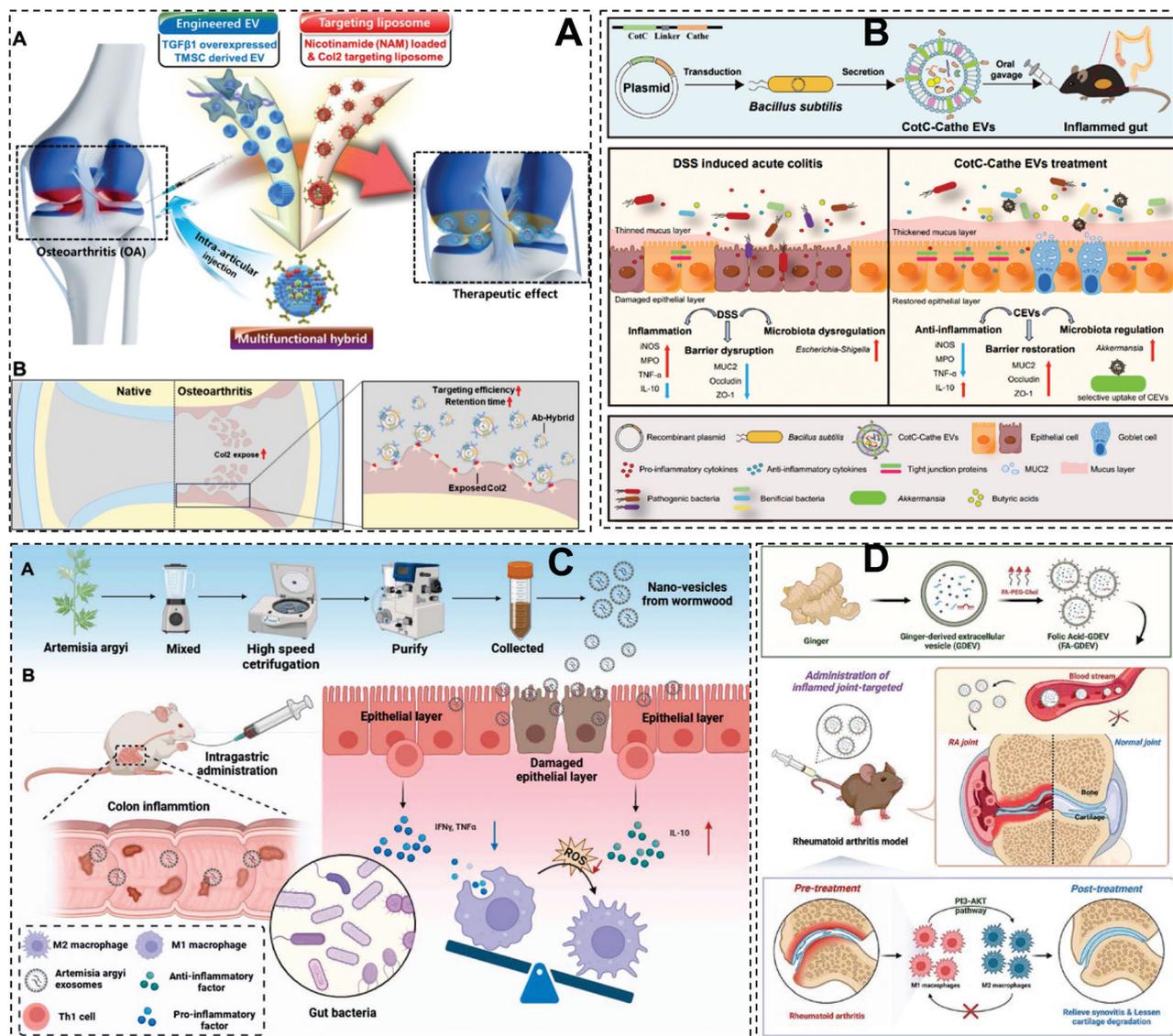
Figure 4 The application of exogenous EVs in Inflammatory diseases. A. The cartilage protection and anti-inflammatory effects of hybrid EVs in OA lesions [119] Copyright 2024, The authors. B. Genetically engineered *Bacillus subtilis*-derived EVs Alleviates Ulcerative Colitis by Restoring Intestinal Barrier and Regulating Gut Microbiota [120]. Copyright 2025, Elsevier B.V. C. Oral administration of *Folium artemisiae* Argyi-derived exosome-like nanovesicles can improve ulcerative colitis by regulating intestinal microorganisms [121]. Copyright 2025, Elsevier GmbH. D. Folic acid-modified ginger-derived EVs for targeted treatment of rheumatoid arthritis by remodeling immune microenvironment via the PI3K-AKT pathway [51]. Copyright 2025, The authors.

are able to restore gut microbiota balance and exhibit no apparent cytotoxicity or systemic toxicity [121].

