(ISSN: 0973-7049(P))

The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES 21(3): S.I (3), 380-423, 2026 www.thebioscan.com

EXOSOMES - BASED DRUG DELIVERY SYSTEMS: EMERGING OPPORTUNITIES FOR TARGETED THERAPEUTICS AND PRECISION MEDICINE

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DOI: https://doi.org/10.63001/tbs.2026.v21.i03.S.I(3).pp380-423

KEYWORDS
Exosomes; Targeted Drug Delivery; Precision Medicine; Nanomedicine.

Article Info
Received: 20.06.2026
Accepted: 08.07.2026
Published: 18.07.2026

Abstract
Exosome-based drug delivery systems have emerged as a promising platform for targeted therapeutics and precision medicine because of their excellent biocompatibility, low immunogenicity, and intrinsic ability to transport diverse therapeutic molecules. Unlike conventional nanocarriers, exosomes efficiently deliver proteins, nucleic acids, and small-molecule drugs while overcoming biological barriers, including the blood–brain barrier. This review highlights the biological characteristics, biogenesis, sources, isolation, purification, and characterization techniques of exosomes. It also discusses recent advances in drug-loading strategies, surface engineering, and exosome modification to improve targeting efficiency and therapeutic performance. Furthermore, the review summarizes the applications of exosome-based drug delivery in cancer, neurological disorders, cardiovascular diseases, inflammatory conditions, infectious diseases, regenerative medicine, and gene therapy. Despite their remarkable therapeutic potential, challenges related to large-scale production, standardization, storage stability, and regulatory approval remain significant barriers to clinical translation. Continued research and technological advancements are expected to facilitate the successful integration of exosome-based therapeutics into personalized healthcare.

1.INTRODUCTION
Drug delivery systems play a vital role in modern medicine by ensuring that therapeutic agents reach their intended target site in a safe, controlled, and efficient manner. Conventional drug delivery methods, including oral, intravenous, and topical administration, often

suffer from limitations such as poor bioavailability, rapid drug degradation, non-specific distribution, and systemic toxicity [1]. These challenges reduce therapeutic efficacy and increase the risk of adverse effects, particularly in the treatment of complex diseases such as cancer, neurodegenerative disorders, cardiovascular diseases, and chronic inflammatory conditions [2]. Consequently, the development of advanced drug delivery systems has become a major focus of pharmaceutical research. Nanotechnology has significantly transformed drug delivery by introducing nanocarriers such as liposomes, polymeric nanoparticles, dendrimers, micelles, and inorganic nanoparticles. These nanocarriers improve drug solubility, protect therapeutic molecules from degradation, and enable controlled drug release [3].

Despite these advantages, synthetic nanocarriers still face several limitations, including immunogenicity, cytotoxicity, limited targeting efficiency, rapid clearance by the immune system, and challenges in large-scale manufacturing. These drawbacks have encouraged researchers to explore naturally derived nanocarriers that offer improved safety and biological compatibility. In recent years, significant progress has been made in the isolation, purification, engineering, surface modification, and drug-loading of exosomes, greatly expanding their potential in clinical applications [4,5]. These advances have improved the ability of exosomes to deliver therapeutic agents more efficiently and selectively to target tissues. As a result, exosome-based therapies have shown promising outcomes in the treatment of cancer, neurological disorders, cardiovascular diseases, infectious diseases, and tissue regeneration. In addition, exosomes can effectively transport therapeutic nucleic acids, including siRNA, miRNA, mRNA, and CRISPR/Cas9 components, making them valuable tools for gene therapy. Their unique biological properties and versatile therapeutic potential have positioned exosomes as an important platform for the development of targeted treatments and personalized medicine [6].

1.1 Overview of Drug Delivery Systems

Drug delivery systems (DDS) play a crucial role in modern medicine by ensuring that therapeutic agents are delivered to the desired site of action in a safe and effective manner [7]. An efficient drug delivery system is designed to improve treatment outcomes by enhancing drug absorption and bioavailability, reducing unwanted side effects, and maintaining the required drug concentration for an extended period. The ultimate goal of a DDS is to deliver the right dose of a drug to the right target at the right time, thereby improving therapeutic effectiveness while minimizing toxicity and improving patient compliance [8]. To overcome these challenges, significant advancements have been made in the development of novel drug delivery systems.

Nanotechnology has played a pivotal role in this progress by introducing nanoscale carriers such as liposomes, polymeric nanoparticles, dendrimers, micelles, and solid lipid nanoparticles. These nanocarriers improve drug stability, enable controlled and sustained drug release, protect therapeutic molecules from premature degradation, and facilitate targeted delivery to diseased tissues [9]. Despite these advantages, many synthetic nanocarriers still encounter limitations such as immunogenicity, cytotoxicity, rapid clearance by the mononuclear phagocyte system, and difficulties in large-scale production.

1.2 Limitations of Conventional Nanocarriers

Conventional nanocarriers, including liposomes, polymeric nanoparticles, dendrimers, micelles, and solid lipid nanoparticles, have played an important role in improving drug delivery by enhancing drug stability, increasing bioavailability, and providing controlled drug release [10]. These nanocarriers have been successfully used to deliver a variety of therapeutic agents and have shown promising results in the treatment of several diseases. However, despite these advantages, their clinical application is still limited by a number of challenges. One of the major concerns associated with conventional nanocarriers is their limited biocompatibility, as some synthetic materials may trigger immune responses or inflammatory reactions after administration. Certain nanomaterials may also exhibit cytotoxicity, which can adversely affect healthy tissues and compromise patient safety [11]. Furthermore, many synthetic nanocarriers are rapidly cleared from the bloodstream by the mononuclear phagocyte system (MPS), reducing their circulation time and limiting the amount of drug that reaches the intended target site.

1.3 Emergence of Exosome-Based Therapeutics

The challenges associated with conventional drug delivery systems have encouraged researchers to explore safer, more efficient, and biologically compatible alternatives. Among these, exosomes have attracted considerable attention as natural nanocarriers because of their unique biological properties and promising therapeutic potential. Exosomes are small extracellular vesicles, typically measuring 30–150 nm in diameter, and are released by almost all cell types.

They play a vital role in cell-to-cell communication by transporting a diverse range of bioactive molecules, including proteins, lipids, messenger RNA (mRNA), microRNA (miRNA), DNA fragments, and other signaling molecules that regulate various physiological and pathological processes [12]. Compared with synthetic nanocarriers, exosomes offer several distinct advantages, including excellent biocompatibility, low immunogenicity, and minimal toxicity, making them highly suitable for therapeutic applications [13]. Their naturally derived lipid membrane helps them evade immune detection, prolongs their circulation time, and enables them to cross biological barriers such as the blood–brain barrier.

1.4 Scope and Objectives of the Review In recent years, exosome-based drug delivery has attracted significant attention as a promising approach for improving the precision and effectiveness of therapeutic interventions. Owing to their natural origin, excellent biocompatibility, and ability to transport a wide range of therapeutic molecules, exosomes have emerged as potential alternatives to conventional drug delivery systems. Their unique properties have created new opportunities for targeted treatment and personalized medicine [14]. Despite these encouraging developments, several scientific and technological challenges must still be addressed before exosome-based therapies can be widely adopted in clinical practice. This article presents a comprehensive overview of the current knowledge on exosome-based drug delivery systems, beginning with their biological characteristics, cellular sources, and methods of isolation and characterization [15].It further examines recent advances in exosome engineering, drug-loading strategies, and the mechanisms responsible for targeted drug delivery. The review also summarizes their therapeutic applications across various diseases, including cancer, neurological, cardiovascular, and inflammatory disorders, while highlighting their growing role in gene delivery and precision medicine. Finally, current challenges, regulatory considerations, and future research directions are

discussed to provide a broader perspective on the clinical potential of exosome-based therapeutics.

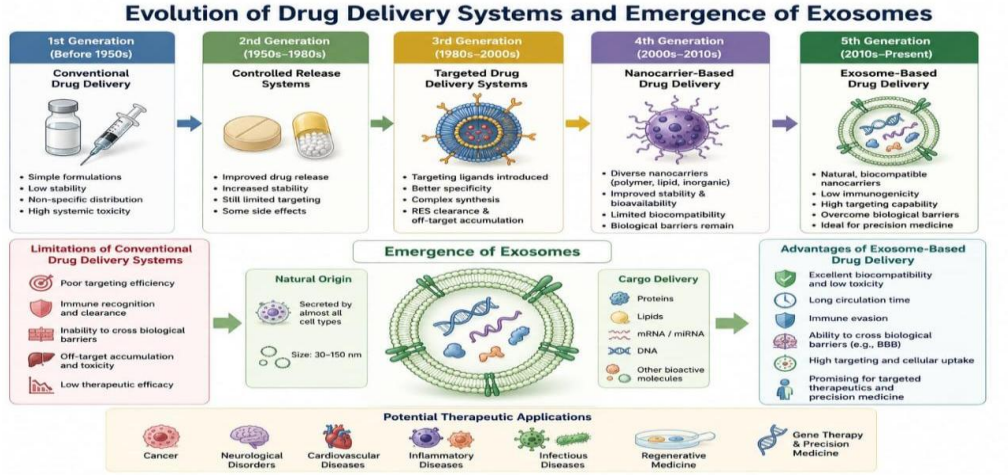

Figure 1: Evolution of Drug Delivery Systems and Emergence of Exosomes.

2. BIOLOGY AND CHARACTERISTICS OF EXOSOMES

2.1 Definition and Classification of Extracellular Vesicles

The definition and classification of EVs remain a topic of great debate. The definition and classification of EVs are still subject of debate. Extracellular vesicles (EVs) are plasma membrane-derived nano-sized extracellular vesicles that are spontaneously secreted by nearly all cell types [16]. A key mechanism for intercellular communication involves the transport of a variety of bioactive molecules, such as proteins, lipids, DNA, messenger RNA (mRNA), and microRNAs (miRNAs), from donor to recipient cells within these vesicles. EVs are generally categorized into three groups: exosomes, microvesicles and apoptotic bodies, based on their size, biogenesis, and biological characteristics. The smallest type of EVs, known as exosomes, are approximately 30–150 nm in size. They are derived from the endosomal pathway and are excreted after the fusion of multivesicular bodies (MVBs) with the plasma membrane [17]. Due to their biogenesis and molecular composition, exosomes are highly stable, low in immunogenicity and have intrinsic ability to carry therapeutic cargo across biological barriers [18]. Microvesicles are comparatively bigger in size ranging from 100–1000 nm and originate from the direct outward budding of the plasma membrane [19]. They are involved in many physiological processes such as cell signaling, inflammation and coagulation. Apoptotic bodies, on the other hand, are the largest sized EVs (500-5000nm) and are produced when cells undergo programmed cell death. These are vesicles filled with various parts of cells and cell nuclei that allow the safe removal of cell debris by phagocytic cells [20].

Table 1: Comparison of Exosomes, Microvesicles, and Apoptotic Bodies

Characteristic	Exosomes	Microvesicles	Apoptotic Bodies
Size	30–150 nm	100–1000 nm	500–5000 nm
Biogenesis	Formed by inward budding of endosomal membranes and released upon fusion of multivesicular bodies (MVBs) with the plasma membrane	Generated by direct outward budding and shedding of the plasma membrane	Produced during programmed cell death (apoptosis) through membrane blebbing and cell fragmentation
Cellular Origin	Most living eukaryotic cells	Most living eukaryotic cells	Cells undergoing apoptosis
Membrane Composition	Lipid bilayer enriched with cholesterol, sphingomyelin, ceramides, and phosphatidylserine	Lipid bilayer resembling the plasma membrane	Lipid bilayer enclosing fragmented cellular components
Major Cargo	Proteins, lipids, mRNA, miRNA, lncRNA, DNA fragments, metabolites	Proteins, lipids, RNA, DNA, cytokines, membrane receptors	Cellular organelles, chromatin, DNA fragments, cytoplasmic proteins
Common Surface Markers	CD9, CD63, CD81, Alix, TSG101, HSP70	Integrins, selectins, CD40, phosphatidylserine	Annexin V-binding phosphatidylserine, histones
Primary Biological Functions	Intercellular communication, immune regulation, tissue repair, gene transfer, drug delivery	Cell signaling, inflammation, coagulation, immune modulation	Clearance of apoptotic debris and maintenance of tissue homeostasis
Isolation Difficulty	Moderate to high due to small size and similarity with other EVs	Moderate	Relatively easy because of larger size
Therapeutic Potential	Excellent; widely investigated as natural drug and gene delivery vehicles	Moderate; useful in biomarker discovery and regenerative medicine	Limited; mainly studied as biomarkers of apoptosis

2.2 Biogenesis of Exosomes

Exosome biogenesis is tightly regulated, an endosomal trafficking pathway. It starts with the inward invagination of the plasma membrane to form early endosomes [21]. These develop into late endosomes where the endosomal membrane continues to bud inward to form many intraluminal vesicles (ILVs), and multivesicular bodies (MVBs). The Endosomal Sorting

Complex Required for Transport (ESCRT) machinery is the main mediator of the sorting of proteins, lipids and nucleic acids into ILVs [22]. Other pathways, such as ceramides, tetraspanins and lipid raft associated proteins, however, have been reported to be involved in exosome formation in an ESCRT independent manner. Once matured, MVBs can fuse with lysosomes and be degraded or with the plasma membrane, which leads to the release of ILVs as exosomes into the extracellular space [23]. Rab GTPases, SNARE proteins and intracellular calcium signaling regulate this secretion process. Exosome biogenesis isn't a fixed, one-size-fits-all process — it shifts depending on what state the parent cell is in, whether that's healthy, stressed, or diseased. When cells encounter stress, like low oxygen, inflammation, oxidative damage, or a lack of nutrients, they tend to ramp up exosome production and change what gets packed inside them [24].

