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Review

Animal-derived vesicles: Nanobridges linking the wisdom of natural evolution with precision medicine

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Abstract:

Animal-derived exosome-like nanoparticles (AELNs), as natural nanocarriers with excellent biocompatibility and cross-species communication capabilities, have attracted increasing attention in tumor therapy. This study aimed to systematically evaluate the biological functions and therapeutic potential of AELNs in cancer treatment. By summarizing recent studies on exosomes derived from animal glandular secretions, tissues, and cells, we analyzed their isolation methods, bioactive components, delivery

pathways, and antitumor mechanisms. The results demonstrated that AELNs exhibited significant tumor-suppressive effects through multiple pathways, including induction of tumor cell apoptosis, inhibition of tumor proliferation and metastasis, modulation of inflammatory tumor microenvironments, and enhancement of chemosensitivity. Notably, camel milk-derived exosomes and oyster mucus-derived exosome-like nanovesicles showed remarkable inhibitory effects on breast cancer and osteosarcoma growth, respectively. Furthermore, animal-derived exosomes displayed superior tissue penetration, targeting ability, and immune evasion capacity compared with plant-derived vesicles. In conclusion, AELNs represent a promising therapeutic platform with excellent biocompatibility, multifunctional bioactivity, and significant antitumor efficacy, highlighting their broad application prospects in precision oncology and nanomedicine.

Keywords: Animal-derived exosome-like nanoparticles, extracellular vesicles, cross-species communication, tumor therapy, regenerative medicine, nanomedicine

INTRODUCTION

Exosomes, specifically referring to disc-shaped lipid bilayer vesicles with diameters ranging from 40 to 100 nm, possess highly diverse components, including nucleic acids, proteins, lipids, amino acids, and metabolic products[1]. Since their initial discovery in 1983 by Harding and Pan *et al.* in rat and sheep reticulocytes, respectively[2,3], and their subsequent formal naming as “exosomes” by Johnstone *et al.* in 1987[4], accumulating evidence has demonstrated that exosomes are involved in the progression of various disease processes and exhibit multiple biological functions, such as antigen presentation, regulation of immune responses, and cytokine transport[5, 6].

Natural exosomes can be broadly classified into animal-derived exosomes and plant-derived exosomes[7]. Traditional studies have primarily focused on exosomes originating from human stem cells and tumor sources. However, with advances in science and technology, increasing attention has been directed toward exosomes derived from non-human sources, such as those from animals and plants, whose potential functional roles and therapeutic prospects are being actively explored. Research on plant-derived exosomes has reached a relatively mature stage, whereas studies on animal-derived exosomes remain limited due to the complexity of their extraction and

the difficulty in obtaining them. For instance, deer antlers, as the only known mammalian organ capable of complete regeneration, have been shown to produce stem cell-derived exosomes that exert significant effects in alleviating human mesenchymal stem cell senescence and osteoarthritis[8]. Additionally, because the process of shell formation in oysters is analogous to human bone formation, exosomes derived from the mantle tissue of oysters have been found to possess promising potential in promoting bone growth and treating osteoporosis[9]. Compared with plant-derived exosomes, animal-derived exosomes exhibit advantages such as higher yield and improved biocompatibility. For example, the number of exosomal particles isolated from oyster mantle tissue can reach as high as 3.14×10^9 particles per gram of mantle tissue[9].Moreover, plant-derived exosomes are limited by seasonal growth patterns and can only be obtained periodically[10,11].

Animal-derived exosomes have attracted considerable attention due to their abundant sources and their potential applications in the fields of biomedicine and nanotechnology. However, there is currently a lack of standardized extraction methods for animal-derived exosomes, and the nano-vesicles obtained often lack clearly defined biological pathways and physicochemical characterization[5]. Therefore, this thesis provides a comprehensive summary of recent research in the field of animal-derived exosomes. In this thesis, animal-derived exosomes are categorized into three types based on their modes of acquisition: those obtained from animal glandular secretions, those isolated through the disruption and separation of animal tissues, and those directly secreted by animal cells. Exosomes derived from animal glandular secretions include those isolated from various animal milk products, such as bovine milk, porcine milk, goat milk, yak milk, and dromedary camel milk[12], as well as exosomes obtained from mucus secreted by Pinctada martensii[13], exosomes extracted from the skin secretions of Bombina maxima[14], and exosomes derived from honey[15]. Exosomes directly isolated from disrupted animal tissues include those derived from oyster mantle tissue[9], egg white[16], regenerative sea anemones[17], and Periplaneta americana[18]. Exosomes directly secreted by animal cells include those derived from deer antler stem cells[8] and myogenic progenitor cells of salamander skeletal muscle[19].

OVERVIEW OF ANIMAL-DERIVED EXOSOME-LIKE NANOVESICLES (AELNS)

Biogenesis of AELNs

The multivesicular body (MVB) pathway is one of the key mechanisms involved in exosome biogenesis in animal cells. The formation of exosomes is closely associated with the continuous dynamics of biological membranes. The plasma membrane undergoes invagination through endocytosis to form early endosomes, followed by the accumulation of bioactive substances within early sorting endosomes (ESEs). Subsequently, under the regulation of the endosomal sorting complex required for transport and other associated proteins, early endosomes mature into late sorting endosomes (LSEs). After a second invagination process, LSEs ultimately develop into multivesicular bodies (MVBs)[7]. According to their functional fate, MVBs can be classified into two categories: secretory MVBs and degradative MVBs. Secretory MVBs are transported to the vicinity of the plasma membrane and fuse with it in a calcium-dependent manner, thereby releasing uniformly sized vesicles into the extracellular space, which are defined as exosomes[20]. In contrast, degradative MVBs undergo fusion with lysosomes following the endocytosis of macromolecules or vesicular structures within the cytoplasm, leading to the degradation of their contents, or they may directly fuse with lysosomes for degradation. Studies have indicated that cholesterol levels are a major determinant of MVB fate. Cholesterol-rich MVBs are targeted and sorted toward the plasma membrane as exocytic vesicles, whereas cholesterol-poor MVBs are directed toward lysosomes for degradation[21, 22]. The mechanisms underlying exosome biogenesis and their hierarchical regulatory processes are highly diverse. Current evidence increasingly supports that Rab family proteins function as molecular switches regulating intracellular vesicle formation and trafficking in eukaryotic cells[23]. However, other studies have reported that certain components, such as tetraspanins and lipid rafts, also contribute to exosome formation[24,25]. Therefore, the precise mechanisms remain a subject of ongoing debate.

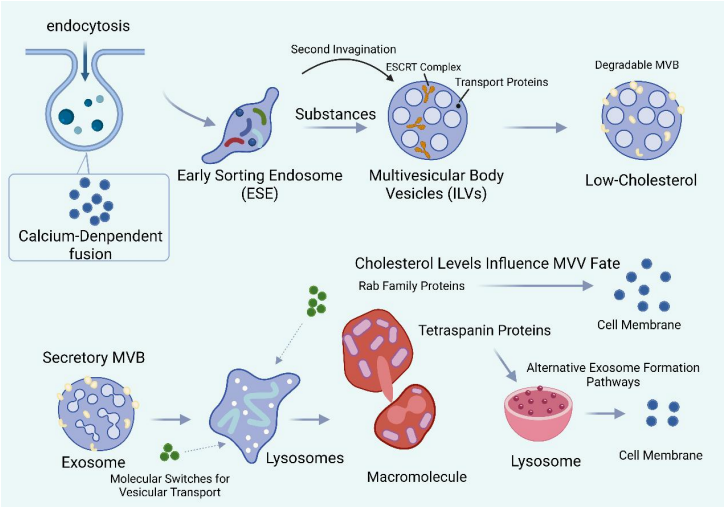

Figure 1. Biogenesis and secretion pathways of animal-derived exosome-like nanoparticles. The schematic illustrates the endocytic formation of early sorting endosomes (ESEs), their maturation into multivesicular bodies (MVBs), and the eventual secretion of exosomes via secretory MVBs, or degradation via lysosomes. Key molecular regulators, including ESCRT complexes, transport proteins, and tetraspanins, are highlighted, demonstrating the intracellular mechanisms controlling exosome formation, cargo sorting, and vesicular release.

Extraction of AELNs

Since animal-derived exosome-like nanoparticles (AELNs) are typically extracted from original animal tissues, a series of pretreatment procedures are generally required prior to exosome isolation to remove impurities and obtain high-purity exosomal vesicles[7]. Depending on the type of exosomes obtained, these pretreatment steps commonly include tissue fragmentation, protease digestion, flow cytometric sorting, and the application of extracellular vesicle inhibitors[26]. The following sections summarize the pretreatment strategies, as well as the separation and purification methods, for different categories of AELNs, together with their respective advantages and disadvantages.