Psoriasis involves keratinocyte overgrowth and Th17 activation. MSC-derived small EVs (sEVs) have immunomodulatory effects but are costly. Plant- and microbe-derived EVs offer affordable alternatives. Huang et al. [122] fused grapefruit EVs with gingival MSC membranes to create CCR6-targeting NVs carrying CX5461. These vesicles cut epidermal thickness by 70% and reduced inflammation in a psoriasis mouse model ($P < 0.001$). The same group used *Parabacteroides goldsteinii* OMVs to inhibit the IL-23/Th17 axis, improving PASI scores without side effects [100].

Non-animal EVs also show promise in joint diseases. *Spirulina platensis* EVs (SP-EVs), which are rich in antioxidants, were loaded into a hydrogel (Rh Gel@SP-EVs) that scavenged ROS and boosted chondrocyte metabolism. This system halved cartilage degradation and reduced IL-1β in an

OA rat model ($P < 0.01$) [123]. Another research team discovered that folic acid-modified ginger-derived extracellular vesicles (FA-GDEVs) selectively target M1 macrophages in inflamed joints via folate receptors (FRs). FA-GDEVs promote the polarization of macrophages toward a reparative M2 phenotype by modulating the PI3K-AKT signaling pathway *in vitro*. *In vivo* studies further demonstrated that FA-GDEVs not only effectively accumulate in affected joints but also significantly alleviate RA symptoms. Based on these findings, the authors proposed that FA-GDEVs represent a promising therapeutic candidate for RA treatment (**Figure 4D**) [51].

Exogenous EVs combine natural targeting, low toxicity, and scalability but face challenges, including standardized production, long-term safety assessment, and mass manufacturing. With ongoing advances, these EVs could revolutionize inflammation-targeted therapies and precision medicine [124].

Challenges of clinical transformation

EVs have demonstrated broad application prospects in various biomedical fields. EVs have emerged as a promising platform in precision medicine research due to excellent biocompatibility, low immunogenicity, and ability to traverse biological barriers. Nevertheless, the translation of EVs from basic research to clinical application is hindered by several critical challenges.

Currently, several challenges dominate the landscape of EV research and application. First, there is limited yield [12]. Existing EV isolation and extraction techniques, such as ultracentrifugation and size-exclusion chromatography, are inefficient and fail to meet the demands of large-scale clinical production. Second, maintenance of bioactivity and *in vivo* stability [12]. EVs are rapidly cleared by the mononuclear phagocyte system, particularly in the liver and lungs, resulting in a short circulation half-life. Moreover, potential structural damage during storage or delivery may compromise the functionality and the underlying mechanisms remain poorly understood, which adds further uncertainty to the therapeutic use. Third, source heterogeneity and purity control [1], especially analytical gaps in non-mammalian EV purity. EVs derived from various biological fluids exhibit significant variability in composition and function. Current separation technologies often struggle to achieve high purity and uniformity, which impedes the development of standardized EV-based drug delivery systems. Critical deficiencies exist in verifying plant-derived EVs purity, particularly in discriminating vesicles from plant-specific contaminants. Although protocols for high-purity *Arabidopsis* EV isolation exist [125], most studies fail to exclude contaminants, obscuring functional interpretations. Standardized validation methods targeting phyto-contaminants are urgently needed [48, 126]. Fourth, while several guidelines, such as the updated MISEV and platforms, like EV-TRACK, have been developed to facilitate reproducibility, these protocols frequently neglect the detailed characterization of immunogenic profiles, which is of crucial importance given that subtle alterations in EV composition could significantly influence immune recognition and clearance [105, 127, 128]. Fifth, although EVs are generally regarded as having low immunogenicity, repeated dosing in preclinical models has been associated with the development of anti-EV IgG antibodies. These antibodies, once present, can opsonize subsequently administered EVs and enhance clearance from the circulation, thereby reducing therapeutic efficacy over time. In view of the above challenges, standardization of isolation, loading, and characterization protocols is necessary to progress toward clinical translation.