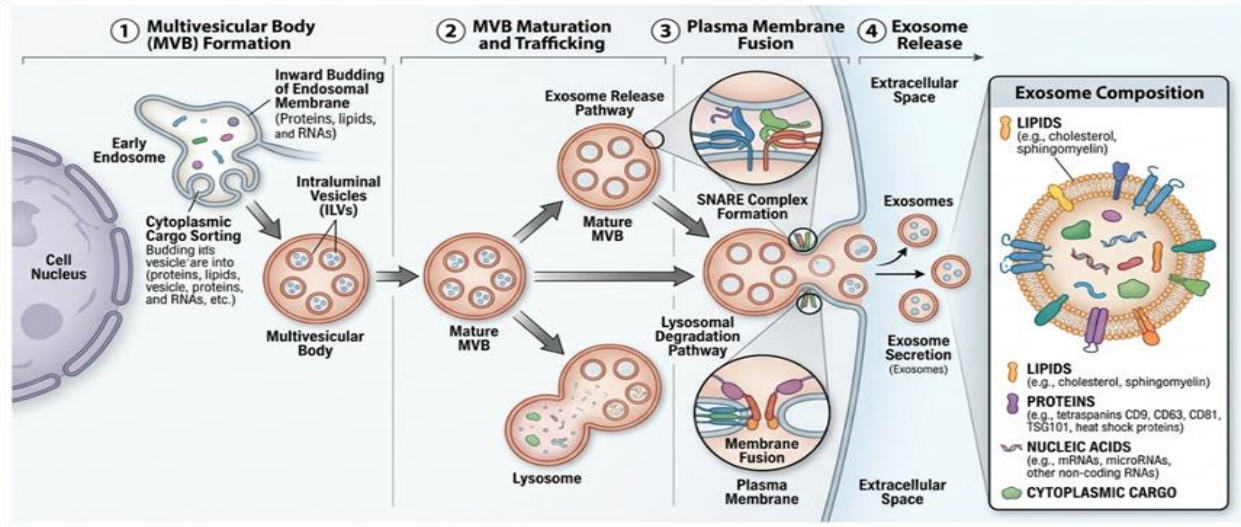

Figure 2: Biogenesis and Release of Exosomes

2.3 Structural Characteristics

The lipids composing the lipid bilayer membrane of exosomes are enriched with cholesterol, sphingomyelin, phosphatidylserine, and ceramides that help stabilize the structure of the exosome and shield its internal contents from enzymatic degradation [25]. They have a membrane that is very similar to the membrane of their host cell, but they also have their own special surface markers that help to identify them and get them into the host cell. The exosome membrane is characterized by the presence of certain proteins such as the tetraspanins CD9, CD63 and CD81, Alix, TSG101, heat shock proteins (HSP70 and HSP90), adhesion molecules etc [26,27]. These proteins are known to be broadly distributed as molecular markers for the identification and characterization of exosomes. The contents of exosomes can vary and include proteins, enzymes, lipids, metabolites, mRNA, microRNA, long non-coding RNA (lncRNA) and even fragments of DNA in some cases. The contents of exosomes

vary from one cell to another and under different physiological conditions, allowing them to modulate many cellular pathways in recipient cells. Their size, stability of their membranes and the protection that they afford to therapeutic molecules increase their usefulness as natural vehicles for drug delivery at the nanoscale [28].

2.4 Physiological Functions

Exosomes are key mediators of intercellular communication, carrying functional biomolecules from neighboring cells to distant cells. Exosomes deliver their molecular contents to the recipient cells, where they modulate the expression of genes, protein production, immune functions, angiogenesis and tissue repair. In physiological conditions, exosomes play regulatory roles in immune surveillance, maintaining tissue homeostasis, neuronal communication, differentiation of stem cells and wound healing. However, under pathological conditions, they can promote disease processes such as tumor growth, metastasis, immune evasion, chronic inflammation, and dissemination of pathogens [29]. This dual function has had many diagnostic and therapeutic research interests. In contrast to numerous synthetic nanocarriers, exosomes have natural targeting properties and very long circulation time with minimal toxicity and biocompatibility. The beneficial biological properties have made exosomes attractive vessels for targeted drug delivery, gene therapy and precision medicine [30].

3.SOURCES OF EXOSOMES

Exosomes are naturally occurring extracellular vesicles secreted by almost all cell types under both physiological and pathological conditions. Their biological composition and therapeutic potential largely depend on the type of parent cell from which they originate [31]. Different sources of exosomes exhibit distinct molecular cargo, including proteins, lipids, nucleic acids, and signaling molecules, which influence their biological functions and clinical applications. Selecting an appropriate exosome source is therefore a critical factor in designing effective drug delivery systems. Among the various sources, mesenchymal stem cells, immune cells, tumor cells, dendritic cells, and plants have received considerable attention because of their unique characteristics and therapeutic advantages. In recent years, exosomes derived from mesenchymal stem cells, dendritic cells, immune cells, tumor cells, and plants have gained significant attention because of their unique advantages in targeted therapeutics, regenerative medicine, immunotherapy, and precision medicine [32].

Exosomes are naturally released by a wide variety of eukaryotic cells and can be found in almost all body fluids, including blood, urine, saliva, cerebrospinal fluid, breast milk, amniotic fluid, lymph, semen, and synovial fluid. Although they were initially considered to be cellular waste products involved in removing unnecessary molecules from cells, research over the past decade has revealed that exosomes play a much more significant role [33]. They are now recognized as important mediators of cell-to-cell communication, transferring a diverse range of bioactive molecules between cells and influencing numerous physiological and pathological processes. This unique ability has made exosomes an attractive platform for therapeutic applications, particularly in targeted drug delivery. The composition and biological functions of exosomes are closely related to the characteristics of the cells from which they originate [34].

3.1 Mesenchymal Stem Cell-Derived Exosomes

Mesenchymal stem cell (MSC)-derived exosomes have attracted considerable attention in recent years because of their strong regenerative capacity and immunomodulatory effects. Among the various types of exosomes, those derived from MSCs are the most widely investigated for therapeutic and drug delivery applications. Mesenchymal stem cells are multipotent adult stem cells that can differentiate into several specialized cell types, including bone cells (osteoblasts), cartilage cells (chondrocytes), fat cells (adipocytes), and muscle cells (myocytes). These stem cells can be obtained from a variety of tissues, such as bone marrow, adipose tissue, umbilical cord, placenta, dental pulp, and Wharton's jelly [35]. Since MSC-derived exosomes retain many of the beneficial characteristics of their parent cells, they can promote tissue repair and facilitate intercellular communication without the potential risks associated with direct stem cell transplantation, such as immune rejection or uncontrolled cell differentiation [36].

3.2 Dendritic Cell-Derived Exosomes

Dendritic cell-derived exosomes (DEXs) have gained considerable attention because of their important role in regulating immune responses. Dendritic cells are specialized immune cells that act as professional antigen-presenting cells, serving as a crucial link between the innate and adaptive immune systems [37,38]. They capture foreign antigens, process them, and present them to T cells, thereby initiating and coordinating immune responses against pathogens and abnormal cells. Exosomes released from dendritic cells inherit many of these immunological functions, making them attractive candidates for immunotherapy and targeted drug delivery. One of the most promising applications of dendritic cell-derived exosomes is in cancer immunotherapy. Rather than directly destroying cancer cells, DEXs help activate the patient's immune system to recognize and eliminate tumor cells [39]. Because they can carry tumor-associated antigens, they have been widely explored as cell-free cancer vaccines capable of inducing targeted anti-tumor immune responses.

Table 2: Classification of Exosome Sources and Their Therapeutic Significance

Sources	Main Characteristics	Advantages	Therapeutic Applications
MSC-derived	Regenerative, anti-inflammatory	High biocompatibility, low immunogenicity	Wound healing, cardiovascular diseases, neurological disorders
Dendritic cell-derived	Antigen presentation	Activate T cells, vaccine potential	Cancer immunotherapy
Immune cell-derived	Immune regulation	Natural targeting of immune cells	Autoimmune diseases, inflammation
Tumor-derived	Tumor-specific cargo	Excellent tumor homing	Cancer diagnosis and targeted therapy
Plant-derived	Bioactive plant	Low toxicity, cost-	Oral drug delivery,

	compounds	effective	inflammatory diseases

3.3 Tumor-Derived Exosomes

Tumor-derived exosomes (TDEs) are small extracellular vesicles released by cancer cells into the tumor microenvironment as well as various body fluids, including blood, urine, and saliva [40]. Like other exosomes, they are surrounded by a lipid bilayer membrane that protects and transports a diverse range of bioactive molecules. What makes TDEs unique is that their molecular composition closely resembles that of the parent tumor cells. As a result, they carry valuable information about the biological and genetic characteristics of the tumor, allowing them to actively participate in communication between cancer cells and their surrounding environment. Owing to these unique properties, tumor-derived exosomes have gained considerable attention as promising biomarkers for cancer diagnosis and as natural nanocarriers for targeted drug delivery [41]. The cargo of tumor-derived exosomes consists of a complex mixture of oncogenic proteins, messenger RNAs (mRNAs), microRNAs (miRNAs), long non-coding RNAs (lncRNAs), DNA fragments, lipids, enzymes, and metabolites [42].

3.4 Immune Cell-Derived Exosomes

Immune cell-derived exosomes (ICDEs) are extracellular vesicles released by various immune cells, including macrophages, T lymphocytes, B lymphocytes, natural killer (NK) cells, neutrophils, and mast cells [43]. These exosomes play a vital role in maintaining immune homeostasis by facilitating communication between immune cells and regulating both innate and adaptive immune responses. By transporting a variety of bioactive molecules, immune cell-derived exosomes influence several physiological processes, including immune activation, inflammation, antigen presentation, and tissue repair [44]. Owing to these unique biological functions, they have emerged as promising candidates for targeted drug delivery and immunotherapy. The molecular cargo of immune cell-derived exosomes reflects the characteristics and activation status of their parent cells. They contain a diverse range of proteins, lipids, cytokines, chemokines, messenger RNAs (mRNAs), microRNAs (miRNAs), enzymes, and surface receptors that regulate cellular signaling and immune responses. These bioactive molecules enable exosomes to transfer important biological information to recipient cells [50].

3.5 Plant-Derived Exosomes

Plant-derived exosomes, commonly known as plant-derived extracellular vesicles (PDEVs) or plant exosome-like nanoparticles (PELNs), have recently gained significant attention as a natural and sustainable alternative to mammalian exosomes in drug delivery [51]. These nanosized vesicles are released by a wide variety of edible and medicinal plants, including ginger, grapefruit, grapes, broccoli, lemon, aloe vera, carrots, and tea leaves. Although their formation differs from that of mammalian exosomes, they possess similar structural characteristics, including a lipid bilayer membrane that protects and transports biologically active molecules [52]. Their natural abundance, excellent safety profile, and ease of production have made them increasingly attractive for biomedical and pharmaceutical applications. From a pharmaceutical perspective, plant-derived exosomes offer several practical advantages [53].

4. ISOLATION AND PURIFICATION

Efficient isolation and purification are a key factor to the successful application of exosomes in diagnostics and drug delivery [54]. Highly pure exosome preparations are still a great challenge because exosomes are present in biological fluids with proteins, lipoproteins, and other extracellular vesicles. An ideal isolation technique should result in high yield, excellent purity, maintain vesicle integrity and be applicable to characterization and therapeutic applications in the downstream process [55]. There are a number of conventional and emerging techniques available and these have their pros and cons.

4.1 Differential Ultracentrifugation

In research laboratories, the most popular and gold standard method for exosome isolation is by differential ultracentrifugation [56,57]. This is a technique that disperses particles according to their size and density by performing sequential centrifugations at progressive centrifugal forces. Low-speed centrifugation is used to collect intact cells and cellular debris, with intermediate speed centrifugation used to collect larger extracellular vesicles. Lastly, the exosomes are pelted by ultracentrifugation at ~100,000 × g. Technique is relatively simple and may be used in processing large volumes of samples. It is however, very time consuming, very expensive to instrument and can co-isolate protein aggregates and other contaminants [58]. An excessive centrifugal force can also interfere with the structure of exosomes.

4.2 Density Gradient Centrifugation

The purity of exosomes can be further enhanced by density gradient centrifugation, which separates vesicles based on their buoyant densities. Samples are applied to density gradients made of sucrose or iodixanol solutions and then ultracentrifuged. The exosomes are known to concentrate in a particular density gradient, enabling efficient separation from proteins and other EVs in the extracellular space. This is more advantageous than the differential ultracentrifugation because it offers higher purity and better structural preservation of the exosomes. However, it is more time-consuming, technically demanding and not as well-suited for general mass isolation.

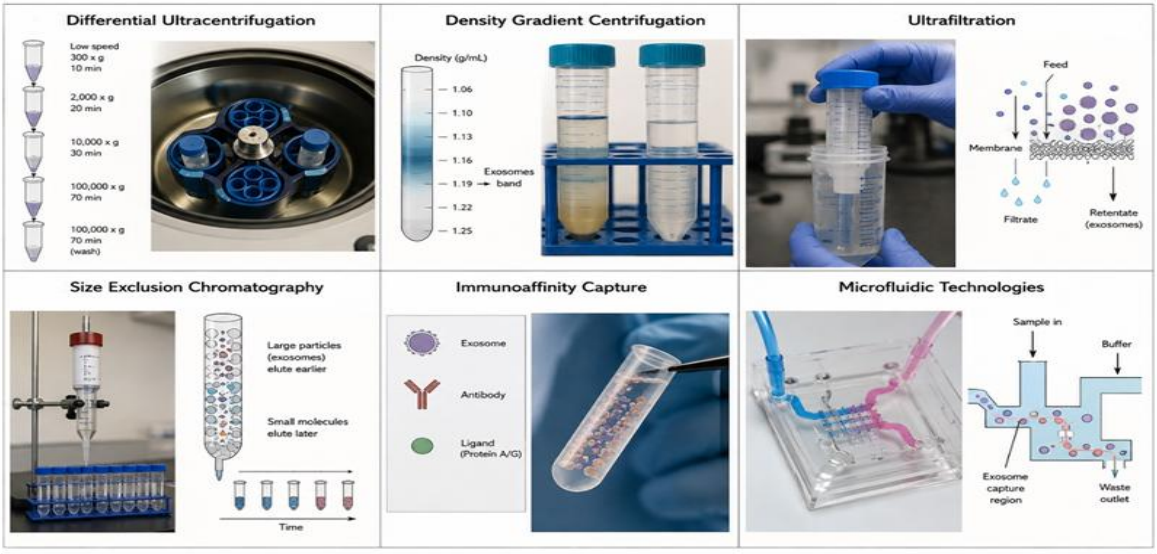

Figure 3: Methods for Isolation and Purification of Exosomes

4.3 Ultrafiltration

Ultrafiltration is a method that uses a membrane with known pore size or molecular weight cut-off to fractionate exosomes based on their size. Smaller molecules are filtered out of samples flowing through these membranes, but exosomes remain [59]. This technique does not demand sophisticated equipment and is quicker than ultracentrifugation. It is especially helpful for the concentration of exosomes prior to purification. However, clogging of the membranes, deforming of vesicles and loss of samples can be encountered, which leads to the lower overall recovery and purity of isolated exosomes [60].

4.4 Size Exclusion Chromatography

Exosomes can be separated from smaller proteins and biomolecules by a process called size exclusion chromatography (SEC), which relies on porous chromatographic beads. The larger particles, like exosomes, do not fit into the pores and will elute first while the smaller molecules will stay inside the pores and elute later. SEC allows preserving the structural and functional integrity of exosomes and provides highly pure preparations with minimal contamination. The method has a number of benefits, but typically results in very dilute samples which need to be concentrated prior to downstream use.

