A 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 (MicroRNA) and lncRNA (Long non-coding RNA) 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 (Clustered Regularly Interspaced Short Palindromic Repeats) gene editing. The aim is to use exosomes as therapeutic drug delivery or carriers as it is much more compatible with the patient's body. Also, it can easily carry the disease information during diagnosis. Our review focuses on two key areas: a) identifying exosomal content from cancer cells as potential diagnostic tools, and b) exploring the possibility of customizing exosomes with therapeutic agents for treatment advantages.

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. These minuscule packages of biological information have been identified as key players in intercellular communication, immune response modulation, and tissue regeneration. 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. 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, 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 (figure 1).

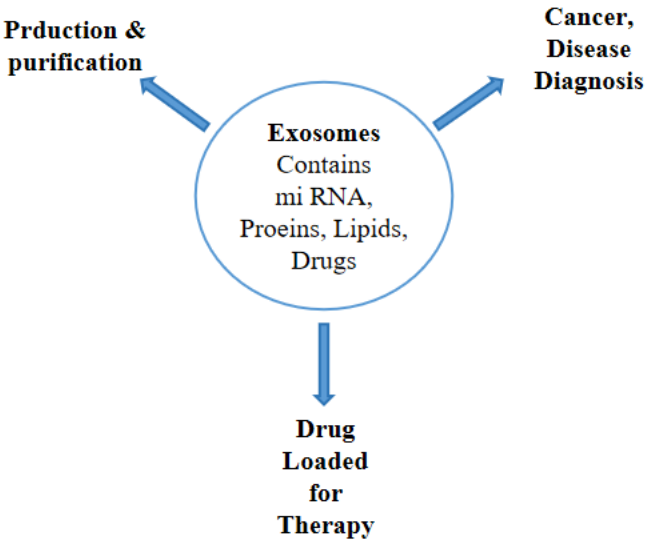

Figure 1 : Exosomes as disease carrier &drug delivery

2) History, Classification Exosome Structure and Composition :

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 (40–100 nm) [34], microvesicles (50–1000 nm) [78,79] and apoptotic bodies (1000–5000 nm) might all be categorised [79].

3) Exosome Structure and Composition :

Exosomes possess an aqueous core and a lipophilic shell, this amphiphilic (hydrophilic and hydrophobic) nature allows them to effectively carry a wide range of molecules, both natural and introduced, within their compartments [50]. These molecules can include proteins, lipids, and even RNA, and are often distinct from the cell where the exosomes originated. Exosomes can carry various proteins originating from the endocytic pathway within the cell. Exosomes, originating from the intracellular budding of multivesicular bodies (MVBs), are enriched with specific protein populations. These populations include membrane transport and fusion proteins: Rab GTPases, annexins, flotillin, and tetraspanins (CD9, CD63, CD81, CD82) [50]. These proteins facilitate the trafficking and fusion of exosomes with recipient cells. Major histocompatibility complexes (MHC I and II): These molecules present antigens on the exosome surface, allowing communication with the immune system [50]. Heat shock proteins (Hsp70, Hsp84, Hsp90): These chaperone proteins contribute to proper protein folding and stability within exosomes. Endosomal sorting complex required for transport (ESCRT) complex components (Alix, TSG101): These proteins play a crucial role in the biogenesis and cargo sorting of exosomes during multivesicular bodies (MVB) formation. Lipid raft-associated proteins like lysobisphosphatidic acid (LBPA) and cholesterol: These components contribute to the unique lipid composition of exosomes, potentially influencing their stability and interaction with target cells [50]. Additionally, they can carry proteins involved in fat metabolism [51]. Beyond these, exosomes can also contain elements of the cellular environment, such as proteins from the extracellular matrix and cell surface (including collagens, integrins, and galectins) [52]. Signalling molecules, cell surface receptors, components of the internal cellular skeleton, metabolic enzymes, and G proteins have also been found within exosomes [53]. This diversity of cargo makes exosomes attractive candidates for drug delivery systems, as they can potentially be loaded with therapeutic molecules and targeted to specific cells.

4) The Normal physiological function of Exosomes:

Exosomes have shown immense potential in various areas of regenerative medicine (Figure 2). 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. These exosomes can enhance cell proliferation, reduce inflammation, and stimulate the formation of new blood vessels, all crucial factors in tissue regeneration.

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. These exosomes contain growth factors and other signalling molecules that promote cell migration and proliferation, leading to faster wound closure and improved tissue remodelling.

By the majority of different body cell type exosomes are released (figure 2) 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. Exosomes are crucial molecules with effector properties that control the body's typical normal physiological processes, including maintenance of stem cells, 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 their involvement in unnecessary protein excretion out of cells, during the life cycle of cells [17,99]. 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 (Major Histocompatibility Complex) class I and II molecules on the cell surface, thereby helping in the activation of CD8+ (cytotoxic T cells or killer T lymphocytes) and CD4+ T cells (Helper T lymphocytes) 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 cells (Natural Killer cells) and CD8+ cell activity, or stimulate monocyte, B, and NK cell activity, thus in early pregnancy, embryos can be protected from the attack of the host immune system, and exosomes also help in embryo implantation. Exosomes are able to transport nucleic acids in addition to proteins and lipids between recipient cells [43]. Mast cell-secreted exosomes contain small RNA and mRNA, 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. Sadri *et al*., 2020, demostrate that dietary breast milk exosomes (BMEs) and their microRNA (miRNA) cargo exhibit bioavailability and accumulate within the placenta and embryo of mice. Furthermore, BME-mediated alterations in gene expression patterns appear to promote embryonic development and survival [63].