Despite these limitations, EVs continue to demonstrate unique advantages as highly efficient and biocompatible drug carriers. Preclinical studies have reported negligible toxicity of EVs in *in vitro* and *in vivo* environments (**Table 2**), reinforcing the safety profile for therapeutic applications [89]. With ongoing advancements in EV-related research and continuous refinement of associated technologies, there is increasing optimism about the future clinical translation of EVs.

Summary and outlook

EVs, as natural carriers of intercellular communication, offer a novel paradigm for disease treatment and regenerative medicine owing to diverse biological origins and unique structural features [21]. Studies have demonstrated that EVs can mediate inter-species and -organ communication by

Table 2 Clinical Trials of Exogenous EVs for Disease Treatment.[a]

ID	Disease	Mode	Intervention	Enrollment	Sponsor	Phase
NCT04493242	ARDS	Treatment	Biological: ExoFlo Other: Intravenous normal saline	102	Direct Biologics, LLC	II
NCT05774509	Non-ischemic Cardiomyopathies	Treatment	Biological: Extracellular vesicle-enriched secretome of cardiovascular progenitor cells differentiated from induced pluripotent stem cells	12	Assistance Pulique-Hôpitaux de Paris	I
NCT05787288	COVID-19	Treatment	Biological: Extracellular vesicles from mesenchymal stem cells	240	First Affiliated Hospital of Wenzhou Medical University	I
NCT06002841	Acute respiratory failure	Treatment	Biological: intravenous treatment with EVs Biological: intravenous treatment with placebo solution	15	D'Or Institute for Research and Education	I II
NCT06242379	Retinitis pigmentosa	Treatment	Biological: GMP compliant-BM-MSC derived sEVs	15	Mahidol University	I II
NCT06825572	Acute/acute-on-chronic Liver failure after liver transplantation	Treatment	Biological: MSC-EVs	30	Third Affiliated Hospital, Sun Yat-Sen University	I

[a]All data from NIH (website: https://clinicaltrials.gov/). ARDS: acute respiratory distress syndrome.

transporting bioactive molecules, such as proteins, lipids, and nucleic acids. For example, milk EVs modulate gut immune homeostasis via microRNA signaling, plant-derived EVs deliver flavonoids and other metabolites to host cells and facilitate antioxidant responses, and microbe-derived EVs can present antigens to activate immune responses within host–microbiota interactions [2]. These findings provide a theoretical foundation for developing EV-based therapeutics from non-mammalian sources.

Notably, plant factories and *in vitro* tissue culture systems are emerging as promising platforms for the large-scale production of plant-derived EVs. These systems offer standardized growth conditions and EV-inducing strategies, enabling sustainable production of EVs with high purity and low cost [129]. In parallel, the rapid advancement of synthetic biology is providing new technological

avenues for the functional design and controlled release of EVs. By engineering donor cells, it is now possible to precisely regulate EV biosynthesis pathways by introducing synthetic regulatory elements to enhance the encapsulation of specific miRNAs or by modulating EV secretion via artificial signaling circuits with spatiotemporal specificity [130]. Artificial intelligence (AI) and machine learning are also reshaping EV research by enabling high-throughput data integration, molecular "fingerprint" identification, disease-associated pattern recognition, and personalized EV design. These computational tools will accelerate the translation of laboratory discoveries into clinically viable products [131].

