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REVIEW

Cutting-Edge Progress in the Acquisition, Modification and Therapeutic Applications of Exosomes for Drug Delivery

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Abstract: Exosomes are vesicles secreted by cells, typically ranging from 30 to 150 nm in diameter, and serve as crucial mediators of intercellular communication. Exosomes are capable of loading various therapeutic substances, such as small molecule compounds, proteins, and oligonucleotides, thereby making them an ideal vehicle for drug delivery. The distinctive biocompatibility, high stability, and targeting properties of exosomes render them highly valuable for future treatments of diseases like cancer and cardiovascular diseases. Despite the potential advantage of exosomes in delivering biologically active molecules, the techniques for the preparation, purification, preservation, and other aspects of stem cell exosomes are not yet mature enough. In this paper, we briefly introduce the composition, biogenesis, and benefits of exosomes, and primarily focus on summarizing the isolation and purification methods of exosomes, the preparation of engineered exosomes, and their clinical applications, to better provide new ideas for the development of exosome drug delivery systems.

Keywords: exosomes, isolation, preservation, drug delivery, engineered exosomes, applications

Introduction

Exosomes, nano-scale vesicles secreted by cells into their extracellular compartments, play a crucial role in various biological processes such as cell recognition and signal transduction through paracrine and autocrine secretion. They carry an abundant cargo of DNA, RNA, proteins, among other molecules, making them a novel cellular communication tool.[1] Exosomes possess excellent biocompatibility, stability, and targeting capabilities,[2] making them a promising candidate for drug delivery systems. Moreover, their versatility allows for engineering and modification in various ways to enhance their therapeutic potential. Consequently, exosomes have emerged as a highly effective drug delivery system that has found widespread applications in the treatment of cardiovascular and cerebrovascular diseases, neurological disorders, tumors, and other medical conditions.[3] With the further advancement of diagnostic and treatment technologies for exosome-related diseases, the two technical hurdles that constrain the fundamental and clinical application of exosomes, namely the separation, purification, and preservation of exosomes, demand greater attention from us.[4] Currently, based on exosome particle size, density, surface markers, etc., researchers have developed a variety of separation and purification techniques, including centrifugation, ultrafiltration, immunoaffinity, microfluidics.[5] To address the preservation challenges, researchers have proposed methods such as freezing, lyophilization, and other storage techniques to maintain the integrity and stability of exosomes to the greatest extent possible.[6]

In this review, we will describe the biogenesis of exosomes and their advantages as drug delivery carriers, summarize isolation and preservation methods along with engineering modification strategies, illustrate the application of exosome-

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mediated targeted drug delivery in clinical disease models, and highlight key translational challenges and opportunities for future exosome development.

Exosome Biogenesis and Composition

Exosomes are small vesicles secreted by cells, typically ranging from 30 to 150 nm in diameter,[7] and are widely found in various biological fluids. Exosome biogenesis is a complex and orderly dynamic process that includes the formation of early endosomes, their maturation into multivesicular bodies (MVBs), and the fusion of MVBs with the cell membrane. Specifically, the cell membrane invaginates inward to form a cup-shaped structure, which further develops into early endosomes. These early endosomes then undergo invagination and protrusion through the vesicle membrane, forming multiple small intraluminal vesicles (ILVs) that selectively encapsulate nucleic acids, proteins, and lipids from the cytoplasm. The resulting multivesicular bodies can either fuse with lysosomes or autophagosomes for degradation, or with the cell membrane to release the internal vesicles, known as exosomes[4,8,9] (Figure 1).

Exosome production is a complex chain of molecular events that requires the collaboration of various protein complexes, this includes both ESCRT-dependent and ESCRT-independent pathways.[10] Together, these factors play a role in ensuring that cells can selectively load molecules into exosomes and regulate the role they play in the exchange of information between cells. It is currently believed that exosome formation is mainly regulated through the ESCRT-dependent pathway, which consists of four complexes: ESCRT-0 (Hrs), ESCRT-I (TSG101, Vps28, Vps37), ESCRT-II (Vps22, Vps36, Vps25), and ESCRT-III (Alix, Vps2).[11] In vivo, ubiquitin markers are recognized by the first ESCRT complex, ESCRT-0, which is enriched at the endosomal membrane and delivers ubiquitinated material to ESCRT-I and ESCRT-II. Tsg101 in ESCRT-I recognizes the disulfide bond and induces the endosomal membrane to be pitted, and then ESCRT-III shears the bud neck to form MVBs.[12] The ESCRT non-dependent pathways mainly include the lipid raft-dependent pathway and the four-membrane transprotein family. The lipid raft-dependent pathway mainly involves ceramide. Ceramide induces the formation of luminal vesicles, facilitates the entry of biomolecules rich in proteins,

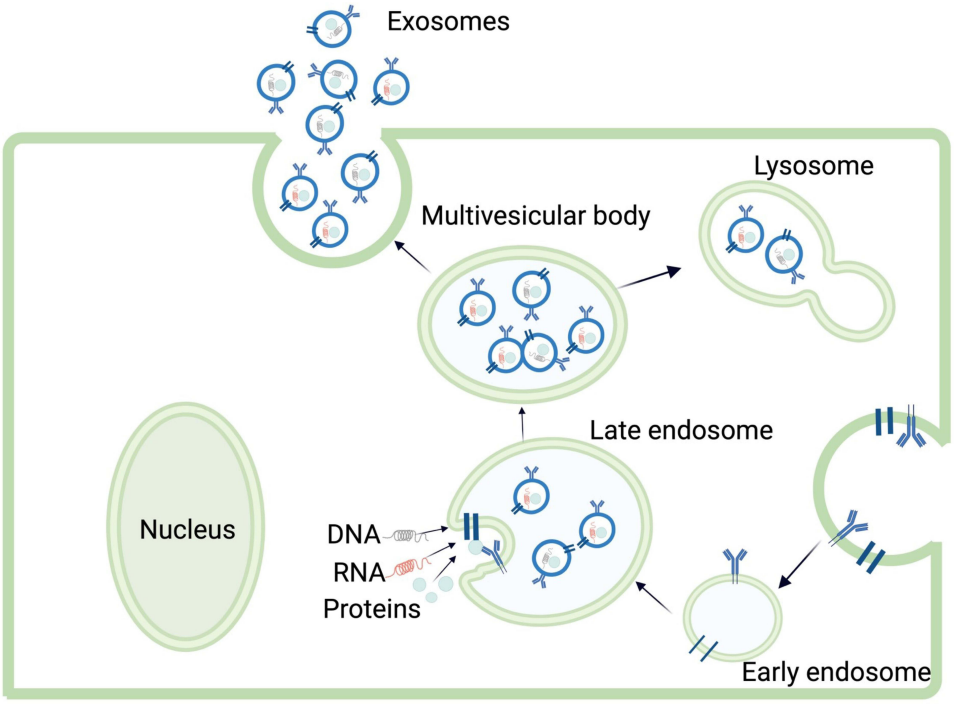

Figure 1 Biogenesis of exosomes. Firstly, the cell membrane invaginates to form a cup-shaped structure, which further forms an early nucleosome; Then, the membrane of the early nucleosome invaginates to encapsulate DNA, RNA, or protein in cytoplasm and buds into vesicles to form the late nucleosome, and then the late nucleosome matures into polycystic bodies; Finally, the cyst fuses with the cell membrane and releases the vesicles to form exosomes Some polyethylene can also be fused with lysosomes and degraded. Created in BioRender. Hu, B. (2025) https://BioRender.com/y22x389.

RNA and lipid rafts into luminal vesicles, and promotes the fusion of luminal vesicles with the cell membrane to release exosome.[13] The tetraspanin family, which includes proteins such as CD63, CD81, and CD9, plays a crucial role in exosome biogenesis and selective cargo loading. Tetraspanins maintain the membrane integrity of multivesicular bodies (MVBs) by forming a meshwork with other membrane proteins.[4,13,14]

The structure of exosome vesicles primarily consists of a phospholipid bilayer encapsulating a variety of biologically active molecules.[15] The phospholipid bilayer, which forms the outer shell of the exosome, is mainly composed of phosphatidylcholine and other phospholipids, with a variety of membrane proteins embedded within it, including tetraspanins (eg, CD63, CD9, CD81), heat-shock proteins (HSPs), and other exosome-specific proteins. These proteins play a crucial role in exosome isolation and signaling.[16] The loaded components of lipid vesicles include a variety of bioactive molecules such as proteins, nucleic acids, metabolites.[17] These molecules interact with the phospholipid bilayer to form a stable structure and endow the exosome with rich biological information, which can be transmitted to other cells to exert specific biological effects.

Isolation and Extraction of Exosomes

Exosomes are nanoscale extracellular vesicles present in complex body fluids and serve as critical mediators of intercellular communication. The isolation and purification of exosomes are essential for subsequent research and clinical applications.[9] Currently, reported methods for exosome isolation and purification include ultracentrifugation, density gradient centrifugation, ultrafiltration, size-exclusion chromatography, polymer precipitation, affinity separation, and microfluidic techniques. Each method possesses distinct advantages and limitations. In this paper, we comprehensively analyze the principles, advantages, disadvantages, separation efficiencies, and applications of each technique (Table 1).

Table 1 Current Methods of Exosome Isolation

Separation Method	Principle	Advantage	Drawbacks	Separation Efficiency	Scope of Application
Ultracentrifugation	Step-by-step separation based on density, size, and dimensions	Uncontaminated by isolation reagents; a large number of exosomes was obtained; relatively mature method; simple operation	Lack of specificity; can be mixed with proteins of similar molecular weight; expensive equipment; time-consuming; repeated centrifugation causes damage to exosomes	High recovery; low specificity	Urine; Serum; cerebrospinal fluid; cell culture media
Density gradient centrifugation	In an inert gradient medium, the particles will reside in a medium of similar density under centrifugal force	Exosomes are virtually free of interfering proteins; relatively pure	Expensive equipment; time-consuming; repeated centrifugation causes exosome damage; not suitable for large-scale preparation	High recovery; low specificity	Urine; Serum; Cerebrospinal fluid; Cell culture media
Ultrafiltration	Differences in particle size between exosomes and other particulate components	Handling of large volume samples; simple operation; no expensive equipment; no chemical reagents; short time; high enrichment; efficiency; high purity	Blockage of membrane pores by large particles; mechanical damage to exosomes	Medium recovery; medium specificity	Urine; Serum; cerebrospinal fluid; cell culture media
Size exclusion chromatography	Differences in particle size between exosomes and other particulate components	Moves only by gravity; retains exosome activity; higher purity; lower cost; no sample prep; less time-consuming	May be doped with impurities of similar size, dilution effect of eluent on exosomes	Medium recovery; medium specificity	Urine; Plasma; Serum; Cerebrospinal fluid; Cell culture media

(*Continued*)

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Table 1 (Continued).

Separation Method	Principle	Advantage	Drawbacks	Separation Efficiency	Scope of Application
Polymer precipitation technology	Hydrophilic polymers alter exosome solubility	Simple to operate; can handle large samples; high throughput	Exosomes are contaminated with other proteins; less pure	High recovery rate; low characterization	Urine; Serum; cerebrospinal fluid; cell culture media
Affinity separation technology	Antigen-specific binding of exosome surface antibodies	High specificity; high purity to ensure the structural integrity and biological activity of exosomes; no need for special equipment; simple operation	Time-consuming; expensive antibody; harsh elution conditions; not suitable for large-scale analysis	Low recovery; high specificity	Urine; Serum; cerebrospinal fluid; cell culture media
Microfluidics	Based on exosome physicochemical or biological properties	Fast and efficient; automated; integrated; high throughput	Requires complex manufacturing technology, not standardized	Low recovery; high specificity	Lack of large-scale trials; not currently used in the clinic

Ultracentrifugation

Ultracentrifugation is a prevalent technique for the isolation of exosomes. By progressively increasing the centrifugal force, exosomes with varying densities, sizes, and morphologies can be effectively separated. Initially, low-speed centrifugation removes debris such as dead cells and large biological particles. Subsequently, ultrahigh-speed centrifugation (>100,000 g) is employed to isolate exosomes, resulting in the collection of exosome pellets,[18] Exosomes can achieve higher purity through the addition of PBS followed by ultrahigh-speed centrifugation to eliminate apoptotic vesicles, proteins, and other contaminants.[19] Due to its advantages of yielding a large volume of exosomes, employing a relatively mature methodology, simplicity in operation, and absence of reagent contamination, this method has been extensively utilized over the past few decades. Specifically, it has been widely applied in the isolation of exosomes from urine, serum, cerebrospinal fluid, and cell culture medium. However, this technique also exhibits certain limitations, including poor specificity, potential contamination with proteins of similar molecular weight, high equipment costs, time-consuming procedures, and damage to exosomes caused by repeated centrifugation.[20–23] In order to further improve the separation efficiency of this classical separation technique by exploring the different physical properties of the objects, several types of centrifugal strategies have now been developed, among which density gradient centrifugation is widely used.[24,25]

Density Gradient Centrifugation

Differences in sedimentation coefficients among biological particles result in their separation within specific inert gradient media. Under the influence of centrifugal force, these particles will be distributed into distinct zones according to their density.[26] This encompasses both rate-zonal centrifugation and isopycnic centrifugation. Presently, these two techniques are utilized in our procedures. Prior to centrifugation, it is necessary to prepare a gradient with progressively increasing density from the top to the bottom of the centrifuge tube. Commonly employed media for this purpose include sucrose and iodixanol.[27] The typical procedure is as follows: after preparing the density gradient medium, the ultra-centrifuged sample is carefully layered on top of the gradient. Upon centrifugation, the sample components will migrate from the top to the bottom according to their densities. Consequently, exosomes, cellular debris, apoptotic bodies, and proteins will stratify into corresponding density layers within the medium.[28,29] Exosomes, on the other hand, reside in their iso-density zone (1.12–1.19 g/mL),[30] without sedimenting at the bottom of the tube. Purified exosomes can be obtained by collecting this specific zone. Exosomes isolated through density gradient centrifugation are nearly devoid of contaminating proteins,[31] purity is comparatively high; however, these methods suffer from several drawbacks including costly equipment, time-consuming processes, and potential damage to exosomes caused by repeated centrifugation. Consequently, they are not suitable for large-scale preparations.[32,33] In view of the prolonged ultracentrifugal force,

which may affect the structure and biological function of the isolated exosomes, other size-based separation methods have been introduced, such as ultrafiltration.

