Review Article

Exosomes: A potential biomarker and therapeutic target for the treatment of diseases

Abstract

Nearly all types of cells release exosomes (30-120 nm, nanosized vesicles), both in diseased and healthy physiological situations. Initially, it was believed that they acted just as "Cellular vacuoles" allowing cells to expel undesired components. Exosome composition and function have been the subject of extensive research since 2007. They are expelled from the majority of cell types and are present in the majority of bodily fluids, such as blood, urine, saliva, breast milk, semen, ascitic fluid, and cell culture media. The study has shown that, the exosomal miRNA (Full name) and lncRNA (Full name) content in diseased patients and healthy persons varies. Scientists have successfully discovered in several studies on disease cells that they work as biomarkers, having particular proteins connected to sick pathology. This is due to their role in expressing RNAs, DNAs, and proteins from cell to cell and their presence in most bodily fluids. Exosome-based diagnostic procedures are now being used for the early diagnosis of cancer, diabetes, neurological illnesses, and other conditions. Exosomes have the ability to traverse the blood-brain barrier and may be utilised to deliver therapeutic agents such as proteins, small compounds, viral gene therapy, RNA treatments, and CRISPR gene editing.

Keywords: Cellular vacuoles, miRNA, lncRNA, ascitic fluid, neurodegenerative, biomarker

1) Introduction

In recent years, scientific research has been uncovering the remarkable potential of exosomes – tiny vesicles secreted by cells – in various fields of medicine and biology. One area where exosomes are showing tremendous promise is animal science (Reference). These minuscule packages of biological information have been identified as key players in intercellular communication, immune response modulation, and tissue regeneration (Reference). As our understanding of exosomes deepens, their applications in animal science continue to expand, holding the promise of revolutionizing diagnostics, therapies, and overall animal health. The majority of eukaryotic cells create exosomes, membrane-bound extracellular vesicles (EVs), in the endosomal compartments . [40] They are phospholipid bilayer-enclosed vesicles and cannot proliferate like a cell. Their main function is to regulate the communications between cells of both normal and diseased states and are required for cell growth and homeostasis in multicellular organisms (Reference). These are ubiquitous in all species and they are found in various biofluids (i.e., blood, urine, cerebrospinal fluid, saliva, breast milk, semen, and ascitic fluid). EVs transport cargo proteins, lipids, nucleic acids or RNA from donor cells to receiver cells via biofluids as well as information [46]. EVs assist in the removal of cellular waste. [27] Extracellular vesicles (EVs) have recently significantly contributed to the study and management of several disorders. In light of the fact that they carry several biomarkers (Reference), EVs are important for diagnostic purposes in many diseases, like cancer, infectious and neurodegenerative diseases, cardiovascular , diseases and pregnancy. [32] A recent study also shows that EVs are capable of acting as naturally occurring drug delivery vehicles.

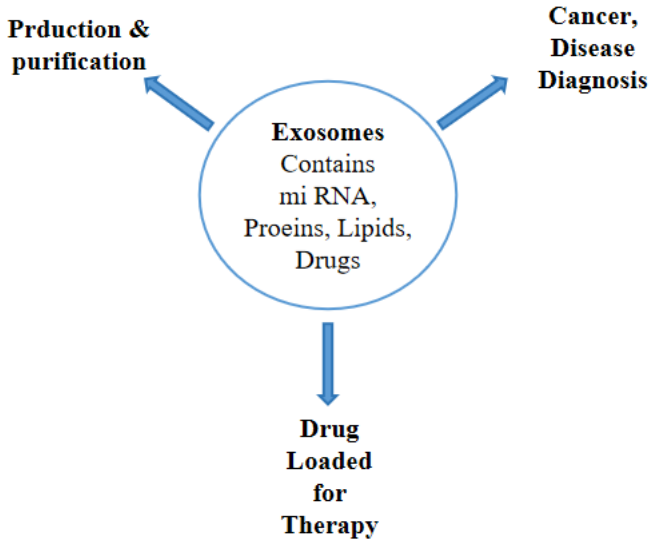

Fig 1: Exosomes as disease carrier & Drug delivery

2) History and Classification:

Rose, Johnstone, and group in 1983 [17, 28] and Stahl and group in 1983 [12] made the initial discovery of exosomes in the growing mammalian reticulocyte (immature red blood cell). Rose, Johnstone, and the group later gave the word "exosomes" to these particles. Extracellular vesicles (EVs) include exosomes as a subclass.