5) 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 (helper T cells) T cell and inhibit the action of NK cells (Natural killer cells) hence inhibiting its cytotoxic activity thus, suppressing the immune response. Those oncogenic proteins and nucleic acids, such as TSG 101 (Tumor Susceptibility Gene 101), viral oncogene proteins and viral materials, and c-Met oncoprotein, are transferred by exosomes derived from cancer cells to create a pro-tumoral microenvironment [2]. These molecules modulate the activity of recipient cells and play key roles in tumorigenesis, cell proliferation, progression, metastasis, and drug resistance [98]. Exosomes were discovered to be responsible for transporting misfolded proteins from unhealthy neurons to nearby cells in neurodegenerative diseases such as Prion disease [101], Alzheimer's disease [100], Parkinson's disease [102], Creutzfeldt-Jakob disease [103] in humans, bovine spongiform encephalopathy in cattle [104], and scrapie in sheep [44]. This spreads the disease from cell to cell. Cells infected with HIV-1 (Human Immunodeficiency Virus Type 1) produce exosomes, which are nonviral nanovesicles. Furthermore, exosomes facilitate viral evasion of host immunity by transporting viral antagonists or proviral/virulence factors to target cells [89,90].

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. Within the tumour microenvironment, where malignant cells organize cellular interactions for their own benefit, exosomes emerge as critical players. These nanovesicles facilitate horizontal information transfer from the tumour to local and distant tissues, promoting tumour growth and metastasis [65]. Exosomes termed "oncosomes," act as vectors for oncogenic cargo, including proteins, lipids, and functional nucleic acids, that can reprogram recipient cells through specific gene expression regulation [66, 67].

ii. Role in Human Breast cancer:

Breast cancer cells and their associated stromal/cancer-associated fibroblasts (CAFs) actively secrete exosomes into the surrounding extracellular space and tumour microenvironment. These exosome-mediated intercellular communication pathways are implicated in a multitude of processes critical for tumour progression [68]. A Study showed that RAB22A, 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) 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. 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. 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].

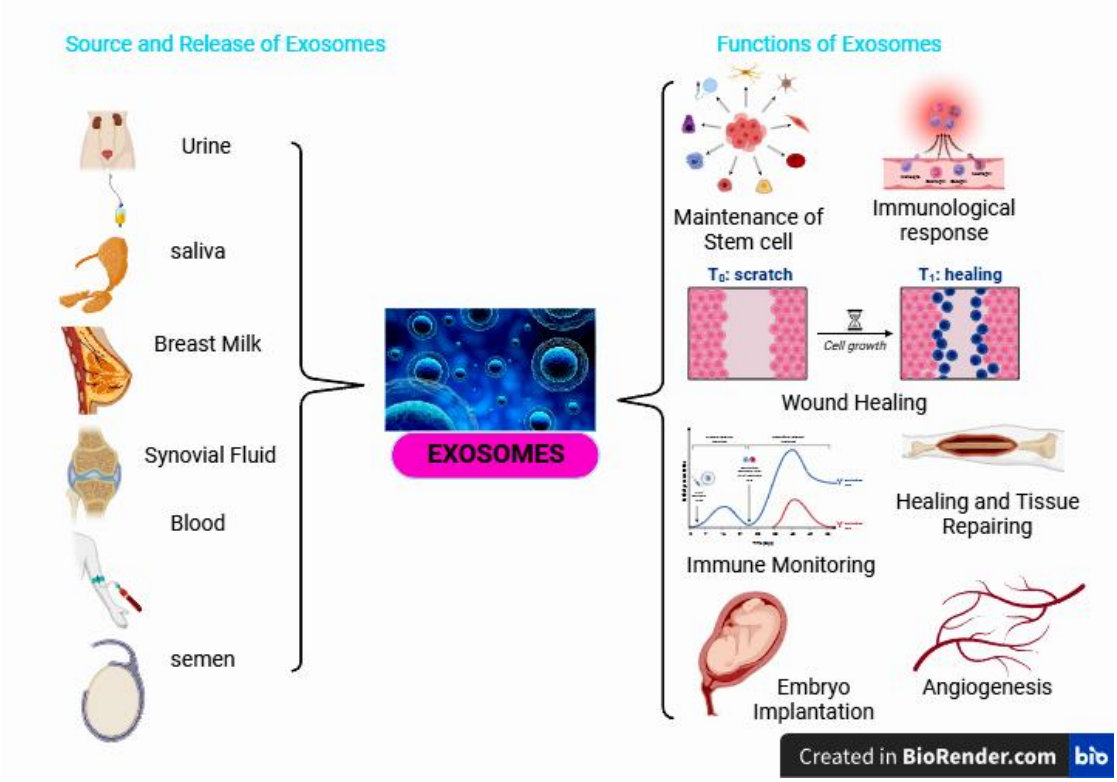

Figure 2 : Sources and release of Exosomes and their functions

6) Therapeutic applications or uses of Exosomes:

Extensive study has been done to explore the potential of exosomes as diagnostic and therapeutic agents 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. They have emerged as a novel and advantageous class of therapeutic delivery systems due to their inherent properties that promote specificity, safety, and stability. Their intrinsic homing capability allows exosomes to navigate complex biological environments and deliver their cargo directly to target cells, even over long distances. This targeted delivery can be particularly beneficial for therapeutics aimed at specific tissues or diseased cells. Furthermore, exosomes offer a unique advantage in their ability to encapsulate a diverse range of therapeutic moieties, including interfering RNA (siRNA) and pharmaceutically active substances [69]. Their natural origin as nanovesicles derived from cells minimizes immunogenicity, leading to a reduced risk of adverse immune reactions [70]. Additionally, exosomes exhibit remarkable stability in circulation, enabling them to travel long distances within the body and persist under various physiological and pathological conditions. This enhanced stability ensures efficient delivery of the therapeutic cargo to the target site. Moreover, the hydrophilic nature of the exosome core makes them particularly suitable for encapsulating water-soluble drugs, which often pose

challenges for conventional delivery methods [71]. Two primary strategies have been explored for loading cargo into exosomes: post-isolation loading and incorporation during exosome biogenesis [72]. These strategies offer researchers flexibility in tailoring the exosome platform for specific therapeutic applications.

a) Disease diagnostics (Biomarkers):

Exosomes act as cellular messengers, shuttling molecules that reflect the health status of their originating cells. This opens doors for their use as biomarkers in disease diagnosis. By analyzing the cargo of exosomes circulating in bodily fluids, researchers might be able to detect diseases like cancer or neurodegenerative disorders at earlier stages, leading to improved treatment outcomes. 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. They are used to identify tumours in individuals with prostate cancer and breast cancer. For instance, swine PRRS (Porcine reproductive and respiratory syndrome) might be detected using PRRSV (Porcine reproductive and respiratory syndrome virus)-infected pigs that have the traditional tetraspanin exosomal markers (CD63 and CD81) [24]. In dairy cows, exosomes contain unique proteins FLOT1 (Flotillin-1) and TSG101 (Tumor susceptibility gene 101) 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) [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 patients may be employed as a possible biomarker [22]. MiR-92b-3p and miR-17-5p 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, miR-320a, miR-423-5p, and miR-92b 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]. Ohno *et al*., 2013 demonstrate that engineered exosomes effectively deliver microRNAs to EGFR-positive breast cancer cells, suggesting a potential new approach to target these tumours with nucleic acid drugs [64].