Ultrafiltration (Separation Technique Based on Differences in Particle Size)

Ultrafiltration holds greater potential for industrial-scale exosome preparation compared to ultracentrifugation. The underlying principle involves passing the sample through one or more membranes with varying pore sizes or molecular weight cut-offs. Impurities smaller than the membrane's pore size are filtered into the filtrate, whereas particles exceeding the pore size in diameter are retained on the membrane surface.[27] The most commonly employed media for membrane filtration are cellulose and its derivatives. Ultrafiltration is driven by electrical charge, low-speed centrifugation, and pressure differentials.[34] Currently, the conventional centrifugation step in ultrafiltration is conducted as follows: as the sample traverses the membrane, a 200 nm pore-size membrane selectively retains larger particles, such as large proteins and apoptotic vesicles. Meanwhile, a 20 nm pore-size membrane effectively intercepts vesicles, including exosomes within the size range of 20–200 nm, while smaller molecules like small proteins pass through and are collected at the bottom. Over the past decade, ultrafiltration has gained increasing popularity due to its capacity to process large sample volumes, operational simplicity, minimal equipment requirements, absence of chemical additives, time efficiency, high enrichment efficacy, and superior purity.[5,35] Nevertheless, several limitations exist, including the occlusion of membrane pores by large particles, mechanical damage to exosomes, and the absence of an effective mechanism for extracting vesicles and proteins.[36,37] A combination of two or more methods, such as ultrafiltration and density gradient centrifugation, can be a good solution to the problem of exosome clogging.[38]

Separation Techniques Based on Differences in Particle Size (Size Exclusion Chromatography SEC)

A physical separation principle based on molecular size differences, commonly referred to as gel filtration chromatography, is utilized. This technique comprises two main components: the stationary phase and the mobile phase. Commonly employed stationary phase media include dextran polymers, agarose, and polyacrylamide.[39] A physical separation principle based on molecular size differences, commonly referred to as gel filtration chromatography, is utilized. This technique comprises two main components: the stationary phase and the mobile phase. Commonly employed stationary phase media include dextran polymers, agarose, and polyacrylamide.[5,35,40] SEC has the advantages of being moved only by gravity, higher purity, higher recovery, low cost, no need for sample pretreatment, and shorter time consuming, but the most appealing feature is that it is capable of retaining the bioactivity of exosomes.[41–44] In recent years, SEC-based exosome isolation techniques have gained increasing popularity owing to their advantages. Nevertheless, the SEC method also encounters certain challenges, such as potential contamination from similarly sized impurities and the dilution effect of the eluent on exosomes.[20] To address the problem that SEC can be doped with impurities of similar size, Gardiner proposed a separation strategy that combines ultrafiltration and SEC, a combination that preserves exosome functionality while greatly improving exosome purity.[45]

Solubility-Based Polymer Precipitation Techniques

The polymer precipitation technique, analogous to ethanol-mediated nucleic acid precipitation, employs highly hydrophilic polymers to interact with water molecules surrounding exosomes. This interaction creates a hydrophobic microenvironment that reduces the solubility of exosomes, leading to their precipitation. Subsequently, centrifugation is utilized to isolate the exosomes.[5,46] Commonly available hydrophilic polymers include ichthyosperm, acetic acid, and organic solvents. Among these, polyethylene glycol (PEG), which is water-soluble and non-toxic, has become the most widely utilized medium for the polymerization and precipitation of exosome.[47–49] Exosomes are initially subjected to pre-treatment to eliminate larger contaminating particles, including cellular debris and apoptotic vesicles. Subsequently, they are incubated with PEG in a 4 °C solution overnight. Finally, exosomes are harvested from the precipitates through low-speed centrifugation.[50] This method has been extensively utilized for the isolation of exosomes from various biological samples, including urine, serum, cerebrospinal fluid, and cell culture medium.[46,51] The advantages of this

technique include its operational simplicity, capability to process large sample volumes, and high throughput. However, it also has limitations, such as potential contamination with non-exosomal proteins and relatively low purity.[52–54] To solve the problem of protein contamination, hydrophobic PEG and hydrophilic dextran can be mixed together to form an aqueous two-phase system, with PEG forming the upper phase and dextran forming the lower phase. By adding PEG and dextran to the exosome solution and centrifuging at low speed, exosomes preferentially aggregate in the dextran phase, while some proteins, macromolecular complexes, etc. preferentially aggregate in the PEG phase, thus improving the recovery of exosomes.[48]

Affinity Separation Techniques Based on Immunoreactivity

Immunoaffinity capture facilitates the isolation of highly purified exosomes through the specific binding of surface antigens on the exosome membrane to their corresponding antibodies.[20] In recent decades, a comprehensive identification and documentation of surface markers on exosomes have been conducted. These markers include the tetraspanin family of proteins (CD9, CD63, CD81, CD82), membrane-associated proteins such as Annexin V, epithelial cell adhesion molecules (EpCAM), lysosome-associated membrane protein-2B (LAMP2B), heat shock proteins, and phospholipases.[55–57] During the process of exosome isolation, antibodies specific to certain markers must be immobilized on carriers such as magnetic beads, silica, or resins.[35,48,58] When exosomes traverse the carriers, they are captured by specific antibodies through antigen-antibody interactions. Following this, an elution step is performed to isolate the exosomes from the carrier-exosome complexes. Immunoaffinity capture offers several advantages, including high specificity and purity, preservation of exosomal structural integrity and biological activity, minimal equipment requirements, and straightforward operation, making it an ideal method for isolating subpopulations of exosomes from specific origins.[59,60] However, this approach also exhibits certain limitations, including time-consuming procedures, high antibody costs, stringent elution conditions, and unsuitability for large-scale isolation.[61] When using immunoaffinity capture methods to isolate exosomes, pretreatment can be combined with polymer precipitation techniques, thus avoiding the use of excessively expensive antibodies.[62]

Microfluidics Based on Fluid Properties

Microfluidics, leveraging its unique fluidic properties, demonstrates significant potential for the separation and purification of exosomes. This technology enables the investigation of the physicochemical characteristics of exosomes at the microscopic level.[63] Microfluidics refers to a system comprising a network of fluidic channels with diameters ranging from tens to hundreds of micrometers. This technology is integrated with fluidic control systems and analytical instrumentation to automate the processes of extracting, detecting, separating, and analyzing biomaterials.[64] Microfluidics is frequently integrated with traditional separation techniques, such as immunoaffinity-based microfluidics for the isolation of highly specific exosomes and size-based microfluidics to enhance the purity of exosome separation.[65,66] Microfluidics leverages the physical (size, density) and chemical (surface antigens) properties of exosomes for their separation. This technology offers significant advantages including rapid and efficient processing, automation, integration, and high throughput, which are revolutionizing the field of exosome isolation and diagnosis. These advancements are particularly valuable for non-invasive disease detection, such as early cancer screening.[67–69] Exosome isolation using microfluidic technology also faces challenges due to the complexity of fabrication techniques and a lack of standardization. These shortcomings necessitate further exploration to develop more efficient and standardized methods.

At present, there are also many emerging and efficient exosome purification systems on the market, for example, EXODUS fully automated exosome extraction system from Shenzhen Huixin Biomedical Technology Company, which utilizes negative pressure oscillation system (NPO) combined with dual-coupled harmonic oscillation system (HO) acting on the nano-ultra-filtration chip to enable the free nucleic acids and proteins and other impurities in the sample to be rapidly removed through the nanopore and sequester the exosomes, thus purifying and enriching the exosomes. Thus, purifying and enriching exosomes. In the future, the integrated and automated design of the equipment will be another new direction of exosome purification, which can quickly process a large number of samples, improve the experimental efficiency, reduce the time and labor costs, and solve the current stage of the exosome preparation of large-scale building problems.[5]

Preservation of Exosomes

The stability of exosomes is critically dependent on their bilayer membrane structure, which safeguards their contents against degradation and denaturation in the extracellular environment.[70] Differences in storage methods can significantly influence multiple characteristics of exosomes, such as the stability of their biological and physical properties, recovery efficiency, diameter distribution, and functional integrity. Presently, common preservation methods for exosomes include cryopreservation and lyophilization, which will be detailed subsequently.

Cryopreservation is a conventional method for preserving exosomes; however, this technique may result in alterations to the morphology and physical properties of exosomes, the formation and aggregation of multilayered vesicles, as well as changes in molecular bioactivity, content, and surface markers due to the freeze-thaw process.[71] Currently, cryopreservation temperatures of 4°C, −20°C, and −80°C are commonly utilized, with −80°C being the most prevalent. At 4°C, exosomes remain unfrozen and do not undergo morphological damage; however, prolonged storage at this temperature results in a decrease in exosome count as well as reductions in the proteins and nucleic acids they contain, leading to diminished biological activity. For instance, plasma-derived exosomes stored at 4°C experience RNA degradation and loss of proteins and nucleic acids,[72] and serum-derived exosomes, which contain miR-122 and miR-14, undergo degradation during preservation at 4°C,[73] Therefore, 4°C is more appropriate for short-term preservation. Storing exosomes at −80°C may affect their morphology due to the process of freezing and thawing; however, the proteins and nucleic acids they contain remain relatively stable, as do their biological activities.[74] It has been demonstrated that milk-derived exosomes stored at −80°C for approximately one month retain their physical and biological properties without alteration.[75] In addition, storage at −80°C has demonstrated a significant effect on the activity of milk-derived extracellular vehicles (EVs).[76] In contrast, plasma-derived exosomal miRNAs remained stable under frozen conditions (−20°C and −80°C) and did not exhibit significant changes over a period of two years.[72] Furthermore, preservation at −80°C is considered the optimal storage environment for semen, urine, milk, blood, and bronchoalveolar lavage fluid.[71,77]

Freeze-drying represents an innovative approach to exosome preservation, offering a cost-effective strategy that extends the shelf life of exosomes while minimally impacting their morphology and content at ambient temperatures. Protective agents such as alginate and mannitol are commonly incorporated during the freeze-drying process. The inclusion of alginate as a bioprotectant enhances protein, cell membrane, and liposome stability, mitigates ice crystal formation, and preserves exosome integrity, stability, and functionality.[70,78] Charoenviriyakul et al found that the absence of a cryoprotectant led to aggregation of B16BL6 melanoma-derived exosomes. The addition of alginate as a cryoprotectant, however, prevented such aggregation during lyophilization. Furthermore, PAGE analysis demonstrated that the proteins and RNAs within the exosomes were effectively protected by the inclusion of alginate as a -cryoprotectant.[70] In addition, mannitol can serve as a cryoprotectant to safeguard exosomes during lyophilization.[79] While lyophilization offers numerous preservation advantages, the duration of exosome stability and its applicability to different types of exosomes remain unclear. This field is still in its preliminary stages and lacks standardized protocols.