Depending on the biogenesis and size of the EVs, exosomes (30–120 nm) [34], microvesicles (120–1000 nm), and apoptotic bodies (1–5 m) might all be categorised (Reference).

3) The Normal physiological function of Exosomes:

Exosomes have shown immense potential in various areas of regenerative medicine. One of the most exciting applications is in tissue regeneration. Exosomes derived from stem cells have been found to promote tissue repair and regeneration in various animal models (Reference). These exosomes can enhance cell proliferation, reduce inflammation, and stimulate the formation of new blood vessels, all crucial factors in tissue regeneration (Reference).

In addition to tissue regeneration, exosomes also hold promise in wound healing. [30] Studies have shown that exosomes derived from platelets, known as platelet-derived exosomes, can accelerate the healing process and improve the quality of healed tissue (Reference). These exosomes contain growth factors and other signals molecules that promote cell migration and proliferation, leading to faster wound closure and improved tissue remodelling (Reference).

By the majority of different body cell type exosomes are released through biological liquids such as synovial fluid, breast milk, blood, urine, saliva, and semen, demonstrating that they are crucial for intercellular interaction and inducing physiological reactions (Reference). Exosomes are crucial molecules with effector properties that control the body's typical normal physiological processes, including maintenance of stem cell, healing and repairing of tissues, and immune monitoring (activating immune response or suppressing inflammatory factors) and blood coagulation by providing the surface for coagulation factors, neovascularization, repairing of damaged tissues, wound healing and angiogenesis. [30] Exosomes are also called a 'garbage bag' due to involving in unnecessary proteins excretion out of cells, during the life cycle of cells. Exosomes are currently understood to have a role in many biological functions, including the maturation of erythrocytes. Exosomes produced by antigen-presenting cells (APCs) can also activate MHC (Full name) class I and II molecules on the cell surface, thereby helping in the activation of CD8 (Full name)+ and CD4 (Full name)+ T cells and the induction of certain immunological responses. [41] Exosomes released by platelets have a role in the inflammatory response by transporting prostaglandins [13]. Exosomes either activate or inhibit regulatory T cell function, reduce NK (Full name)and CD8+ cell activity, or stimulate monocyte, B, and NK cell activity, thus in early pregnancy embryo can be protected from the attack of the host immune system, exosomes also help in embryo implantation (Reference). Exosomes are able to transport nucleic acids in addition to proteins and lipids between recipient cells [43]. Mast cell-secreted exosomes contain small RNA (Full name) and mRNA (Full name), which are then transferred to specific recipient cells and translated into the recipient cell. In general, the actions of exosomes promote target-specific cell-to-cell communication throughout the body.

4) Role in diseased conditions in humans and animals:

Exosomes have been linked to multiple common disorders in addition to normal biological functions. Exosomes' macromolecular constituents are important for cellular processes and pathological conditions like cancer, neurological disorders, angiogenesis, inflammation, and immunological responses [16]. In cancer, tumour-excreted exosomes help to grow tumours more rapidly and help in spreading. They inhibit the CD4 (Full name) T cell and inhibit the action of NK cells hence inhibiting its cytotoxic activity thus, suppressing the immune response. Those oncogenic proteins and nucleic acids, such as TSG 101 (Full name), viral oncogene proteins and viral materials, and c-Met oncoprotein, are transferred by exosomes [2] derived from cancer cells to create a pro-tumorial microenvironment. These molecules modulate the activity of recipient cells and play key roles in tumorigenesis (Reference), cell proliferation, progression, metastasis, and drug resistance(Reference). Exosomes were discovered to be responsible for transporting misfolded proteins from unhealthy neurons to nearby cells in neurodegenerative diseases such as Prion disease, Alzheimer's disease, Parkinson's disease (Reference), Creutzfeldt-Jakob disease in humans, bovine spongiform encephalopathy in cattle, and scrapie in sheep [44]. This spreads the disease from cell to cell. Cells infected with HIV-1 (Full name) produce exosomes, which are nonviral nanovesicles. The latter are intracellularly formed 50–100 nm lipid bilayer vesicles that result from endosome membrane invagination.