4.5 Immunoaffinity Capture

Immunoaffinity capture is a highly specific method of isolation, based on antibodies targeted to surface proteins of the exosomes identified as CD9, CD63, and CD81. These antibodies are attached to magnetic beads or chromatography matrices which are used to selectively capture target exosomes. This allows for high specificity and purity and is especially useful for biomarker discovery and clinical studies. The method is, however, quite costly, dependent on the availability of antibodies, and in general not suitable for the isolation of large amounts of exosomes.

4.6 Microfluidic Technologies

The microfluidic technologies are the newest method for fast exosome isolation that is based on miniaturized technology that brings together principles like immunoaffinity, filtration, acoustic separation or electrophoresis onto a single platform. They are able to process small sample volumes, with fast processing times and high sensitivity. Microfluidic devices have demonstrated great potential in the field of point-of-care diagnostics and personalized medicine. However, most of these platforms are still in the developmental phase and standardization, scaling and cost of manufacturing still pose several major issues for broad clinical use.

Table 3: Comparison of Exosome Isolation Techniques

Isolation Technique	Principle	Advantages	Limitations	Typical Applications
Differential Ultracentrifugation	Separation based on particle size and density using sequential centrifugation	Widely used, suitable for large sample volumes	Time-consuming, requires expensive equipment, lower purity	Basic research and laboratory-scale isolation

Density Gradient Centrifugation	Separation based on buoyant density using sucrose or iodixanol gradients	High purity and better preservation of exosomes	Labor-intensive and lengthy procedure	High-quality exosome purification
Ultrafiltration	Separation using membrane filters with defined pore sizes	Rapid, simple, cost-effective	Membrane clogging and possible vesicle damage	Sample concentration and preliminary isolation
Size Exclusion Chromatography (SEC)	Separation according to particle size using porous beads	High purity, maintains exosome integrity	Diluted fractions require concentration	Functional studies and therapeutic applications
Immunoaffinity Capture	Antibody-based capture of exosome surface markers	Highly specific and pure isolates	Expensive and low sample yield	Biomarker studies and clinical diagnostics
Microfluidic Technologies	Miniaturized separation using physical or immunological principles	Fast, sensitive, requires small sample volume	Limited scalability and high development cost	Point-of-care diagnostics and precision medicine

5. CHARACTERIZATION OF EXOSOMES

After isolation and purification, detailed characterization of exosomes is crucial to verify the identity, purity, morphology, and biological functions of exosomes [61]. Characterization verifies that isolated vesicles contain the expected molecular markers, morphology and cargo and are free of contamination with other extracellular vesicles or protein aggregates and have the characteristic size and morphology of exosomes. Characterization of the physicochemical properties of exosomes is especially relevant when they are used as therapeutic agents because they directly affect the stability, biodistribution, cellular uptake and effectiveness of drug delivery. Therefore, a multi-technique approach is generally used to obtain a confident characterization, involving physical, biochemical and molecular [62].

5.1 Particle Size Analysis

One of the hallmarks of exosomes is their particle size, which falls in the range of 30–150 nm. The size of the particles can be used to differentiate exosomes from larger extracellular vesicles and to gain insights into the quality and homogeneity of the sample. Nanoparticle Tracking Analysis (NTA) is among the most common methods for determination of particle size [63]. It observes the Brownian motion of individual vesicles illuminated by a laser and determines their size using the Stokes–Einstein equation. In addition to measuring particle size distribution, NTA offers particle concentration, and is therefore extremely useful for exosome yield evaluation. Another popular method is Dynamic Light Scattering (DLS). It measures fluctuations in intensity of scattered light by particles in solution to estimate

particle size. DLS is fast and easy to perform, but can be affected by larger particles or aggregates and is therefore best applied to relatively homogeneous samples [64]. The precise particle size measurement is essential as it could impact the circulation time, biodistribution, cellular uptake, and therapeutic effects of exosome-based drug delivery systems.

5.2 Zeta Potential Measurement

Zeta potential is an important indicator of the colloidal stability of exosomes, because it indicates the electrical charge on the surface of the exosomes. It describes the electrostatic interactions between suspended particles in a liquid phase and affects the tendency of the particles to aggregate during the course of the storage and circulation processes. Typically, the zeta potential is measured by electrophoretic light scattering. Exosomes usually have a slight negative surface charge from the phospholipids and membrane associated proteins. Stable exosome suspensions usually have good electrostatic repulsion to prevent particle aggregation. In addition to stability testing, zeta potential also affects the interaction between exosomes and the target cell. Surface charge is a crucial parameter for any therapeutic application of exosomes, as it influences cellular uptake, circulation time and biodistribution.

5.3 Morphological Characterization

Morphological analysis is direct visualization of exosomes and it can confirm size, shape and structural integrity of the exosomes. The electron microscopy-based techniques are still the standard used for morphology characterization [65].

Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM), with its unparalleled high resolution, is the most commonly used imaging method for characterization of exosomes. Gives a way to visualize individual exosomes and verify its nanoscale size and membrane-bounded structure. The exosomes frequently look like a cup in TEM images, but this shape is thought to be an artefact created during the preparation of the sample and during the dehydration process and not necessarily the natural shape of the exosomes.

Scanning Electron Microscopy (SEM)

The Scanning Electron Microscopy (SEM) gives detailed information about the external surface morphology and topography of exosomes. SEM is useful to study particle distribution and surface characteristics as it produces three-dimensional images of the surface unlike TEM. Its resolution is usually lower, though, than TEM in the examination of internal structures.

Atomic Force Microscopy (AFM)

The advanced imaging technique called Atomic Force Microscopy (AFM) provides high resolution 3D images under near physiological conditions. AFM does not require much sample preparation and can measure the height, surface roughness, elasticity, and mechanical properties of particles, which is not possible in electron microscopy. This method is especially suitable to assess the integrity of a structure after drug loading or surface modification.

The three techniques, TEM, SEM and AFM, complement each other in giving information on the morphology of exosomes and to deeper understanding of their structural characterization.

5.4 Molecular Characterization

Exosomes cannot be uniquely identified by physical characterization. For this reason, molecular characterization is carried out to identify specific proteins in exosomes and to measure the purity of the samples.

Western Blotting

The standard method for the identification of exosome-specific protein markers is western blotting. The tetraspanins CD9, CD63 and CD81, as well as Alix, TSG101 and heat shock proteins like HSP70 are frequently detected markers. At the same time, negative markers, like calnexin, can be studied, to verify the absence of cellular contaminants.

Flow Cytometry

Exosomal surface proteins can be analyzed quantitatively using flow cytometry with the help of fluorescently labelled antibodies. While flow cytometers have some drawbacks in analyzing nanosized vesicles, recent developments such as bead-assisted flow cytometry and high-resolution flow cytometers have improved the analysis of exosomes. This method is especially useful for analyzing various cell source exosomes.

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is a fast and sensitive technique for detecting the antigen-antibody interaction of protein molecules in exosomes [66]. It has been used widely for the quantification of biomarkers (including CD63, CD81) and has become particularly important for the diagnosis of disease and clinical biomarker research.

These molecular methods confirm the identity of exosomes and can give important data on the protein expression profiles and the purity of the sample.

5.5 Omics-Based Characterization

The current omics advances have substantially furthered the comprehension of the biology of exosomes by permitting the detailed examination of their molecular content. Proteomics analyzes the full proteome of exosomes, such as by liquid chromatography–mass spectrometry (LC-MS/MS). These studies have identified many proteins that participate in cell signaling, the regulation of the immune system, and disease progression. Transcriptomics studies exosomal nucleic acids such as messenger RNA (mRNA), microRNA (miRNA), long non-coding RNA (lncRNA) and circular RNA (circRNA). They modulate gene expression in recipient cells and have become a target of considerable interest as diagnostic markers and therapeutic compounds [67]. In lipidomic, the researchers are examining the variety of lipids within exosomes, such as cholesterol, phospholipids, ceramides and sphingolipids. Lipidomic can be applied to gain insights into exosome function and therapeutic performance because membrane lipids affect vesicle stability, membrane fusion and cellular uptake. Exosomes are composed of metabolites, many of which are small molecules, and metabolomics is a rapidly developing branch of research that involves studying the metabolites in exosomes [68]. These metabolites are a measure of the metabolic functions of the donor cells and could be used as new biomarkers for different kind of disease. A combination of various omics platforms provides a detailed molecular portrait of exosomes and greatly improves their use for precision medicine, biomarker identification, and drug delivery [69].

Table 4: Characterization Techniques for Exosomes

Technique	Principle	Information Obtained	Advantages	Limitations
Nanoparticle Tracking Analysis (NTA)	Tracks Brownian motion of individual particles	Particle size distribution and concentration	High accuracy, quantitative analysis	Requires specialized equipment; affected by sample impurities
Dynamic Light Scattering (DLS)	Measures fluctuations in scattered light intensity	Average particle size and size distribution	Rapid, simple, non-destructive	Less accurate for heterogeneous samples and aggregates
Zeta Potential Analysis	Measures surface electrical charge	Surface charge and colloidal stability	Predicts stability and aggregation tendency	Sensitive to sample preparation and buffer conditions
Transmission Electron Microscopy (TEM)	Electron beam passes through thin specimen	Morphology, size, membrane structure	High-resolution imaging	Expensive, labor-intensive, preparation artifacts possible
Scanning Electron Microscopy (SEM)	Electron beam scans specimen surface	Surface morphology and topography	Three-dimensional surface visualization	Lower internal structural resolution than TEM
Atomic Force Microscopy (AFM)	Mechanical probe scans particle surface	Three-dimensional morphology, surface roughness, mechanical properties	Minimal sample preparation, operates under near-physiological conditions	Slow analysis and relatively expensive instrumentation
Western Blotting	Immunodetection of specific proteins	Detection of exosomal markers (CD9, CD63, CD81, Alix, TSG101)	Highly specific and widely accepted	Semi-quantitative and time-consuming
Flow Cytometry	Fluorescent antibody-based detection	Surface marker expression and exosome populations	Multiparametric analysis	Conventional instruments have limited sensitivity for

				nanosized vesicles
ELISA	Antigen–antibody interaction	Quantification of exosomal proteins	Highly sensitive and suitable for clinical studies	Limited to selected protein targets
Proteomics (LC-MS/MS)	Mass spectrometry-based protein analysis	Comprehensive protein profile	High sensitivity and large-scale analysis	High cost and complex data interpretation
Transcriptomics	Sequencing of exosomal RNA	mRNA, miRNA, lncRNA and circRNA profiling	Biomarker discovery and gene expression analysis	Requires high-quality RNA and bioinformatics expertise
Lipidomics & Metabolomics	Mass spectrometry-based molecular profiling	Lipid and metabolite composition	Provides insight into membrane composition and metabolic state	Expensive instrumentation and complex analysis

6. Drug Loading Strategies in Exosomes

The therapeutic applications of exosomes greatly rely on the capacity of the vesicles to encapsulate and bind active molecules to target cells with high efficiency. The loading of the drug is an important step in the preparation of EBDs systems because it is responsible for the therapeutic cargo carried, loading efficiency, stability and the therapeutic effect of the exosomes. Several loading strategies have been created for the delivery of small molecule drugs, proteins, peptides, nucleic acids and gene-editing tools into exosomes [70]. These techniques can be broadly divided into passive loading, active loading, surface modification and exosome engineering methods. The choice of the loading strategy is dependent on the physicochemical properties of the therapeutic agent, the loading efficiency desired and the intended clinical application [71].

6.1 Passive Loading Methods

Passive loading is the easiest and less disruptive method of loading therapeutic agents into exosomes. One approach involves placing purified exosomes in a controlled environment with the desired drug, then allowing the drug molecules to pass through the lipid bilayer of the exosomes and enter the vesicles [72]. Hydrophobic drugs tend to show better loading efficiency because they tend to easily partition into the lipid membrane, while the hydrophilic compounds show poor encapsulation. One of the alternative passive methods is to pre-treat (incubate) donor cells with therapeutic agents before exosome isolation. In exosome biogenesis, the cells naturally incorporate into the newly formed vesicles a portion of the drug inside. This technique is especially suitable for endogenous loading of some drugs, proteins and nucleic acids in the exosomes without disrupting them. Passive loading has several benefits, including simplicity, low cost, little equipment and preservation of

membrane structure. The whole loading efficiency, however, is still low compared to other nanocarriers, particularly for large biomolecules like proteins and RNA, so it has restricted applications in therapy that demands high drug concentrations.

6.2 Active Loading Methods

The active loading methods temporarily disrupt the exosomal membrane and cause the therapeutic agent to penetrate the membrane more effectively. These methods usually lead to better encapsulation efficiency than passive loading, but can damage the membranes if not properly optimized.

Electroporation

One of the most popular active loading methods for introducing nucleic acids is electroporation. In this way, small interfering RNA (siRNA), messenger RNA (mRNA), microRNA (miRNA), and plasmid DNA are introduced into the exosomal membrane through transient nanopores. Once the electrical field is switched off, the membrane reform, trapping the therapeutic cargo inside the vesicles. RNA-based therapeutics have become the method of choice due to high loading efficiency with electroporation. However, high electrical stimulation can cause RNA aggregation or cause damage to the cell membrane and decrease biological activity.

Sonication

Sonication is a method for briefly breaking open the phospholipid bilayer of exosomes. During sonication, mechanical shear forces increase the permeability of membranes allowing the diffusion of the drugs to the inside of the vesicles. After incubation, the membrane slowly returns to its normal structure. This is the general method for obtaining higher loading efficiencies than passive incubation, and is especially effective with hydrophobic drugs but excessive sonication can cause the membrane proteins to change or damage the membrane structure of exosomes, so the operating conditions should be carefully optimized.

Extrusion

In the extrusion method, the exosomes and therapeutic agents are mechanically pressed against the membrane multiple times to repeatedly pass through the membrane containing pores of specific size. The result is a disruption in the membrane that allows cargo to mix with the contents of the exosome prior to membrane reassembly. Extrusion gives rise to a fairly uniform particle size distribution and high drug loading efficiency. In this process, however, the mechanical stress generated can change the composition of the membrane and modify some biological properties of the exosomes.

Freeze–Thaw Method

The freeze–thaw method is a series of fast freezing and slow thawing. The formation of ice crystals temporarily breaks the lipid bilayer and allows therapeutic molecules to penetrate the vesicles prior to the resealing process. This process is low cost and would not need any special equipment and would be applicable for laboratory-scale applications. Repeated freeze–thaw cycles may, however, cause vesicle aggregation and decrease the loading efficiency from the vesicles compared to electroporation or sonication. In summary, active loading methods offer a higher encapsulation efficiency and need to be optimized to avoid compromising the structure and function of exosomes.