Heterogeneity is observed among exosomes derived from different sources, and their stability exhibits considerable variation under identical preservation conditions. For instance, exosomes isolated from sperm can be stored at −80°C for up to 30 years without significant alterations in morphology, physical properties, or nucleic acid and protein composition,[77] While bronchoalveolar lavage fluid-derived exosomes exhibit significant alterations in protein abundance and volume when frozen compared to fresh samples.[71] Furthermore, varying levels of acidity and alkalinity significantly influence the preservation and functionality of exosomes. Acidic conditions result in a higher yield of isolated proteins and nucleic acids, as well as enhanced cellular uptake of exosomes. Specifically, HEK 293T cells produce exosomes with protein and nucleic acid concentrations that are fivefold higher in acidic environments compared to neutral conditions,[80] In alkaline environments, however, the concentrations are significantly lower. Exosomes derived from human urine are isolated under acidic conditions, which facilitates the maintenance of their stability and integrity, resulting in a higher yield of total RNA and minimized protein degradation.[81]

Based on the current evidence, storage at −80°C appears to be the most favorable condition for preserving biological fluids and isolated exosomes. For short-term storage of exosomes, a temperature of 4°C is recommended. Additionally, the addition of cryoprotectants or adjustment of pH can help maintain the integrity and biological activities of exosomes.

Further research is warranted to better understand how storage conditions affect exosome particles, functions, and activities, which will contribute to the development of improved exosome storage strategies and comprehensive cryopreservation methods.

Advantages of Exosomes as Drug Delivery Systems

Biocompatibility

Exosomes, as endogenous substances, are not recognized, captured, or cleared by the mononuclear phagocyte system in the body.[82] Usman et al introduced antisense oligonucleotides into erythrocyte-derived exosomes via electroporation and demonstrated that these exosome-loaded antisense oligonucleotides significantly inhibited breast cancer cell growth and were non-immunogenic in vivo.[83] In contrast, synthetic nanocarriers can induce an immune response in the body after multiple drug administrations, leading to rapid clearance by the immune system and potential adverse immune reactions such as allergic responses.[84–86]

Biostability

Exosomes exhibit excellent biostability, enabling prolonged circulation in vivo, even under inflammatory conditions, where they offer robust protection for therapeutic agents.[87] Moreover, the expression of CD47 on the exosomal membrane effectively prevents clearance by the mononuclear phagocyte system, thereby extending their circulation time in vivo. Additionally, surface modification of exosomes can further enhance their in vivo circulation duration.[88] Exosomes derived from macrophages were functionalized with aminoethyl anisamide polyethylene glycol (AA-PEG), which significantly diminishes their recognition and internalization by the monocyte-macrophage system (MPS), thereby preventing rapid clearance and markedly extending their circulation time in vivo.[89,90] Asparagus phylum-extracted nanovesicles (ACNV) have been shown to inhibit hepatocellular carcinoma proliferation through activation of the apoptotic pathway; however, the cytosolic effects mediated by the mononuclear phagocyte system result in rapid exosome clearance from the bloodstream. Modification of ACNV with distearoyl-phosphatidylethanolamine-polyethylene glycol 2000 (DSPE-PEG2000) altered its pharmacokinetic profile, leading to enhanced in vivo circulation time.[91,92]

Targeted

Exosomes are specifically recognized by signaling molecules, such as receptors on the cell membrane surface or associated proteins in the extracellular matrix, and subsequently bind to the surface of target cells. This recognition facilitates directed migration of exosomes to specific tissues or organs, enabling the delivery of therapeutic molecules (eg, miRNAs) to their intended sites while minimizing degradation.[93] Moreover, exosomes derived from certain cellular sources possess intrinsic targeting capabilities. For instance, exosomes from central nervous system (CNS) cells can traverse the blood-brain barrier and target specific neurons, whereas exosomes from hypoxic tumor cells are preferentially recruited to hypoxic tumor tissues.[94–96] Furthermore, surface modification of exosomes can be employed to enhance their targeting capabilities. For instance, the conjugation of cyclic RGD peptides to natural exosomes significantly improves their tumor-targeting ability. This functionalization facilitates the enhanced accumulation of chemotherapeutic agents, such as DOX, within the tumor microenvironment.[97] Macrophage-derived exosomes engineered with the AS1411 aptamer exhibit efficient blood-brain barrier (BBB) penetration and precise cancer cell targeting capabilities.[98]

In addition to exhibiting excellent biocompatibility, biostability, and targeting capabilities, exosomes also possess superior permeability. As lipid-coated vesicles, they can effectively traverse the blood-brain barrier, cytoplasmic membranes, and other biological barriers, thereby achieving efficient drug delivery.[96] Exosomes are distinguished by their remarkable modifiability, which can be influenced by the characteristics of their source cells, thereby modulating their properties and functions. By manipulating the gene expression profiles of exosome-producing cells, it is possible to regulate both the secretion and composition of exosomes. For instance, certain studies have demonstrated that overexpression of genes encoding specific membrane proteins or drug-carrying proteins can enhance the cargo capacity of exosomes for therapeutic agents or specific molecules. Furthermore, exosomes can undergo modifications through

chemical, biological, immunological, and physical approaches, such as chemical or enzymatic alterations of functional molecules on their surface to impart targeted biological effects. This versatility in modification enhances the flexibility and controllability of exosome-based drug delivery systems, facilitating the implementation of personalized medicine.[90,99–101]

In addition to exosomes, there are several carriers that can be used for drug delivery. Lipid nanoparticles (LNP) is a synthetic liposome. Although it can efficiently encapsulate nucleic acids and achieve rapid delivery (eg, mRNA vaccines), its cationic lipid and PEG lipid components may trigger immune reactions or hepatotoxicity, in addition to its organ targeting and penetration limitations.[102,103] In contrast, exosomes, as natural vesicles secreted by cells, have low immunogenicity, natural targeting, and excellent biocompatibility, which can cross complex barriers and reduce the risk of off-targeting.[96] In addition, exosomes can carry a variety of endogenous active molecules, and will become a safer and more precise next-generation delivery system after further optimization of drug-carrying efficiency through engineering modification.

Engineered Exosomes

Engineered exosomes are vesicles that have undergone surface modifications and encapsulation of therapeutic molecules. As an innovative drug delivery platform, engineered exosomes have shown considerable promise in diverse fields such as oncology, cardiology, stomatology, neurology, and tissue regeneration and repair.[104]

Engineered Exosome Preparation

Despite the widespread application of natural exosomes in systemic disease therapy, their inherent limitations, including low yield, impurities, and inadequate targeting capabilities, have constrained their therapeutic efficacy. Conversely, advancements in engineered exosome technology offer a more comprehensive approach to disease diagnosis and treatment. This technology leverages the inherent properties of exosomes, such as biocompatibility, natural carriage of bioinformatic molecules, and low immunogenicity, and enhances their functionalities through engineering techniques. These enhancements include specific targeting, improved drug loading capacity, and resistance to organismal clearance. Commonly employed methods for preparing engineered exosomes can be categorized into two main types: cell-based engineering and direct exosome modification. Cell-based engineering encompasses genetic manipulation, alteration of cell culture conditions, and pharmacological treatments, while direct exosome modification includes biological, immunological, physical, chemical modifications, and the conjugation of biomaterials. These approaches refine and specify the functional capabilities of exosomes.

In the field of cellular engineering, researchers have concentrated on genetically modifying parental cells of exosomes to enhance the expression of specific proteins or signaling molecules. This approach aims to improve the targeting accuracy and therapeutic efficacy of exosomes. Modified exosomes can be engineered during their biosynthesis to incorporate functional components such as surface peptides or targeting ligands, thereby increasing their affinity for target cells.[105] Alvarez-Erviti et al were the first to demonstrate that exosomes secreted by plasmid-transfected dendritic cells, when fused with neuron-specific rabies virus glycoprotein and exosomal lysosome-associated membrane protein 2b (Lamp2b), could deliver siRNA specifically to the brain. The fusion of a target protein with Lamp2b facilitates its localization on the exosome surface, setting a precedent in this field.[106] Modifying cell culture conditions represents an effective engineering strategy that can indirectly influence the yield and characteristics of exosomes by adjusting parameters such as the composition of the culture medium, cell density, or oxygen concentration.[107] This method not only enhances the purity of exosome isolation but also improves the stability and biological activity of the encapsulated therapeutic agents. Under hypoxic conditions, Patton et al demonstrated that culturing pancreatic cancer cells resulted in an increased release of exosomes with a reduced average particle size, thereby providing favorable conditions for the adaptive growth of these cells.[108] The strategy of co-incubating parental cells with drugs facilitates the natural encapsulation of drug components into the exosome interior during exosome biogenesis, thereby eliminating the need for additional drug loading steps and preserving the bioactivity and biocompatibility of the drug. In inflammation-related autoimmune and inflammatory diseases as well as cancer, co-incubation of curcumin with EL-4 (mouse lymphoma cell)-derived exosomes has been shown to enhance the therapeutic efficacy of exosomes.[109] From this point of view, genetic

engineering technology can not only successfully construct highly targeted engineered exosomes, but also significantly increase the drug loading capacity of engineered exosomes to achieve precision therapy, but the biosafety of genetic engineering still needs more research to examine. However, the biological safety of genetic engineering still needs more research. Although changing the cell growth conditions is an effective method to increase the production of exosomes, the degree of increase in the production is not enough to become a major method for mass production of exosomes.

The advancement of physical processing techniques has opened new avenues for engineering exosomes, enabling more precise therapeutic targeting and enhanced safety in the presence of external physical stimuli such as magnetic fields, laser irradiation, ultrasound, and electroporation. For instance, exosomes derived from human umbilical cord mesenchymal stem cells (HUCMSC) have been successfully ultrasonicated to encapsulate silver nanoparticles (AgNPs), thereby acquiring antimicrobial properties and promoting wound healing.[110] Additionally, neutrophil-derived exosomes modified with superparamagnetic iron oxide nanoparticles (SPIONs) have demonstrated selective accumulation at tumor sites when subjected to external magnetic fields.[111] Furthermore, the loading of tumor necrosis factor (TNF-α) and SPION-modified exosomes markedly enhances cancer-targeting efficacy under external magnetic fields while reducing in vitro and in vivo toxicity.[112] Additionally, Wan et al demonstrated that electroporation-mediated loading of Cas9 ribonucleoprotein (RNP) into exosomes purified from hepatic stellate cells facilitated efficient cytoplasmic delivery of RNP, promoting specific accumulation in hepatic tissues and enabling tissue-specific gene therapy in the liver.[113] Engineered exosomes prepared by physical methods have better biosafety by reducing the incorporation of intermediate mediators other than drugs, but they are overly dependent on preparation methods and equipment.

In addition, biological, immunological, and chemical modifications are employed to enhance the spatial targeting capabilities of exosomes. TLyp-1 peptide-modified exosomes exhibit high transfection efficiency in lung cancer treatment due to the selective binding of TLyp-1 peptide to neuropilin-1 (NRP1) and neuropilin-2 (NRP2).[114–116] Anti-CD3 and anti-EGFR (epidermal growth factor receptor) antibody-engineered exosomes facilitate the binding of T cells to cancer cells, thereby enabling precise therapy.[117] The tumor microenvironment (TME) is characterized by relative acidity, likely resulting from elevated glycolysis rates and increased lactate production, which facilitates tumor-targeted modification of engineered exosomes. Paclitaxel (PTX), a common chemotherapeutic agent, can be encapsulated with NaHCO3 in exosomes. Upon endocytosis by cancer cells, this combination rapidly generates carbon dioxide bubbles, creating an acidic environment that promotes effective drug release.[118] By surface modification, the engineered exosomes are more targeted, but the receptor-ligand reaction binding is weaker and the drug adhesion is not very strong during drug delivery.

Furthermore, researchers in the fields of biology, immunology, and chemical modification have achieved significant advancements in the biomaterial loading of exosomes. These developments facilitate disease treatment by utilizing various types of biomaterials, such as hydrogel scaffolds or dressings, to provide structural support and enhance exosome delivery.[119,120] Specifically, a bio-responsive polyethylene glycol (PEG)/DNA hybrid hydrogel system was developed and loaded with exosomes derived from human apical papillary stem cells (SCAP-Exo). This system has demonstrated efficacy in promoting bone regeneration under both normal and diabetic conditions.[121] Biomaterials loaded with exosomes have better targeting, slow drug release, and can be combined with other therapeutic means to realize multifunctional collection, but the safety and high cost of biomaterials limit their large-scale clinical application. Comparison of advantages and disadvantages regarding engineered exosome preparation methods (Table 2).

Engineered Exosome Applications and Advances

Exosomes have demonstrated extensive potential as drug delivery vehicles in the treatment of specific diseases. Through targeted engineering and modification, exosomes can facilitate efficient and precise drug delivery. Research has indicated that exosomes can serve as carriers for anticancer agents, anti-inflammatory compounds, and genetic materials, thereby enabling the treatment of tumors, inflammatory conditions, cardiovascular disorders, neurological ailments, and other diseases (Figure 2).