Preprocessing

Prior to the extraction of exosomes derived from animal tissues, the surface of the animal tissue must first be cleaned to remove superficial contaminants. The tissue is then washed with phosphate-buffered saline (PBS) to eliminate ions and elements

present in tap water. Subsequently, tissue fragmentation is typically performed to accurately obtain the target tissue, followed by pretreatment with specific proteases to remove contaminating proteins and thereby facilitate the purification of the extracted exosomes. For example, during the preparation of exosomes derived from oyster mantle tissue, fresh oysters are first manually dissected to obtain mantle tissue. After washing with PBS, the tissue is cut into small pieces on ice and then digested with papain at 37 °C. Only after the removal of various protein impurities can further purification and isolation of exosomes be performed[8]. For exosomes derived from animal cells, pretreatment requires the separation of target cells from tissues, followed by purification using flow cytometry and related protein characterization methods. In the pretreatment process of deer antler stem cell-derived exosomes, newly formed antlers are typically manually isolated, after which collagenase III is used to dissociate the tissue into single-cell suspensions, and flow cytometry is employed to sort antlerogenic stem cells. In the characterization of salamander myogenic precursor A1 cells, a 22/18 blastemal marker antibody is used to confirm cell identity[19]. In studies of exosomes derived from regenerative sea anemones, the organisms are first thoroughly washed with sufficient ice-cold sterile PBS, followed by ultrasonic treatment using a Q125 sonicator (Qsonica). The sonicator is set to 20 kHz at full amplitude, with a sonication duration of 10 seconds and intervals of 20 seconds, for a total of 30 min[17]. Due to the greater complexity of animal tissue composition, the isolation and extraction of exosomes derived from these sources are more challenging. This thesis summarizes the pretreatment steps required prior to the isolation of various types of animal-derived exosomes, with the aim of facilitating subsequent research in this field.

Isolation and purification

Following pretreatment, certain superficial and relatively large impurities can be effectively removed. However, during the isolation and processing stages, additional contaminants may still be present in exosome preparations, including cellular debris, proteins, host cell DNA, and non-exosomal extracellular vesicles. These impurities can adversely affect the purity and functional properties of exosomes, thereby necessitating further purification. Commonly employed purification methods include ultracentrifugation, density gradient centrifugation, ultrafiltration, commercial kit-based methods, polymer-based precipitation, and immunoaffinity purification. Owing to the distinct physicochemical properties of different animal-derived exosome-like

nanoparticles (AELNs), it is often necessary to apply different techniques or a combination of multiple methods to achieve efficient isolation and obtain exosomes at high concentration and purity[27].

The principle of ultracentrifugation is based on the separation of different components according to their sedimentation rates in a homogeneous suspension. This method involves a series of centrifugation cycles with varying centrifugal forces and durations to isolate exosomes based on differences in their density and size relative to other components in the sample. Generally, preliminary low-speed centrifugation steps are performed prior to ultracentrifugation to remove intact cells and cellular debris. Typically, centrifugation at 10,000 × g allows for the separation of apoptotic bodies and larger vesicles; approximately 35,000 × g is used to isolate medium-sized vesicles; and 100,000 × g is suitable for smaller extracellular vesicles[12]. This method enables the isolation of exosomes in relatively high yield without contamination from separation reagents. However, it also presents several limitations, including relatively low purity, high equipment cost, time-consuming procedures, and the potential to disrupt exosomal structure, which may affect downstream analyses[12,28].

Density gradient centrifugation combines ultracentrifugation with density gradient principles for separation. The primary purification approaches involve the use of sucrose (discontinuous) or iodixanol (continuous) density gradients. Different exosomes sediment into distinct density fractions, thereby enabling effective separation. For example, the isolation and purification of exosomes derived from oyster mantle tissue have been performed using differential ultracentrifugation combined with a sucrose density gradient. In this procedure, the pellet obtained after centrifugation at 100,000 × g is resuspended in PBS and subjected to a sucrose gradient (8%, 30%, 45%, and 60%), followed by centrifugation at 150,000 × g for 70 min at 4 °C. The fraction located between 30% and 45% is collected and designated as OME[9]. This method yields exosomes with relatively high purity[29]. However, the limitations of this approach are similar to those of ultracentrifugation and may be even more pronounced[12]. The procedure involves complex and time-consuming preparation steps, is highly dependent on operator expertise as well as specialized instrumentation, and is associated with relatively low extracellular vesicle recovery efficiency.

Polymer-based precipitation typically employs polyethylene glycol (PEG) as a medium to reduce the solubility of exosomes, thereby enabling their collection under centrifugation conditions[7,30]. Rider *et al.*[30] adapted a PEG-based viral isolation method for the purification of exosomes and other extracellular vesicles. This approach, referred to as ExtraPEG, yields total protein and RNA from isolated vesicles in quantities and qualities sufficient for proteomic and sequencing analyses, demonstrating that this method is superior to ultracentrifugation in terms of purity and recovery efficiency for applications such as biomarker discovery and diagnostics. Polymer-based precipitation is relatively simple to perform, requires a shorter processing time, and is suitable for handling large-volume samples. However, residual polymers generated during the process are difficult to remove and may interfere with subsequent experimental analyses.

Immunoaffinity chromatography (IAC) is a separation and purification technique based on the specific binding between antibodies and ligands. The ligands are typically high-abundance proteins expressed on the exosomal membrane, such as members of the tetraspanin superfamily and ESCRT-associated proteins, as well as common exosomal surface components, or unique molecules that facilitate the selective recognition of exosomes derived from specific cellular origins[31]. Compared with ultracentrifugation, IAC exhibits higher specificity, sensitivity, purity, and yield, and also enables both qualitative and quantitative analysis of exosomes. However, exosomes obtained through immunoaffinity chromatography generally require relatively stringent storage conditions and are therefore not suitable for large-scale isolation[32].

Size-exclusion chromatography (SEC) operates on the principle that macromolecules are unable to enter the pores of the gel matrix and are therefore eluted along the interstitial spaces between porous gel particles with the mobile phase, whereas smaller molecules are retained within the gel pores and are subsequently eluted. The application of SEC is characterized by its rapidity, simplicity, and low cost[33]. Exosomes isolated by this method generally retain intact structures and exhibit relatively uniform size distributions[34], with minimal adverse effects on their biological properties; however, co-isolation of particles with similar sizes may occur, leading to reduced purity[7]. In the extraction of exosomes from Bombina maxima skin secretions, differential centrifugation combined with SEC has been employed for separation and purification[14].

In the isolation of bovine milk-derived exosomes, SEC is one of the most commonly used methods[33]. Nevertheless, due to the presence of a large amount of proteins in whey, and the fact that some proteins fall within the same size range as extracellular vesicles, the use of SEC alone is not sufficient for effective separation[35].

Membrane filtration using filters with different pore sizes and molecular weight cut-offs (MWCO) is employed for separation[36]. Particles larger than the MWCO are retained, whereas particles smaller than the MWCO, including exosomes, are collected. Ultrafiltration serves as an effective concentration method and is often used in combination with other exosome isolation techniques[37]. However, shear forces generated during the process may cause structural damage to exosomes, and may also lead to aggregation, entrapment of vesicles within membrane pores, or adsorption of exosomes onto the membrane surface, resulting in sample loss.

In addition to conventional isolation methods, a variety of commercial kits are currently available, such as the exoEasy Maxi Kit (QIAGEN), MagCapture™ Exosome Isolation Kit PS (Wako), and the Minute™ High-Efficiency Exosome Precipitation Kit (Invent)[7]. n the extraction of exosomes derived from regenerative sea anemones, the ExoVista Exosome Isolation Kit (PerciaVista Biotechnology Co., Iran) has also been utilized[38]. Kit-based methods are time-efficient, provide relatively high yields, and preserve exosomal integrity effectively[39]. However, these kits are relatively expensive, and their extraction performance may vary considerably.

Table 1. Methods for isolation and purification of animal-derived extracellular vesicle-like particles and their yields.