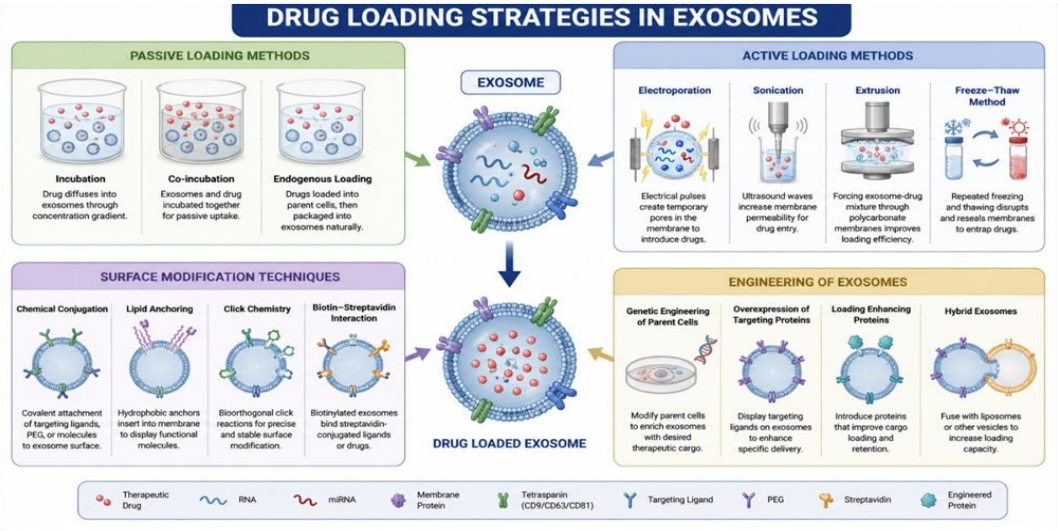

Figure 4: Drug loading strategies in Exosomes

6.3 Surface Modification Techniques

In addition to the ability of encapsulating drugs in its interior, the surface of exosomes can be chemically modified to enhance targeting, circulation time and therapeutic efficacy. The goal of surface modification is to increase the interaction between exosomes and target tissues, while decreasing off-target effects [73]. Chemical modification includes the modification of the exosomal membrane with targeting ligands, antibodies, peptides and polymers including polyethylene glycol (PEG). These ligands bind specifically to receptors which are over-expressed on the diseased cells leading to better targeting of drugs. Another technique for surface engineering involves genetically modifying donor cells. Donor cells are genetically modified to contain the genes encoding membrane proteins linked to targeting peptides, which then end up naturally on the surface of the exosomes. One such engineered exosome shows targeting efficiency while preserving membrane integrity of the exosome [74]. Surface modification is a critical and essential technique to improve the specificity and biodistribution of cells, especially in cancer therapy, neurological disorders, and precision medicine. However, too much modification can change the natural biological properties of exosomes, or make the process too complicated.

6.4 Engineering of Exosomes

Novel technologies in biotechnology have allowed the establishment of modified exosomes that show improved therapeutic activity. The engineering of exosomes includes modification of the source cells prior to the production of exosomes or/and modification of the purified exosomes to enhance the therapeutic activity, loading or targeting of drugs. Pre-isolation engineering involves the genetic engineering of the cell donor to ensure that therapeutic proteins, RNAs or targeting ligands are naturally packaged into exosomes during biogenesis. This approach guarantees stable cargo loading and maintains exosomes' natural property. Post-isolation engineering is a process that alters purified

exosomes through physical, chemical, or biological means. Functionalization of exosomes for targeted biomedical applications is achieved by approaches like click chemistry, lipid insertion, membrane fusion and nanoparticle hybridization. In terms of therapeutic applications, engineered exosomes have demonstrated great potential for delivery of chemotherapeutic drugs, anti-inflammatory agents, proteins, nucleic acids, CRISPR/Cas9 components and vaccines. In addition, the fusion of exosomes with synthetic nanoparticles has led to the development of hybrid delivery systems, achieving an association of high loading capacity and biocompatibility of nanomaterials and exosomes. Although exosome engineering offers exciting opportunities for personalized medicine, challenges such as large-scale manufacturing, reproducibility, storage stability, and regulatory approval remain to be addressed before widespread clinical translation.

Table 5: Drug Loading Techniques and Their Advantages

Drug Loading Method	Principle	Suitable Cargo	Advantages	Limitations
Passive Incubation	Drug molecules diffuse across the exosomal membrane during incubation	Small hydrophobic drugs	Simple, inexpensive, preserves membrane integrity	Low loading efficiency, unsuitable for large biomolecules
Donor Cell Incubation (Endogenous Loading)	Donor cells are incubated with therapeutic agents before exosome isolation	Small molecules, proteins, nucleic acids	Natural cargo incorporation, maintains exosome stability	Difficult to control loading amount; lower efficiency
Electroporation	Electrical pulses create temporary membrane pores for drug entry	siRNA, miRNA, mRNA, DNA	High loading efficiency for nucleic acids	May cause RNA aggregation and membrane damage
Sonication	Ultrasonic waves temporarily disrupt the membrane	Hydrophobic drugs, proteins	Higher loading efficiency than passive methods	Possible alteration of membrane proteins and vesicle structure
Extrusion	Mechanical force through membrane filters promotes cargo incorporation	Small molecules, proteins, nucleic acids	Uniform particle size and good loading efficiency	Mechanical stress may affect membrane composition
Freeze–Thaw Cycles	Repeated freezing and thawing temporarily	Small molecules and proteins	Simple and inexpensive	Vesicle aggregation and relatively lower efficiency

	increase membrane permeability			
Surface Modification	Attachment of ligands, antibodies, or polymers to the exosomal membrane	Targeting molecules	Improved tissue specificity and prolonged circulation	Increased preparation complexity and production cost
Genetic Engineering	Modification of donor cells to produce cargo- or ligand-enriched exosomes	Therapeutic proteins, RNA, targeting peptides	Stable cargo loading and enhanced targeting	Technically demanding, regulatory challenges

7. Mechanisms of Exosome-Mediated Drug Delivery

The versatility of exosomes in encapsulating a wide range of therapeutic agents is not the only intriguing aspect of their therapeutic potential; their efficient and targeted delivery to the recipient cells is equally captivating. Exosomes have native biological characteristics that prevent them from being recognized by the immune system, remain in the bloodstream longer, and specifically bind to target tissues, which makes them more advantageous in comparison to other synthetic nanocarriers [75]. The membrane proteins and lipids that are naturally present in their cells allow them to communicate efficiently with one another, which is why they make great drug delivery vehicles. To best use exosomes in a precision medicine, it is crucial to understand the mechanisms by which they recognize, enter and release their cargo in target cells.

7.1 Cellular Uptake Mechanisms

The initial stage of drug delivery via exosomes is the recognition and binding of exosomes to recipient cells. Specific proteins, lipids and glycoproteins on the exosomal membrane mediate this interaction, such as tetraspanins, integrins and adhesion molecules. These surface elements engage complementary receptors which are expressed on target cells, which gives rise to tissue specificity and cellular selectivity. After attaching, exosomes enter the cells through various cellular uptake mechanisms. Pathway choice is related to the size of the exosomes, membrane composition, origin of the donor cell, and the physiologic state of the recipient cell. This is crucial since it directly affects the amount of therapeutic cargo that will be delivered into the target cell in an efficient manner.

7.2 Endocytosis Pathways

It is believed that the main way exosomes enter recipient cells is via endocytosis. Endocytic pathways leading to the internalization of exosomes include different endocytic processes. One of the best characterized pathways is the clathrin-mediated endocytosis. One of them involves the binding to cell surface receptors and uptake via clathrin-coated vesicles. This receptor-mediated process allows for the selective uptake of exosomes with particular ligands. Flask-shaped plasma membrane invaginations called caveolae play a role in caveolin-mediated endocytosis. This pathway is especially

significant in endothelial cells and is involved in the efficient transport of materials in the cell, while reducing the degradation of materials in the lysosomes. Non-specific uptake mechanism called macropinocytosis involves the formation of large vesicles of the plasma membrane that are able to internalize extracellular fluid and suspended exosomes. This pathway is commonly seen in immune cells and some types of cancer.

7.3 Membrane Fusion

The mechanism of exosome endocytosis is not the only way by which they can be taken up by target cells; exosomes can also fuse directly with the plasma membrane of target cells. In the process of membrane fusion, the lipid bilayer of the exosome fuses with the cell membrane, and therapeutic cargo is released immediately into the cytoplasm. Direct membrane fusion provides a mechanism for efficient delivery, which avoids endosomal trafficking and minimizes the risk of degradation in lysosomes. The pathway is especially suitable for the introduction of fragile therapeutic agents like messenger RNA (mRNA), small interfering RNA (siRNA), proteins, and components of CRISPR/Cas9 into the cytoplasm, where these molecules function. Fusogenic proteins, membrane fluidity, membrane pH and membrane lipid composition all affect the efficiency of membrane fusion.

7.4 Intracellular Drug Release

Therapeutic cargo needs to be properly released in the recipient cell after internalization to have a biological effect. The endocytosed exosomes pass through early and late endosomes. Others fuse with the endosomal membrane and the contents of the exosomes are released into the cytoplasm before they are destroyed by the lysosomes. The therapeutic molecules contained within the capsule react to the cell's targets as per their mechanism of action once they are released. Small molecule drugs interact with intracellular proteins or enzymes, proteins restore or modify cellular functions, and nucleic acid therapeutics such as mRNA, microRNA (miRNA), small interfering RNA (siRNA), and gene editing components, can control gene expression or protein synthesis. Efficient intracellular release is one of the major determinants of therapeutic success. If endosomal escape is incomplete, the therapeutic efficacy is reduced due to lysosomal degradation of the cargo. Consequently, improving intracellular release remains an active area of research in exosome engineering and targeted drug delivery.

8. THERAPEUTIC APPLICATION

Exosome-based drug delivery systems have emerged as one of the most promising advances in modern nanomedicine because of their ability to transport therapeutic molecules safely and efficiently to specific target tissues [76]. Unlike conventional drug carriers, exosomes are naturally produced by living cells and possess excellent biocompatibility, low immunogenicity, prolonged circulation time, and the ability to cross biological barriers such as the blood–brain barrier. These unique characteristics enable exosomes to deliver a wide range of therapeutic cargo, including small-molecule drugs, proteins, peptides, messenger RNAs (mRNAs), microRNAs (miRNAs), small interfering RNAs (siRNAs), DNA, and CRISPR/Cas9 components with improved precision and minimal off-target effects. Their versatility has expanded their applications across numerous medical fields, including oncology, neurology, cardiovascular medicine, regenerative medicine, infectious diseases, and gene therapy. One of the major advantages of exosome-based therapeutics is their natural ability to facilitate cell-to-cell communication. By transferring biologically active molecules to recipient cells, exosomes can regulate gene expression, cellular signaling

pathways, immune responses, and tissue repair processes [77].

8.1 Cancer Therapy

Cancer remains one of the leading causes of morbidity and mortality worldwide, despite significant advances in diagnosis and treatment. Conventional therapeutic approaches, including chemotherapy, radiotherapy, surgery, and immunotherapy, have improved patient outcomes but continue to face several limitations. Chemotherapeutic drugs often lack target specificity, resulting in damage to healthy tissues and causing severe side effects such as myelosuppression, cardiotoxicity, neurotoxicity, and gastrointestinal complications. In addition, poor drug solubility, rapid systemic clearance, multidrug resistance, and inadequate penetration into solid tumors further reduce the effectiveness of conventional anticancer therapies [78]. These challenges have created a growing need for targeted drug delivery systems that can improve therapeutic efficacy while minimizing systemic toxicity. Exosome-based drug delivery has emerged as a promising strategy to overcome many of these limitations.

8.1.1 Chemotherapy Delivery

Chemotherapy remains one of the most widely used treatment strategies for various types of cancer. Although chemotherapeutic drugs such as doxorubicin, paclitaxel, cisplatin, gemcitabine, methotrexate, and docetaxel have shown significant clinical benefits, their therapeutic effectiveness is often limited by poor target specificity, rapid systemic clearance, low bioavailability, and severe dose-dependent toxicity. Because these drugs circulate throughout the body, they can damage healthy tissues alongside cancer cells, leading to adverse effects such as cardiotoxicity, nephrotoxicity, neurotoxicity, bone marrow suppression, and gastrointestinal complications. In addition, the development of multidrug resistance (MDR) remains a major challenge, reducing the long-term success of chemotherapy.

8.1.2 Immunotherapy

Cancer immunotherapy has transformed the landscape of cancer treatment by harnessing the body's immune system to recognize and eliminate malignant cells. Unlike conventional chemotherapy, which directly destroys rapidly dividing cells, immunotherapy enhances the natural immune response against tumors, resulting in improved specificity and long-lasting therapeutic effects. Although approaches such as immune checkpoint inhibitors, monoclonal antibodies, adoptive cell therapy, and cancer vaccines have shown remarkable clinical success, their effectiveness is often limited by immune-related adverse effects, poor targeting efficiency, treatment resistance, and variability in patient response. These limitations have encouraged the development of innovative drug delivery systems capable of improving the precision and efficacy of immunotherapeutic agents.

8.1.3 Gene Therapy

Gene therapy has emerged as a promising approach for treating cancer by targeting the underlying genetic abnormalities responsible for tumor initiation, progression, and resistance to therapy. Unlike conventional treatments that mainly destroy cancer cells, gene therapy aims to modify or regulate the expression of specific genes involved in disease development. Therapeutic strategies include gene silencing, gene replacement, gene editing, and the delivery of tumor-suppressor genes, all of which have the potential to provide more precise and long-lasting treatment. However, the successful clinical

application of gene therapy largely depends on the availability of safe and efficient delivery systems capable of protecting fragile nucleic acids and transporting them to target cells.