i. Role in cancer spread:

Exosomes could contribute in several ways to the development of cancer. They can alter both the local tumour environment and the overall environment to promote the development, spread, and early stages of metastasis in cancer cells [14, 15]. Tumour cells can share ontogenically active proteins via exosomes inside the tumour microenvironment.

ii. Role in Human Breast cancer:

RAB22A (Full name), a membrane-bound protein that functions as a molecular switch to integrate intracellular signalling and membrane trafficking events, was discovered to be expressed by hypoxia-inducible factors, which, in turn, increased the release of microvesicles during studies of human breast carcinoma cell lines (MCF-7, MDA-MB-231, and MDA-MB-435 (Full name for all)) in low oxygen environments. Because of this, RAB22A may be a viable new therapeutic target for stopping the invasion and metastasis of cancer cells. [33]

iii. Role in Neurodegenerative diseases:

The formation of amyloid plaques in the brain is a hallmark of Alzheimer's disease (AD) [5]. There is evidence that these amyloid β (Aβ) molecules travel to other neuronal cells in the patient's brain through exosomes (Reference). This conclusion has been further supported by the discovery that these molecules are physically linked to exosomes. In addition, it has been demonstrated that the exosomal marker Alix is concentrated in the brains of AD patients as compared to the control (healthy) individuals, in whom Alix is almost nonexistent [1]. Another neurological pathogenesis associated with exosomes is Parkinson's disease (Reference). A rise in -synuclein accumulation has been linked to the disease's development. The accumulation lag time is shortened when neuroblastoma exosomes are present. Exosomes offer a favourable environment for the accumulation of -synuclein, which is stimulated by the exosomes' lipids [11].

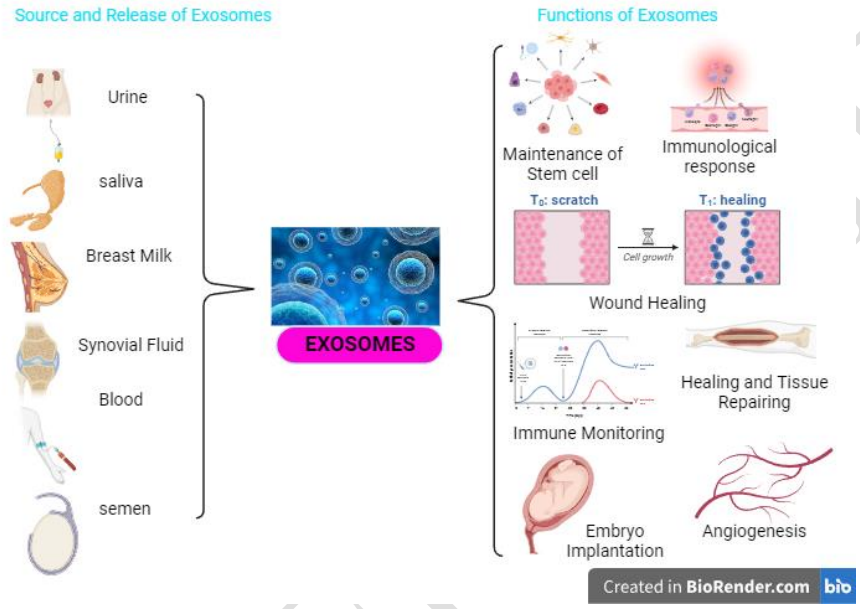

Fig 2: Sources and release of Exosomes and their function

5) Therapeutic applications or uses of Exosomes:

Extensive study has been done to explore the potential of exosomes as diagnostic and therapeutic agents (Reference) since they can be extracted from practically any cell, are engaged in cell-to-cell communication, and play a role in both healthy and pathological processes.

a) Disease diagnostics (Biomarkers):