Animal source	Initial amount	Initial treatment	Isolation method	RAF
Apis mellifera honey	Diluted sample (1:20)	Dilution, centrifugation	UC+GUC	[40]
Aulactinia stella	1.5 g per individual	ICW washing, PBS suspension	US+DC+Precip	[17]
Bee products	NR	Centrifugation	UC	[15]

Bombina maxima	NR	NR	DC+SEC	[14]
Bovine colostrum	NR	Filtration, centrifugation	UC	[41]
Bovine milk	NR	Centrifugation, filtration	UC	[42]
Bovine milk	1 mL	Centrifugation, filtration	Precip	[43]
Bovine milk	NR	Centrifugation, enzymatic treatment, filtration	UC	[44]
Chicken eggs	Egg yolk (1:10 dilution)	Dilution, centrifugation	DC+GUC	[45]
Cobra venom	NR	Centrifugation	UC	[46]
Cooked pork	250 g	Boiling, filtration, centrifugation	UC	[47]
Deer antler MSCs	NR	NR	UC	[8]
Guinea pig blood	NR	Centrifugation, incubation	UC	[48]
Human breastmilk	NR	Centrifugation, fractionation	NR	[49]
Oyster mantle	NR	Washing, tissue digestion, centrifugation	NR	[9]
Periplaneta americana	NR	Soaking, centrifugation	UC	[18]
Reticulocytes	NR	Centrifugation	UC	[4]
Yak milk	NR	NR	UC	[50]

Composition of AELNs

Extracellular vesicles (EVs) carry a wide range of bioactive molecules, including proteins, nucleic acids, lipids, and carbohydrates[51]. Under physiological conditions, exosomes typically mediate the transfer of functional biomolecules between cells, such as mRNA, microRNA, long non-coding RNA, circular RNA, and DNA[1], thereby facilitating intercellular communication[52]. Compared with plant-derived exosomes, animal-derived exosomes contain a greater diversity and abundance of functional components. Although their safety profile is relatively lower, they exhibit greater potential therapeutic value[5]. Analyses of functional components in various animal-derived exosomes, including lipidomics, untargeted metabolomics, and proteomics, have identified numerous molecules that are associated with the treatment of related diseases. Animal-derived exosome-like nanoparticles (AELNs) naturally carry abundant functional constituents and can exert direct or indirect effects on the human body across multiple biological contexts[53]. Different types of AELNs exert their functions through diverse classes of biomolecules, which will be systematically summarized in the table.

Lipids

Lipids not only constitute the structural framework of exosomes but also actively participate in key biological processes, including their biogenesis, maintenance of stability, intercellular communication, and targeted delivery[54]. The core structure of exosomes consists of a phospholipid bilayer membrane, in which lipids play a critical role in determining their functional properties and cellular uptake capacity[55]. Animal-derived extracellular exosomes are enriched in various lipid species, including sphingolipids, cholesterol, phosphatidylserine, and ceramides. These features contribute to enhanced membrane fluidity and optimized lipid raft structures, facilitating fusion with animal cell membranes. Their lipid composition is consistent with functions related to mammalian cell communication and immune regulation. In contrast, lipids present in plant-derived exosome-like nanoparticles (PDENs) include glycolipids, phytosterols, and phosphatidic acid, reflecting the membrane characteristics of plant cells and their involvement in physiological processes such as stress responses and pathogen defense[56,57]. Analytical techniques such as thin-layer chromatography, gas-liquid chromatography, and mass spectrometry have been widely applied for the identification and characterization of lipids in exosomes[58].

Lipidomic analysis of exosomes derived from oyster mantle tissue indicates that the major lipid classes present in OME include phosphatidylcholine (PC, ~24.8%), phosphatidylethanolamine (PE, ~23.1%), phosphatidylserine (PS, ~14.6%), and lysophosphatidylcholine (LPC, ~14.3%). Among these, the relatively high content of phosphatidylcholine (PC) contributes to enhanced membrane fluidity and structural stability of the exosomal membrane[9].

Protein

Animal-derived extracellular exosomes are enriched in a variety of functional proteins, which are primarily involved in intercellular communication, immune regulation, and target recognition. In recent years, the study of exosomal proteins has become a central focus for elucidating their functional mechanisms and exploring their clinical applications. Through techniques such as mass spectrometry-based proteomics, Western blotting, and single-cell sequencing, thousands of proteins have been systematically identified within exosomes, including membrane proteins (e.g., tetraspanin family members such as CD9, CD63, and CD81) and luminal proteins (e.g., heat shock proteins and cell cycle regulatory proteins). According to the current version of the exosome content database ExoCarta (http://www.exocarta.org), a total of 9,769 proteins have been identified in exosomes derived from various cell types and multiple organisms[59]. Exosomal proteins exhibit diverse biological functions, including targeting/adhesion, anti-apoptotic activity, membrane fusion, signal transduction, metabolism, and structural dynamics[55,60].

In studies of exosomes derived from oyster mantle tissue, mass spectrometry combined with LC-MS/MS was employed to identify and quantify the protein composition of OME. The results indicated that a total of 3,468 proteins were identified in OME. Gene Ontology (GO) analysis revealed that these proteins are mainly enriched in biological functions and processes such as binding, catalytic activity, organismal systems, cellular processes, and metabolic processes[61]. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis further demonstrated that the top 20 proteins involved in osteogenic differentiation-related signaling pathways all play confirmed roles in the regulation of osteogenesis[9]. Proteomic analysis of exosomes derived from deer antler stem cells showed that, among the 1,432 identified proteins, a distinct proteomic profile was observed in exosomes derived from antler blastema progenitor cells (EVsABPC)

compared with exosomes derived from embryonic mouse neural stem cells (EVsNSC). Notably, 79 upregulated proteins in EVsABPC were found to be involved in physiological processes such as wound healing (including PPIA, ANXA5, and TIMP1), synaptic vesicle maturation, and immune responses[62].

Although protein composition appears broadly similar across species, available proteomic data from exosomes isolated from murine and human dendritic cells (DCs) indicate that approximately 80% of proteins are conserved between the two species[55, 63]. However, exosomes secreted by different cell types exhibit distinct protein profiles[64], and the protein composition of exosomes from a given cell type also differs from that of their parent cells. In studies of exosomes derived from Bombina maxima[14]， omparison with the protein composition of its skin secretions revealed the presence of 62 extracellular vesicle (EV)-specific proteins within exosomes. Among these, stress-related heat shock proteins constitute the majority and contribute to ensuring proper protein synthesis and degradation, as well as maintaining normal cellular function under stress conditions such as heat, oxidative stress, nitrosative stress, and bacterial infection[65]. These findings suggest that EVs may play a critical role in maintaining the homeostasis of protein synthesis and degradation during stress responses. Such variations also provide potential biomarkers and therapeutic targets for disease diagnosis, progression monitoring, and treatment. At present, among transmembrane proteins, CD63, CD81, and CD9 have been identified as established protein markers of mammalian cell-derived exosomes. In contrast, although the protein composition and functions of plant-derived extracellular vesicles have been partially characterized, specific markers remain insufficiently defined[66].

Nucleic acids

All exosomes share characteristic components derived from their donor cells, including genetic materials such as DNA, tRNA, mRNA, microRNA (miRNA), and small and long non-coding RNAs (sncRNA and lncRNA, respectively)[51, 55]. As important mediators of intercellular communication, animal-derived exosomes exhibit highly complex and dynamically adaptable nucleic acid profiles. Among these, miRNAs have been demonstrated to play a central role in the therapeutic effects of most extracellular vesicle-based treatments[67,68]. In bovine milk-derived exosomes, miRNA components

such as miR-219, miR-338, and miR-4334 have been shown to inhibit lipopolysaccharide (LPS)-induced apoptosis and pro-inflammatory cytokine secretion in intestinal epithelial cells by targeting the p53 and NF-κB signaling pathways[69]. In exosomes derived from chicken egg yolk, a large number of miRNAs, including miR-22 and miR-200a, have been identified, which exhibit protective effects against oxidative stress-induced apoptosis[45, 70]. Furthermore, miR-22 and miR-200a have been reported to suppress inflammatory activation and intestinal epithelial cell apoptosis, thereby inhibiting the onset and progression of inflammatory bowel disease (IBD)[71]. In studies on egg white-derived exosomes, treatment with proteinase K and RNase revealed that the RNase-treated group exhibited significantly reduced cell viability, along with increased expression levels of IL-8, IL-1β, IL-6, TNF-α, Tp53, NF-κB, and FAS. These findings indicate that, compared with proteins, miRNA-22 exerts a more pronounced therapeutic effect in LPS-induced intestinal epithelial injury[16]. In investigations of wound healing promoted by exosomes derived from deer antler stem cells, favorable outcomes have been associated with specific microRNAs present in AMSC-Exo[8]. For instance, miR-21-5p enhances cell proliferation and migration by targeting SPRY2 and promotes angiogenesis and granulation tissue formation by inducing macrophage polarization toward the M2 phenotype. Owing to their ability to regulate gene expression, miRNAs represent a promising avenue for disease treatment and serve as powerful tools for therapeutic intervention.