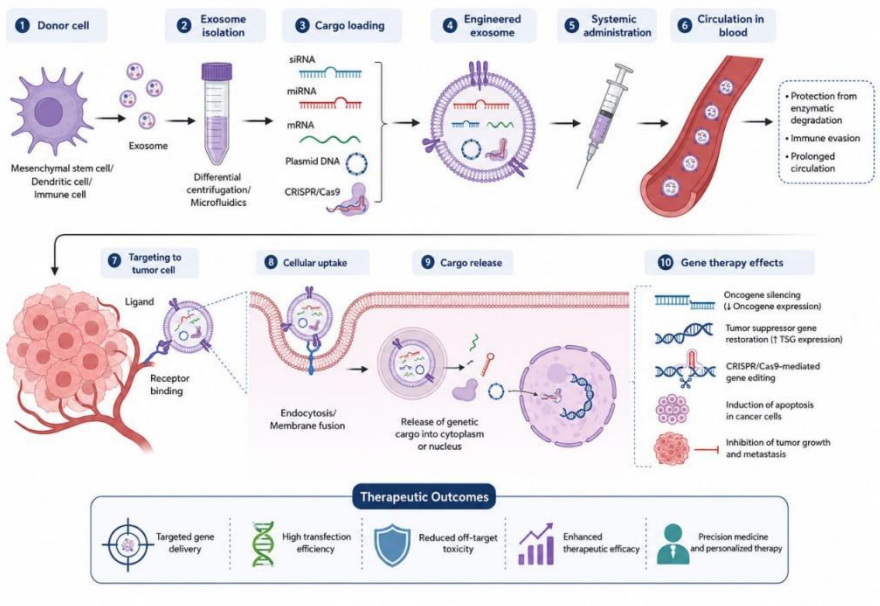

Figure 5: Exosome-Based Delivery of Therapeutic Nucleic Acids for Cancer Treatment

8.2 Neurological Disorder

Neurological disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), stroke, multiple sclerosis (MS), glioblastoma, epilepsy, and traumatic brain injury (TBI), are among the leading causes of disability and mortality worldwide. Despite considerable advances in neuroscience and pharmacology, effective treatment remains a major challenge because many therapeutic agents are unable to reach the brain in sufficient concentrations [79]. One of the primary obstacles is the blood–brain barrier (BBB), a highly selective physiological barrier that protects the central nervous system (CNS) from harmful substances while simultaneously limiting the entry of many potentially beneficial drugs. As a result, conventional drug delivery systems often exhibit poor brain penetration, low bioavailability, and reduced therapeutic efficacy [80].

8.2.1 Alzheimer's disease

Alzheimer's disea**se (**AD) is the most common neurodegenerative disorder and the leading cause of dementia worldwide. It is characterized by progressive memory loss, cognitive decline, impaired learning ability, and behavioral changes that gradually interfere with daily activities. The pathological hallmarks of Alzheimer's disease include the accumulation of amyloid-beta (Aβ) plaques, intracellular neurofibrillary tangles composed of hyperphosphorylated tau proteins, chronic neuroinflammation, oxidative stress, and progressive neuronal loss. Despite decades of research, current treatment options provide only symptomatic relief and are unable to halt or reverse disease progression. One of the major obstacles in developing effective therapies is the blood–brain barrier (BBB), which restricts the delivery of many therapeutic agents to the brain.

8.2.2 Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease and primarily affects movement and motor coordination. The disease is characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra, resulting in a significant reduction in dopamine levels within the brain. Common clinical manifestations include resting tremors, muscle rigidity, bradykinesia (slowness of movement), postural instability, and impaired balance. As the disease progresses, many patients also develop non-motor symptoms such as cognitive decline, depression, sleep disturbances, and autonomic dysfunction. Although medications such as levodopa and dopamine agonists help manage symptoms, they do not prevent neuronal degeneration or halt disease progression.

8.2.3 Brain-targeted delivery

One of the greatest challenges in the treatment of neurological disorders is the effective delivery of therapeutic agents to the brain. The blood–brain barrier (BBB**)** serves as a highly selective physiological barrier that protects the central nervous system from harmful substances while also restricting the passage of many drugs. Although this protective function is essential for maintaining brain homeostasis, it significantly limits the effectiveness of conventional drug delivery systems. As a result, many promising therapeutic agents fail to reach the brain in adequate concentrations, reducing their clinical efficacy in the treatment of neurological diseases.

8.3 Cardiovascular Diseases

Cardiovascular diseases (CVDs), including myocardial infarction, heart failure, ischemic heart disease, atherosclerosis, hypertension, and stroke, remain the leading cause of morbidity and mortality worldwide. Despite significant advances in pharmacological therapies, interventional cardiology, and surgical procedures, the regeneration of damaged cardiac tissue continues to be a major clinical challenge [81]. Conventional drug therapies often suffer from poor tissue specificity, rapid systemic clearance, limited retention at the site of injury, and undesirable side effects. Furthermore, the heart has a limited regenerative capacity, making it difficult to restore normal cardiac function after injury. These limitations have encouraged the development of targeted drug delivery systems capable of improving therapeutic efficacy while minimizing systemic toxicity. Exosome-based drug delivery has emerged as a promising therapeutic strategy for cardiovascular diseases because of the unique biological properties of exosomes [82]. As naturally occurring extracellular vesicles, exosomes possess excellent biocompatibility, low immunogenicity, and the ability to transport a wide range of bioactive molecules directly to injured tissues.

8.4 Inflammatory Diseases

Inflammatory diseases comprise a broad group of acute and chronic disorders characterized by persistent activation of the immune system and excessive production of inflammatory mediators. Conditions such as rheumatoid arthritis (RA), inflammatory bowel disease (IBD), psoriasis, asthma, systemic lupus erythematosus (SLE), and sepsis are associated with prolonged inflammation that gradually damages healthy tissues and impairs normal organ function [83]. Although conventional therapies, including corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs), immunosuppressants, and biological agents, have significantly improved disease management, their long-term use is often accompanied by systemic side effects, poor tissue

specificity, frequent dosing, and an increased risk of infections

These limitations have encouraged the development of targeted drug delivery systems that can selectively modulate inflammation while minimizing adverse effects. Exosome-based drug delivery has emerged as a promising therapeutic approach for inflammatory diseases because of the unique biological characteristics of exosomes. As naturally secreted extracellular vesicles, exosomes possess excellent biocompatibility, low immunogenicity, and the ability to transport a wide variety of bioactive molecules between cells [84].

8.5 Infectious Diseases

Infectious diseases caused by bacteria, viruses, fungi, and parasites continue to pose a major global health challenge despite significant advances in antimicrobial therapies and vaccination programs. The emergence of antimicrobial resistance, frequent viral mutations, limited drug bioavailability, and the inability of many therapeutic agents to reach infected tissues effectively have reduced the success of conventional treatment strategies. In addition, prolonged use of antibiotics and antiviral drugs can lead to systemic toxicity, disruption of the normal microbiota, and the development of drug-resistant pathogens [85]. These challenges have created an urgent need for innovative drug delivery systems that can improve therapeutic efficacy while minimizing adverse effects. Exosome-based drug delivery has emerged as a promising approach for the treatment of infectious diseases because of the natural biological properties of exosomes.

8.6 Regenerative Medicine

Regenerative medicine is an emerging field that focuses on repairing, replacing, or regenerating damaged tissues and organs to restore their normal structure and function. It combines advances in stem cell biology, tissue engineering, biomaterials, and molecular medicine to develop innovative therapies for diseases that have limited treatment options. Conditions such as bone and cartilage injuries, myocardial infarction, spinal cord injury, liver disease, kidney disease, skin wounds, and neurodegenerative disorders often involve irreversible tissue damage, making regeneration a major clinical challenge [86]. Although stem cell therapy has shown promising results, concerns related to immune rejection, tumor formation, low cell survival, and ethical issues have limited its widespread clinical application. One of the most promising applications of exosome-based regenerative therapy is in cardiac tissue repair following myocardial infarction. Cardiac injury caused by ischemia results in extensive loss of cardiomyocytes and impaired heart function [87].

8.7 Precision Medicine Applications

Precision medicine is transforming modern healthcare by shifting from a "one-size-fits-all" approach to treatment strategies that are tailored to the unique genetic, molecular, and clinical characteristics of each patient. By integrating information from genomics, proteomics, metabolomics, and other molecular profiling techniques, precision medicine aims to identify the most appropriate therapy for individual patients while minimizing adverse effects and improving treatment outcomes [88]. However, the success of precision medicine largely depends on the availability of efficient drug delivery systems capable of transporting therapeutic agents specifically to diseased cells without affecting healthy tissues.

Table 6. Therapeutic Applications of Exosome-Based Drug Delivery Systems

Disease/Conditions	Therapeutic Cargo Delivered	Mechanism of action	Major Therapeutic Benefits
Cancer Therapy	Doxorubicin, Paclitaxel, Cisplatin, Gemcitabine, siRNA, miRNA, CRISPR/Cas9	Targeted drug delivery, gene silencing, apoptosis induction	Enhanced tumor targeting, reduced systemic toxicity, improved therapeutic efficacy
Chemotherapy Delivery	Chemotherapeutic drugs (Doxorubicin, Paclitaxel, Methotrexate, Cisplatin)	Controlled drug release and improved intracellular uptake	Higher drug bioavailability, reduced multidrug resistance, minimized side effects
Cancer Immunotherapy	Tumor antigens, cytokines, immune checkpoint inhibitors, dendritic cell-derived exosomes	Activation of T cells, NK cells, and antigen presentation	Enhanced antitumor immune response and prolonged immune memory
Gene Therapy	siRNA, miRNA, mRNA, CRISPR/Cas9, plasmid DNA	Regulation of disease-related genes and gene editing	Precise gene modulation with reduced off-target effects
Alzheimer's Disease	siRNA, miRNA, neuroprotective drugs, antioxidant molecules	Reduction of amyloid-β plaques, inhibition of tau pathology, neuroprotection	Improved cognitive function and reduced neuroinflammation
Parkinson's Disease	Dopamine, catalase, GDNF, therapeutic RNAs	Protection of dopaminergic neurons and reduction of α-synuclein aggregation	Improved motor function and neuronal survival
Brain-Targeted Delivery	Drugs, proteins, peptides, siRNA, miRNA, CRISPR/Cas9	Crossing the blood–brain barrier and targeted neuronal delivery	Enhanced CNS drug delivery with minimal systemic toxicity
Cardiovascular Diseases	Growth factors, miRNAs, proteins, regenerative molecules	Angiogenesis, anti-apoptotic activity, cardiac tissue repair	Improved cardiac function and reduced fibrosis after myocardial infarction
Inflammatory Diseases	Anti-inflammatory cytokines, miRNAs,	Suppression of inflammatory pathways	Reduced inflammation, tissue protection, and

	siRNAs	and immune modulation	immune homeostasis
Infectious Diseases	Antibiotics, antiviral drugs, antimicrobial peptides, siRNA	Targeted antimicrobial delivery and immune regulation	Improved pathogen clearance and reduced drug resistance
Regenerative Medicine	Growth factors, miRNAs, proteins, stem cell-derived exosomes	Cell proliferation, angiogenesis, extracellular matrix remodeling	Accelerated tissue repair and regeneration
Precision Medicine	Personalized drugs, nucleic acids, CRISPR/Cas9, biomarker molecules	Patient-specific targeted therapy and molecular diagnosis	Personalized treatment, reduced adverse effects, improved clinical outcomes

9. EXOSOMES FOR NUCLEIC ACID DELIVERY

The unique physicochemical and biological characteristics of exosomes have made them attractive biological nanocarriers for therapeutic nucleic acid delivery [89]. Small interfering RNA (siRNA), microRNA (miRNA), messenger RNA (mRNA) and CRISPR/Cas9 gene-editing systems represent very specific nucleic acid therapeutics that can be used to treat different genetic or acquired diseases by interfering with gene expression. However their clinical utilization is limited by their susceptibility to nucleases, inadequate cellular uptake, short circulation half-life and immune recognition. The main limitation of the above is that nucleic acids are sensitive to enzyme degradation in blood and are also difficult to deliver into the cells [90]. Exosomes possess a natural phospholipid bilayer membrane which protects its nucleic acids against enzymatic degradation and delivers them into cells efficiently.

They are superior to many synthetic delivery systems because of their low immunogenicity, excellent biocompatibility, capacity to penetrate biological barriers like the blood–brain barrier and their inherent cell-targeting capability. As a result, the use of exosome-mediated delivery of nucleic acid has attracted significant attention in the field of cancer treatment, treatment of other diseases such as regeneration diseases, neurological diseases, infectious diseases, and precision medicine [91]. Exosomes can be sensitized to tissue-specific targeting as well as loaded with various nucleic acid cargoes via passive or active loading and thus modified to achieve improved therapeutic efficacy while reducing systemic toxicity.

9.1 siRNA Delivery

Small interfering RNA (siRNA) is a double-stranded RNA molecule of ~21-23 base pairs that is responsible for sequence-specific degradation of its complementary messenger RNA (mRNA) via the RNA interference (RNAi) pathway. siRNA has been found as a powerful therapeutic approach in the treatment of cancer, viral infections, inflammatory diseases and inherited genetic disorders by selectively silencing disease-associated genes [92]. However, due to their hydrophilic property and negative surface charge, naked siRNA cannot be retained in the blood and has excessively high renal clearance and low cellular uptake.

Exosomes have shown to be highly promising as natural delivery vehicles for siRNA. They have a lipid bilayer membrane, which protects siRNA molecules from nuclease-mediated degradation and extends their circulation time in vivo. Furthermore, exosomes also contain membrane proteins like CD9, CD63, CD81 and integrins that enable receptor-mediated uptake into recipient cells [93]. These biological properties make the delivery efficiency into the cells much more efficient than the traditional liposomes and polymeric nanoparticles. There are a number of strategies that have been used to encapsulate siRNA into exosomes.

9.2 miRNA Delivery

MicroRNAs (miRNAs) are small (~18–25 nucleotides) endogenous RNA molecules that post-transcriptionally regulate gene expression through binding to the target messenger RNA (mRNA) through its complementary region. This interaction can lead to the degradation of the target mRNA or iRISK, thereby acting as a regulator of different biological processes, including cell proliferation, differentiation, apoptosis, angiogenesis, immune regulation and metabolism [94]. The dysregulation of miRNA expression has been linked to different diseases such as cancer, cardiovascular, neurodegenerative, diabetes and inflammatory. Therefore, the possibility of miRNA replacement therapy and anti-miRNA-based drugs has been suggested as promising treatment strategies for diseases. Multiple factors such as low stability in biological fluids, facile degradation by RNases, low cellular uptake and non-specific biodistribution limit the therapeutic utility of miRNAs. Because of these properties, exosomes have been the subject of much interest as natural miRNA nanocarriers, which could deliver miRNAs to target cells with ease while protecting them from the enzymatic degradation [95].

Additionally, alsolsonic miRNAs have demonstrated therapeutic effects in inflammatory conditions, hepatofibrosis, pulmonary fibrosis and metabolic diseases by regulating immune responses and re-stabilizing the cellular homeostasis. Although these encouraging results have been produced, there are some issues to overcome before more general use in the clinic can be made [96]. In order to support clinical translation, standardization of exosome isolation and purification, standard procedures for loading, large-scale manufacturing, quality control and long-term safety. Evaluations are necessary. Ongoing novel developments in exosome engineering and targeting modalities are likely to have a positive impact on the therapeutic efficacy and applications of exosome-encapsulated miRNA in precision medicine.