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. 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. In dairy cows, exosomes contain unique proteins FLOT1 and TSG101 suggesting that the animal is suffering from uterine disease or metabolic disorder [3].

7) 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. For example, in neurodegenerative diseases such as Parkinson's and Alzheimer's [80], exosomes can deliver therapeutic molecules directly to affected brain cells, potentially slowing down disease progression or even reversing damage [80]. Similarly, in heart failure, exosomes derived from cardiac cells can promote cardiac repair and regeneration, offering new hope for patients with this debilitating condition [81]. By releasing growth factors, proteins, miRNA, mRNA, non-coding RNA, and lipids, exosomes have been demonstrated to have the therapeutic potential to stimulate tissue regeneration. For instance, exosomes derived from mesenchymal stem cells (MSCs) and endothelial progenitor cells have been shown to repair heart tissue and neovascularization [82] in a myocardial infarction [84] and kidney injury model [83]. RNAs were transported through exosomes reported in 2007 [43]. Lung-trapped MSCs release TSG-6, an anti-inflammatory protein that is helpful for myocardial infarction and is transported via exosomes [20]. Exosomes generated from MSCs 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[72]. 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, and atypical severe acute respiratory syndrome are among the diseases for which exosomes are promising candidates for vaccines [47].

i. Exosomes as Diagnostics and Treatment of Inflammatory Brain Disorders:

The development of CNS (Central Nervous System) treatments as neuro medicine 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 [69]. Anti-inflammatory drugs like

curcumin or JSI124 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 [49].

CSF-Derived Exosomes: While the isolation of exosomes from cerebrospinal fluid (CSF) has demonstrated potential, limitations in acquiring samples from CSF biobanks have hampered extensive biomarker characterization [85]. However, emerging evidence suggests CSF exosomes hold promise for diagnosing neurodegenerative disorders. For instance, elevated levels of tau phosphorylated at Thr181 (AT270), a well-established biomarker for Alzheimer's disease (AD), have been observed in exosomes isolated from the CSF of patients with mild AD symptoms [35]. Additionally, Exosome Diagnostics (US) has filed a patent [36] outlining a method for detecting neurodegenerative diseases and brain cancer (glioblastoma) through the measurement of RNAs (mRNA, miRNA, siRNA, or shRNA) associated with CSF-derived exosomes. The reported examples highlight the use of nucleic acids corresponding to APP, Aβ42, BACE1, and tau (alone or combined) as biomarkers for AD [86] and other neurodegenerative diseases, while EGFR variants were identified as potential markers for glioblastoma and other brain cancers [87].

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 [73].

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 [74]. 16 hours after the LPS injection, the IL-6 and TNF- 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/6j mice.

8) Agents used as exosomal content for drug delivery:

There are mainly three types: the classification of agents loaded within exosomes is shown in Figure 3:
a) **Small molecules**: eg Doxorubicin (DOX) [42], Paclitaxel [19] chemotherapy medications that are frequently employed to treat a variety of malignancies. However 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.
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 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].

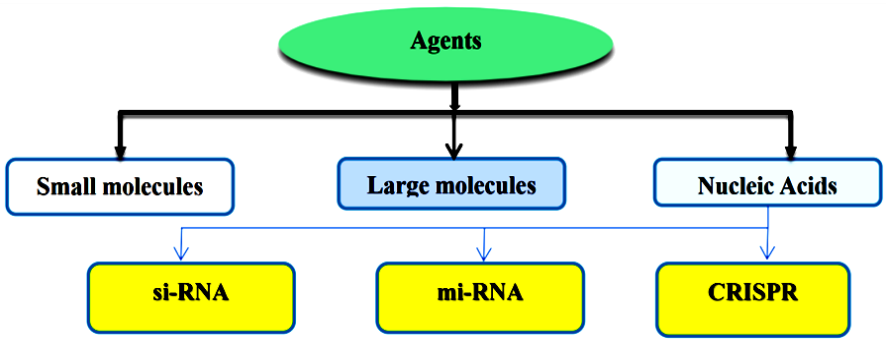

Figure 3: Exosomes as drug delivery system

i) Types of methods perform to collect and purify exosomes: The different types of collection and purification methods are shown in table 1.

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]. Table 2 shows different relative centrifugal force (g) 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	4ºC	Remove cells,
2.	2000g	10 min	4ºC	Dead cells,
3.	10,000g	30 min	4ºC	Cellular debris
4.	100,000g	1 hr 10 min	4ºC	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 purposes exosomes are collected in many different methods and purifying 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 [91]. 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.

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 [92].

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 [92].

iv) Drug loading techniques: mostly applied in pharmaceutical companies [88].