Table 2. Physical characteristics of animal-derived extracellular vesicle-like nanoparticles.

Animal source	Size (nm, NTA)	Particle concentration (NTA)	Buffer	RAF
Apis mellifera honey	~120-190 nm	NR	PBS	[40]
Aulactinia stella	~99.38 nm	NR	PBS	[17]
Bee products	~120-190 nm	NR	PBS	[15]
Bombina maxima	—	NR	PBS	[14]

Bovine colostrum	~30-150 nm	NR	PBS	[41]
Bovine milk	~100-150 nm	NR	PBS	[42]
Bovine milk	~80-190 nm	NR	PBS	[43]
Bovine milk	~30-200 nm	NR	PBS	[44]
Chicken eggs	~90-130 nm	6.3×10^{10} particles/mL	PBS	[45]
Cobra venom	~100-150 nm	NR	PBS	[46]
Cooked pork	~20-200 nm	NR	PBS	[47]
Deer antler MSCs	~100 nm	NR	NR	[8]
Guinea pig blood	~50 nm	NR	PBS	[48]
Human breastmilk	NR	NR	NR	[49]
Oyster mantle	~30-300 nm	NR	PBS	[9]
Periplaneta americana	~105nm	2.33×10^{9} particles/mL	PBS	[18]
Reticulocytes	~40-100 nm	NR	PBS	[4]
Yak milk	~50-200 nm	NR	NR	[50]

ADMINISTRATION ROUTES AND *IN VIVO* BIODISTRIBUTION CHARACTERISTICS OF ANIMAL-DERIVED EXOSOMES

Animal-derived exosomes achieve tissue-targeted delivery through diverse administration routes, and their *in vivo* distribution is closely associated with their physicochemical properties and physiological barriers. Oral administration, intravenous injection, and local delivery represent the primary strategies, and different routes significantly influence the biodistribution efficiency and therapeutic potential of exosomes.

Oral delivery has emerged as an important strategy due to its non-invasive nature[72]. Notably, extracellular vesicles (EVs) and their RNA cargo are resistant to acidic conditions in the gastric environment[49,73]. his stability is attributed to milk-derived

extracellular vesicles (mEVs), which provide protection for labile cargo against the harsh conditions of the gastrointestinal tract, enabling their entry into systemic circulation and subsequent modulation of gene expression in distal cells. Following different administration routes, vesicles have been observed to accumulate in organs such as the liver, spleen, and brain, with vesicle-specific miRNAs distributed across various tissues, including the intestinal mucosa, spleen, liver, heart, and brain. For example, after oral gavage, the signal of miR-320a at 6 h is significantly higher in the liver, spleen, and kidneys compared with control groups, while miR-34a and miR-155-5p are enriched in the brain and spleen, respectively. In contrast, following intravenous injection, miR-320a is detected at lower levels in the kidneys, lungs, and spleen[42]. After oral administration, honey-derived exosomes (HEc-EVs) rapidly localize to intestinal epithelial cells and traverse the intestinal barrier via microvillus-mediated transport mechanisms, with detectable signals observed in multiple organs, including the liver, spleen, and lungs, within 72 h[40]. In studies of chicken egg yolk-derived exosomes, it has been demonstrated that egg-derived small extracellular vesicles (sEVs) and their RNA cargo can be absorbed via the oral route and distributed to organs such as the intestine, brain, and lungs in mice. The observed accumulation in the brain suggests that sEVs may cross the blood-brain barrier or indirectly influence central nervous system function through the gut-brain axis[45].

Intravenous administration is the most commonly used delivery route[74], enabling therapeutic cells or exosomes to be distributed systemically via the circulatory system, thereby allowing simultaneous action on multiple targets and organs. However, this approach is associated with high rates of organ clearance and limited targeting specificity[75]. Following tail vein injection, deer antler-derived exosomes preferentially accumulate at sites of spinal cord injury, guided by inflammatory chemokine gradients, reaching concentrations at the target site that are 2.3-fold higher than those in plasma within 72 h[62]. Exosomes derived from mesenchymal stem cells (MSCs), when administered intravenously, exhibit natural homing capabilities and low immunogenicity[76], allowing for systemic distribution and targeted enrichment at lesions associated with systemic lupus erythematosus (SLE). By delivering bioactive molecules such as microRNAs, these exosomes regulate immune cell functions, including promoting regulatory T cell (Treg) differentiation and inhibiting B cell activation, thereby achieving systemic immunomodulation[77]. However,

mammalian-derived exosomes (e.g., those derived from bovine mammary epithelial cells) are readily internalized by liver sinusoidal endothelial cells following intravenous administration, leading to reduced drug loading efficiency. Consequently, surface modification strategies are often required to enhance transmembrane transport efficiency and improve therapeutic efficacy[78].

Local administration maximizes the biological activity of exosomes while minimizing systemic exposure, thereby achieving multiple benefits, including targeted and sustained release as well as low immunogenicity. For example, when honey-derived exosome-based gel formulations are applied to cutaneous wounds, exosomes penetrate into the dermal layer through intercellular spaces between keratinocytes and continuously release miR-21 over a period of up to 7 days, thereby promoting fibroblast proliferation. Similarly, cockroach-derived exosomes, when locally injected into diabetic ulcer sites, are internalized by macrophages within 72 h and significantly downregulate the expression of the pro-inflammatory cytokine TNF-α[18]. Bone marrow mesenchymal stem cell-derived exosomes (BMSC-Exos), when administered to the surface of injured spinal cord tissue, enable sustained release at the lesion site, ensuring prolonged retention and targeted accumulation within the spinal cord injury (SCI) region. Moreover, as a cell-free therapeutic strategy, this mode of administration avoids the risk of immune rejection associated with stem cell transplantation. In addition, owing to their inherently low immunogenicity, exosomes can safely traverse the blood-spinal cord barrier and effectively target central nervous system (CNS) injury sites[79].

The *in vivo* biodistribution of exosomes is jointly influenced by particle size, membrane surface proteins, and cellular uptake characteristics[1, 23]. Studies have demonstrated that honey-derived exosomes can be internalized by intestinal epithelial cells and exert biological activity[15]. Exosomes derived from antler stem cells exhibit prolonged retention at sites of spinal cord injury, suggesting a potential capacity for immune evasion[80]. In addition, Periplaneta americana-derived exosomes have been shown to promote tissue repair through modulation of the local inflammatory microenvironment[18]. These findings indicate that exosomes derived from different animal sources may possess distinct tissue distribution patterns and biological behaviors.

Animal-derived exosomes achieve multidimensional biodistribution through distinct administration strategies[81]. Oral administration primarily targets the gastrointestinal tract and gut microbiota regulation, whereas intravenous injection is more suitable for systemic therapy but requires optimization of targeting efficiency[82]. In contrast, local delivery enhances therapeutic efficacy at the site of injury or disease. Future studies should focus on carrier engineering strategies, including biomimetic modifications and stimulus-responsive systems[83], as well as the development of *in vivo* dynamic tracking technologies such as live imaging[84], in order to further improve the precision and safety of exosome-based therapies.

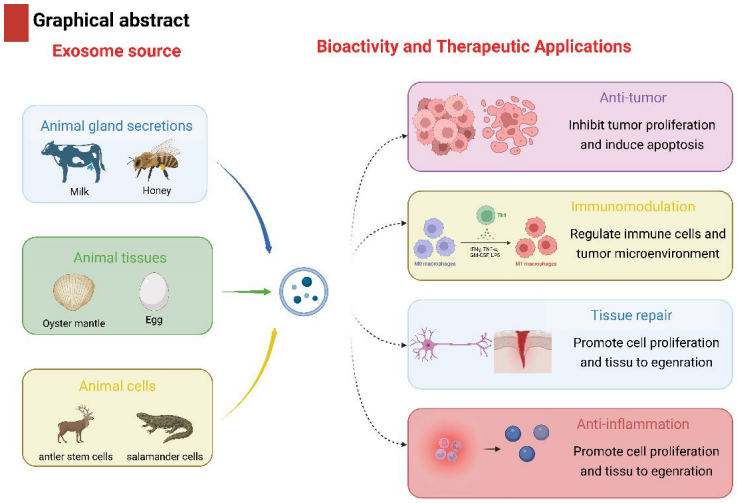

Figure 2. The sources and bioactivities of animal-derived exosome-like nanoparticles (AELNs). The diagram summarizes the main animal sources, including glandular secretions (e.g., milk and honey), tissues (e.g., oyster mantle and egg), and animal cells (e.g., antler stem cells and salamander cells), alongside their corresponding therapeutic applications, including anti-tumor, immunomodulatory, tissue repair, and anti-inflammatory effects.