9.3 mRNA Delivery

The therapeutic potential of messenger RNA (mRNA) has revolutionized the treatment of various diseases, with the ability for transient protein expression in target cells. Compared to DNA-therapy, mRNA does not become part of the genome of its host, which makes it a safer choice in gene therapy. Once delivered into the cytoplasm, mRNA becomes a target for translation by the host cellular machinery into functional proteins that can be used to replace faulty proteins, to trigger immune responses or to direct tissue regeneration. Given the unprecedented success of mRNA vaccines for COVID-19, the field of mRNA-based therapeutics for cancer, infectious diseases, genetic disorders and regenerative medicine are rapidly gaining traction. But there are some problems with clinical use of mRNA. Unprotected, free mRNA molecules that exist in

physiological conditions are very vulnerable to the effects of RNase. They are not very stable in physiological conditions, have a large molecular size and are negatively charged, a factor that hampers their uptake by cells and might trigger innate immune responses. However, conventional delivery approaches, including lipid nanoparticles and viral vectors, have drawn a few limitations, including cytotoxicity, immunogenicity, and limited tissue specificity.

9.4 CRISPR/Cas9 Delivery Systems

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) combined with CRISPR-associated protein 9 (Cas9) has brought revolution in the field of genome editing for precise and efficient editing and modification of genome. The technology is based on a single-guide RNA (sgRNA), which guides the Cas9 enzyme to a specific sequence of DNA, and the Cas9 endonuclease [97]. Cas9 binds to the sequence, creating a double-stranded break in the target DNA that facilitates targeted deletion, repair or insertion of the DNA within the cell through repair processes. Due to the specificity and versatility of its function, CRISPR/Cas9 has become an attractive targeted treatment for inherited genetic diseases, cancer, and infectious and neurodegenerative diseases. Although it has great therapeutic promise, the use of CRISPR/Cas9 in therapy is hampered by key challenges in the safe and efficient delivery of the gene editing components [98]. The drawbacks of viral vectors include immunogenicity, insertional mutagenesis, low cargo capacity and safety concerns, while their advantages include high efficiency of delivery. The advantages of viral vectors include high efficiency in delivery and the disadvantages include immunogenicity, insertional mutagenesis, low capability of delivery of cargoes and potential safety issues.

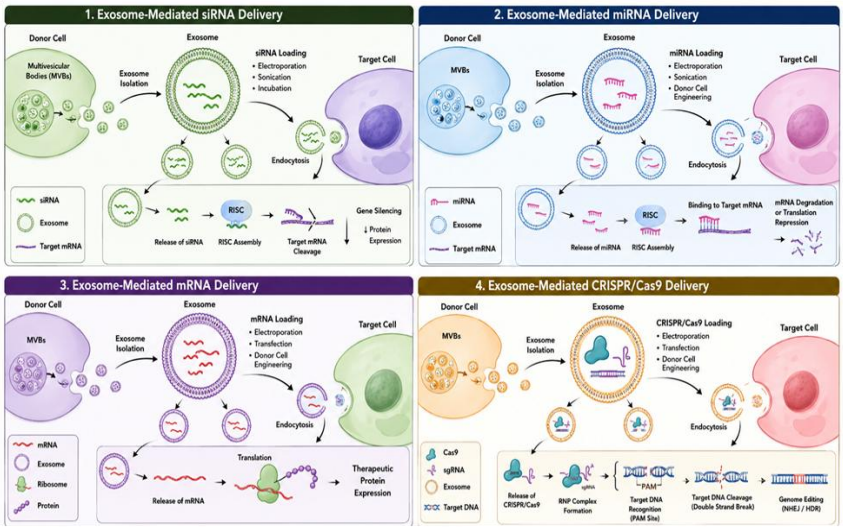

Figure 6: Exosome-Mediated Gene Delivery Platforms

1O. CLINICAL TRANSLATION AND COMMERCIAL DEVELOPMENT

10.1 Clinical Trials of Exosome-Based Therapeutics

Physicians use exosome-based therapeutics in clinical trials. Exosome based therapeutics is being used clinically [99]. The translation of these exosome-based drug delivery systems from the laboratory to the clinic has increased tremendously in recent years, due to their potential unique biological characteristics such as biocompatibility, low immunogenicity, intrinsic targeting ability, and the ability to transport therapeutic molecules [100]. There have been numerous preclinical studies that illustrate the potential of exosomes as natural nanocarriers for delivery of drugs, proteins, nucleic acids and gene-editing components. The positive results have sparked the growth of clinical trials focused on therapeutic approaches targeting other diseases using exosomes, such as cancer, cardiovascular diseases, neurological diseases, inflammatory conditions and regenerative medicine [101]. While many studies are still in early clinical development, preliminary findings show that exosome-based therapeutics offer several advantages over conventional approaches to drug delivery, such as increased drug bioavailability, reduced systemic toxicity, and improved efficacy [102]. However, larger scale randomized controlled trials are needed to obtain critical data on the long-term safety, optimal dosage, PK, BD and efficacy of exosome-based formulations for routine clinical use.

10.2 Current Commercial Products

Given the speed at which the exosome field has evolved, there is great interest from the Biotech and Pharma sectors. Many companies around the world are advancing research into the use of exosomes for therapeutic, diagnostic technologies, regenerative medicine treatments and targeted drug delivery [103]. The main areas of commercial interest are with engineered exosomes for the delivery of small molecule drugs, proteins, peptides, messenger RNA (mRNA), micro-RNA (miRNA) and small interfering RNA (siRNA). Additionally, exosome-based liquid biopsy platforms are being created to detect disease-specific biomarkers in the exosomes found in circulating blood in order to diagnose and monitor degenerative diseases, such as cancer, neurodegenerative diseases, and cardiovascular diseases, at early stages.

10.3 Industrial Scale-Up Strategies

One of the biggest hurdles for commercialization of therapeutic exosomes is their large-scale production. Traditional isolation practices like differential ultracentrifugation are only suitable for industrial scale production, which requires a lot of time and effort [104]. To ensure a higher production efficiency of exosomes retaining their biological activity and purity, alternative purification techniques, such as tangential flow filtration (TFF), size-exclusion chromatography (SEC), immunoaffinity separation (IAF) and microfluidic technologies have been developed. In vivo or in vitro cell culture systems based on bioreactor have proven to be promising systems for the scale-up production of exosomes. Such systems allow for cultivation of producer cells in a continuous fashion in a controlled environment, which would result in the production of more exosomes, and lower manufacturing costs. The use of automatic purification systems and Good Manufacturing Practice (GMP) factory is indispensable to guarantee the purity of the product, sterility, reproducibility and batch-to-batch consistency [105].

10.4 Manufacturing Challenges

Despite great advances in technology, there are still some manufacturing problems that need to be solved for the commercialization of exosome therapeutics. A drawback is the limited exosome production that can be achieved from cell culture

limiting the production of a large amount. Aside from the diversity of the cells, the methods of isolation and culture, heterogeneity of exosomes also makes purification and quality-control more complicated. Consistency in loading production batches with cargo is also difficult, as is retaining the biological activity [106]. However, another crucial consideration related to the limitations and the potential to alter the morphology of exosomes, decrease membrane integrity, and affect therapeutic efficacy would be storage stability, whereby repeated freeze-thaw cycles would harm exosomes. Technologies that improve the efficiency of preservation systems, cryoprotectants and standardized storage conditions will therefore be crucial to preserve product quality [107].

Table 7: Recent Clinical Trials on Exosome-Based Therapeutics (2020–2026)

Clinical Trial / NCT ID	Disease/Indication	Exosome Source	Therapeutic Cargo	Phase / Status
NCT03608631	Metastatic pancreatic cancer (KRASG12D mutation)	Mesenchymal stem cell (MSC)-derived exosomes	KRASG12D siRNA	Phase I (Active/Completed)
NCT01159288	Unresectable non-small cell lung cancer	Dendritic cell-derived exosomes	Tumor antigen-loaded exosomes	Phase II (Completed)
NCT01294072	Colon cancer	Plant-derived exosomes	Curcumin	Phase I (Recruiting/Completed)
MSC-derived exosome trials (2020–2026)	COVID-19/ARDS MSC-derived exosomes Native exosomes Phase I/II	MSC-derived exosomes	Bioactive proteins and miRNAs	Phase I/II
MSC-derived exosome trials	Osteoarthritis	MSC-derived exosomes	Growth factors	Phase I/II
MSC-derived exosome trials	Chronic wound healing	MSC-derived exosomes	Native exosomes	Early clinical evaluation
MSC-derived exosome trials	Graft-versus-host disease	MSC-derived exosomes	Native exosomes	Phase I/II

11. SAFETY, TOXICITY, AND REGULATORY CONSIDERATIONS

An understanding of the safety, toxicity, pharmacokinetics, and regulatory issues needs to be addressed before the clinical translation of exosome-based drug delivery systems can be achieved [108]. Exosomes have many benefits over synthetic nanoparticles, such as high biocompatibility, low immunogenicity, and intrinsic targeting action, but certain problems about their biodistribution, long-term toxicity, consistency of their manufacturing scale, and standardization in the regulatory agencies remain not trivial [109,110]. The rigorous preclinical and clinical testing program is highlighted by regulatory agencies, aimed at ensuring product quality, safety, and effectiveness before use in a clinical setting.

11.1 Immunogenicity

There are several key benefits of exosomes, such as their comparatively minimal immunogenicity compared to viral vectors and man-made nanoparticles. Due to the natural secretion by host cells, exosomes are generally considered immune "self," thus decreasing the possibility of immune rejection [111]. They carry therapeutic cargo through their lipid bilayer membrane, and reduce activation of inflammatory pathways. The immunogenicity varies significantly depending on the source, purification techniques and characteristics of the donor cells, as well as the composition of surface proteins. MSCs derived exosomes are recognized with good immunomodulatory activities and their use in therapeutic purposes is in great research focus. In the case of a tumor cell-derived exosome, it could be that immunogenic proteins or bioactive molecules in the exosomes enable unwanted immune responses; for allogeneic exosomes, it's the possibility that such proteins or molecules are present in the exosomes [112].

11.2 Biodistribution and Pharmacokinetics

The biodistribution and the pharmacokinetic characteristics of exosomes are important parameters to understand and optimize the therapeutic efficacy and reduce off-targeted effects. Exosomes are systemically distributed and are mainly internalized by organs in the reticuloendothelial system (RES), such as liver, spleen, lungs and kidneys. They do not determine their distribution because of particle size, surface proteins, lipid composition, route of administration or physiological status of recipient. Many synthetic nanoparticles are rapidly degraded or cleared by immune mechanisms, whereas exosomes have a long circulation time because their biological membrane helps to prevent this [113].They can get into target cells via receptor-mediated endocytosis, phagocytosis, macropinocytosis, and/or direct membrane fusion, and can deliver therapeutic cargo to the cells efficiently within. Advanced imaging technologies are widely used to explore exosome biodistribution and pharmacokinetics in vivo, such as fluorescence imaging, positron emission tomography (PET) and magnetic resonance imaging (MRI).

11.3 Toxicological Evaluation

Expanding the toxicological assessment of therapeutics based on exosomes is an essential condition of their development. Toxicity studies involve the repeated administration of the compound, such as repeated oral dose toxicity, organ toxicity, haematological changes, immunotoxicity, genotoxicity, reproductive toxicity and potential carcinogenicity. While most pre-clinical works indicate very good biocompatibility and almost absence of any systemic toxicity, there could be differences in safety performances, depending on the cellular

source and purification processes used, on the amount of drug formulation administered, and on the type of therapeutic cargo. Contamination with cell debris, leftover proteins, endotoxins, viruses, or any unwanted nucleic acids which can happen in production processes can be a potential safety problem.

11.4 FDA and EMA Regulatory Perspectives

As clinical development progresses, regulatory guidelines on the use of exosomes as therapeutics continue to progress. In the U.S., Food and Drug Administration (FDA) is generally a regulatory body that governs exosome products designed to treat diseases, which then must undergo extensive preclinical studies, Investigational New Drug (IND) applications, and clinical testing in phases, before they can be marketed. The FDA has also released public safety recommendations that at this present time, there are no FDA-approved products for clinical use as exosomes, and unsupported therapeutic claims should be avoided regarding the use of such unapproved exosomes. The European Medicines Agency (EMA) also assesses exosome therapeutics within the framework of the regulatory system for medicinal products.

11.5 Quality Control Requirements

To ensure the safety, efficacy and replicability of exosome-based therapeutics, it is essential to have a product quality control system in place. For particle size distribution, particle concentration, particle morphology, sterility, purity, potency, particle identity, cargo and biological activity, standardized analytical methods are required. Methodologies for characterization include: nanoparticle tracking analysis (NTA), western blotting, flow cytometry, enzyme-linked immunosorbent assay (ELISA), omics analysis, and transmission electron microscopy (TEM). For clinical-grade exosome production, some important quality attributes include batch-to-batch consistency, stability testing, and testing for endotoxins, microbial contamination and storage validation.

Table 8: Regulatory Guidelines and Safety Considerations

Parameter	Safety and Regulatory Consideration	Importance
Immunogenicity	Evaluate immune responses and cytokine release after administration; prefer low-immunogenic exosome sources such as MSC-derived exosomes.	Minimizes adverse immune reactions and improves patient safety.
Biodistribution	Assess tissue distribution, target-organ accumulation, circulation time, and clearance using validated imaging and pharmacokinetic studies	Ensures efficient targeting while reducing off-target toxicity.

Toxicological Evaluation	Perform acute, chronic, genotoxicity, immunotoxicity, and tumorigenicity studies before clinical use.	Demonstrates overall safety and supports regulatory approval.
FDA Requirements	Products are regulated as biological products and require preclinical studies, IND submission, cGMP-compliant manufacturing, and clinical trials before approval. No exosome therapeutic is currently	Ensures quality, safety, efficacy, and regulatory compliance
EMA Requirements	Exosome therapeutics are generally evaluated within the framework for advanced biologics/ATMPs, requiring GMP compliance, quality documentation, and clinical evidence.	Standardizes product development and ensures patient safety in Europe.

12.CHALLENGES AND LIMITATIONS

While the advantages of exosome-based drug delivery systems have been undeniable, the field has not yet realized many of these systems could be broadly used in the clinic, given the numerous scientific, technical, and regulatory hurdles [114]. There still are great challenges with regards to isolation and purification, loading the drugs, storage stability, standardization and scale-up for commercialization. These constraints will need to be overcome by technological advancements, harmonization of protocols, and regulatory efforts, if exosome therapeutics can be sufficiently translated for widespread clinical use [115].

12.1 Isolation and Purification Challenges

Isolation and purification of exosomes are some of the most important hurdles that must be addressed in exosome research. Standard approaches like differential ultracentrifugation, density gradient centrifugation, ultrafiltration and precipitation methods typically yield low amounts of material and can lead to less purity as the proteins, lipoproteins or other extracellular vesicles are co-isolated [116]. Microfluidic and Immunoaffinity capture technologies enable higher specificity, but are economically and technically difficult to scale up for industrial manufacture. The variability in the procedures used to isolate from one laboratory to another compounds the lack of reproducibility and comparison of the research results. Hence, the need for the evolution of the fast, affordable, highly productive, and standardized cell isolation methods continues to be important for research and for clinical use.