9) Advantages of exosomes as a drug delivery vehicle & diagnostic purpose :

Personalized medicine: Exosomes can be derived from a variety of cell types, allowing for the development of personalized therapies. For instance, exosomes derived from a patient's own healthy cells could be used to treat their specific disease. This personalized approach has the potential to be more effective and have fewer side effects compared to traditional therapies. Detecting tumour mutations through pathological analysis has significantly contributed to the development of personalized therapies for lung cancer patients, leading to increased survival rates [54,55]. **Long circulating half-life** [59]**:** Exosomes naturally possess a long lifespan in circulation due to their unique lipid bilayer composition. This allows them to travel throughout the body and deliver their cargo for extended periods, potentially increasing the efficacy of therapeutic molecules. **Long-distance travellers:** Unlike traditional drug delivery systems, engineered exosomes synergize with ultrasound irradiation, demonstrating a combination of favourable properties: high biocompatibility, extended blood circulation, precise tumour targeting, controlled drug release, and minimal damage to surrounding healthy tissues [60]. **Reduced immunogenicity:** Exosomes exhibit distinct advantages as drug delivery vehicles compared to traditional nanocarriers such as liposomes, metal-based nanoparticles, and polymer nanomaterials. Exosomes are naturally biocompatible and exhibit low immunogenicity, meaning they are less likely to trigger an immune response in the body [58]. These advantages include improved bioavailability, reduced non-targeted cytotoxicity, and minimized immunogenicity. This is a significant advantage compared to synthetic drug carriers, which can often be rejected by the immune system. **Inherent targeting capabilities:** In immunotherapy, engineered exosomes derived from immune cells and tumour cells, serving as immune mediators, have attracted increasing attention due to their inherent characteristics. This inherent targeting ability eliminates the need for complex modifications, simplifying therapeutic development and potentially leading to more precise treatment delivery [57]. **Easily crossing barriers:** Exosomes have a remarkable ability to cross biological membranes [70] barriers, such as the blood-brain barrier, which restricts the passage of most drugs and therapies. This unique property makes them ideal for delivering therapeutic molecules to the central nervous system, a challenging feat for traditional methods [56].

10) Challenges in exosome isolation:

Exosomes hold tremendous promise for various applications, but their isolation presents several significant challenges. Here's a breakdown of the key disadvantages: **Sample Viscosity:** Isolating exosomes from highly viscous samples like blood or serum can be difficult, leading to lower yields. This is because viscous solutions hinder the separation techniques typically used for exosomes. **Centrifugation Sensitivity:** The application of force may result in the deformation and fragmentation of large vesicles, potentially skewing the results of downstream analyses [60]. The isolation process often relies on centrifugation, and even slight variations in time or speed can significantly impact the yield and purity of exosomes. Maintaining precise control over these parameters adds complexity. **Exosome Size Alteration:** Many isolation techniques involve attaching membranes or tags to exosomes for easier separation. This can alter their natural size and potentially affect their function or ability to interact with target cells. **Liposome Contamination:** Differential ultracentrifugation, a commonly employed technique for exosome isolation, faces limitations due to the inherent heterogeneity of the exosome population and significant size overlap with other extracellular vesicles (EVs). This overlap often leads to co-isolation of contaminating EVs and potential losses of desired exosome subpopulations [65]. Liposomes, which are similar in size and structure to exosomes, can contaminate isolated samples. This can make it challenging to obtain pure exosome preparations, which are crucial for therapeutic applications. **Scalability Issues** [88]**:** Techniques like immunological separation, while effective for small-scale isolation, become impractical for processing large volumes needed for clinical applications. **Time-consuming with high cost and complex procedures** [96,97]**:** Current isolation methods can be quite lengthy, hindering large-scale production and potentially affecting the integrity of the exosomes due to prolonged handling. Ultracentrifugation-based exosome isolation methods are hindered by several drawbacks, including high initial equipment costs, complex protocols, extended processing times, and substantial labour requirements. Additionally, their limited portability restricts their use at the point-of-care. Moreover, high-speed centrifugation forces can potentially damage exosomes, compromising their integrity and functionality for

downstream analyses [61,62]. Developing and maintaining sophisticated isolation techniques can be expensive and require specialized equipment and expertise.

11) The overall aim of research in exosomes

Exosomes hold immense potential that extends far beyond their exciting application as drug delivery systems. Here's a breakdown of their broader implications: It can be broadly divided into two main areas: **1. Diagnostics:** To understand the role of exosomes in various diseases by analyzing their cargo (proteins, RNA, etc.) and how it differs between healthy and diseased states [95]. To develop exosomes as biomarkers for early detection and diagnosis of diseases like cancer, diabetes, and neurological disorders. To leverage the accessibility of exosomes in bodily fluids for non-invasive diagnostic tests. Cell-to-Cell Communication [94]: By studying the unique properties and cargo of exosomes, scientists can gain valuable insights into how cells communicate with each other. This knowledge could be crucial for understanding normal physiological processes as well as the development and progression of diseases. Additionally, it might lead to the development of new methods to manipulate cell communication for therapeutic purposes. **2. Therapeutics:** To exploit the unique properties of exosomes, such as their biocompatibility, long circulation time, and ability to cross barriers [71], as drug delivery vehicles. To develop methods to load exosomes with therapeutic agents like proteins, small molecules, or even gene editing tools (CRISPR) [93]. To create personalized therapies by using exosomes derived from a patient's own cells. Long-distance Communication between Organ Systems: Exosomes act as a sophisticated communication network within the body. They can travel long distances, carrying messages from one organ system to another. This newfound understanding of exosome-mediated communication paves the way for a deeper exploration of how different organs interact and influence each other's function. It might also lead to the development of novel therapeutic strategies that target these communication pathways to treat complex diseases.

12) 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 technology advances, exosome-based therapies may become mainstream in veterinary medicine, offering safer and more effective treatment options for animals.

13) Discussion:

Exosomes hold an important area of research with immense potential for therapeutic applications. Their relative ease of isolation and manipulation, both in terms of content and surface molecules, makes them highly attractive. The lipid and protein composition of exosomes naturally enhances their stability and longevity in circulation, further solidifying their role as ideal carriers. Beyond their biocompatibility and lack of toxicity, exosomes exhibit target specificity, minimizing unintended effects. Exosomes, due to their small size (30–120 nm) and minimal safety concerns, are emerging as a promising next-generation drug delivery system. Additionally, large-scale exosome production is feasible, making them more cost-effective and ethically sound compared to MSC (mesenchymal stem cells) therapy. While the potential of exosomes for therapeutics is undeniable, further research is crucial to ensure their safe and effective clinical application. A comprehensive understanding of their composition, immune responses they evoke, and methods to load them with therapeutic cargo without altering their natural properties is essential. Scaling up exosome isolation for clinical use requires overcoming existing limitations. Standardizing protocols for exosome manipulation, isolation, and characterization is paramount to translating this exciting technology from the bench to the bedside.