Table 2 Comparison of Advantages and Disadvantages of Engineered Exosome Preparation Methods

	Cell Engineering	**Physical Methods**	**Surface Modification**	**Biomaterial Loading**
Advantages	High biosafety, genetically engineered cell lines can be frozen and then reused	High biosafety, especially for hydrophilic drug loading	Strong site targeting	Contributes to better targeted drug delivery and release
Drawbacks	Higher cost, more complicated process, low efficiency of drug loading	Requires membrane-breaking facilities, which may impair exosome properties	Higher technical and equipment requirements, difficult to operate	Difficult to operate, high safety requirements for biomaterials

Tumor Related Diseases

Engineered exosomes, as an innovative drug delivery platform and an ideal modality for clinical therapeutic applications, can significantly enhance anti-tumor efficacy in cancer treatment and augment the effectiveness of conventional therapies such as radiotherapy, photothermal therapy, photodynamic therapy, and gene therapy. In preclinical studies, engineered exosomes are typically administered intravenously to target tumor sites and deliver their cargo, including chemotherapeutic agents, non-coding RNAs, and immune molecules, directly into the tumor microenvironment, thereby inducing tumor cell apoptosis.

Chemotherapy usually leads to systemic adverse effects such as myelosuppression.[122,123] Through the exosome-mediated delivery of chemotherapeutic agents, enhanced anti-tumor efficacy can be achieved while minimizing systemic

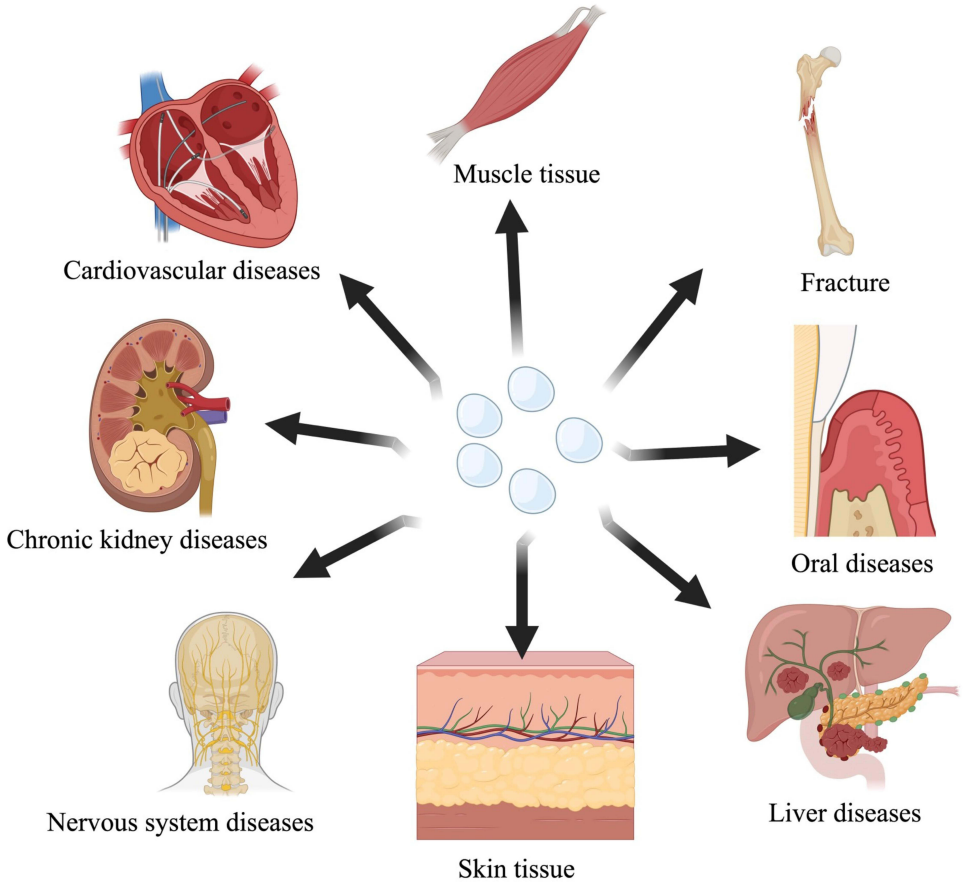

Figure 2 Clinical application of extracellular vesicle therapy. Created in BioRender. Hu, B. (2025) https://BioRender.com/b12c371.

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toxic effects.[111] In breast cancer, the encapsulation of paclitaxel (PTX) within exosomes effectively prevents recurrence and metastasis, and significantly inhibits tumor growth in murine models of breast cancer.[118,124] Macrophage-derived exosomes loaded with paclitaxel (PTX) and conjugated with aminoethyl anisamide polyethylene glycol (AA-PEG) were evaluated in a murine lung cancer metastasis model.[89] The findings indicated that the presence of PEG hindered the recognition and internalization of exosomes by the mononuclear phagocyte system (MPS), consequently prolonging the circulation time of exosomes in vivo.[90]

Gene therapy for oncology involves the modification of DNA or RNA to treat cancer. One common approach is to load non-coding RNA into engineered exosomes, which are then delivered to the tumor site to exert therapeutic effects.[125] In gliomas, exosomes loaded with miR-29a-3p have been shown to inhibit tumor migration and angiogenesis by targeting ROBO1.[126] In hepatocellular carcinoma, engineered exosomes modified with miR-199a effectively deliver this microRNA to hepatocellular carcinoma cells, thereby significantly enhancing their sensitivity to Adriamycin in vitro and promoting the antitumor effects of Adriamycin on hepatocellular carcinoma in vivo.[127]

Photodynamic therapy (PDT) is a minimally invasive treatment modality for cancer that primarily employs photosensitizers.[128] Recent studies have demonstrated that engineered exosomes can effectively deliver Rose Bengal, a photosensitizer[129] in conjunction with Erastin, an inducer of ferroptosis[130] to a murine model of hepatocellular carcinoma, thereby inducing iron-dependent cell death in tumor cells. Acoustic kinetic therapy (AKT), another ROS-mediated cancer treatment, utilizes ultrasound and acoustic sensitizers to target and destroy tumors.[131] When combined with engineered exosomes, AKT enables more controlled drug release and enhanced precision in tumor targeting. The presence of the blood-brain barrier (BBB) and hypoxic tumor microenvironment poses significant challenges to conventional AKT efficacy. However, macrophage-derived exosomes modified with the AS1411 aptamer exhibit robust BBB penetration and targeting capabilities, thereby enhancing therapeutic outcomes,[132] and the exosomes loaded with acoustic sensitizers (ICGs) and catalase-loaded silica nanoparticles (CATSiO2) can effectively treat tumors.[133] Additionally, the oxygen generated from the catalase-catalyzed decomposition of hydrogen peroxide significantly improves the hypoxic tumor microenvironment.

Cardiovascular Disease

Cardiovascular diseases (CVD) is a collective term for heart diseases and cardiovascular diseases, and CVD has always been the leading cause of mortality, with 40% of deaths in the population being attributed to CVD.[134] Consequently, enhancing the therapeutic efficacy for CVD is of paramount importance. Current treatment modalities for CVD, including pharmacological interventions, interventional procedures, and open surgeries, have significantly improved patient outcomes. However, these treatments are associated with certain complications. Recent advancements in exosome research have revealed their potential in mitigating inflammation, promoting ventricular remodeling, and facilitating neovascularization, thereby offering promising therapeutic avenues for CVD.[135,136]

Myocardial Infarction

Acute myocardial infarction (AMI) is a severe cardiovascular condition characterized by sustained ischemia and hypoxia in the coronary arteries. Emerging evidence indicates that exosomes play a crucial role in both the diagnosis and therapeutic approaches for AMI.[137] Su et al conducted a comprehensive analysis of miRNA expression profiles in exosomes derived from 6 patients with acute myocardial infarction (AMI) and 6 patients with stable coronary artery disease (SCAD). They observed that the expression levels of exosomal miR-3, miR-4, miR-507, miR-656, and miR-1915-3p were significantly reduced in AMI specimens compared to SCAD specimens. This finding suggests that these specific exosomal miRNAs may serve as potential biomarkers for the diagnosis of AMI.[138] A growing body of evidence indicates that exosomes derived from various tissues can suppress cardiac inflammatory responses, exert immunomodulatory effects, and mitigate myocardial injury via distinct molecular mechanisms. For instance, Pan et al demonstrated that miR-146a, contained within exosomes derived from adipose stem cells, mitigates inflammatory responses and enhances the local microenvironment by inhibiting the release of interleukin 6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-alpha (TNF-α). Furthermore, it alleviates myocardial cell injury induced by acute myocardial infarction through the downregulation of early growth response factor 1 (EGR1).[139]

Atherosclerosis

Atherosclerosis (AS) is characterized by a chronic inflammatory response, with macrophages serving as key mediators in the formation and progression of atherosclerotic plaques.[140] Macrophages, which are versatile immune cells, can be categorized into classically activated macrophages (M1-type) and alternatively activated macrophages (M2-type) based on their functional properties and activation states. Research has demonstrated that the stimulation of both murine and human macrophages with oxidized low-density lipoprotein (oxLDL), followed by the isolation and characterization of exosomes, reveals that exosomal miR-146a contributes to the development of AS by inhibiting cell migration, promoting macrophage retention within the vascular wall, and inducing oxidative stress.[141,142] MSC-derived exosomes can also be activated via the miR-let7/HMGA2/NF-κB pathway, which induces macrophage polarization from the M1 to the M2 phenotype, exerts anti-inflammatory effects, and regulates the progression of atherosclerosis.[143]

Diseases of the Nervous System

Low intracerebral drug concentrations have posed a significant challenge in the advancement of therapeutic strategies for central nervous system (CNS) diseases. The existence of the blood-brain barrier (BBB) and its selective permeability restricts the effective entry of pharmacological agents into the brain.[144] In recent years, a growing body of research has indicated that exosomes may serve as promising natural drug delivery systems (DDSs). Firstly, exosomes exhibit minimal immunogenicity, thereby enhancing drug efficacy and minimizing adverse reactions;[145] Secondly, their unique phospholipid-membrane-protein structure facilitates efficient intracellular delivery of therapeutic agents into target cells.[146] Additionally, exosomes demonstrate remarkable stability, which prevents phagocytosis by macrophages and extends the half-life of the drug;[147] Most importantly, the lipid bilayer of exosomes can assist in crossing natural barriers such as the blood-brain barrier (BBB), thereby improving the bioavailability of central nervous system (CNS) therapeutic drugs.[148,149]

Alzheimer's Disease

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder, characterized by progressive cognitive decline and memory impairment.[150] Silymarin (silibinin, Slb) has demonstrated therapeutic potential in AD by inhibiting amyloid-β (Aβ) aggregation and suppressing astrocyte activation; however, its clinical application is limited due to low bioavailability.[151] In this study, Huo et al investigated the efficacy of exosome-encapsulated silymarin (Exo-Slb) in treating Alzheimer's disease. Specifically, Huo et al encapsulated Slb within macrophage-derived exosomes (Exo-Slb), which upon entering the brain, selectively bind to Aβ1-42 and inhibit the aggregation of Aβ1-42. Additionally, Exo-Slb is internalized into astrocytes, where it regulates the nuclear factor κB (NF-κB) pathway to suppress astrocyte activation, thereby mitigating astrocyte-mediated neuronal damage and effectively alleviating cognitive deficits in AD mice.[152] Lee et al demonstrated that exosomes derived from adipose stem cells significantly reduced the expression levels of Aβ42 and Aβ40, as well as the Aβ42/Aβ40 ratio in neural stem cells of Alzheimer's disease mice. Furthermore, these exosomes were capable of modulating the Aβ42/Aβ40 ratio. Additionally, exosomes from adipose stem cells of Alzheimer's disease mice could inhibit apoptosis in neural stem cells, effectively repair synapses damaged by Aβ in the brain, and consequently regulate neural function. These findings suggest that exosomes can potentially intervene in Alzheimer's disease progression by modulating apoptosis and promoting synaptic growth.[153]

Parkinson's Disease (PD)

Parkinson's disease is the second most common neurodegenerative disease.[154] In addition to conventional diagnostic criteria such as patient history, bradykinesia, and muscle tone assessment, the measurement of exosome levels can serve as an auxiliary tool for diagnosis and identification of therapeutic targets. Alpha-synuclein (α-syn), a primary component of Lewy bodies (LBs), contributes to mitochondrial dysfunction, increased oxidative stress, and degeneration of dopaminergic neurons through its aggregation. Consequently, strategies aimed at reducing α-syn expression represent promising therapeutic approaches to mitigate the progression of Parkinson's disease (PD).[155] Stuendl et al investigated exosomes in the cerebrospinal fluid (CSF) of patients with Parkinson's disease. Their findings

demonstrated that CSF-derived exosomes from these patients could induce the oligomerization of soluble α-Syn in target cells, leading to further disease progression. This suggests that interventions targeting CSF-derived exosomes-induced α-Syn oligomerization may effectively modulate disease development.[156] Clinically, Parkinson's disease (PD) is commonly managed through dopamine injections. However, the blood-brain barrier (BBB) significantly hinders the efficient delivery of dopamine to the brain. To address this issue, drug delivery systems (DDS) that can directly encapsulate dopamine are required for effective treatment.[157] While antisense oligonucleotides (ASOs) have shown promise in various clinical applications, including the treatment and management of PD, their ability to reduce the expression of α-synuclein (α-syn) remains limited by challenges in safely and effectively delivering ASOs to neurons. Yang J et al developed a safe and efficient delivery method for ASOs by utilizing exosomes derived from bone marrow mesenchymal stem cells (hbmMSC) to encapsulate ASO4 (Exo-ASO4). It was demonstrated that Exo-ASO4 can effectively penetrate the brain parenchyma of Parkinson's disease (PD) model mice, leading to a significant reduction in the expression levels of α-synuclein. Consequently, the use of exosomes to deliver exogenous ASO4 into the brain markedly ameliorates dopaminergic neuronal degeneration and motor dysfunction in PD model mice.[158]

Other Diseases

Stem cell exosomes have demonstrated significant potential in disease-specific therapies. For instance, in the treatment of diabetes, stem cell exosomes can markedly enhance blood glucose regulation and mitigate tissue damage by modulating insulin secretion and alleviating insulin resistance.[159] In skin photoaging repair, stem cell exosomes can promote collagen synthesis and cell proliferation, reducing wrinkles and hyperpigmentation.[160,161] In oral and maxillofacial disorders, stem cell exosomes can assist in the management of conditions such as periodontitis, endodontitis, and oral cancer, contributing to both diagnosis and treatment[162] (Table 3).