THERAPEUTIC EFFECTS

Recent studies have shown that certain animals possess intrinsic specialized functions—for example, deer antlers represent the only fully regenerable organ in mammals[8], and adult salamanders are capable of regenerating entire limbs, as well as significant portions of the jaw, retina, and heart after injury[85]. Exosomes derived from

these animals exhibit close biological associations with their source organisms, not only contributing to endogenous physiological processes but also reflecting their unique regenerative capacities. For instance, in studies of Bombina maxima, exosomes derived from skin secretions were found to be enriched in heat shock proteins (HSPs), redox enzymes such as Prdx6, and antimicrobial peptides (AMPs). These vesicles can be internalized by fibroblasts via endocytic pathways, suggesting that extracellular vesicles (EVs) may play important roles in maintaining skin homeostasis in amphibians, including water balance, respiration, immune defense, and antioxidative functions[14]. Moreover, increasing evidence indicates that animal-derived exosomes play critical roles in cross-species interactions. In particular, studies on milk-derived exosomes—exemplified by bovine milk—have demonstrated that these vesicles can be absorbed by osteoporotic mouse models through multiple administration routes, including oral delivery and intravenous injection, thereby alleviating osteoporosis-related phenotypes[86].

Exosomes are widely present in edible animals, plants, and microorganisms. These nanoscale vesicles carry diverse bioactive cargos derived from their parent organisms and can serve as delivery vehicles to transport these molecules into host cells[87], They are capable of mediating cross-kingdom regulation by efficiently transferring molecular signals among different biological systems, including animals, plants, and bacteria, thereby modulating the biological activities of recipient cells[88]. Animal-derived exosome-like nanoparticles (AELNs) exert multifunctional biological effects—including anti-inflammatory, antitumor, antioxidant, and pro-regenerative activities—and play roles across multiple systems such as the skin, skeletal, digestive, and nervous systems. Consequently, they exhibit substantial therapeutic potential in a wide range of human diseases, including osteoporosis, osteoarthritis, inflammatory bowel disease, and spinal cord injury. Compared with conventional stem cell-based therapies, which have shown transformative promise in treating age-related disorders such as diabetes, heart failure, and Parkinson's disease[8], AELNs offer several distinct advantages, including higher stability, improved biocompatibility, lower immunogenicity and toxicity, scalable production, and tunable targeting capabilities. In addition, AELNs demonstrate certain advantages over plant-derived exosomes in terms of functional complexity and therapeutic potential[89]. This thesis further summarizes animal-derived exosomes from three main sources—animal tissues, animal cells, and

animal glandular secretions—and systematically outlines their shared characteristics and specific applications in anti-inflammatory, antitumor, regenerative, and antimicrobial contexts. Detailed classifications are presented in tabular form.

Anti-inflammatory effects

Inflammation is a complex response of the host immune system to injury, infection, or foreign substances, characterized by the accumulation of large numbers of neutrophils and fundamental alterations in tissue metabolism[90], as well as the release of pro-inflammatory cytokines (such as IL-1β and TNF-α), immune cell infiltration, and the initiation of tissue repair processes. Exosomes, as natural nanoscale carriers, play a pivotal role in intercellular communication. The bioactive components they transport—including proteins, nucleic acids, and lipids—enable precise modulation of inflammatory responses. Animal-derived exosomes exhibit distinct advantages in anti-inflammatory therapy due to their abundant sources and high biocompatibility. Evidence indicates that these vesicles exert anti-inflammatory effects primarily by regulating the nuclear factor-κB (NF-κB) signaling pathway, promoting macrophage polarization toward the anti-inflammatory M2 phenotype, and downregulating the expression of pro-inflammatory cytokines.

It has been reported that exosomes derived from milk and egg white exhibit significant anti-inflammatory activity in intestinal inflammation models[41,44,50,91]. Specifically, bovine milk-derived exosomes containing TGF-β1 can inhibit Th17 cell differentiation and promote the proliferation of Treg cells, thereby reducing the expression of pro-inflammatory cytokines such as IL-6 and TNF-α and effectively alleviating colitis (IBD). In addition, yak milk-derived exosomes reduce the levels of IL-1β and TNF-α by inhibiting the PI3K/Akt signaling pathway, further supporting their anti-inflammatory properties. These findings suggest that milk-derived exosomes maintain intestinal immune homeostasis through the coordinated regulation of multiple signaling pathways. Exosomes derived from antler stem cells[8] have demonstrated notable anti-inflammatory potential in models of osteoarthritis and spinal cord injury. Through intra-articular administration, these vesicles deliver bioactive molecules that downregulate upstream regulators of pro-inflammatory cytokines (such as TNF, IL1B, and IFNG), thereby inhibiting NF-κB signaling activity while simultaneously promoting TGF-β1-mediated anti-inflammatory responses[92]. This mechanism, characterized by

the suppression of inflammation associated with degenerative conditions, not only facilitates cartilage regeneration but also achieves systemic anti-inflammatory effects by restoring the balance between Th17 and Treg cell populations. Furthermore, as reported by Shijie Yang *et al.*[62], engineered antler stem cell-derived exosomes can inhibit the activation of the NF-κB signaling pathway by reducing the expression of phosphorylated IκBα (p-IκBα) and p65 (p-p65). This modulation promotes the phenotypic transition of macrophages from the pro-inflammatory M1 phenotype to the anti-inflammatory and tissue-repair-associated M2 phenotype.

Exosomes derived from Periplaneta americana[18] (PA-ELNs) have demonstrated significant anti-inflammatory effects in diabetic wound models. These vesicles alleviate local inflammation and promote tissue repair by regulating macrophage polarization from the pro-inflammatory M1 phenotype toward the reparative M2 phenotype, thereby suppressing the excessive secretion of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6[93].

Notably, the anti-inflammatory effects of exosomes are not universally consistent. For example, miR-2904 contained in cobra venom-derived exosomes has been reported to activate the TLR4/NF-κB signaling pathway, resulting in the upregulation of IL-1β and TNF-α expression and the induction of pro-inflammatory responses[46]. This contrasting finding highlights the considerable heterogeneity of exosomal cargo and underscores the importance of carefully evaluating the origin and biological functions of exosomes prior to their therapeutic application.

Antitumor effects

Although studies specifically investigating the antitumor effects of animal-derived exosomes remain relatively limited, these vesicles have demonstrated unique advantages in cancer therapy as natural nanocarriers. According to the classification described above, the antitumor activities of animal-derived exosomes are primarily associated with exosomes originating from glandular secretions. Emerging evidence suggests that exosomes derived from different species exert antitumor effects through distinct molecular mechanisms. Mammalian milk-derived exosomes, such as camel milk exosomes, can suppress tumor progression by inducing tumor cell apoptosis, reducing oxidative stress, and remodeling the inflammatory tumor microenvironment.

Both oral and local administration of these exosomes have been shown to significantly inhibit the growth of breast cancer xenografts, while local injection exhibits greater tumor accumulation efficiency compared with systemic administration[94]. Exosome-like nanovesicles derived from marine organisms exhibit additional mechanistic characteristics. For example, mucus-derived exosomes from Pinctada martensii inhibit osteosarcoma cell proliferation by targeting the ERK1/2 and Wnt signaling pathways, while simultaneously promoting the osteogenic differentiation of mesenchymal stem cells[13]. Notably, animal-derived exosomes are also capable of delivering functional miRNAs for precise molecular regulation. Milk-derived exosomal miR-2478 has been reported to inhibit melanogenesis in melanoma cells by suppressing the Rap1a-PI3K/Akt signaling axis, and its cross-species regulatory activity has also been validated in human cells[43,95]. In terms of therapeutic strategies, bovine milk-derived exosomes have been demonstrated to remodel the metabolic profile of neuroblastoma cells and enhance chemosensitivity through the inhibition of glycolysis and the Wnt/β-catenin signaling pathway50[94].