14) 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 and human well-being. This exploration of the exosomal microenvironment holds significant promise for the development of novel strategies that can benefit both animals and humans.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.

2.

3.

15) References

1. Aguzzi A, Rajendran L. The transcellular spread of cytosolic amyloids, prions, and prionoids. Neuron. 2009;64(6):783-90.
2. Ailawadi S, Wang X, Gu H, Fan GC. Pathologic function and therapeutic potential of exosomes in cardiovascular disease. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2015;1852(1):1-1.
3. Almughlliq FB, Koh YQ, Peiris HN, Vaswani K, Holland O, Meier S, Roche JR, Burke CR, Crookenden MA, Arachchige BJ, Reed S. Circulating exosomes may identify biomarkers for cows at risk for metabolic dysfunction. Scientific Reports. 2019;9(1):13879.
4. Almughlliq FB, Koh YQ, Peiris HN, Vaswani K, McDougall S, Graham EM, Burke CR, Arachchige BJ, Reed S, Mitchell MD. Proteomic content of circulating exosomes in dairy cows with or without uterine infection. Theriogenology. 2018;114:173-9.
5. Bellingham SA, Guo BB, Coleman BM, Hill AF. Exosomes: vehicles for the transfer of toxic proteins associated with neurodegenerative diseases?. Frontiers in physiology. 2012;3:124.
6. Cai M, He H, Jia X, Chen S, Wang J, Shi Y, Liu B, Xiao W, Lai S. Genome-wide microRNA profiling of bovine milk-derived exosomes infected with Staphylococcus aureus. Cell Stress and Chaperones. 2018;23:663-72.
7. Cheruvanky A, Zhou H, Pisitkun T, Kopp JB, Knepper MA, Yuen PS, Star RA. Rapid isolation of urinary exosomal biomarkers using a nanomembrane ultrafiltration concentrator. American Journal of Physiology-Renal Physiology. 2007;292(5):F1657-61.
8. Faruqu FN, Xu L, Al-Jamal KT. Preparation of exosomes for siRNA delivery to cancer cells. JoVE (Journal of Visualized Experiments). 2018; 5(142):e58814.
9. Gong C, Tian J, Wang Z, Gao Y, Wu X, Ding X, Qiang L, Li G, Han Z, Yuan Y, Gao S. Functional exosome-mediated co-delivery of doxorubicin and hydrophobically modified microRNA 159 for triple-negative breast cancer therapy. Journal of nanobiotechnology. 2019;17(1):1-8.
10. Goren Y, Kushnir M, Zafrir B, Tabak S, Lewis BS, Amir O. Serum levels of microRNAs in patients with heart failure. European journal of heart failure. 2012;14(2):147-54.

11. Grey M, Dunning CJ, Gaspar R, Grey C, Brundin P, Sparr E, Linse S. Acceleration of α-synuclein aggregation by exosomes. Journal of Biological Chemistry. 2015;290(5):2969-82.
12. Harding C, Stahl P. Transferrin recycling in reticulocytes: pH and iron are important determinants of ligand binding and processing. Biochemical and biophysical research communications. 1983;113(2):650-8.
13. Heijnen HF, Schiel AE, Fijnheer R, Geuze HJ, Sixma JJ. Activated Platelets Release Two Types of Membrane Vesicles: Microvesicles by Surface Shedding and Exosomes Derived From Exocytosis of Multivesicular Bodies and□-Granules. Blood, The Journal of the American Society of Hematology. 1999;94(11):3791-9.
14. Hood JL, Pan H, Lanza GM, Wickline SA. Paracrine induction of endothelium by tumor exosomes. Laboratory investigation. 2009;89(11):1317-28.
15. Hood JL, San RS, Wickline SA. Exosomes released by melanoma cells prepare sentinel lymph nodes for tumor metastasis. Cancer research. 2011;71(11):3792-801.
16. Howitt J, Hill AF. Exosomes in the pathology of neurodegenerative diseases. Journal of Biological Chemistry. 2016;291(52):26589-97.
17. Johnstone RM, Adam M, Hammond JR, Orr L, Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). Journal of Biological Chemistry. 1987;262(19):9412-20.
18. Kang JH, Irwin DJ, Chen-Plotkin AS, Siderowf A, Caspell C, Coffey CS, Waligórska T, Taylor P, Pan S, Frasier M, Marek K. Association of cerebrospinal fluid β-amyloid 1-42, T-tau, P-tau181, and α-synuclein levels with clinical features of drug-naive patients with early Parkinson disease. JAMA neurology. 2013;70(10):1277-87.
19. Kim MS, Haney MJ, Zhao Y, Yuan D, Deygen I, Klyachko NL, Kabanov AV, Batrakova EV. Engineering macrophage-derived exosomes for targeted paclitaxel delivery to pulmonary metastases: in vitro and in vivo evaluations. Nanomedicine: Nanotechnology, Biology and Medicine. 2018;14(1):195-204.
20. Lee RH, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL, Semprun-Prieto L, Delafontaine P, Prockop DJ. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell stem cell. 2009;5(1):54-63.
21. Lin Y, Wu J, Gu W, Huang Y, Tong Z, Huang L, Tan J. Exosome–liposome hybrid nanoparticles deliver CRISPR/Cas9 system in MSCs. Advanced Science. 2018;5(4):1700611.
22. Liu T, Zhang X, Gao S, Jing F, Yang Y, Du L, Zheng G, Li P, Li C, Wang C. Exosomal long noncoding RNA CRNDE-h as a novel serum-based biomarker for diagnosis and prognosis of colorectal cancer. Oncotarget. 2016;7(51):85551.
23. Livshits MA, Khomyakova E, Evtushenko EG, Lazarev VN, Kulemin NA, Semina SE, Generozov EV, Govorun VM. Isolation of exosomes by differential centrifugation: Theoretical analysis of a commonly used protocol. Scientific reports. 2015;5(1):17319.
24. Montaner-Tarbes S, Pujol M, Jabbar T, Hawes P, Chapman D, Portillo HD, Fraile L, Sánchez-Cordón PJ, Dixon L, Montoya M. Serum-derived extracellular vesicles from African swine fever virus-infected pigs selectively recruit viral and porcine proteins. Viruses. 2019;11(10):882.
25. Munagala R, Aqil F, Jeyabalan J, Gupta RC. Bovine milk-derived exosomes for drug delivery. Cancer letters. 2016;371(1):48-61.
26. Oskouie MN, Aghili Moghaddam NS, Butler AE, Zamani P, Sahebkar A. Therapeutic use of curcumin-encapsulated and curcumin-primed exosomes. Journal of cellular physiology. 2019;234(6):8182-91.
27. Oves M, Qari HA, Felemban NM, Khan AA, Rehan M, Tabrez S, Ahmed F, Haque A, Khan MS, Khan JM, Husain FM. Exosomes: a paradigm in drug development against cancer and infectious diseases. Journal of Nanomaterials. 2018;2018.
28. Pan BT, Johnstone RM. Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: selective externalization of the receptor. Cell. 1983;33(3):967-78.
29. Patil SM, Sawant SS, Kunda NK. Exosomes as drug delivery systems: a brief overview and progress update. European Journal of Pharmaceutics and Biopharmaceutics. 2020;154:259-69.
30. Qin J, Xu Q. Functions and application of exosomes. Acta Pol Pharm. 2014;71(4):537-43.
31. Rout ED, Webb TL, Laurence HM, Long L, Olver CS. Transferrin receptor expression in serum exosomes as a marker of regenerative anaemia in the horse. Equine veterinary journal. 2015;47(1):101-6.
32. Schorey JS, Cheng Y, Singh PP, Smith VL. Exosomes and other extracellular vesicles in host–pathogen interactions. EMBO reports. 2015;16(1):24-43.