Table 3 Engineered Exosome Applications

Disease	Disease Models	Preparation Strategies	Loaded Drugs	Research Results
Neoplasms	Osteosarcoma (benign tumor composed of bone-like material)	Genetic engineering	miR-199a	Bone marrow-derived MSC-Exos can target transfer miR-25-3p to osteosarcoma cells and inhibit the expression of its target genes after overexpression, thereby inducing OS cell formation and migration, and regulating OS cell proliferation, migration, and invasive ability.[163]
	Lymphoma	Drug co-incubation	PTX, ICG	Improved accumulation of ICG and PTX drugs in tumors, inhibited tumor growth, and explored the triple-engineered exosome treatment modality of chemotherapy, photothermal, and photosensitization.[164]
Cardiovascular disease	Myocardial infarction	Changing cell culture conditions	Not mentioned	Hypoxia increased the level of angiogenic miRNAs and decreased the level of fibrillar miRNAs in exosomes, and exosomes under hypoxic conditions induced tubulogenesis and enhanced myocardial protection.[165]
	Myocardial infarction	Genetic engineering	miR-146a	Adipose stem cell-derived exosome miR-146a inhibits inflammation and improves the local microenvironment by inhibiting the release of interleukin 6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor α (TNF-α), and attenuates myocardial cell injury caused by acute myocardial infarction by down-regulating early growth response factor 1 (EGR1).[139]

(*Continued*)

Table 3 (Continued).

Disease	Disease Models	Preparation Strategies	Loaded Drugs	Research Results
Diseases of the nervous system	Ischemic stroke	Drug co-incubation	Edaravone (Edv)	Macrophage-derived exosomes containing Edv (Exo + Edv) significantly increased the intracerebral bioavailability of Edv, showing higher maximum blood concentrations and greater area under the drug-time curve with a prolonged half-life. Under the mediation of exosomes, more Edv reached the ischemic sites and localized to neuronal cells and microglia, thus reducing neuronal cell death and promoting the transition of microglia from M1 to M2 phenotype, and enhancing neuroprotective effects.[166]
	Multiple sclerosis	Addition of drugs to cell culture	IFN-γ	Engineered exosomes, reduced demyelination, attenuated neuroinflammation and induced tolerance, inhibited T cells and induced Treg proliferation in vitro.[167]
Tissue regeneration and repair	Diabetes	Biomaterials loading	Antioxidant Polyurethane (PUAO)	A wound dressing consisting of antioxidant polyurethane (PUAO) loaded with adipose mesenchymal stem cell exosomes enhances diabetic wound healing by promoting faster wound closure, enhanced collagen deposition, faster re-epithelialization, increased angiogenesis and reduced oxidative stress.[168]
Oral Diseases	Oral cancer	Co-incubation	miRNA-34a	Cholesterol-modified miRNA-34a was loaded into exosomes of HEK293T cells by co-incubation and eventually taken up by oral squamous carcinoma cells. Exosomes loaded with miRNA-34a inhibited the proliferation, migration and invasion of oral squamous cell carcinoma cells by down-regulating SATB6 expression.[169]

Summary and Outlook

Traditional drug delivery systems predominantly rely on exogenous carriers. Although nanoscale delivery vehicles have emerged, practical applications continue to face multiple challenges, including concerns over carrier safety, in vivo heterogeneity, and low bioavailability.[85] Exosomes exhibit excellent biocompatibility and minimal immunogenicity. Through engineered modifications, they can target specific therapeutic sites and load designated drugs, achieving targeted transport and controlled release. This not only enhances drug bioavailability and therapeutic efficacy but also offers broad prospects for development.[96] Especially for the engineering modification of exosomes, compared with the inefficiency of cell engineering, direct modification of purified natural exosomes can directly, efficiently and rapidly obtain a large number of engineered exosomes, and reduce the uncertainty in the process of isolation and purification of exosomes, so as to make the engineered exosomes obtained more homogeneous. Engineered exosomes have been used in the research of tumours, cardiovascular diseases, neurological diseases and other fields, and have demonstrated superior therapeutic effects. Moreover, the application of exosomes in clinical medicine, bioengineering, regenerative medicine, cosmetics, and medical aesthetics is currently under active exploration.

Nevertheless, exosomes continue to encounter numerous challenges in practical applications. For instance, establishing a standardized protocol for exosome isolation, production, and preservation remains a critical issue. Additionally, addressing the in vivo heterogeneity of exosomes is essential to ensure consistent activity and therapeutic efficacy during application. Improving drug loading efficiency is another significant challenge. Furthermore, effective clinical translation while ensuring the safety of translational applications is crucial.[104] These challenges not only affect the yield of exosomes, but also limit the homogeneity of their quality. In the future, we may be able to realize the efficient separation of exosomes by developing new exosome separation technologies, such as the fully automated exosome extraction system based on microfluidic chip EXODUS of Shenzhen Huixin Biomedical Technology Company, and the automated and integrated exosome purification purifier of Wuhan JAU Biotechnology Company; and to maintain the stability and long-term activity of exosomes by exploring the appropriate cryopreservation additives. In summary, despite the

multitude of challenges in exploring exosomes, the opportunities are undoubtedly greater. With deeper insights into exosome biology, innovative discoveries in preparation and modification techniques, and further optimization of targeting, stability, and drug delivery systems, the potential of exosomes in biomedicine will be increasingly realized.

Abbreviations

HSPs, heat-shock proteins; MVBs, multivesicular bodies; ILVs, intraluminal vesicles; ESCRT, endosomal sorting complex required for transport; PEG, polyethylene glycol; EpCAM, epithelial cell adhesion molecules; LAMP2B, lysosome-associated membrane protein-2B; EVs, extracellular vesicles; AA-PEG, aminoethyl anisamide polyethylene glycol; ACNV, Asparagus phylum-extracted nanovesicles; NPO, negative pressure oscillation system; HO, harmonic oscillation system; DSPE-PEG2000, distearoyl-phosphatidylethanolamine-polyethylene glycol 2000; CNS, central nervous system; BBB, blood-brain barrier; LNP, Lipid nanoparticles; HUCMSC, human umbilical cord mesenchymal stem cells; AgNPs, encapsulate silver nanoparticles; SPIONs, superparamagnetic iron oxide nanoparticles; TNF-α, tumor necrosis factor; RNP, ribonucleoprotein; NRP1, neuropilin-1; NRP2, neuropilin-2; TME, the tumor microenvironment; PTX, paclitaxel; PEG, polyethylene glycol; SCAP-Exo, exosomes derived from human apical papillary stem cells; MPS, mononuclear phagocyte system; PDT, Photodynamic therapy; AKT, Acoustic kinetic therapy; CATSiO2, catalase-loaded silica nanoparticles; CVD, Cardiovascular diseases; AMI, Acute myocardial infarction; SCAD, stable coronary artery disease; IL-6, interleukin 6; IL-1β, interleukin-1β; EGR1, early growth response factor 1; AS, Atherosclerosis; ox-LDL, oxidized low-density lipoprotein; DDSs, drug delivery systems; AD, Alzheimer's disease; Exo-Slb, exosome-encapsulated silymarin; NF-κB, nuclear factor κB.

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Disclosure

The authors declare no conflicts of interest in this work.

References

1. Yamamoto T, Kosaka N, Ochiya T. Latest advances in extracellular vesicles: from bench to bedside. *Sci Technol Adv Mater*. 2019;20(1):746–757. doi:10.1080/14686996.2019.1629835
2. Simpson RJ, Jensen SS, Lim JWE. Proteomic profiling of exosomes: current perspectives. *Proteomics*. 2008;8(19):4083–4099. doi:10.1002/pmic.200800109
3. Ma T, Chen Y, Chen Y, et al. MicroRNA-132, delivered by mesenchymal stem cell-derived exosomes, promote angiogenesis in myocardial infarction. *Stem Cells Int*. 2018;2018:3290372. doi:10.1155/2018/3290372
4. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science*. 2020;367(6478):eaau6977. doi:10.1126/science.aau6977
5. Yang D, Zhang W, Zhang H, et al. Progress, opportunity, and perspective on exosome isolation - efforts for efficient exosome-based theranostics. *Theranostics*. 2020;10(8):3684–3707. doi:10.7150/thno.41580
6. Kusuma GD, Barabadi M, Tan JL, Morton DAV, Frith JE, Lim R. To protect and to preserve: novel preservation strategies for extracellular vesicles. *Front Pharmacol*. 2018;9:1199. doi:10.3389/fphar.2018.01199
7. Zhang Y, Bi J, Huang J, Tang Y, Du S, Li P. Exosome: a review of its classification, isolation techniques, storage, diagnostic and targeted therapy applications. *Int J Nanomed*. 2020;15:6917–6934. doi:10.2147/IJN.S264498
8. Wang Y, Jiang M, Zheng X, et al. Application of exosome engineering modification in targeted delivery of therapeutic drugs. *Biochem Pharmacol*. 2023;215:115691. doi:10.1016/j.bcp.2023.115691
9. Willms E, Cabañas C, Mäger I, Wood MJA, Vader P. Extracellular vesicle heterogeneity: subpopulations, isolation techniques, and diverse functions in cancer progression. *Front Immunol*. 2018;9:738. doi:10.3389/fimmu.2018.00738
10. Vietri M, Radulovic M, Stenmark H. The many functions of ESCRTs. *Nat Rev Mol Cell Biol*. 2020;21(1):25–42. doi:10.1038/s41580-019-0177-4
11. Mosesso N, Nagel MK, Isono E. Ubiquitin recognition in endocytic trafficking - with or without ESCRT-0. *J Cell Sci*. 2019;132(16):jcs232868. doi:10.1242/jcs.232868
12. Colombo M, Moita C, van Niel G, et al. Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles. *J Cell Sci*. 2013;126(Pt 24):5553–5565. doi:10.1242/jcs.128868
13. Trajkovic K, Hsu C, Chiantia S, et al. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science*. 2008;319(5867):1244–1247. doi:10.1126/science.1153124