Compared with plant-derived exosomes, animal-derived vesicles exhibit superior tissue penetration and greater specificity in immune regulation. Although plant-derived exosomes possess intrinsic lipid membrane stability and prolonged circulation properties[5], the secondary metabolites they carry may trigger host immune responses. In contrast, surface proteins expressed on animal-derived exosomes, such as CD47, enable them to evade macrophage phagocytosis through the “don’t eat me” signaling pathway, a mechanism similarly exploited by tumor cells to escape immune surveillance via CD47 overexpression[96]. Nevertheless, animal-derived exosome-based therapies still face multiple challenges. The lack of standardized isolation and purification procedures results in significant batch-to-batch heterogeneity[5]. In addition, their nanoscale size may limit penetration efficiency within solid tumors, while long-term systemic administration could potentially induce autoimmune responses. Furthermore, although several studies have demonstrated tumor growth inhibition, clinical translation remains restricted by the unclear *in vivo* biodistribution of exosomes and the narrow therapeutic dosage window. For example, the antitumor activity of PMMENs, including inhibition of proliferation, migration, and invasion as well as induction of apoptosis, increased in a concentration-dependent manner within the range of 50-200 μg/mL; however, higher concentrations (≥ 500 μg/mL) may exert cytotoxic effects on normal

cells such as osteoblasts11[13]. Future studies should focus on the engineering modification of exosomes, including membrane functionalization to enhance targeting efficiency, optimization of delivery systems such as biomimetic nanoparticle hybrids, and the establishment of organoid-based platforms for therapeutic efficacy evaluation.

Growth-promoting and regenerative effects

Animal-derived exosome-like nanovesicles (AELNs), as natural nanoscale messengers, have demonstrated significant growth-promoting and regenerative effects across multiple systems, including skeletal, neural, and cutaneous tissues. These vesicles carry unique bioactive molecular repertoires acquired during the evolution of their source organisms and are capable of exerting cross-species regulatory effects on mammalian cells. By delivering functional cargoes such as proteins and microRNAs (miRNAs), AELNs systematically modulate the biological behavior of recipient cells and reshape the tissue microenvironment, thereby providing novel therapeutic strategies for orthopedics and regenerative medicine[9].

Regulation of skeletal system repair and metabolic homeostasis:

In the regulation of skeletal system repair and metabolic homeostasis, AELNs exhibit dual mechanisms involving the direct stimulation of bone formation and the systemic modulation of bone metabolism. Among them, oyster mantle-derived exosomes (OMEs) have been extensively investigated. OMEs not only directly promote osteoblast differentiation *in vitro*, but also preferentially accumulate in bone tissue following oral administration *in vivo*. By simultaneously activating the PI3K/Akt/β-catenin signaling pathway in osteoblasts and suppressing the NF-κB pathway in osteoclast precursors, OMEs exert bidirectional regulatory effects characterized by enhanced osteogenesis and inhibited osteoclastogenesis, thereby effectively promoting bone regeneration in animal models of osteoporosis and bone defects[9]. In addition, milk- and colostrum-derived exosomes have also demonstrated therapeutic potential in improving bone metabolism. Oral administration of these exosomes alleviated glucocorticoid-induced osteoporosis, and their mechanisms may involve not only the direct inhibition of osteoclast differentiation, but also systemic regulation through remodeling of the gut microbiota. These findings further highlight the potential significance of the “gut-bone axis” in mediating the biological functions of AELNs[86].

Regeneration and functional reconstruction of the nervous system:

In the field of nervous system regeneration and functional reconstruction, exosomes derived from organisms with extraordinary regenerative capacity exhibit unique therapeutic advantages. Extracellular vesicles derived from antler reserve mesenchyme progenitor cells (EVsABPC) are enriched with unique proteins associated with nervous system development and can significantly promote neural stem cell proliferation and neuronal axonal growth. Within the complex microenvironment of spinal cord injury, engineered ACPP@EVsABPC can specifically target the lesion site and synergistically enhance neural regeneration through the coordinated activation of the Wnt/β-catenin and Hippo/YAP signaling pathways. Simultaneously, these vesicles strongly induce macrophage polarization toward the anti-inflammatory M2 phenotype by suppressing the NF-κB signaling pathway, thereby alleviating the inflammatory microenvironment that inhibits regeneration and ultimately promoting substantial recovery of motor function[80]. In addition, salamander A1 exosomes deliver mitochondria-associated proteins, such as TOM70, to enhance oxidative phosphorylation in recipient cells and support rapid axonal growth. Although their regenerative mechanisms rely partly on amphibian-specific metabolic characteristics, including anaerobic glycolysis, partial functional reconstruction has still been observed in murine models.

Wound healing and anti-aging effects in skin and soft tissues

In the context of skin and soft tissue wound healing as well as anti-aging therapy, multiple AELNs accelerate tissue repair through multi-target regulatory mechanisms. Exosome-like nanoparticles derived from the American cockroach (PA-ELNs) can be internalized by endothelial cells and fibroblasts, thereby promoting their proliferation and migration and ultimately accelerating wound healing in diabetic mouse models[18]. Antler mesenchymal stem cell-derived exosomes (AMSC-Exo) exhibit more comprehensive regenerative effects. The key molecule miR-21-5p carried by these exosomes regulates the STAT3 signaling pathway, enhancing cellular activity and angiogenesis while simultaneously optimizing collagen remodeling by promoting the transition from type III collagen to type I collagen, ultimately achieving high-quality wound healing with reduced scar formation60[97]. In addition, bovine colostrum-derived exosomes and regeneration-associated sea anemone-derived exosomes have demonstrated anti-photoaging effects and the ability to promote fibroblast migration, respectively, further broadening the potential applications of AELNs in skin repair and

regeneration.

The above studies reveal that exosomes mediate regenerative repair through a three-tiered regulatory network encompassing the “organelle-tissue-organ” axis. At the cellular level, exosomes precisely deliver genetic information to regulate recipient cell phenotypes; at the tissue level, they remodel the immune-stromal interactive microenvironment; and at the organ level, they integrate metabolic and biomechanical signaling pathways. Notably, the evolutionary conservation of cross-species communication provides novel insights for xenogeneic therapeutic applications. For example, porcine skeletal muscle-derived exosomes have been shown to mediate cross-species transfer of miRNAs, thereby inducing metabolic syndrome-like phenotypes in mice61[47]. In addition, the oral stability and immunomodulatory properties of gland-derived exosomes further expand their translational potential. For instance, bovine milk-derived exosomes can improve gut microbiota diversity, including increasing the abundance of Lactobacillus species, enhance the expression of tight junction proteins, and restore intestinal epithelial barrier integrity[12,86]. Future studies should focus on elucidating the spatiotemporal dynamics of exosomal cargo composition and the mechanisms underlying exosome-host microenvironment interactions, thereby advancing the development of precision regenerative medicine based on natural nanovesicles.

Antibacterial and antiviral effects

In addition to their anti-inflammatory, antitumor, growth-promoting, and regenerative properties, animal-derived exosomes—particularly those isolated from bovine milk, goat milk, and bee-derived products such as honey and royal jelly—have been reported to exhibit significant antibacterial and antiviral activities, providing new opportunities for the development of novel therapeutic strategies. The following sections will discuss the mechanisms underlying the antibacterial and antiviral effects of milk-derived exosomes from cattle and goats, as well as exosomes derived from bee products.

Milk-derived exosomes from mammals such as cattle and sheep (mEVs) are enriched with immunomodulatory factors, including transforming growth factor-β1 (TGF-β1) and interleukin-10 (IL-10), as well as antimicrobial peptides such as lysozyme and lactoferrin. These bioactive components can suppress pathogen colonization by

regulating intestinal immune homeostasis[12]. For instance, bovine milk-derived exosomes have been shown to reduce the expression of pro-inflammatory cytokines, including IL-6 and TNF-α, while promoting the secretion of anti-inflammatory factors, thereby alleviating intestinal inflammatory injury in dextran sulfate sodium (DSS)-induced murine colitis models[41,98]. In addition, mEVs maintain intestinal mucosal barrier integrity through the delivery of regulatory sequences such as miR-200a-3p, which suppress the expression of the chemokine CXCL9 induced by pathogenic bacteria, including Escherichia coli[99]. Exosome-like vesicles derived from bee products, including honey and royal jelly (HEc-EVs), contain antimicrobial peptides such as major royal jelly protein 1 (MRJP1), defensin-1, and jellein-3, which exert bactericidal effects by disrupting bacterial cell membrane integrity[40]. tomic force microscopy (AFM) revealed that Staphylococcus aureus treated with honey-derived exosome-like vesicles (HEc-EVs) exhibited cell wall shrinkage and perforation, impaired cell division, and a significant reduction in elastic modulus[15]. *In vitro* experiments further demonstrated that the minimum inhibitory concentration (MIC) of HEc-EVs against methicillin-resistant Staphylococcus aureus (MRSA) was 0.05:1 (vesicle number/colony-forming unit), and that HEc-EVs effectively inhibited biofilm formation[15]. Royal jelly-derived exosomes suppress bacterial growth through modulation of bacterial metabolic pathways. Specifically, the MRJP1 protein carried by these exosomes can bind to bacterial ATP synthase, thereby disrupting energy metabolism. Simultaneously, they induce elevated levels of reactive oxygen species (ROS), resulting in DNA damage and cell cycle arrest[15]. Studies on Streptococcus mutans further demonstrated that royal jelly-derived exosomes downregulate the expression of glucosyltransferases (gtfB/C), inhibit extracellular polysaccharide synthesis, and consequently impair biofilm formation[40].