In the future, the application of exogenous EVs is expected to be revolutionized by the integration of AI and synthetic biology (**Figure 5**). By leveraging AI to analyze a patient's
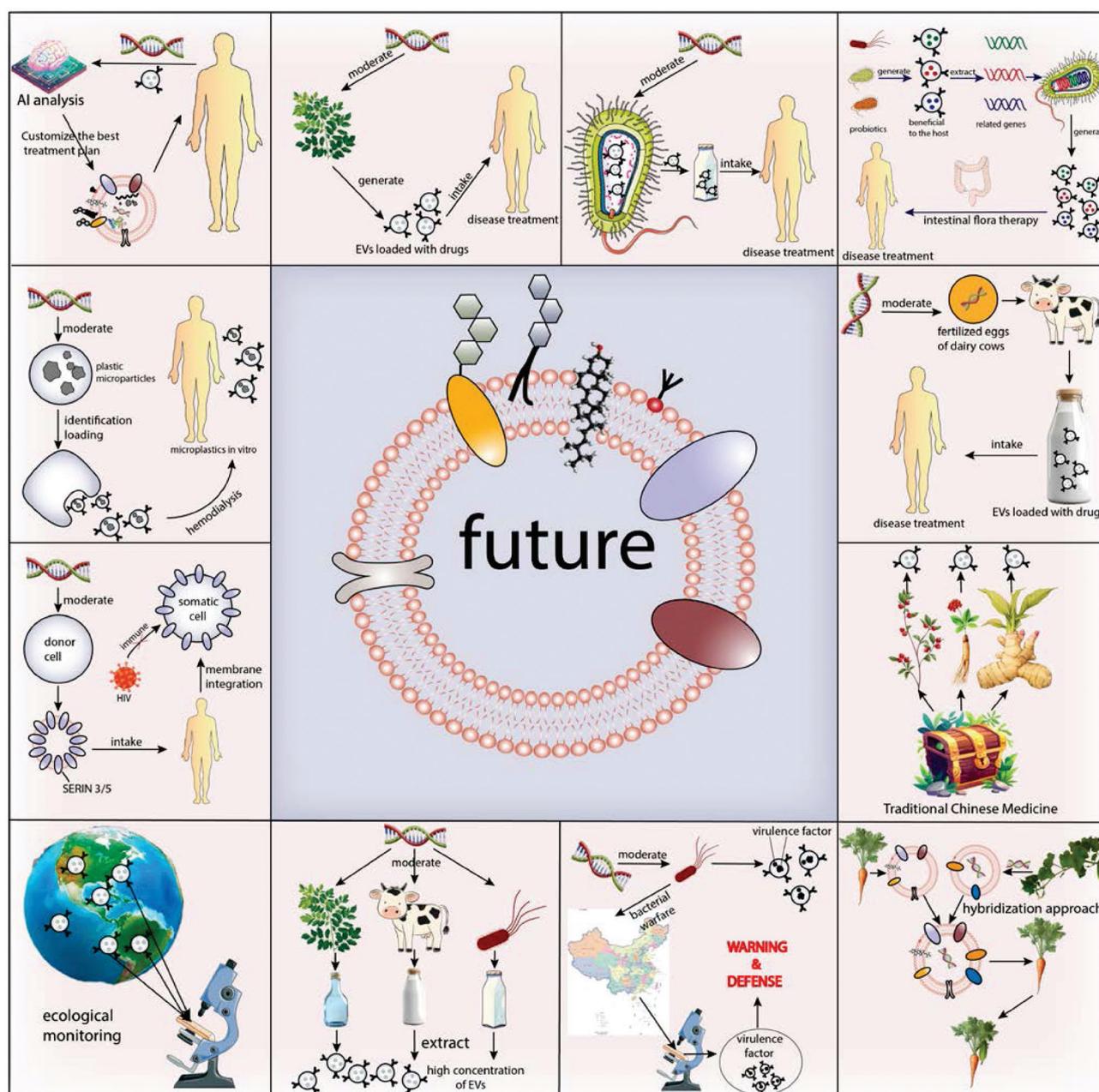

Figure 5 The outlook of exogenous EVs in translational medicine. Created with the Adobe Illustrator. Map Review Number for the Map of China: GS (2022) No. 4308.

genomic information and EV membrane architecture, it will be possible to identify the optimal lipid composition, membrane proteins, and bioactive cargo, thereby enabling the design of fully customized, high-performance therapeutic EVs for truly personalized medicine [9]. Furthermore, by introducing specifically engineered DNA into plants or microbial cell factories, these organisms could be programmed to naturally produce medicinally active EVs during growth or metabolism. Such an approach would allow patients to achieve therapeutic effects through simple ingestion or administration, dramatically reducing production costs and improving accessibility. In addition, EVs hold great promise as sensitive and highly specific biomarkers for the early diagnosis and risk stratification of major diseases, paving the way for a paradigm shift in healthcare-from treatment to a comprehensive "prediction–prevention–intervention" model.

Author contributions

Muran Bai, Zhongshi Li, and Tianren Shi contributed equally. They are contributors in writing the manuscript and responsible for the visualization. Xiangling Li, Jia Li, Jian Ma, and Lei Huang are responsible for review the manuscript. Zhijin Fan is responsible for conceptualization and review the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

Zhijin Fan is an Editorial Board Member of BIO Integration. He was not involved in the peer-review or handling of the manuscript. The other 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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