Exosomes contain and express infectious RNA and proteins, enabling the diagnosis of infectious disorders as well as the detection of active and latent types of intracellular infection (Reference). They are used to identify tumours in individuals with prostate cancer and breast cancer (Reference). For instance, swine PRRS (Full name) might be detected using PRRSV-infected pigs that have the traditional tetraspanin exosomal markers (CD63 (Full name) and CD81 (Full name)). [24] In dairy cows, exosomes contain unique proteins FLOT1 (Full name) and TSG101 (Full name) suggesting that the animal is suffering from uterine disease or metabolic disorder [4]. Staphylococcus aureus infected bovine mastitis-derived exosomes showed 5 mi RNAs (miR-2339, miR-213p, miR-23a, miR-365-3p and miR-92a (Full name)) [6]. Horses with anaemia may have a marker for regeneration due to TfR1 expression in serum exosomes. [31] The lncRNA colorectal neoplasia differentially expressed-h (CRNDE-h) found in the serum exosomes of CRC (Full name) patients may be employed as a possible biomarker. [22] MiR-92b-3p and miR-17-5p (Full name) may also be used as potential circulating biomarkers for the early detection of swine pregnancy [48]. Patients with heart failure (HF) have been shown to have elevated levels of the miRNAs miR-22 (Full name), miR-320a, miR-423-5p (Full name), and miR-92b (Full name) in their blood and serum exosomes [10]. These miRNAs can be employed as specific biomarkers for the diagnosis and prognosis of systolic HF. A-synuclein (Alzheimer's disease) has also been identified as a biomarker of neurodegeneration in cerebrospinal fluid, in addition to A-, T-, and P-Tau (Parkinson's disease) . [18]

b) **Biomarker of metabolic disorder in cows:**

Dairy cows are most susceptible to metabolic diseases during the lactation to pregnancy period. Circulating exosomes may act as biomarkers to detect at-diseased cows to improve the productivity and health of the cows (Reference). Circulating exosomes are produced by a variety of organs and tissues that affect the behaviour of target tissues or cells. Exosomes in circulation provide a sensitive liquid biopsy of the physiological state (Reference). In dairy cows, exosomes contain unique proteins FLOT1 (Full name) and TSG101 (Full name) suggests that the animal is suffering from uterine disease or metabolic disorder [3].

6) Drug delivery vehicle (Therapeutic agents)

Exosomes are not limited to promoting tissue regeneration and wound healing. They also have the potential to treat a wide range of diseases (Reference). For example, in neurodegenerative diseases such as Parkinson's and Alzheimer's, exosomes can deliver therapeutic molecules directly to affected brain cells, potentially slowing down disease progression or even reversing damage (Reference). Similarly, in heart failure, exosomes derived from cardiac cells can promote cardiac repair and regeneration, offering new hope for patients with this debilitating condition (Reference).

By releasing growth factors, proteins, miRNA, mRNA, non-coding RNA (Full name all), and lipids, exosomes have been demonstrated to have the therapeutic potential to stimulate tissue regeneration (Reference). For instance, exosomes from stem cells and endothelial progenitor cells have been shown to repair heart tissue and neovascularization in a myocardial infarction and kidney injury model. RNAs were transported through exosomes reported in 2007 [43]. Lung-trapped MSCs (Full name) release TSG-6 (Full name), an anti-inflammatory protein that is helpful for myocardial infarction and is transported via exosomes. [20] Exosomes generated from MSCs (Full name) encourage cell division and prevent skin cells from dying. Many anticancer drugs may be used via the exosomal carrier. Due to biocompatibility, they're easily absorbed and deliver drugs successfully. Many anticancer drugs, anti-inflammatory drugs, and anti-neurodegenerative disease drugs are successfully inserted inside the exosomes or bound with exosomes. Infections including toxoplasmosis, diphtheria, TB (Full name), and atypical severe acute respiratory syndrome are among the diseases for which exosomes are promising candidates for vaccines. [47]

i. Treatment of Inflammatory Brain Disorders:

The development of CNS (Full name)treatments has been complicated by the blood-brain barrier, which prevents the practical application of potentially potent medicinal medicines in the treatment of several brain cell disorders where inflammation plays a causal role. Anti-inflammatory drugs like curcumin or JSI124 (Full name) are successfully carried to the brain via exosomes (intranasally) without producing noticeable adverse effects [49]. They reported that intranasal administration of Exo-cur (exosome encapsulated curcumin) resulted in a significant decrease in the number of microglial cells and that Exo-JSI124 (exosome encapsulated cucurbitacin I) enhanced tumour apoptosis and a parallel decrease in disease development in all models (Reference).

ii. Gene therapy

Gene therapy can be performed using exosomes. Exosomes' transport capability can be employed to create anticancer treatments. The immune-stimulatory effects of anticancer medicines or RNAs may be reduced, and their hydrophilic properties may make it easier for them to pass cell membranes during gene therapy. [35]

iii. **Anti-inflammatory activity:**

According to Sun et al. (2010), exosomal curcumin can lessen the production of IL-6 and TNF- in LPS-induced (i/p) mice. [36] 16 hours after the LPS (Full name) injection, the IL-6 (Full name) and TNF (Full name)- concentrations in the sera were assessed using an ELISA assay. Exosomal curcumin (@4mg/kg) and LPS (@18.5mg/kg, Sigma-Aldrich) were administered intraperitoneally to C57BL/6 (Full name)j mice.

iv. **Agents used as exosomal content for drug delivery:** There are mainly 3 types:

a) **Small molecules**: eg Doxorubicin (DOX) [42], Paclitaxel [19] chemotherapy medications that are frequently employed to treat a variety of malignancies. But prolonged use of DOX can cause cytotoxicity. Paclitaxel [45]: a chemotherapeutic drug used to treat ovarian cancer as well as lung cancer, breast cancer, and pancreatic cancer (Reference).

b) **Large Molecules:** eg. Curcumin, Antioxidant Curcumin: Studies are showing that the anti-inflammatory, antioxidant, endothelial, neuroprotective, and anticancer effects of curcumin-primed and curcumin-encapsulated exosomes are mediated by several intercellular and regulatory signalling systems [26,36]. Rhodamine-12 [45], Withaferin [25] use to control tumour growth.

c) Nucleic Acids: si-RNA is utilised in genetic treatment to disrupt target genes [8]. In order to regulate post-transcriptional gene expression, mi-RNA [9] binds to complementary regions on targeted mRNA. Exosomes are employed as therapeutic delivery systems for miRNA since they contain miRNA naturally. CRISPR/Cas9 (Full name) systems have been utilised by researchers in recent years to cure a variety of genetic disorders, including cancer, by correcting, eliminating, or silencing specific genetic abnormalities linked to the disease. [21]

7) Classification of agents loaded within exosomes:

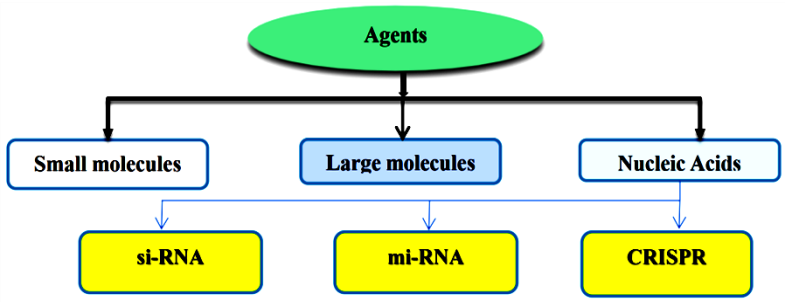

Fig. 3 Exosomes as drug delivery system

i) Types of methods perform to collect and purify exosomes

Table 1: Methods of Exosomes Collection and Purification:

Methods	Mechanisms	Specificity
Differential centrifugation	centrifugal force-based	Exosome isolation from bodily fluids is a common practice. [23]
Size exclusion chromatography	based on the force of gravity and material porosity	With this technique, particular beads are used for a particular size, and centrifugal force-sensitive vesicles are used to isolate them. This approach allows the simultaneous running of several biological samples [7].
Filtration	based on the porosity and membrane materials	Separate exosomes from soluble chemicals and tiny particles very easily [7].
Polymer-based precipitation	based on precipitation and polymer materials	Precipitation benefits include the use of neutral pH and minimal effect on separate exosomes [37].
Immunological separation	on the basis of antibody-receptor interaction	techniques for characterising and quantifying a protein implicated with specific exosome subtypes. [38]