Virus-specific mechanisms underlying antiviral activity

Bovine milk-derived exosomes can target the NS3 protein of dengue virus (DENV), thereby inhibiting its RNA helicase activity and suppressing viral replication. Quantitative real-time PCR analysis demonstrated that treatment with goat milk-derived exosomes (GME) reduced DENV RNA copy numbers by 66.4%. Moreover, heat inactivation or RNase treatment abolished the antiviral effect, confirming an RNA-dependent mechanism[100]. In addition, GME inhibited the adsorption of Newcastle disease virus (NDV-K) by interfering with the interaction between viral

envelope proteins and host cell receptors. However, no significant inhibitory effect was observed against HIV-1, suggesting that the antiviral activity of GME exhibits virus-specific characteristics.

Animal-derived exosomes exert antibacterial and antiviral effects through multiple mechanisms of action. Milk-derived exosomes primarily function through immune regulation and metabolic intervention, whereas exosomes derived from honey and other bee products rely mainly on antimicrobial peptides for direct bactericidal activity. In contrast, virus-specific inhibitory effects are associated with mechanisms involving RNA interference and protein blockade. The intrinsic advantages of animal-derived exosomes, including their resistance to the gastrointestinal environment and low immunogenicity, make them promising candidates for use as efficient drug delivery vehicles[101].

Table 3. Animal-derived extracellular vesicle-like nanoparticles as therapeutic agents.

Animal source	Contents	Cellular accumulation	Application	Biological function	RAF
Apis mellifera honey	Protein, RNA	Immune cells	Anti-inflammatory	Modulate immune response	[40]
Aulactinia stella	Protein, RNA	Fibroblasts / skin cells	Anti-inflammatory	Reduce inflammatory responses	[17]
Bee products	Protein	MSCs	Therapeutic	Inhibit bacterial growth, suppress biofilm formation, promote cell	[15]

				migration	
Bombina maxima	Protein, RNA, DNA	Fibroblasts	Wound healing	Promote fibroblast proliferation and migration	[14]
Bovine colostrum	Proteins, RNA	Keratinocytes, melanocytes, fibroblasts	Skin repair	Promote proliferation, reduce oxidative stress, inhibit melanin production	[41]
Bovine milk	Proteins, RNA	Liver, spleen, brain	Drug delivery	Deliver miRNA cargos, regulate gene expression, enable cross-species communication	[42]
Bovine milk	Proteins, RNA	NR	Skin whitening	Deliver miRNA cargos, inhibit melanogenesis, regulate gene expression	[43]
Bovine milk	Proteins, RNA	Intestine (colon)	Ulcerative colitis treatment	Regulate immune response, inhibit inflammation,	[44]

				restore gut microbiota, rebalance Treg/Th17 cells	
Chicken eggs	Lipids, protein, RNA	Immune cells / macrophages	Anti-inflammatory	Regulate gut microbiota and inflammation	[45]
Cobra venom	Protein, miRNA	Fibroblasts, endothelial cells	Wound healing	Promote proliferation, migration, angiogenesis and ECM remodeling	[46]
Cooked pork	Proteins, RNA	Intestinal cells	Metabolic disorder model	Induce insulin resistance, alter glucose metabolism, promote lipid accumulation	[47]
Deer antler MSCs	Protein, lipid, RNA	Mesenchymal stem cells, joint cells	Anti-aging, osteoarthritis	Anti-senescence, pro-proliferation, anti-inflammatory, cartilage repair	[8]
Guinea pig blood	Protein	NR	Mechanistic study	Regulate membrane	[48]

				phospholipid asymmetry during maturation	
Human breastmilk	miRNAs	Intestinal epithelial cells, immune cells	Infant development, immune protection	Regulate gene expression, modulate immune system, support development	[49]
Oyster mantle	Proteins, lipids, RNA	Osteoblasts, osteoclasts	Bone regeneration	Promote proliferation, inhibit differentiation, regulate bone homeostasis	[9]
Periplaneta americana	Protein, RNA	Fibroblasts, endothelial cells, macrophages	Wound healing	Promote cell proliferation, enhance cell migration, inhibit inflammation, regulate autophagy	[18]
Reticulocytes	Protein, lipids	Blood circulation	Mechanistic study	Remove membrane proteins, mediate protein sorting,	[4]

				regulate membrane remodeling	
Yak milk	Proteins, RNA	Intestinal epithelial cells	Anti-inflammatory	Promote proliferation, reduce inflammation, enhance barrier function	[50]

SAFETY EVALUATION AND TOXICITY ANALYSIS OF ANIMAL-DERIVED EXOSOMES

Safety evaluation of animal-derived exosome-like nanoparticles (AELNs) represents a critical foundation for their clinical translation. Current studies have conducted preliminary investigations into the biocompatibility and potential toxicity of various AELNs using both *in vitro* and *in vivo* models. However, due to differences in exosome sources and heterogeneity in experimental design, the safety profiles of AELNs should be interpreted and evaluated according to their specific origins.

Exosome-like nanoparticles derived from Periplaneta americana (PA-ELNs) showed no observable acute or chronic toxic effects following oral administration in a rat diabetic wound model. The treatment group exhibited enhanced skin tissue regeneration and significantly reduced inflammatory cytokine levels, including TNF-α and IL-6. However, the study lacked blood biochemical analyses, such as liver and kidney function assessments, as well as histopathological evaluation of major organs to support systemic safety. Similarly, oyster mantle-derived exosomes (OMEs) isolated from Crassostrea hongkongensis did not induce abnormal body weight changes or pathological damage to the liver and kidneys in ovariectomized mice following daily oral administration of 25-50 mg/kg for six weeks. Nevertheless, immune responses and hematological toxicity markers were not evaluated. These findings suggest that animal-derived exosomes may exhibit favorable biocompatibility due to their intrinsically low immunogenicity, although standardized toxicological validation

remains necessary. In healthy mouse models, goat milk-derived exosomes (goat milk exosomes) did not alter liver function indicators, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), nor did they affect inflammation-related proteins such as C-reactive protein and albumin, according to plasma biochemical analyses. Notably, only triglyceride levels were significantly reduced (treatment group: 144.13 ± 52.43 mg/dL *vs.* control group: 228.14 ± 86.85 mg/dL), suggesting a potential metabolic regulatory effect rather than toxicity. In cytotoxicity assays, bovine colostrum-derived exosomes (ColM-exos) could be isolated at yields several dozen times higher than those obtained from cell-derived exosomes from the same sample volume, while exhibiting no detectable cytotoxicity even at high concentrations. Studies investigating the anti-osteosarcoma effects of exosomes derived from the mucus of Pinctada martensii demonstrated no significant toxicity toward 143B osteosarcoma cells, HepG2 hepatocellular carcinoma cells, or other tested cell lines at concentrations ≤ 200 μg/mL. In addition, no abnormalities in body weight or organ pathology were observed in animal experiments. However, long-term oral administration of pork-derived exosomes (PMEs), equivalent to 5 g of pork per day, induced metabolic disturbances in mice, including insulin resistance, hepatic lipid accumulation, and excessive weight gain. These effects were hypothesized to result from exosome-associated miRNAs, such as miR-1 and miR-133a-3p, which may disrupt host homeostasis through modulation of lipid metabolism pathways. Deer-derived exosomes, including EVsABPC and AnSC-exos, showed no reported acute adverse reactions following local injection in osteoarthritis and trauma models. However, data regarding immunogenicity and long-term toxicity remain unavailable. Although salamander A1 cell-derived exosomes (A1-EVs) have demonstrated protective effects against oxidative stress-induced cardiomyocyte injury, their dose-dependent toxicity and host immune responses have not yet been evaluated. Notably, parasite-derived exosomes, such as Schistosoma japonicum egg-derived exosomal Sja-miR-71a, have shown therapeutic potential in antifibrotic applications; however, their pathogenicity and potential immunomodulatory adverse effects remain unclear.