33. Schwartz SL, Cao C, Pylypenko O, Rak A, Wandinger-Ness A. Rab GTPases at a glance. Journal of cell science. 2007;120(22):3905-10.
34. Simons M, Raposo G. Exosomes–vesicular carriers for intercellular communication. Current opinion in cell biology. 2009;21(4):575-81.
35. Saman S, Kim W, Raya M, Visnick Y, Miro S, Saman S, Jackson B, McKee AC, Alvarez VE, Lee NC, Hall GF. Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid in early Alzheimer disease. Journal of biological chemistry. 2012 Feb 1;287(6):3842-9.
36. Skog JK, Russo L. Cerebrospinal fluid assay. Patent No US20150038335. Washington, DC: US Patent and Trademark Office. 2015.
37. Takov K, Yellon DM, Davidson SM. Comparison of small extracellular vesicles isolated from plasma by ultracentrifugation or size-exclusion chromatography: yield, purity and functional potential. Journal of extracellular vesicles. 2019;8(1):1560809.
38. Tauro BJ, Greening DW, Mathias RA, Ji H, Mathivanan S, Scott AM, Simpson RJ. Comparison of ultracentrifugation, density gradient separation, and immunoaffinity capture methods for isolating human colon cancer cell line LIM1863-derived exosomes. Methods. 2012;56(2):293-304.
39. Théry C, Amigorena S, Raposo G, Clayton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. Current protocols in cell biology. 2006;30(1):3-22.
40. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, Antoniou A, Arab T, Archer F, Atkin-Smith GK, Ayre DC. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. Journal of extracellular vesicles. 2018;7(1):1535750.
41. Théry C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. Nature reviews immunology. 2002;2(8):569-79.
42. Tian Y, Li S, Song J, Ji T, Zhu M, Anderson GJ, Wei J, Nie G. A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy. Biomaterials. 2014;35(7):2383-90.
43. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nature cell biology. 2007;9(6):654-9.
44. Vella LJ, Greenwood DL, Cappai R, Scheerlinck JP, Hill AF. Enrichment of prion protein in exosomes derived from ovine cerebral spinal fluid. Veterinary immunology and immunopathology. 2008;124(3-4):385-93.
45. Yang T, Martin P, Fogarty B, Brown A, Schurman K, Phipps R, Yin VP, Lockman P, Bai S. Exosome delivered anticancer drugs across the blood-brain barrier for brain cancer therapy in Danio rerio. Pharmaceutical research. 2015;32:2003-14.
46. Zaborowski MP, Balaj L, Breakefield XO, Lai CP. Extracellular vesicles: composition, biological relevance, and methods of study. Bioscience. 2015;65(8):783-97.
47. Zacharias E, Milton G, Momen-Heravi F, Hu J, Zhang X, Wu Y. Therapeutic uses of exosomes. J Circulat Biomarkers. 2013;1(1).
48. Zhou C, Cai G, Meng F, Xu Z, He Y, Hu Q, Zheng E, Huang S, Xu Z, Gu T, Hu B. Deep-sequencing identification of MicroRNA biomarkers in serum exosomes for early pig pregnancy. Frontiers in Genetics. 2020;11:536.
49. Zhuang X, Xiang X, Grizzle W, Sun D, Zhang S, Axtell RC, Ju S, Mu J, Zhang L, Steinman L, Miller D. Treatment of brain inflammatory diseases by delivering exosome encapsulated anti-inflammatory drugs from the nasal region to the brain. Molecular Therapy. 2011;19(10):1769-79.
50. Ren J, He W, Zheng L, Duan H. From structures to functions: insights into exosomes as promising drug delivery vehicles. Biomaterials science. 2016;4(6):910-21.
51. Kourembanas S. Exosomes: vehicles of intercellular signaling, biomarkers, and vectors of cell therapy. Annual review of physiology. 2015;77:13-27.
52. Record M, Carayon K, Poirot M, Silvente-Poirot S. Exosomes as new vesicular lipid transporters involved in cell–cell communication and various pathophysiologies. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids. 2014;1841(1):108-20.
53. Dutta S, Reamtong O, Panvongsa W, Kitdumrongthum S, Janpipatkul K, Sangvanich P, Piyachaturawat P, Chairoungdua A. Proteomics profiling of cholangiocarcinoma exosomes: A potential role of oncogenic protein