12.2 Low Drug Loading Efficiency

Another major drawback is that therapeutic cargo loading into exosomes are not efficient. Most of passive loading techniques result in low encapsulation efficiency and electroporation, sonication, extrusion, and freeze–thaw cycles, are some of the active loading approaches, which can likely cause some disruption to the exosomal membrane or cargo integrity. Additionally, loading efficiencies vary from molecule to molecule in terms of size, charge and physicochemical properties. Current challenges involve creating optimized loading strategies and engineered exosomes with the ability to selectively load therapeutic agents, to boost the efficiency of drug delivery and to influence therapeutic outcomes.

12.3 Storage and stability problems

The storage of structurally intact and biologically active exosomes is still a great challenge. The membrane of the exosomes may be disrupted, aggregation, cargo degradation, and reduced therapeutic efficacy may occur due to temperature extremes and repeated freeze and thaw, as well as during extended storage. Several factors have been examined to enhance the stability, such as appropriate storage media, cryoprotective agents and lyophilization procedures, but there is no universally accepted procedure for successful storage. It is desirable for the optimized exosome product storage conditions to be determined with long-term stability studies [117].

12.4 Standardization Problems

Important hurdles to the clinical translation is the lack of standardized protocols for isolation, characterization, quantitation and quality assessment of exosomes. All these differences related to the donor cell origin, culture conditions, purification methods, analytical tools and cargo loading protocols lead to high variability of exosome quality and biological activity. Furthermore, the absence of standard standards for convenient reference standards makes quality control and regulatory approval more difficult. To effectively establish standard rules for exosome production, characterization, and therapeutic evaluation, collaboration has to be developed among researchers, regulatory authorities, and industry.

12.5 Large-Scale Manufacturing Challenges

The commercial production of clinical grade exosomes will need scalable, reproducible and cost-effective manufacturing processes. However, in current production methods, exosome yield is low and labor-intensive and difficult to scale production purification procedures are used. It is technically difficult to maintain batch-to-batch consistency for large-scale manufacturing of products that must be sterile, pure, potent and biologically active. Good Manufacturing Practice (GMP) standards add to the complexity and cost of production. Expansion of bioreactor technology capabilities, automation of purification systems, AI-aided production and automation of production platforms are anticipated to enhance the efficiency of production and promote commercialization of exosome-based therapeutics.

13. EMERGING TRENDS AND FUTURE PERSPECTIVES

13.1 Artificial Intelligence in Exosome Engineering

Artificial intelligence (AI) and machine learning (ML) are increasingly being integrated into

exosome research to optimize exosome engineering, cargo selection, and therapeutic design. AI algorithms can analyze large biological datasets to identify suitable donor cells, predict therapeutic cargo loading, optimize targeting ligands, and improve manufacturing efficiency [118]. Machine learning also assists in biomarker discovery, disease diagnosis, and prediction of treatment outcomes. Furthermore, AI-driven automation can enhance quality control, reduce manufacturing variability, and accelerate the clinical development of exosome-based therapeutics.

13.2 Hybrid Exosome–Nanoparticle Systems

Hybrid exosomes–nanoparticle systems combine the natural advantages of exosomes with the functional properties of synthetic nanoparticles. These hybrid carriers improve drug-loading efficiency, structural stability, controlled drug release, imaging capability, and targeted delivery. Nanomaterials such as liposomes, polymeric nanoparticles, gold nanoparticles, magnetic nanoparticles, and mesoporous silica nanoparticles have been successfully integrated with exosomes to develop multifunctional delivery systems. These hybrid platforms have demonstrated promising applications in cancer therapy, gene delivery, bioimaging, and theranostics.

13.3 Personalized Exosome-Based Therapies

Personalized medicine aims to provide individualized treatment based on a patient's genetic, molecular, and clinical characteristics. Patient-derived exosomes have emerged as promising carriers for personalized drug delivery because they exhibit excellent biocompatibility, minimal immunogenicity, and improved targeting efficiency. Personalized exosome-based therapies enable the delivery of customized drugs, proteins, siRNA, miRNA, mRNA, and gene-editing components according to individual disease profiles. This approach has shown significant potential in cancer treatment, rare genetic disorders, and regenerative medicine, supporting the development of precision therapeutics with improved clinical outcomes.

13.4 Smart and Stimuli-Responsive Exosomes

Smart exosomes are engineered to release their therapeutic cargo only in response to specific internal or external stimuli, thereby improving treatment precision and reducing systemic side effects. Stimuli-responsive exosomes can respond to changes in pH, temperature, enzyme activity, redox conditions, ultrasound, magnetic fields, or light irradiation. These intelligent delivery systems enable controlled drug release at disease sites while minimizing exposure to healthy tissues. Ongoing research focuses on developing multifunctional exosomes capable of simultaneous targeted delivery, controlled release, diagnostic imaging, and therapeutic monitoring for advanced biomedical applications.

13.5 Exosomes in Precision Medicine

Exosomes are expected to play a central role in the future of precision medicine due to their unique ability to deliver therapeutic agents with high specificity while simultaneously serving as diagnostic biomarkers. Their natural targeting properties allow efficient transport of drugs, nucleic acids, proteins, and gene-editing tools to diseased tissues, reducing off-target toxicity and improving therapeutic efficacy [119]. In addition, exosomes isolated from patient body fluids can provide valuable molecular information for early disease diagnosis, prognosis, and treatment monitoring through liquid biopsy technologies. Continued advancements in exosome engineering, artificial intelligence, gene editing, and nanotechnology are expected to accelerate the clinical translation of exosome-based precision medicine and establish exosomes as an essential component of next-generation personalized healthcare.

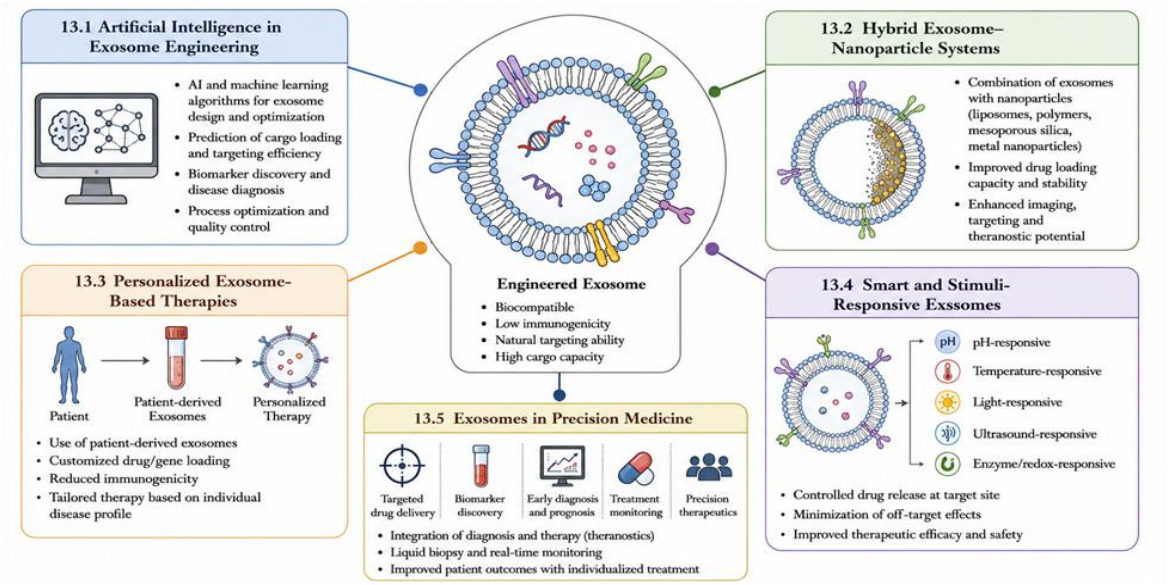

Figure 7: Future Trends in Exosome-Based Drug Delivery

14. CONCLUSION

Exosomes have become one of the most exciting developments in nanomedicine, offering a natural alternative to synthetic drug carriers. Because they come from the body's own cells, they're well-tolerated, don't trigger strong immune reactions, stay in circulation longer, and can even cross tough barriers like the blood-brain barrier. They can also carry a wide range of therapeutic cargo, from small drug molecules to proteins and gene-editing tools. This review covered the basics of exosome biology, how they're sourced and isolated, how they're engineered, and how they're used therapeutically [120]. Engineering methods, like modifying their surface or genetically altering the cells that produce them, have made these exosomes more precise and effective, improving how well they target disease sites while cutting down on side effects. This has opened doors in cancer treatment, neurological and cardiovascular disease, infections, regenerative medicine, and gene therapy.

Beyond drug delivery, exosomes are also showing real promise as biomarkers, helping detect diseases early and monitor treatment through liquid biopsies. Combined with gene therapy, they're paving the way toward more personalized treatment approaches. That said, real challenges remain. There's still no standardized way to isolate, purify, characterize, or manufacture exosomes at scale, and questions around safety, biodistribution, and regulatory approval need more research before these therapies can become mainstream. Solving these problems will take close collaboration between scientists, clinicians, regulators, and industry. Looking forward, progress in nanotechnology, molecular biology, and AI should help push engineered exosomes further, making them more targeted and easier to manufacture at scale [121]. Future work needs to focus on standardizing production and running larger clinical trials to prove these therapies work across different diseases. Overall, exosome-based drug delivery represents a genuinely transformative approach to medicine. As research continues to close the remaining gaps, these therapies are likely to play a major role in how we diagnose and treat disease going forward, making healthcare more precise and more personal.

REFERENCES

1. Gurung S, Perocheau D, Touramanidou L, Baruteau J. The exosome journey: from biogenesis to uptake and intracellular signalling. Cell Communication and Signaling. 2021;19:47.

2. Butreddy A, Kommineni N, Dudhipala N. Exosomes as Naturally Occurring Vehicles for Delivery of Biopharmaceuticals: Insights from Drug Delivery to Clinical Perspectives. Nanomaterials. 2021;11(6):1481.

3. Gutierrez-Millan C, Calvo Díaz C, Lanao JM, Colino CI. Advances in Exosomes-Based Drug Delivery Systems. Macromolecular Bioscience. 2021;21:e2000269.

4. Kučuk N, Primožič M, Knez Ž, Leitgeb M. Exosomes Engineering and Their Roles as Therapy Delivery Tools, Therapeutic Targets, and Biomarkers. International Journal of Molecular Sciences. 2021;22(17):9543.

5. Massey AE, Malik S, Sikander M, et al. Clinical Implications of Exosomes: Targeted Drug Delivery for Cancer Treatment. International Journal of Molecular Sciences. 2021;22(10):5278.

6. Kugeratski FG, Kalluri R. Exosomes as mediators of immune regulation and immunotherapy in cancer. The FEBS Journal. 2021;288(1):10–35.

7. de Toledo MA, et al. Challenges in the development and establishment of exosome-based drug delivery systems. Journal of Controlled Release. 2021;329:894–906

8. Rezaie J, Feghhi M, Etemadi T. Exosomes in clinical trials: perspectives, challenges and opportunities. Cell Communication and Signaling. 2022.

9. Chen H, Wang L, Zeng X, et al. Exosomes, a new star for targeted delivery. Frontiers in Cell and Developmental Biology. 2021;9

10. Sun Y, Liu G, Zhang K, et al. Mesenchymal Stem Cell-Derived Exosomes for Drug Delivery. Stem Cell Research & Therapy. 2021.

11. Batrakova EV, Kim MS. Using exosomes, naturally equipped nanocarriers, for drug delivery. Journal of Controlled Release. 2015;219:396–405.

12. Vader P, Mol EA, Pasterkamp G, Schiffelers RM. Extracellular vesicles for drug delivery.

Advanced Drug Delivery Reviews. 2016;106:148–156.

13. Luan X, Sansanaphongpricha K, Myers I, Chen H, Yuan H, Sun D. Engineering exosomes as refined biological nanoplatforms for drug delivery. Acta Pharmacologica Sinica. 2017;38:754–763.

14. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. Science. 2020;367(6478):eaau6977.

15. Pegtel DM, Gould SJ. Exosomes. Annual Review of Biochemistry. 2019;88:487–514.:

16. Tang Y, Zhou Y, Li HJ. Advances in mesenchymal stem cell exosomes: a review. Stem Cell Res Ther. 2021;12:71.

17. Lee BC, Kang I, Yu KR. Therapeutic features and updated clinical trials of mesenchymal stem cell (MSC)-derived exosomes. J Clin Med. 2021;10(4):711.

18. Quiñones-Vico MI, Sanabria-de la Torre R, Sánchez-Díaz M, Sierra-Sánchez Á, et al. The role of exosomes derived from mesenchymal stromal cells in dermatology. Front Cell Dev Biol. 2021;9:647012. 19. Hassanpour M, Rezaie J, Nouri M, Panahi Y. New therapeutic approaches of mesenchymal stem cells-derived exosomes. J Biomed Sci. 2021;28:39.

20. Hassanzadeh A, Rahman HS, Markov A, et al. Mesenchymal stem/stromal cell-derived exosomes in regenerative medicine and cancer: overview of development, challenges, and opportunities. Stem Cell Res Ther. 2021;12:297.

21. Hade MD, Suire CN, Suo Z. Mesenchymal stem cell-derived exosomes: applications in regenerative medicine. Cells. 2021;10(8):1959.

22. Luo T, von der Ohe J, Hass R. MSC-derived extracellular vesicles in tumors and therapy. Cancers (Basel). 2021;13(20):5212.

23. Hosseini R, Asef-Kabiri L, Yousefi H, et al. The roles of tumor-derived exosomes in altered differentiation, maturation and function of dendritic cells. Mol Cancer. 2021;20:83.

24. Karamanidou T, Tsouknidas A. Plant-derived extracellular vesicles as therapeutic nanocarriers. Int J Mol Sci. 2022;23(1):191.

25. Zhao R, Chen X, Song H, Bie Q, Zhang B. Dual role of MSC-derived exosomes in tumor development. Stem Cells Int. 2020;2020:8844730.

26. El Andaloussi S, Mäger I, Breakefield XO, Wood MJA. Extracellular vesicles: Biology and emerging therapeutic opportunities. Nat Rev Drug Discov. 2013;12(5):347–357.

27. Yáñez-Mó M, Siljander PRM, Andreu Z, Zavec AB, Borràs FE, Buzas EI, et al. Biological properties of extracellular vesicles and their physiological functions. J Extracell Vesicles. 2015;4:27066.

28. Raposo G, Stoorvogel W. Extracellular vesicles: Exosomes, microvesicles, and friends. J Cell Biol. 2013;200(4):373–383.