14. Perez-Hernandez D, Gutiérrez-Vázquez C, Jorge I, et al. The intracellular interactome of tetraspanin-enriched microdomains reveals their function as sorting machineries toward exosomes. *J Biol Chem*. 2013;288(17):11649–11661. doi:10.1074/jbc.M112.445304
15. Jeppesen DK, Fenix AM, Franklin JL, et al. Reassessment of exosome composition. *Cell*. 2019;177(2):428–445.e18. doi:10.1016/j.cell.2019.02.029
16. Gholami farashah MS, Javadi M, Mohammadi A, Soleimani Rad J, Shakouri SK, Roshangar L. Bone marrow mesenchymal stem cell's exosomes as key nanoparticles in osteogenesis and bone regeneration: specific capacity based on cell type. *Mol Biol Rep*. 2022;49(12):12203–12218. doi:10.1007/s11033-022-07807-1
17. Record M, Carayon K, Poirot M, Silvente-Poirot S. Exosomes as new vesicular lipid transporters involved in cell-cell communication and various pathophysiologies. *Biochim Biophys Acta*. 2014;1841(1):108–120. doi:10.1016/j.bbalip.2013.10.004
18. Cvjetkovic A, Lötvall J, Lässer C. The influence of rotor type and centrifugation time on the yield and purity of extracellular vesicles. *J Extracell Vesicles*. 2014;3(1). doi:10.3402/jev.v3.23111
19. Chen W, Cao R, Su W, et al. Simple and fast isolation of circulating exosomes with a chitosan modified shuttle flow microchip for breast cancer diagnosis. *Lab Chip*. 2021;21(9):1759–1770. doi:10.1039/d0lc01311k
20. Doyle LM, Wang MZ. Overview of extracellular vesicles, their origin, composition, purpose, and methods for exosome isolation and analysis. *Cells*. 2019;8(7):727. doi:10.3390/cells8070727
21. Muller L, Hong CS, Stolz DB, Watkins SC, Whiteside TL. Isolation of biologically-active exosomes from human plasma. *J Immunol Methods*. 2014;411:55–65. doi:10.1016/j.jim.2014.06.007
22. Livshits MA, Khomyakova E, Evtushenko EG, et al. Isolation of exosomes by differential centrifugation: theoretical analysis of a commonly used protocol. *Sci Rep*. 2015;5(1):17319. doi:10.1038/srep17319
23. Hiemstra TF, Charles PD, Gracia T, et al. Human urinary exosomes as innate immune effectors. *J Am Soc Nephrol*. 2014;25(9):2017–2027. doi:10.1681/ASN.2013101066
24. Zhu J, Liu B, Wang Z, et al. Exosomes from nicotine-stimulated macrophages accelerate atherosclerosis through miR-21-3p/PTEN-mediated VSMC migration and proliferation. *Theranostics*. 2019;9(23):6901–6919. doi:10.7150/thno.37357
25. Schuldner M, Dörsam B, Shatnyeva O, et al. Exosome-dependent immune surveillance at the metastatic niche requires BAG6 and CBP/p300-dependent acetylation of p53. *Theranostics*. 2019;9(21):6047–6062. doi:10.7150/thno.36378
26. Street JM, Koritzinsky EH, Glispie DM, Yuen PST. Urine Exosome Isolation and Characterization. *Methods Mol Biol*. 2017;1641:413–423. doi:10.1007/978-1-4939-7172-5_23
27. Théry C, Witwer KW, Aikawa E, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the international society for extracellular vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles*. 2018;7(1):1535750. doi:10.1080/20013078.2018.1535750
28. Tran PHL, Wang T, Yin W, et al. Development of a nanoamorphous exosomal delivery system as an effective biological platform for improved encapsulation of hydrophobic drugs. *Int J Pharm*. 2019;566:697–707. doi:10.1016/j.ijpharm.2019.06.028
29. Tran PHL, Wang T, Yin W, et al. Aspirin-loaded nanoexosomes as cancer therapeutics. *Int J Pharm*. 2019;572:118786. doi:10.1016/j.ijpharm.2019.118786
30. Dash M, Palaniyandi K, Ramalingam S, Sahabudeen S, Raja NS. Exosomes isolated from two different cell lines using three different isolation techniques show variation in physical and molecular characteristics. *Biochim Biophys Acta Biomembr*. 2021;1863(2):183490. doi:10.1016/j.bbamem.2020.183490
31. Seo K, Yoo JH, Kim J, et al. Ginseng-derived exosome-like nanovesicles extracted by sucrose gradient ultracentrifugation to inhibit osteoclast differentiation. *Nanoscale*. 2023;15(12):5798–5808. doi:10.1039/d2nr07018a
32. Zhu L, Sun HT, Wang S, et al. Isolation and characterization of exosomes for cancer research. *J Hematol Oncol*. 2020;13(1):152. doi:10.1186/s13045-020-00987-y
33. Chen BY, Sung CWH, Chen C, et al. Advances in exosomes technology. *Clin Chim Acta*. 2019;493:14–19. doi:10.1016/j.cca.2019.02.021
34. Lu Y, Eguchi T, Sogawa C, et al. Exosome-based molecular transfer activity of macrophage-like cells involves viability of oral carcinoma cells: size exclusion chromatography and concentration filter method. *Cells*. 2021;10(6):1328. doi:10.3390/cells10061328
35. Li P, Kaslan M, Lee SH, Yao J, Gao Z. Progress in exosome isolation techniques. *Theranostics*. 2017;7(3):789–804. doi:10.7150/thno.18133
36. Cheruvanky A, Zhou H, Pisitkun T, et al. Rapid isolation of urinary exosomal biomarkers using a nanomembrane ultrafiltration concentrator. *Am J Physiol Renal Physiol*. 2007;292(5):F1657–1661. doi:10.1152/ajprenal.00434.2006
37. Taylor DD, Shah S. Methods of isolating extracellular vesicles impact down-stream analyses of their cargoes. *Methods*. 2015;87:3–10. doi:10.1016/j.ymeth.2015.02.019
38. Patel GK, Khan MA, Zubair H, et al. Comparative analysis of exosome isolation methods using culture supernatant for optimum yield, purity and downstream applications. *Sci Rep*. 2019;9(1):5335. doi:10.1038/s41598-019-41800-2
39. Ruysschaert T, Marque A, Duteyrat JL, Lesieur S, Winterhalter M, Fournier D. Liposome retention in size exclusion chromatography. *BMC Biotechnol*. 2005;5(1):11. doi:10.1186/1472-6750-5-11
40. Suthar J, Parsons ES, Hoogenboom BW, Williams GR, Guldin S. Acoustic immunosensing of exosomes using a quartz crystal microbalance with dissipation monitoring. *Anal Chem*. 2020;92(5):4082–4093. doi:10.1021/acs.analchem.9b05736
41. Guan S, Yu H, Yan G, Gao M, Sun W, Zhang X. Characterization of urinary exosomes purified with size exclusion chromatography and ultracentrifugation. *J Proteome Res*. 2020;19(6):2217–2225. doi:10.1021/acs.jproteome.9b00693
42. Karimi N, Cvjetkovic A, Jang SC, et al. Detailed analysis of the plasma extracellular vesicle proteome after separation from lipoproteins. *Cell Mol Life Sci*. 2018;75(15):2873–2886. doi:10.1007/s00018-018-2773-4
43. Gámez-Valero A, Monguió-Tortajada M, Carreras-Planella L, Franquesa ML, Beyer K, Borràs FE. Size-exclusion chromatography-based isolation minimally alters extracellular vesicles' characteristics compared to precipitating agents. *Sci Rep*. 2016;6(1):33641. doi:10.1038/srep33641
44. Navajas R, Corrales FJ, Paradela A. Serum exosome isolation by size-exclusion chromatography for the discovery and validation of preeclampsia-associated biomarkers. *Methods Mol Biol*. 2019;1959:39–50. doi:10.1007/978-1-4939-9164-8_3
45. Witwer KW, Buzás EI, Bemis LT, et al. Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. *J Extracell Vesicles*. 2013;2(1). doi:10.3402/jev.v2i0.20360

46. Soares Martins T, Catita J, Martins Rosa I, AB da Cruz E Silva O, Henriques AG. Exosome isolation from distinct biofluids using precipitation and column-based approaches. *PLoS One*. 2018;13(6):e0198820. doi:10.1371/journal.pone.0198820
47. Zeringer E, Barta T, Li M, Vlassov AV. Strategies for isolation of exosomes. *Cold Spring Harb Protoc*. 2015;2015(4):319–323. doi:10.1101/pdb.top074476
48. Konoshenko MY, Lekchnov EA, Vlassov AV, Laktionov PP. Isolation of extracellular vesicles: general methodologies and latest trends. *Biomed Res Int*. 2018;2018:8545347. doi:10.1155/2018/8545347
49. García-Romero N, Madurga R, Rackov G, et al. Polyethylene glycol improves current methods for circulating extracellular vesicle-derived DNA isolation. *J Transl Med*. 2019;17(1):75. doi:10.1186/s12967-019-1825-3
50. Dou YQ, Kong P, Li CL, et al. Smooth muscle SIRT1 reprograms endothelial cells to suppress angiogenesis after ischemia. *Theranostics*. 2020;10(3):1197–1212. doi:10.7150/thno.39320
51. Wan Z, Zhao L, Lu F, et al. Mononuclear phagocyte system blockade improves therapeutic exosome delivery to the myocardium. *Theranostics*. 2020;10(1):218–230. doi:10.7150/thno.38198
52. György B, Módos K, Pállinger E, et al. Detection and isolation of cell-derived microparticles are compromised by protein complexes resulting from shared biophysical parameters. *Blood*. 2011;117(4):e39–48. doi:10.1182/blood-2010-09-307595
53. Kalra H, Adda CG, Liem M, et al. Comparative proteomics evaluation of plasma exosome isolation techniques and assessment of the stability of exosomes in normal human blood plasma. *Proteomics*. 2013;13(22):3354–3364. doi:10.1002/pmic.201300282
54. Li J, Zhang Y, Dong PY, Yang GM, Gurunathan S. A comprehensive review on the composition, biogenesis, purification, and multifunctional role of exosome as delivery vehicles for cancer therapy. *Biomed Pharmacother*. 2023;165:115087. doi:10.1016/j.biopha.2023.115087
55. Chen C, Skog J, Hsu CH, et al. Microfluidic isolation and transcriptome analysis of serum microvesicles. *Lab Chip*. 2010;10(4):505–511. doi:10.1039/b916199f
56. Hong CS, Muller L, Boyiadzis M, Whiteside TL. Isolation and characterization of CD34+ blast-derived exosomes in acute myeloid leukemia. *PLoS One*. 2014;9(8):e103310. doi:10.1371/journal.pone.0103310
57. Zhang Y, Liu Y, Liu H, Tang WH. Exosomes: biogenesis, biologic function and clinical potential. *Cell Biosci*. 2019;9(1):19. doi:10.1186/s13578-019-0282-2
58. Poellmann MJ, Nair A, Bu J, Kim JKH, Kimple RJ, Hong S. Immunoavidity-based capture of tumor exosomes using poly(amidoamine) dendrimer surfaces. *Nano Lett*. 2020;20(8):5686–5692. doi:10.1021/acs.nanolett.0c00950
59. Zarovni N, Corrado A, Guazzi P, et al. Integrated isolation and quantitative analysis of exosome shuttled proteins and nucleic acids using immunocapture approaches. *Methods*. 2015;87:46–58. doi:10.1016/j.ymeth.2015.05.028
60. Park J, Park JS, Huang CH, et al. An integrated magneto-electrochemical device for the rapid profiling of tumour extracellular vesicles from blood plasma. *Nat Biomed Eng*. 2021;5(7):678–689. doi:10.1038/s41551-021-00752-7
61. Batrakova EV, Kim MS. Using exosomes, naturally-equipped nanocarriers, for drug delivery. *J Control Release*. 2015;219:396–405. doi:10.1016/j.jconrel.2015.07.030
62. Kornilov R, Puhka M, Mannerström B, et al. Efficient ultrafiltration-based protocol to deplete extracellular vesicles from fetal bovine serum. *J Extracell Vesicles*. 2018;7(1):1422674. doi:10.1080/20013078.2017.1422674
63. Gholizadeh S, Shehata Draz M, Zarghooni M, et al. Microfluidic approaches for isolation, detection, and characterization of extracellular vesicles: current status and future directions. *Biosens Bioelectron*. 2017;91:588–605. doi:10.1016/j.bios.2016.12.062
64. Pishbin E, Sadri F, Dehghan A, et al. Recent advances in isolation and detection of exosomal microRNAs related to Alzheimer's disease. *Environ Res*. 2023;227:115705. doi:10.1016/j.envres.2023.115705
65. Wang Y, Li Q, Shi H, et al. Microfluidic Raman biochip detection of exosomes: a promising tool for prostate cancer diagnosis. *Lab Chip*. 2020;20(24):4632–4637. doi:10.1039/d0lc00677g
66. Wang Z, Wu H-J, Fine D, et al. Ciliated micropillars for the microfluidic-based isolation of nanoscale lipid vesicles. *Lab Chip*. 2013;13(15):2879–2882. doi:10.1039/c3lc41343h
67. Suwatthanarak T, Thiodorus IA, Tanaka M, et al. Microfluidic-based capture and release of cancer-derived exosomes via peptide-nanowire hybrid interface. *Lab Chip*. 2021;21(3):597–607. doi:10.1039/d0lc00899k
68. Ding L, Liu X, Zhang Z, et al. Magnetic-nanowaxberry-based microfluidic ExoSIC for affinity and continuous separation of circulating exosomes towards cancer diagnosis. *Lab Chip*. 2023;23(6):1694–1702. doi:10.1039/d2lc00996j
69. Zhang P, Zhou X, He M, et al. Ultrasensitive detection of circulating exosomes with a 3D-nanopatterned microfluidic chip. *Nat Biomed Eng*. 2019;3(6):438–451. doi:10.1038/s41551-019-0356-9
70. Charoenviriyakul C, Takahashi Y, Nishikawa M, Takakura Y. Preservation of exosomes at room temperature using lyophilization. *Int J Pharm*. 2018;553(1–2):1–7. doi:10.1016/j.ijpharm.2018.10.032
71. Maroto R, Zhao Y, Jamaluddin M, et al. Effects of storage temperature on airway exosome integrity for diagnostic and functional analyses. *J Extracell Vesicles*. 2017;6(1):1359478. doi:10.1080/20013078.2017.1359478
72. Ge Q, Zhou Y, Lu J, Bai Y, Xie X, Lu Z. miRNA in plasma exosome is stable under different storage conditions. *Molecules*. 2014;19(2):1568–1575. doi:10.3390/molecules19021568
73. Aiso T, Takigami S, Yamaki A, Ohnishi H. Degradation of serum microRNAs during transient storage of serum samples at 4°C. *Ann Clin Biochem*. 2018;55(1):178–180. doi:10.1177/0004563217704233
74. Jeyaram A, Jay SM. Preservation and storage stability of extracellular vesicles for therapeutic applications. *AAPS J*. 2017;20(1):1. doi:10.1208/s12248-017-0160-y
75. Agrawal AK, Aqil F, Jeyabalan J, et al. Milk-derived exosomes for oral delivery of paclitaxel. *Nanomedicine*. 2017;13(5):1627–1636. doi:10.1016/j.nano.2017.03.001
76. Munagala R, Aqil F, Jeyabalan J, Gupta RC. Bovine milk-derived exosomes for drug delivery. *Cancer Lett*. 2016;371(1):48–61. doi:10.1016/j.canlet.2015.10.020
77. Welch JL, Madison MN, Margolick JB, et al. Effect of prolonged freezing of semen on exosome recovery and biologic activity. *Sci Rep*. 2017;7(1):45034. doi:10.1038/srep45034
78. Bosch S, de Beaurepaire L, Allard M, et al. Trehalose prevents aggregation of exosomes and cryodamage. *Sci Rep*. 2016;6(1):36162. doi:10.1038/srep36162