transferring in cancer progression. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2015;1852(9):1989-99.
54. Ung TH, Madsen HJ, Hellwinkel JE, Lencioni AM, Graner MW. Exosome proteomics reveals transcriptional regulator proteins with potential to mediate downstream pathways. Cancer science. 2014;105(11):1384-92.
55. De Mello RA, Madureira P, Carvalho LS, Araujo A, O'Brien M, Popat S. EGFR and KRAS mutations, and ALK fusions: current developments and personalized therapies for patients with advanced non-small-cell lung cancer. Pharmacogenomics. 2013;14(14):1765-77.
56. Wood MJ, O'Loughlin AJ, Lakhal S. Exosomes and the blood–brain barrier: implications for neurological diseases. Therapeutic delivery. 2011;2(9):1095-9.
57. Zhang M, Hu S, Liu L, Dang P, Liu Y, Sun Z, Qiao B, Wang C. Engineered exosomes from different sources for cancer-targeted therapy. Signal transduction and targeted therapy. 2023;8(1):124.
58. Srivastava A, Amreddy N, Razaq M, Towner R, Zhao YD, Ahmed RA, Munshi A, Ramesh R. Exosomes as theranostics for lung cancer. Advances in cancer research. 2018;139:1-33.
59. Xu M, Yang Q, Sun X, Wang Y. Recent advancements in the loading and modification of therapeutic exosomes. Frontiers in Bioengineering and Biotechnology. 2020;8:586130.
60. Batrakova EV, Kim MS. Using exosomes, naturally-equipped nanocarriers, for drug delivery. Journal of Controlled Release. 2015;219:396-405.
61. Li P, Kaslan M, Lee SH, Yao J, Gao Z. Progress in exosome isolation techniques. Theranostics. 2017;7(3):789.
62. Jeppesen DK, Hvam ML, Primdahl-Bengtson B, Boysen AT, Whitehead B, Dyrskjøt L, Ørntoft TF, Howard KA, Ostenfeld MS. Comparative analysis of discrete exosome fractions obtained by differential centrifugation. Journal of extracellular vesicles. 2014;3(1):25011.
63. Sadri M, Shu J, Kachman SD, Cui J, Zempleni J. Milk exosomes and miRNA cross the placenta and promote embryo survival in mice. Reproduction. 2020;160(4):501-9.
64. Ohno SI, Takanashi M, Sudo K, Ueda S, Ishikawa A, Matsuyama N, Fujita K, Mizutani T, Ohgi T, Ochiya T, Gotoh N. Systemically injected exosomes targeted to EGFR deliver antitumor microRNA to breast cancer cells. Molecular therapy. 2013 Jan 1;21(1):185-91.
65. Atay S, Godwin AK. Tumor-derived exosomes: A message delivery system for tumor progression. Communicative & integrative biology. 2014;7(1):e28231.
66. Rak J, Guha A. Extracellular vesicles–vehicles that spread cancer genes. Bioessays. 2012;34(6):489-97.
67. Martins VR, Dias MS, Hainaut P. Tumor-cell-derived microvesicles as carriers of molecular information in cancer. Current opinion in oncology. 2013;25(1):66-75.
68. Lowry MC, Gallagher WM, O'Driscoll L. The role of exosomes in breast cancer. Clinical chemistry. 2015;61(12):1457-65.
69. Aryani A, Denecke B. Exosomes as a nanodelivery system: a key to the future of neuromedicine?. Molecular neurobiology. 2016;53:818-34.
70. Ha D, Yang N, Nadithe V. Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges. Acta Pharmaceutica Sinica B. 2016;6(4):287-96.
71. Jiang XC, Gao JQ. Exosomes as novel bio-carriers for gene and drug delivery. International journal of pharmaceutics. 2017;521(1-2):167-75.
72. van der Meel R, Fens MH, Vader P, Van Solinge WW, Eniola-Adefeso O, Schiffelers RM. Extracellular vesicles as drug delivery systems: lessons from the liposome field. Journal of controlled release. 2014;195:72-85.
73. Steinbichler TB, Dudás J, Skvortsov S, Ganswindt U, Riechelmann H, Skvortsova II. Therapy resistance mediated by exosomes. Molecular cancer. 2019;18(1):1-1.
74. Sun D, Zhuang X, Xiang X, Liu Y, Zhang S, Liu C, Barnes S, Grizzle W, Miller D, Zhang HG. A novel nanoparticle drug delivery system: the anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes. Molecular therapy. 2010;18(9):1606-14.
75. Shao R. YKL-40: A potential biomarker and therapeutic target for breast cancer diagnosis and therapy. British Journal of Medicine and Medical Research. 2012;2(3):358-72.
76. Alghifari RM, Alhusayni NI, Alyamani ZF, Sabban A, Almoghrabi Y, Bakheit KH. The Role of Biochemical Markers in the Prediction of Preeclampsia. International Journal of Biochemistry Research & Review. 2023;32(8):39-47.