Current studies generally emphasize the advantages of animal-derived exosome-like nanoparticles (AELNs) as cell-free therapeutic agents, particularly their ability to avoid transplant rejection. However, comprehensive toxicological evidence remains insufficient, as analyses of acute and subchronic toxicity, organ biodistribution, and

immunogenicity are still lacking. Although certain exosomes, such as those derived from goat milk and bovine colostrum, have demonstrated preliminary safety based on basic biochemical and physiological indicators, the potential metabolic interference associated with complex exosomal components, including miRNAs and lipids, warrants further investigation. In particular, it is essential to distinguish the dose threshold between therapeutic functional effects and toxic responses. Future studies should integrate standardized evaluation frameworks, such as ISO 10993, together with multi-omics approaches, to systematically characterize the clinical safety profile of AELNs.

SUMMARY AND PERSPECTIVES

Animal-derived exosome-like nanoparticles (AELNs), as natural nanocarriers mediating cross-species signal transmission, have demonstrated unique therapeutic advantages in fields such as skeletal regeneration, neural repair, and immune regulation. However, the rapid development of this field urgently requires systematic reflection and critical reconstruction from three key dimensions: molecular mechanisms, clinical translation, and theoretical paradigms.

Molecular mechanisms and evolutionary paradoxes underlying cross-species signal transmission

The cross-species bioactivity of animal-derived exosome-like nanoparticles (AELNs) challenges the traditional immunological framework regarding the incompatibility of xenogeneic biomolecules. For example, antler mesenchymal stem cell-derived exosomes (AMSC-Exo) promote skin repair through the miR-21-5p/STAT3 signaling axis, a mechanism highly similar to that of mammalian mesenchymal stem cell-derived exosomes. This functional conservation may result from convergent evolutionary selection of critical signaling pathways under evolutionary pressure, such as the central role of the Wnt/β-catenin pathway in both antler regeneration and human bone metabolism. Furthermore, exosomes derived from marine invertebrates such as oysters (OMEs) have been shown to regulate osteogenic differentiation in mammals through activation of the PI3K/Akt signaling pathway, suggesting the existence of previously unrecognized conserved molecular docking mechanisms across phylogenetically distant species. Such mechanisms may be mediated by specific sphingomyelin-to-cholesterol ratios within the lipid bilayer or by mimicry-related structures of transmembrane

proteins.

However, this phenomenon has also raised critical concerns regarding how the nucleic acid components of xenogeneic exosomes, particularly miRNAs, evade recognition by mammalian RNA interference and innate immune surveillance systems. Recent studies have shown that Periplaneta americana-derived exosome-like nanoparticles (PA-ELNs) can mimic the surface characteristics of host-derived exosomes through glycosylation modifications on the exosomal membrane, such as α-2,6-linked sialic acid residues, thereby avoiding Toll-like receptor 7/8 (TLR7/8)-mediated immune recognition. Although this "molecular mimicry" strategy may enhance biocompatibility, it may also increase the risk of misrecognition of pathogen-associated molecular patterns (PAMPs). Consequently, there is an urgent need to establish predictive models for evaluating the immunogenicity of cross-species exosomes.

The interplay between biological origin and engineering modification

The compositional heterogeneity of natural animal-derived exosome-like nanoparticles (AELNs) serves as both the foundation of their multifunctionality and a major obstacle to clinical standardization. For example, although bovine milk-derived exosomes are enriched in anti-inflammatory components such as miR-219 and miR-338, proteomic analyses have revealed batch-to-batch variations of up to 30%. This variability is likely attributable to the combined effects of individual animal differences, breeding conditions, and isolation methodologies. In contrast, plant-derived exosome-like nanoparticles (PELNs) can achieve higher purity through mechanical disruption owing to the presence of plant cell walls; however, this advantage is often accompanied by the loss of intrinsic targeting capability.

Current engineering strategies attempt to balance the preservation of natural properties with functional optimization. For instance, antler-derived exosomes modified with DSPE-PEG/ACPP achieved targeted accumulation at sites of spinal cord injury, with targeting efficiency increased by 2.6-fold compared with unmodified exosomes. However, the loss of surface CD47 molecules during the modification process may compromise their intrinsic immune evasion capability. This trade-off phenomenon, in which enhancement of one function may occur at the expense of another, suggests that future engineering approaches should focus on establishing multiparametric

optimization models rather than relying on linear modifications aimed at a single functional objective.

Spatiotemporal dynamics of metabolic reprogramming and microenvironmental remodeling

The therapeutic efficacy of animal-derived exosome-like nanoparticles (AELNs) is highly dependent on their spatiotemporal distribution characteristics. Following oral administration, honey-derived exosome-like vesicles (HEc-EVs) exhibit a gradient distribution pattern across the liver, spleen, and lungs within 72 h. This organ tropism is largely determined by specific integrin subtypes expressed on the exosomal surface, with αvβ5 mediating pulmonary targeting and α6β4 promoting intestinal retention. However, most existing studies rely primarily on static biodistribution analyses and fail to account for the dynamic clearance processes of exosomes. For example, egg-derived exosomes (IgY-Exos), due to the absence of CD47 expression, exhibit a circulatory half-life that is only approximately one-third that of synthetic liposomes. Therefore, the development of real-time imaging technologies based on radioisotope labeling or quantum dot tracking will be essential for elucidating the pharmacokinetic behavior and metabolic dynamics of AELNs.

Bioethical and ecological challenges associated with large-scale production

Marine invertebrates provide a representative example of the large-scale production potential of animal-derived exosome-like nanoparticles (AELNs). Approximately 3.14×10^{10} exosomes can be isolated from each gram of oyster mantle tissue, highlighting their considerable commercial applicability. However, the ecological burden associated with intensive aquaculture practices, such as coastal eutrophication, has not yet been incorporated into current evaluation frameworks. More importantly, although deer antlers possess ethical advantages as renewable organs, their growth is highly dependent on seasonal hormonal regulation, and industrial-scale expansion may lead to alterations in exosomal composition. The establishment of heterologous expression systems based on synthetic biology, such as recombinant expression of antler-specific miRNAs in Chinese hamster ovary (CHO) cells, may provide a promising strategy for balancing production yield and product quality.

Reconstruction of theoretical paradigms: from “drug delivery” to “ecological

medicine”

Current studies largely regard animal-derived exosome-like nanoparticles (AELNs) as independent functional entities, while overlooking their ecological significance within the original organisms from which they are derived. For example, skin-derived exosomes from Bombina maxima are enriched in heat shock proteins and antimicrobial peptides, which fundamentally constitute part of the symbiotic defense system of amphibian skin. When such exosomes are applied as anti-inflammatory agents, they may inadvertently disrupt the allelopathic interaction networks within the human microbiota. Therefore, future AELN research should incorporate an ecological medicine perspective to evaluate their cascading effects on the interaction network among humans, microorganisms, and the environment. Achieving this objective will require the development of dynamic systems models integrating multi-omics analyses.

CONCLUSION AND PERSPECTIVES

The therapeutic potential of animal-derived exosome-like nanoparticles (AELNs) is fundamentally dependent on a profound understanding of the mechanisms underlying cross-species molecular communication. Future research should therefore focus on: (1) elucidating the evolutionary selection principles governing conserved signaling modules; (2) developing intelligent engineering platforms that balance preservation of natural biological properties with functional enhancement; and (3) establishing ecological and ethical evaluation systems spanning the entire life cycle of AELNs. Only by overcoming the limitations of current reductionist research paradigms can the translational leap of AELNs from laboratory research to clinical application be fully realized. Achieving this goal will require deep interdisciplinary integration among biology, materials science, and computational science. This field is currently approaching a critical point of paradigm transformation, and future breakthroughs may fundamentally redefine the boundaries of our understanding of material and energy exchange among living systems.

DECLARATIONS

Authors’ contributions

Contributed equally to this work and performed the experiments and analyzed the data: YH.L, XT.W, ZK.C;

Conceived and designed the study: YH.L, XT.W, ZK.C, L.F;

Provided technical and material support: PL.X, C.Y;

Drafted the manuscript: YH.L, XT.W;

Revised the manuscript: ZL.C, ZY.L, L.F;

Supervised the study: L.F, ZY.L;

All authors read and approved the final manuscript.

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Not applicable.

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Consent for publication

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