79. Bari E, Perteghella S, Catenacci L, et al. Freeze-dried and GMP-compliant pharmaceuticals containing exosomes for acellular mesenchymal stromal cell immunomodulant therapy. *Nanomedicine*. 2019;14(6):753–765. doi:10.2217/nnm-2018-0240
80. Ban JJ, Lee M, Im W, Kim M. Low pH increases the yield of exosome isolation. *Biochem Biophys Res Commun*. 2015;461(1):76–79. doi:10.1016/j.bbrc.2015.03.172
81. Zhao Y, Chen K, Li H, Wu H. Effect of pH on the isolation of urinary exosome. *Int Urol Nephrol*. 2017;49(1):165–169. doi:10.1007/s11255-016-1408-7
82. Tang TT, Wang B, Wu M, et al. Extracellular vesicle-encapsulated IL-10 as novel nanotherapeutics against ischemic AKI. *Sci Adv*. 2020;6(33): eaaz0748. doi:10.1126/sciadv.aaz0748
83. Usman WM, Pham TC, Kwok YY, et al. Efficient RNA drug delivery using red blood cell extracellular vesicles. *Nat Commun*. 2018;9(1):2359. doi:10.1038/s41467-018-04791-8
84. Haque S, Whittaker MR, McIntosh MP, Pouton CW, Kaminskas LM. Disposition and safety of inhaled biodegradable nanomedicines: opportunities and challenges. *Nanomedicine*. 2016;12(6):1703–1724. doi:10.1016/j.nano.2016.03.002
85. Sun L, Wu Q, Peng F, Liu L, Gong C. Strategies of polymeric nanoparticles for enhanced internalization in cancer therapy. *Colloids Surf B Biointerfaces*. 2015;135:56–72. doi:10.1016/j.colsurfb.2015.07.013
86. Dams ET, Laverman P, Oyen WJ, et al. Accelerated blood clearance and altered biodistribution of repeated injections of sterically stabilized liposomes. *J Pharmacol Exp Ther*. 2000;292(3):1071–1079. doi:10.1016/S0022-3565(24)35391-1
87. Armstrong JPK, Holme MN, Stevens MM. Re-engineering extracellular vesicles as smart nanoscale therapeutics. *ACS Nano*. 2017;11(1):69–83. doi:10.1021/acsnano.6b07607
88. Kamerkar S, LeBleu VS, Sugimoto H, et al. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. *Nature*. 2017;546(7659):498–503. doi:10.1038/nature22341
89. Kim MS, Haney MJ, Zhao Y, et al. Engineering macrophage-derived exosomes for targeted paclitaxel delivery to pulmonary metastases: in vitro and in vivo evaluations. *Nanomedicine*. 2018;14(1):195–204. doi:10.1016/j.nano.2017.09.011
90. Kooijmans SA, Fliervoet LA, van der Meel R, et al. PEGylated and targeted extracellular vesicles display enhanced cell specificity and circulation time. *J Control Release*. 2016;224:77–85. doi:10.1016/j.jconrel.2016.01.009
91. Zhang L, He F, Gao L, et al. Engineering exosome-like nanovesicles derived from asparagus cochinchinensis can inhibit the proliferation of hepatocellular carcinoma cells with better safety profile. *Int J Nanomed*. 2021;16:1575–1586. doi:10.2147/IJN.S293067
92. Blanco E, Shen H, Ferrari M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nat Biotechnol*. 2015;33 (9):941–951. doi:10.1038/nbt.3330
93. Elliott RO, He M. Unlocking the power of exosomes for crossing biological barriers in drug delivery. *Pharmaceutics*. 2021;13(1):122. doi:10.3390/pharmaceutics13010122
94. Shi M, Sheng L, Stewart T, Zabetian CP, Zhang J. New windows into the brain: central nervous system-derived extracellular vesicles in blood. *Prog Neurobiol*. 2019;175:96–106. doi:10.1016/j.pneurobio.2019.01.005
95. Jung KO, Jo H, Yu JH, Gambhir SS, Pratx G. Development and MPI tracking of novel hypoxia-targeted theranostic exosomes. *Biomaterials*. 2018;177:139–148. doi:10.1016/j.biomaterials.2018.05.048
96. Saint-Pol J, Gosselet F, Duban-Deweer S, Pottiez G, Karamanos Y. Targeting and crossing the blood-brain barrier with extracellular vesicles. *Cells*. 2020;9(4):851. doi:10.3390/cells9040851
97. Kang C, Han P, Lee JS, Lee D, Kim D. Anchor, spacer, and ligand-modified engineered exosomes for trackable targeted therapy. *Bioconjug Chem*. 2020;31(11):2541–2552. doi:10.1021/acs.bioconjchem.0c00483
98. Wu T, Liu Y, Cao Y, Liu Z. Engineering macrophage exosome disguised biodegradable nanoplatform for enhanced sonodynamic therapy of glioblastoma. *Adv Mater*. 2022;34(15):e2110364. doi:10.1002/adma.202110364
99. Li S, Wu Y, Ding F, et al. Engineering macrophage-derived exosomes for targeted chemotherapy of triple-negative breast cancer. *Nanoscale*. 2020;12(19):10854–10862. doi:10.1039/d0nr00523a
100. Milbank E, Dragano NRV, González-García I, et al. Small extracellular vesicle-mediated targeting of hypothalamic AMPKα1 corrects obesity through BAT activation. *Nat Metab*. 2021;3(10):1415–1431. doi:10.1038/s42255-021-00467-8
101. Fuhrmann G, Serio A, Mazo M, Nair R, Stevens MM. Active loading into extracellular vesicles significantly improves the cellular uptake and photodynamic effect of porphyrins. *J Control Release*. 2015;205:35–44. doi:10.1016/j.jconrel.2014.11.029
102. Rurik JG, Tombácz I, Yadegari A, et al. CAR T cells produced in vivo to treat cardiac injury. *Science*. 2022;375(6576):91–96. doi:10.1126/science.abm0594
103. August A, Attarwala HZ, Himansu S, et al. A Phase 1 trial of lipid-encapsulated mRNA encoding a monoclonal antibody with neutralizing activity against Chikungunya virus. *Nat Med*. 2021;27(12):2224–2233. doi:10.1038/s41591-021-01573-6
104. Erana-Perez Z, Igartua M, Santos-Vizcaino E, Hernandez RM. Genetically engineered loaded extracellular vesicles for drug delivery. *Trends Pharmacol Sci*. 2024;45(4):350–365. doi:10.1016/j.tips.2024.02.006
105. Zhang M, Hu S, Liu L, et al. Engineered exosomes from different sources for cancer-targeted therapy. *Signal Transduct Target Ther*. 2023;8 (1):124. doi:10.1038/s41392-023-01382-y
106. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nature Biotechnol*. 2011;29(4):341–345. doi:10.1038/nbt.1807
107. Liu W, Li L, Rong Y, et al. Hypoxic mesenchymal stem cell-derived exosomes promote bone fracture healing by the transfer of miR-126. *Acta Biomater*. 2020;103:196–212. doi:10.1016/j.actbio.2019.12.020
108. Patton MC, Zubair H, Khan MA, Singh S, Singh AP. Hypoxia alters the release and size distribution of extracellular vesicles in pancreatic cancer cells to support their adaptive survival. *J Cell Biochem*. 2020;121(1):828–839. doi:10.1002/jcb.29328
109. Sun D, Zhuang X, Xiang X, et al. A novel nanoparticle drug delivery system: the anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes. *Mol Ther*. 2010;18(9):1606–1614. doi:10.1038/mt.2010.105
110. Yue H, Yuan L, Zhang W, Zhang S, Wei W, Ma G. Macrophage responses to the physical burden of cell-sized particles. *J Mat Chem B*. 2018;6 (3):393–400. doi:10.1039/c7tb01673e
111. Zhang J, Ji C, Zhang H, et al. Engineered neutrophil-derived exosome-like vesicles for targeted cancer therapy. *Sci Adv*. 2022;8(2):eabj8207. doi:10.1126/sciadv.abj8207