Among all the methods differential-centrifugation is the most commonly used and traditional method. [23] Below table for exosome collection based on the centrifugation method:

Table 2: Four consequent centrifugation steps for exosome isolation [39]:

Sl no	Centrifugation steps	Duration	Temperature	Purpose
1.	300g	10 min	4°C	Remove cells,
2.	2000g	10 min	4°C	Dead cells,
3.	10,000g	30 min	4°C	Cellular debris
4.	100,000g	1 hr 10 min	4°C	Collect exosome fraction

A repeated 100,000 g centrifugation of the re-suspended pellet is frequently employed to separate the exosome preparation from the lower mobility fractions, usually from free proteins.

ii) **Exosome purification:** Both for disease detection and disease therapy purpose exosomes are collected in many different methods (Reference) and purifying (Reference) them is the essential stage for further clinical application and basic research. Based on the size, shape, density, and surface proteins of exosomes, many exosome separation techniques have been developed, including differential centrifugation, size-exclusion chromatography, filtration, polymer-based precipitation, and immunological separation techniques (Reference). To further the study of exosomes and their use, it is necessary to further develop these techniques, which have both benefits and drawbacks.

iii) Exosome drug loading:

Therapeutic agents must efficiently be loaded into the exosomes, for effective use as a drug delivery system of exosomes. Exosomes contain therapeutic substances that can either be actively loaded or passively loaded/encapsulated (Reference).

1. **Passive loading:** These techniques are actually simple and basic. Both drug incubation with sample exosomes and drug incubation with donor cells might result in passive loading. [29] By passive loading, Munagala and his colleagues have been able to encapsulate chemotherapeutic and chemopreventive drugs including paclitaxel, doxorubicin, and withaferin in exosomes derived from bovine milk. [25]

2. **Active loading:** Actively disrupt of exosome membrane so the compound can diffuse inside. Mainly used for loading large molecules. Active drug loading can be performed by:

i) Sonication: In order to enable the drug to penetrate into exosomes without damaging membrane-bound proteins, the exosomes' membrane integrity is impaired. [29]

ii) Extrusion: It is done by a syringe-based lipid extruder, disrupts the membrane and enhances drug diffusion.

iii) Electroporation: In this technique Cells are subjected to an electrical field to improve the cell membrane's permeability, enabling the introduction of compounds, medications, or DNA within the cell (Reference).

iv) Drug conjugation techniques: mostly applied in pharma industries (Reference).

8) Future Expectations

The future of exosome research in animal science is filled with promise. Researchers are working on refining techniques for isolating and characterizing exosomes, which will enhance our understanding of their biological functions and potential applications. As the technology advances, exosome-based therapies may become mainstream in veterinary medicine, offering safer and more effective treatment options for animals.

9) Conclusion:

Exosomes are unlocking new dimensions in our understanding of animal biology and health. Their ability to facilitate intercellular communication, modulate immune responses, and carry therapeutic payloads holds great promise for the field of animal science. Exosomes have great biocompatibility, making them interesting as therapeutic carriers. However, there are risks and issues, such as immunosuppression. and the development of tumours. Recent research demonstrates that one of the most popular medication delivery techniques uses both liposomes and polymeric nanoparticles, and they're causing toxicity, immunogenicity, less biocompatibility and long-distance travel issues. Because exosomes are able to carry several kinds of cargo molecules, including bioactive proteins, lipids, and nucleic acids, they have become an essential medication delivery method. and specific drugs for a variety of disorders, including solid tumours, bone regeneration, cardiac diseases, Parkinson's disease, and others. As research progresses and technology evolves, exosomes may very well reshape the landscape of diagnostics, treatments, and overall animal well-being. The journey into this microscopic realm promises exciting discoveries that will benefit animals and humans alike.

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