77. Zhou SS, Jin JP, Wang JQ, Zhang ZG, Freedman JH, Zheng Y, Cai L. miRNAS in cardiovascular diseases: potential biomarkers, therapeutic targets and challenges. Acta Pharmacologica Sinica. 2018 Jul;39(7):1073-84.
78. Bebelman MP, Smit MJ, Pegtel DM, Baglio SR. Biogenesis and function of extracellular vesicles in cancer. Pharmacology & therapeutics. 2018;188:1-1.
79. Kul Y, Erbaş O. Exosomes: Classification, Isolation, and Therapeutic Applications in Various Diseases. Journal of Experimental and Basic Medical Sciences. 2022;3(1):006-12.
80. Thomas L, Florio T, Perez-Castro C. Extracellular vesicles loaded miRNAs as potential modulators shared between glioblastoma, and Parkinson's and Alzheimer's diseases. Frontiers in Cellular Neuroscience. 2020 Nov 4;14:590034.
81. Kurtzwald-Josefson E, Zeevi-Levin N, Rubchevsky V, Bechar Erdman N, Schwartz Rohaker O, Nahum O, Hochhauser E, Ben-Avraham B, Itskovitz-Eldor J, Aravot D, D. Barac Y. Cardiac fibroblast-induced pluripotent stem cell-derived exosomes as a potential therapeutic mean for heart failure. International Journal of Molecular Sciences. 2020 Sep 29;21(19):7215.
82. Aurich H, Sgodda M, Kaltwaßer P, Vetter M, Weise A, Liehr T, Brulport M, Hengstler JG, Dollinger MM, Fleig WE, Christ B. Hepatocyte differentiation of mesenchymal stem cells from human adipose tissue in vitro promotes hepatic integration in vivo. Gut. 2009 Apr 1;58(4):570-81.
83. Morigi M, Imberti B, Zoja C, Corna D, Tomasoni S, Abbate M, Rottoli D, Angioletti S, Benigni A, Perico N, Alison M. Mesenchymal stem cells are renotropic, helping to repair the kidney and improve function in acute renal failure. Journal of the American Society of Nephrology. 2004 Jul 1;15(7):1794-804.
84. Lai RC, Arslan F, Lee MM, Sze NS, Choo A, Chen TS, Salto-Tellez M, Timmers L, Lee CN, El Oakley RM, Pasterkamp G. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. Stem cell research. 2010 May 1;4(3):214-22.
85. Hornung S, Dutta S, Bitan G. CNS-derived blood exosomes as a promising source of biomarkers: opportunities and challenges. Frontiers in Molecular Neuroscience. 2020 Mar 19;13:38.
86. Dong Z, Gu H, Guo Q, Liu X, Li F, Liu H, Sun L, Ma H, Zhao K. Circulating small extracellular vesicle-derived miR-342-5p ameliorates beta-amyloid formation via targeting beta-site APP cleaving enzyme 1 in Alzheimer's disease. Cells. 2022 Nov 29;11(23):3830.
87. Naryzhny S, Volnitskiy A, Kopylov A, Zorina E, Kamyshinsky R, Bairamukov V, Garaeva L, Shlikht A, Shtam T. Proteome of glioblastoma-derived exosomes as a source of biomarkers. Biomedicines. 2020 Jul 16;8(7):216.
88. Ahn SH, Ryu SW, Choi H, You S, Park J, Choi C. Manufacturing therapeutic exosomes: from bench to industry. Molecules and cells. 2022 May 1;45(5):284-90.
89. Arenaccio C, Chiozzini C, Columba-Cabezas S, Manfredi F, Affabris E, Baur A, Federico M. Exosomes from human immunodeficiency virus type 1 (HIV-1)-infected cells license quiescent CD4+ T lymphocytes to replicate HIV-1 through a Nef-and ADAM17-dependent mechanism. Journal of virology. 2014 Oct 1;88(19):11529-39.
90. Arenaccio C, Chiozzini C, Columba-Cabezas S, Manfredi F, Federico M. Cell activation and HIV-1 replication in unstimulated CD4+ T lymphocytes ingesting exosomes from cells expressing defective HIV-1. Retrovirology. 2014 Dec;11:1-6.
91. Chen J, Li P, Zhang T, Xu Z, Huang X, Wang R, Du L. Review on strategies and technologies for exosome isolation and purification. Frontiers in bioengineering and biotechnology. 2022 Jan 5;9:811971.
92. Xi XM, Xia SJ, Lu R. Drug loading techniques for exosome-based drug delivery systems. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2021 Feb 25;76(2-3):61-7.
93. Duan L, Ouyang K, Wang J, Xu L, Xu X, Wen C, Xie Y, Liang Y, Xia J. Exosomes as targeted delivery platform of CRISPR/Cas9 for therapeutic genome editing. ChemBioChem. 2021 Dec 10;22(24):3360-8.
94. Dragomir M, Chen B, Calin GA. Exosomal lncRNAs as new players in cell-to-cell communication. Translational cancer research. 2018 Mar;7(Suppl 2):S243.
95. Samanta S, Rajasingh S, Drosos N, Zhou Z, Dawn B, Rajasingh J. Exosomes: new molecular targets of diseases. Acta Pharmacologica Sinica. 2018 Apr;39(4):501-13.
96. Liang LG, Kong MQ, Zhou S, Sheng YF, Wang P, Yu T, Inci F, Kuo WP, Li LJ, Demirci U, Wang S. An integrated double-filtration microfluidic device for isolation, enrichment and quantification of urinary extracellular vesicles for detection of bladder cancer. Scientific reports. 2017 Apr 24;7(1):46224.
97. Merchant ML, Rood IM, Deegens JK, Klein JB. Isolation and characterization of urinary extracellular vesicles: implications for biomarker discovery. Nature Reviews Nephrology. 2017 Dec;13(12):731-49.

98. Sousa D, Lima RT, Vasconcelos MH. Intercellular transfer of cancer drug resistance traits by extracellular vesicles. Trends in molecular medicine. 2015 Oct 1;21(10):595-608.
99. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. Journal of Cell Biology. 2013 Feb 18;200(4):373-83.
100. Dinkins MB, Dasgupta S, Wang G, Zhu G, Bieberich E. Exosome reduction in vivo is associated with lower amyloid plaque load in the 5XFAD mouse model of Alzheimer's disease. Neurobiology of aging. 2014 Aug 1;35(8):1792-800.
101. Février B, Vilette D, Laude H, Raposo G. Exosomes: a bubble ride for prions?. Traffic. 2005 Jan;6(1):10-7.
102. Wu X, Zheng T, Zhang B. Exosomes in Parkinson's disease. Neuroscience bulletin. 2017 Jun;33:331-8.
103. Hill AF. Exosomes in Neurological Disease. Current Medical Literature: Neurology. 2009 Jun 1;25(2).
104. Bellingham SA, Guo BB, Coleman BM, Hill AF. Exosomes: vehicles for the transfer of toxic proteins associated with neurodegenerative diseases?. Frontiers in physiology. 2012 May 3;3:124.