29. Johnstone RM. Revisiting the road to the discovery of exosomes. Blood Cells Mol Dis. 2005;34(3):214–219.

30. 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. Nat Cell Biol. 2007;9(6):654–659

31. Théry C, Zitvogel L, Amigorena S. Exosomes: Composition, biogenesis and function. Nat Rev Immunol. 2002;2(8):569–579.

32. Wang Q, Zhuang X, Mu J, Deng ZB, Jiang H, Zhang L, et al. Delivery of therapeutic agents by nanoparticles made of grapefruit-derived lipids. Nat Commun. 2013;4:1867.

33. Kavya ANVL, Subramanian S, Ramakrishna S. Therapeutic applications of exosomes in various diseases: A review. Biomaterials Advances. 2022;134:112579.

34. Chen Z, Xiong M, Tian J, Song D, Duan S, Zhang L, et al. Encapsulation and assessment of therapeutic cargo in engineered exosomes: A systematic review. J Nanobiotechnology. 2024;22:18.

35. Tan F, Li X, Wang Z, Li J, Shahzad K, Zheng J, et al. Clinical applications of stem cell-derived exosomes. Signal Transduct Target Ther. 2024;9:17.

36. Kim HI, Park J, Zhu Y, Wang X, Han Y, Zhang D, et al. Recent advances in extracellular vesicles for therapeutic cargo delivery. Exp Mol Med. 2024;56:836–849.

37. Lee KWA, Chan LKW, Hung LC, Phoebe LKW, Park Y, Yi KH. Clinical Applications of Exosomes: A Critical Review. Int J Mol Sci. 2024;25(14):7794

38. Palakurthi SS, Shah B, Kapre S, Charbe N, Immanuel S, Pasham S, et al. A comprehensive review of challenges and advances in exosome-based drug delivery systems. Nanoscale Adv. 2024;6:5803–5826.

39. Fan X, Zhang Y, Liu W, Shao M, Gong Y, Wang T, et al. A comprehensive review of engineered exosomes from the preparation strategy to therapeutic applications. Biomater Sci. 2024;12:3500–3521

40. Pang JL, Shao H, Xu XG, Lin ZW, Chen XY, et al. Targeted drug delivery of engineered mesenchymal stem/stromal cell-derived exosomes in cardiovascular disease: Recent trends and future perspectives. Front Bioeng Biotechnol. 2024;12.

41. Alghamdi BS, Alghamdi SA, et al. Insight into the Functional Dynamics and Challenges of Exosomes in Pharmaceutical Innovation and Precision Medicine. Pharmaceutics. 2024;16(6):709.

42. Gurunathan S, Kang MH, Jeyaraj M, Qasim M, Kim JH. Review of the Isolation, Characterization, Biological Function, and Multifarious Therapeutic Approaches of Exosomes. Cells. 2019;8(4):307

43. Zhang Y, Liu Y, Liu H, Tang WH. Exosomes: Biogenesis, Biologic Function and Clinical Potential. Cell & Bioscience. 2019;9:19

44. Popowski KD, Lutz H, Hu S, George A, Dinh PU, Cheng K. Exosome therapeutics for lung regenerative medicine. Journal of Extracellular Vesicles. 2020;9(1):1785161.

45. Théry C, Witwer KW, Aikawa E, et al. Minimal Information for Studies of Extracellular Vesicles 2018 (MISEV2018). J Extracell Vesicles. 2018;7:1535750.

46. Mathieu M, Martin-Jaular L, Lavieu G, Théry C. Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. Nat Cell Biol. 2019;21:9–17.

47. Raposo G, Stahl PD. Extracellular vesicles: a new communication paradigm? Nat Rev Mol Cell Biol. 2019;20:509–510.

48. Alzhrani GN, Alanazi ST, Alsharif SY, et al. Exosomes: Isolation, characterization, and biomedical applications. Cell Biochem Funct. 2021;45:1807–1831. (Also referenced as Alzhrani GN, et al. 2021).

49. Wang X, Xia J, Yang L, et al. Recent progress in exosome research: isolation, characterization and clinical applications. Cancer Gene Ther. 2023;30:1051–1065.

50. Agarwal P, Anees A, Kumar P, et al. A Comprehensive Review on Exosome: Recent Progress and Outlook. Pharm Nanotechnol. 2024.

51. Chaudhari AP, Budayr OM, Bonacquisti EE, et al. The status of extracellular vesicles as drug carriers and therapeutics. Nat Rev Bioeng. 2026.

52. Kurian TK, Banik S, Gopal D, et al. Elucidating Methods for Isolation and Quantification of Exosomes: A Review. Mol Biotechnol. 2021;63:249–266.

53. Bano R, Ahmad F, Mohsin M. A perspective on the isolation and characterization of extracellular vesicles from different biofluids. RSC Adv. 2021;11:19598–19615.

54. Sani M, et al. Unveiling exosomes: Cutting-edge isolation techniques and their therapeutic potential. J Cell Mol Med. 2024.

55. Zhang Y, et al. Recent developments in isolating methods for exosomes. TrAC Trends Anal Chem. 2023.

56. Konoshenko MY, et al. Isolation of extracellular vesicles: General methodologies and latest trends.

57. Li P, Kaslan M, Lee SH, et al. Progress in exosome isolation techniques.

58. Ludwig AK, Giebel B. Exosomes: small vesicles participating in intercellular communication.

59. Xu R, Rai A, Chen M, et al. Extracellular vesicles in cancer — implications for isolation.

60. Gardiner C, et al. Techniques used for the isolation and characterization of extracellular vesicles.

61. Lobb RJ, Becker M, Wen SW, et al. Optimized exosome isolation protocol for cell culture supernatant and human plasma.

62. Witwer KW, Goberdhan DCI, et al. Updating extracellular vesicle characterization standards.

63. Dragovic RA, et al. Sizing and phenotyping of cellular vesicles using Nanoparticle Tracking Analysis.

64. Gardiner C, et al. Techniques for extracellular vesicle characterization.

65. Coumans FAW, et al. Methodological guidelines for extracellular vesicle characterization.

66. Van der Pol E, et al. Particle size analysis of extracellular vesicles.

67. Lässer C, et al. Proteomic characterization of exosomes.

68. Zhang H, Freitas D, Kim HS, et al. Identification of distinct nanoparticle populations among extracellular vesicles.

69. Mehryab F, Rabbani S, Shahhosseini S, et al. Exosomes as a next-generation drug delivery system: An update on drug loading approaches. Acta Biomater. 2020.

70. Gündüz M, et al. Exosomes: Large-scale production, isolation, drug loading efficiency, and biodistribution and uptake. J Control Release. 2022.

71. Wu J, Li R, et al. Exosome Augmentation Technologies for Drug Delivery and Disease Treatment. Biomater Res. 2026.

72. Kim MS, Haney MJ, Zhao Y, et al. Development of exosome-based drug delivery systems.

73. Elsharkasy OM, et al. Extracellular vesicles as drug delivery systems.

74. Tian T, Zhang HX, He CP, et al. Surface-functionalized exosomes for targeted drug delivery.

75. Johnsen KB, Gudbergsson JM, et al. A comprehensive overview of exosomes as drug delivery vehicles.

76. Murphy DE, et al. Engineering extracellular vesicles for targeted drug delivery.

77. Mulcahy LA, Pink RC, Carter DRF. Routes and mechanisms of extracellular vesicle uptake.

78. Tian T, Zhu YL, Hu FH, et al. Dynamics of exosome internalization and intracellular trafficking.

79. Costa Verdera H, Gitz-Francois JJ, Schiffelers RM, Vader P. Cellular uptake of extracellular vesicles.

80. McAndrews KM, et al. Mechanisms of extracellular vesicle uptake in cancer.

81. Joshi BS, de Beer MA, et al. Endocytosis and membrane fusion of extracellular vesicles.

82. O'Brien K, Breyne K, Ughetto S, et al. RNA delivery by extracellular vesicles.

83. Zhang Y, Liu Q, Zhang X, Huang H, Tang S, Chai Y, Xu Z, Li M, Chen X, Liu J, Yang C. Recent advances in exosome-mediated nucleic acid delivery for cancer therapy.

84. Amiri A, Bagherifar R, Ansari Dezfouli E, Kiaie SH, Jafari R, Ramezani R. Exosomes as bio-inspired nanocarriers for RNA delivery: preparation and applications.

85. Guo J, Zhang J, Han X, et al. Extracellular vesicles as delivery vehicles for therapeutic nucleic acids in cancer gene therapy: Progress and challenges. PMC.

86. Almohaimeed HM, Waggas HA, Altowaijri OM, Abdulhakim JA, Mohammedsaleh ZM, Almars AI, et al. Exosome precision engineering: A comprehensive method for targeted gene and drug delivery. ScienceDirect / Frontiers. 2026.

87. Wang Y, Li P, Zhao W, et al. Engineering exosomes for RNA-based therapeutic delivery.

88. Li X, Chen Y, Zhang L, et al. Exosome-mediated siRNA delivery for targeted cancer therapy.

89. Chen J, Liu H, Wang X, et al. Exosome-based mRNA delivery systems for precision medicine.

90. Zhao L, Sun Y, Zhang H, et al. Advances in CRISPR/Cas9 delivery using engineered exosomes.

91. Liu X, Wang C, Zhang Z, et al. MicroRNA-loaded exosomes as therapeutic nanocarriers for human diseases.

92. Wu H, Yang Y, Li J, et al. Extracellular vesicle-based delivery systems for nucleic acid therapeutics.

93. Rezaie J, Feghhi M, Etemadi T. A review on exosomes application in clinical trials: Perspective, questions, and challenges. PubMed. 2022.

94. Rahnama M, Heidari M, Poursalehi Z, Golchin A. Global Trends of Exosomes Application in Clinical Trials: A Scoping Review. 2024.

95. Verma N, Arora S. Navigating the Global Regulatory Landscape for Exosome-Based

Therapeutics: Challenges, Strategies, and Future Directions. Pharmaceutics. 2025.

96. Matsuzaka Y, Yashiro R. Exosomes as Therapeutic and Diagnostic Tools: Advances, Challenges, and Future Directions. 2025.

97. Cheng CH, Hao WR, Cheng TH. Stem Cell Exosomes: New Hope for Recovery from Diabetic Brain Hemorrhage. PMC. 2024.

98. Rahnama M, Golchin A, et al. Clinical Translation of Exosome-Based Therapeutics: Current Progress and Future Prospects. 2024.

99. Park SJ, Jung HI. Critical Challenges and Future Direction in Extracellular Vesicle Research and Commercialization. 2025.

100. Verma N, Arora S. Quality Control and Regulatory Harmonization for Exosome-Based Therapeutics. 2025.

101. Yoo KH, Ha DH, Kim JO, et al. Quality and Safety Considerations for Therapeutic Products Based on Extracellular Vesicles. Pharmaceutics. 2024.

102. Limongi T, Milla P, Stella B, Arpicco S. Regulatory Challenges and Opportunities for Cell-Derived Extracellular Vesicles in Pharmaceutical Development: A European and Global Perspective. Journal of Extracellular Vesicles. 2026.

103. Kim H, Lee J, Park S, et al. Regulation of Exosomes as Biologic Medicines: Regulatory Challenges Faced in Exosome Development and Manufacturing Processes. 2024.

104. Almohaimeed HM, Waggas HA, Altowaijri OM, et al. Therapeutic Potential of Extracellular Vesicles: From Biogenesis, Isolation and Molecular Characterization to Addressing Translational Gaps and Regulatory Barriers. International Journal of Molecular Sciences. 2025.

105. Maher IA, et al. Regulatory, Ethical, and Safety Considerations of Exosome-Based Therapies in Dermatology. Dermatological Reviews. 2026.

106. Steć A, et al. Toxicology and Biodistribution of Plant-Derived Extracellular Vesicles for Drug Delivery: Quality Control, Safety Mechanisms, and Translational Testing Priorities. Toxicology Letters. 2026.

107. Limongi T, Milla P, Stella B, Arpicco S. Safety Evaluation and Regulatory Perspectives for Extracellular Vesicle-Based Therapeutics. Journal of Extracellular Vesicles. 2026.

108. Serrano A, et al. Exosome-Based Drug Delivery: A Next-Generation Platform for Cancer, Infection, Neurological and Immunological Diseases, Gene Therapy and Regenerative Medicine. Pharmaceutics. 2025.

109. Liu Y, Zhang Y, et al. Progress, challenges, and prospects of small extracellular vesicles isolation and characterization. Journal of Holistic Integrative Pharmacy. 2024.

110. Kim J, Lee H, et al. Manufacturing Therapeutic Exosomes: From Bench to Industry. Molecules and Cells. 2022.

111. Miceli V, et al. Extracellular vesicles, RNA sequencing, and bioinformatic analyses: Challenges, solutions, and recommendations. Journal of Extracellular Vesicles.

112. Wójcik M, et al. Human Blood Exosomes: Isolation and Characterization Methods, Variability, and the Need for Standardized Protocols—A Review. Biomedicines.

113. Zhang X, Li Y, et al. Recent advances in scalable exosome production: Challenges and

innovations. Chinese Journal of Plastic and Reconstructive Surgery. 2025.

114. Singh R, et al. Exosomal bioactive molecules: Molecular regulation, functional heterogeneity, and translational implications in disease diagnostics and therapeutics. Clinical and Experimental Medicine. 2026. (Also referenced under identical title in 2025).

115. Kaur G, et al. Most recent advances and applications of extracellular vesicles in tackling neurological challenges. Medicinal Research Reviews. 2024.

116. Zhang Y, Chen X, et al. From bench to bedside: The promise and roadblocks of extracellular vesicle therapeutics. Advanced Drug Delivery Reviews. 2025.

117. Picchio V, Pontecorvi V, Dhori X, Bordin A, Floris E, Cozzolino C, et al. The emerging role of artificial intelligence applied to exosome analysis: From cancer biology to other biomedical fields. Life Sciences. 2025.

118. Mendonca SR, Bangera PD, Keerikkadu M, Tippavajhala VK, Rathnanand M, et al. Multifunctional Engineering of Exosomes for Precision Therapeutics: Strategies for Targeted Delivery, Barrier Evasion, and Clinical Translation. Pharmaceutical Research. 2025.

119. Chao T, Zhao J, Gao R, Wang H, Guo J, Gao Z, et al. Exosome Surface Modification and Functionalization: A Narrative Review of Emerging Technologies and Their Application Potential in Precision Medicine. Advanced Technology in Neuroscience. 2025.

120. Dai X, Ye Y, He F. Emerging Innovations on Exosome-Based Onco-Therapeutics. Frontiers in Immunology. 2022.

121. Preetam S, Lata S, Aditi, Bora J, Thapliyal S, Mehta S, et al. Exosome-Driven Biohybrid Nanorobots: Bridging Nature and Nanotechnology in Bi