112. Zhuang M, Chen X, Du D, et al. SPION decorated exosome delivery of TNF-α to cancer cell membranes through magnetism. *Nanoscale*. 2020;12(1):173–188. doi:10.1039/c9nr05865f
113. Wan T, Zhong J, Pan Q, Zhou T, Ping Y, Liu X. Exosome-mediated delivery of Cas9 ribonucleoprotein complexes for tissue-specific gene therapy of liver diseases. *Sci Adv*. 2022;8(37):eabp9435. doi:10.1126/sciadv.abp9435
114. Teesalu T, Sugahara KN, Kotamraju VR, Ruoslahti E. C-end rule peptides mediate neuropilin-1-dependent cell, vascular, and tissue penetration. *Proc Natl Acad Sci USA*. 2009;106(38):16157–16162. doi:10.1073/pnas.0908201106
115. Kawakami T, Tokunaga T, Hatanaka H, et al. Neuropilin 1 and neuropilin 2 co-expression is significantly correlated with increased vascularity and poor prognosis in nonsmall cell lung carcinoma. *Cancer*. 2002;95(10):2196–2201. doi:10.1002/cncr.10936
116. Roth L, Agemy L, Kotamraju VR, et al. Transtumoral targeting enabled by a novel neuropilin-binding peptide. *Oncogene*. 2012;31(33):3754–3763. doi:10.1038/onc.2011.537
117. Fan M, Liu H, Yan H, et al. A CAR T-inspiring platform based on antibody-engineered exosomes from antigen-feeding dendritic cells for precise solid tumor therapy. *Biomaterials*. 2022;282:121424. doi:10.1016/j.biomaterials.2022.121424
118. Nguyen Cao TG, Kang JH, Kim W, et al. Engineered extracellular vesicle-based sonotheranostics for dual stimuli-sensitive drug release and photoacoustic imaging-guided chemo-sonodynamic cancer therapy. *Theranostics*. 2022;12(3):1247–1266. doi:10.7150/thno.65516
119. Xiao S, Xiao C, Miao Y, et al. Human acellular amniotic membrane incorporating exosomes from adipose-derived mesenchymal stem cells promotes diabetic wound healing. *Stem Cell Res Ther*. 2021;12(1):255. doi:10.1186/s13287-021-02333-6
120. Tao SC, Guo SC, Li M, Ke QF, Guo YP, Zhang CQ. Chitosan wound dressings incorporating exosomes derived from MicroRNA-126-overexpressing synovium mesenchymal stem cells provide sustained release of exosomes and heal full-thickness skin defects in a diabetic rat model. *Stem Cells Translational Medicine*. 2017;6(3):736–747. doi:10.5966/sctm.2016-0275
121. Jing X, Wang S, Tang H, et al. Dynamically Bioresponsive DNA Hydrogel Incorporated with Dual-Functional Stem Cells from Apical Papilla-Derived Exosomes Promotes Diabetic Bone Regeneration. *ACS Appl Mater Interfaces*. 2022;14(14):16082–16099. doi:10.1021/acsami.2c02278
122. Xu RH, Zhang Y, Pan H, et al. Efficacy and safety of weekly paclitaxel with or without ramucirumab as second-line therapy for the treatment of advanced gastric or gastroesophageal junction adenocarcinoma (RAINBOW-Asia): a randomised, multicentre, double-blind, Phase 3 trial. *Lancet Gastroenterol Hepatol*. 2021;6(12):1015–1024. doi:10.1016/S2468-1253(21)00313-7
123. Conroy T, Bosset JF, Etienne PL, et al. Neoadjuvant chemotherapy with FOLFIRINOX and preoperative chemoradiotherapy for patients with locally advanced rectal cancer (UNICANCER-PRODIGE 23): a multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol*. 2021;22(5):702–715. doi:10.1016/S1470-2045(21)00079-6
124. Huang H, Shao L, Chen Y, et al. Synergistic strategy with hyperthermia therapy based immunotherapy and engineered exosomes-liposomes targeted chemotherapy prevents tumor recurrence and metastasis in advanced breast cancer. *Bioeng Transl Med*. 2022;7(2):e10284. doi:10.1002/btm2.10284
125. Anguela XM, High KA. Entering the modern era of gene therapy. *Annu Rev Med*. 2019;70(1):273–288. doi:10.1146/annurev-med-012017-043332
126. Zhang Z, Guo X, Guo X, et al. MicroRNA-29a-3p delivery via exosomes derived from engineered human mesenchymal stem cells exerts tumour suppressive effects by inhibiting migration and vasculogenic mimicry in glioma. *Aging*. 2021;13(4):5055–5068. doi:10.18632/aging.202424
127. Lou G, Chen L, Xia C, et al. MiR-199a-modified exosomes from adipose tissue-derived mesenchymal stem cells improve hepatocellular carcinoma chemosensitivity through mTOR pathway. *J Exp Clin Cancer Res*. 2020;39(1):4. doi:10.1186/s13046-019-1512-5
128. Xie J, Wang Y, Choi W, et al. Overcoming barriers in photodynamic therapy harnessing nano-formulation strategies. *Chem Soc Rev*. 2021;50(16):9152–9201. doi:10.1039/d0cs01370f
129. Wang B, Wang JH, Liu Q, et al. Rose-bengal-conjugated gold nanorods for in vivo photodynamic and photothermal oral cancer therapies. *Biomaterials*. 2014;35(6):1954–1966. doi:10.1016/j.biomaterials.2013.11.066
130. Dixon SJ, Lemberg KM, Lamprecht MR, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell*. 2012;149(5):1060–1072. doi:10.1016/j.cell.2012.03.042
131. Kennedy JE. High-intensity focused ultrasound in the treatment of solid tumours. *Nat Rev Cancer*. 2005;5(4):321–327. doi:10.1038/nrc1591
132. Yuan D, Zhao Y, Banks WA, et al. Macrophage exosomes as natural nanocarriers for protein delivery to inflamed brain. *Biomaterials*. 2017;142:1–12. doi:10.1016/j.biomaterials.2017.07.011
133. Song G, Chen Y, Liang C, et al. Catalase-loaded TaOx nanoshells as bio-nanoreactors combining high-Z element and enzyme delivery for enhancing radiotherapy. *Adv Mater*. 2016;28(33):7143–7148. doi:10.1002/adma.201602111
134. Ma LY, Chen WW, Gao RL, et al. China cardiovascular diseases report 2018: an updated summary. *J Geriatr Cardiol*. 2020;17(1):1–8. doi:10.11909/j.issn.1671-5411.2020.01.001
135. Shi C, Ulke-Lemée A, Deng J, Batulan Z, O'Brien ER. Characterization of heat shock protein 27 in extracellular vesicles: a potential anti-inflammatory therapy. *FASEB J*. 2019;33(2):1617–1630. doi:10.1096/fj.201800987R
136. Huang P, Wang L, Li Q, et al. Combinatorial treatment of acute myocardial infarction using stem cells and their derived exosomes resulted in improved heart performance. *Stem Cell Res Ther*. 2019;10(1):300. doi:10.1186/s13287-019-1353-3
137. Pan W, Zhu Y, Meng X, Zhang C, Yang Y, Bei Y. Immunomodulation by exosomes in myocardial infarction. *J Cardiovasc Transl Res*. 2019;12(1):28–36. doi:10.1007/s12265-018-9836-7
138. Su J, Li J, Yu Q, et al. Exosomal miRNAs as potential biomarkers for acute myocardial infarction. *IUBMB Life*. 2020;72(3):384–400. doi:10.1002/iub.2189
139. Pan J, Alimujiang M, Chen Q, Shi H, Luo X. Exosomes derived from miR-146a-modified adipose-derived stem cells attenuate acute myocardial infarction-induced myocardial damage via downregulation of early growth response factor 1. *J Cell Biochem*. 2019;120(3):4433–4443. doi:10.1002/jcb.27731
140. Koelwyn GJ, Corr EM, Erbay E, Moore KJ. Regulation of macrophage immunometabolism in atherosclerosis. *Nat Immunol*. 2018;19(6):526–537. doi:10.1038/s41590-018-0113-3
141. Nguyen MA, Karunakaran D, Geoffrion M, et al. Extracellular vesicles secreted by atherogenic macrophages transfer MicroRNA to inhibit cell migration. *Arterioscler Thromb Vasc Biol*. 2018;38(1):49–63. doi:10.1161/ATVBAHA.117.309795

142. Zhang YG, Song Y, Guo XL, et al. Exosomes derived from oxLDL-stimulated macrophages induce neutrophil extracellular traps to drive atherosclerosis. *Cell Cycle*. 2019;18(20):2674–2684. doi:10.1080/15384101.2019.1654797
143. Li J, Xue H, Li T, et al. Exosomes derived from mesenchymal stem cells attenuate the progression of atherosclerosis in ApoE-/- mice via miR-let7 mediated infiltration and polarization of M2 macrophage. *Biochem Biophys Res Commun*. 2019;510(4):565–572. doi:10.1016/j.bbrc.2019.02.005
144. Elsharkasy OM, Nordin JZ, Hagey DW, et al. Extracellular vesicles as drug delivery systems: why and how? *Adv Drug Deliv Rev*. 2020;159:332–343. doi:10.1016/j.addr.2020.04.004
145. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJA. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat Biotechnol*. 2011;29(4):341–345. doi:10.1038/nbt.1807
146. Soares AR, Martins-Marques T, Ribeiro-Rodrigues T, et al. Gap junctional protein Cx43 is involved in the communication between extracellular vesicles and mammalian cells. *Sci Rep*. 2015;5(1):13243. doi:10.1038/srep13243
147. Wan Y, Wang L, Zhu C, et al. Aptamer-conjugated extracellular nanovesicles for targeted drug delivery. *Cancer Res*. 2018;78(3):798–808. doi:10.1158/0008-5472.CAN-17-2880
148. Gassama Y, Favereaux A. Emerging roles of extracellular vesicles in the central nervous system: physiology, pathology, and therapeutic perspectives. *Front Cell Neurosci*. 2021;15:626043. doi:10.3389/fncel.2021.626043
149. Basso M, Bonetto V. Extracellular vesicles and a novel form of communication in the brain. *Front Neurosci*. 2016;10:127. doi:10.3389/fnins.2016.00127
150. Forner S, Baglietto-Vargas D, Martini AC, Trujillo-Estrada L, LaFerla FM. Synaptic impairment in Alzheimer's disease: a dysregulated symphony. *Trends Neurosci*. 2017;40(6):347–357. doi:10.1016/j.tins.2017.04.002
151. Bai D, Jin G, Zhang D, et al. Natural silibinin modulates amyloid precursor protein processing and amyloid-β protein clearance in APP/PS1 mice. *J Physiol Sci*. 2019;69(4):643–652. doi:10.1007/s12576-019-00682-9
152. Huo Q, Shi Y, Qi Y, Huang L, Sui H, Zhao L. Biomimetic silibinin-loaded macrophage-derived exosomes induce dual inhibition of Aβ aggregation and astrocyte activation to alleviate cognitive impairment in a model of Alzheimer's disease. *Mater Sci Eng C Mater Biol Appl*. 2021;129:112365. doi:10.1016/j.msec.2021.112365
153. Lee M, Ban JJ, Yang S, Im W, Kim M. The exosome of adipose-derived stem cells reduces β-amyloid pathology and apoptosis of neuronal cells derived from the transgenic mouse model of Alzheimer's disease. *Brain Res*. 2018;1691:87–93. doi:10.1016/j.brainres.2018.03.034
154. Kalia LV, Lang AE. Parkinson's disease. *Lancet*. 2015;386(9996):896–912. doi:10.1016/S0140-6736(14)61393-3
155. Rocha EM, De Miranda B, Sanders LH. Alpha-synuclein: pathology, mitochondrial dysfunction and neuroinflammation in Parkinson's disease. *Neurobiol Dis*. 2018;109(Pt B):249–257. doi:10.1016/j.nbd.2017.04.004
156. Stuendl A, Kunadt M, Kruse N, et al. Induction of α-synuclein aggregate formation by CSF exosomes from patients with Parkinson's disease and dementia with Lewy bodies. *Brain*. 2016;139(Pt 2):481–494. doi:10.1093/brain/awv346
157. Silindir Gunay M, Yekta Ozer A, Chalon S. Drug delivery systems for imaging and therapy of parkinson's disease. *CN*. 2016;14(4):376–391. doi:10.2174/1570159X14666151230124904
158. Yang J, Luo S, Zhang J, et al. Exosome-mediated delivery of antisense oligonucleotides targeting α-synuclein ameliorates the pathology in a mouse model of Parkinson's disease. *Neurobiol Dis*. 2021;148:105218. doi:10.1016/j.nbd.2020.105218
159. Li Q, Hu W, Huang Q, et al. MiR146a-loaded engineered exosomes released from silk fibroin patch promote diabetic wound healing by targeting IRAK1. *Signal Transduct Target Ther*. 2023;8(1):62. doi:10.1038/s41392-022-01263-w
160. Gao W, Zhang Y, Yuan L, Huang F, Wang YS. Long non-coding RNA H19-overexpressing exosomes ameliorate UVB-induced photoaging by upregulating SIRT1 via sponging miR-138. *Photochem Photobiol*. 2023;99(6):1456–1467. doi:10.1111/php.13801
161. You Y, Tian Y, Yang Z, et al. Intradermally delivered mRNA-encapsulating extracellular vesicles for collagen-replacement therapy. *Nat Biomed Eng*. 2023;7(7):887–900. doi:10.1038/s41551-022-00989-w
162. Ren J, Jing X, Liu Y, et al. Exosome-based engineering strategies for the diagnosis and treatment of oral and maxillofacial diseases. *J Nanobiotechnol*. 2023;21(1):501. doi:10.1186/s12951-023-02277-4
163. Yoshida A, Fujiwara T, Uotani K, et al. Clinical and functional significance of intracellular and extracellular microRNA-25-3p in osteosarcoma. *Acta Med Okayama*. 2018;72(2):165–174. doi:10.18926/AMO/55857
164. Kim S, Kang JH, Nguyen Cao TG, et al. Extracellular vesicles with high dual drug loading for safe and efficient combination chemo-phototherapy. *Biomater Sci*. 2022;10(11):2817–2830. doi:10.1039/d1bm02005f
165. Gray WD, French KM, Ghosh-Choudhary S, et al. Identification of therapeutic covariant microRNA clusters in hypoxia-treated cardiac progenitor cell exosomes using systems biology. *Circ Res*. 2015;116(2):255–263. doi:10.1161/CIRCRESAHA.116.304360
166. Li F, Zhao L, Shi Y, Liang J. Edaravone-loaded macrophage-derived exosomes enhance neuroprotection in the rat permanent middle cerebral artery occlusion model of stroke. *Mol Pharm*. 2020;17(9):3192–3201. doi:10.1021/acs.molpharmaceut.0c00245
167. Riazifar M, Mohammadi MR, Pone EJ, et al. Stem cell-derived exosomes as nanotherapeutics for autoimmune and neurodegenerative disorders. *ACS Nano*. 2019;13(6):6670–6688. doi:10.1021/acsnano.9b01004
168. Shiekh PA, Singh A, Kumar A. Exosome laden oxygen releasing antioxidant and antibacterial cryogel wound dressing OxOBand alleviate diabetic and infectious wound healing. *Biomaterials*. 2020;249:120020. doi:10.1016/j.biomaterials.2020.120020
169. Deng W, Meng Y, Wang B, et al. In vitro experimental study on the formation of microRNA-34a loaded exosomes and their inhibitory effect in oral squamous cell carcinoma. *Cell Cycle*. 2022;21(16):1775–1783. doi:10.1080/15384101.2022.2070832