

Exosomes: Biogenesis, Isolation Methods, Advantages, Therapeutic Applications and Future Perspectives — A Review**Jitendra Jat¹, Rahul Sepat¹, Dr. Aniket Singh²****¹Scholar Sri Balaji College of Pharmacy, Jaipur, Rajasthan, India****²Associate Professor, Sri Balaji College of Pharmacy, Jaipur, Rajasthan, India**

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Abstract:

Exosomes are nanosized extracellular vesicles released by many cell types and are increasingly recognized as important mediators of intercellular communication. These membrane-enclosed vesicles contain diverse biological molecules, including proteins, lipids, messenger RNA (mRNA), microRNA (miRNA), long non-coding RNA, DNA fragments, metabolites, and other bioactive substances. Their ability to transfer biological information between cells has stimulated considerable interest in their use as biomarkers, therapeutic agents, and natural nanocarriers for drug and gene delivery. Exosomes originate primarily from the endosomal pathway through the formation of intraluminal vesicles within multivesicular bodies, which subsequently fuse with the plasma membrane and release vesicles into the extracellular environment. Several techniques have been developed for exosome or small extracellular vesicle isolation, including differential ultracentrifugation, density-gradient centrifugation, size-exclusion chromatography, ultrafiltration, polymer-based precipitation, immunoaffinity capture, and microfluidic approaches. Each technique has advantages and limitations related to recovery, purity, processing time, cost, scalability, and preservation of vesicle integrity. Exosomes possess several properties desirable for drug delivery, including biocompatibility, nanoscale dimensions, protection of cargo, cellular uptake, and the possibility of surface engineering for targeted delivery. They have therefore been investigated for applications in cancer, neurological disorders, cardiovascular diseases, inflammatory conditions, regenerative medicine, vaccination, gene therapy, and precision medicine. Despite promising preclinical findings, clinical translation is limited by challenges involving source selection, isolation, characterization, manufacturing, cargo loading, stability, biodistribution, safety, scalability, and regulatory standardization. This review summarizes the biology and composition of exosomes, major isolation and characterization approaches, their advantages, pharmaceutical and biomedical applications, current limitations, and future perspectives. Continued development of standardized and scalable manufacturing and analytical approaches may establish exosome-based systems as important platforms in next-generation nanomedicine.

Keywords: Exosomes; extracellular vesicles; drug delivery; nanomedicine; exosome isolation; targeted delivery; biomarkers; gene therapy; regenerative medicine.

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Introduction

The development of effective drug delivery systems is an important objective of modern pharmaceutical research. Conventional dosage forms and synthetic nanocarriers can provide substantial therapeutic benefits; however, limitations such as poor aqueous solubility, rapid degradation, nonspecific distribution, systemic toxicity, inadequate cellular uptake, and limited delivery of biological macromolecules continue to stimulate the search for improved delivery technologies. Natural extracellular vesicles, particularly exosomes, have emerged as promising biological nanocarriers because they are produced by cells and naturally participate in intercellular communication. Exosomes are small membrane-enclosed extracellular vesicles commonly described in the

approximate size range of 30–150 nm. They contain proteins, lipids, and nucleic acids and can transfer their molecular cargo to recipient cells. Their composition can reflect the physiological or pathological state of the parent cell, which has stimulated interest in their use as biomarkers as well as therapeutic vehicles. Exosome research has expanded considerably since the recognition that these vesicles are not merely cellular waste products but biologically active mediators of cell-to-cell communication. Exosomes can interact with recipient cells through receptor–ligand interactions, membrane fusion, endocytosis, phagocytosis, and other uptake mechanisms. Following uptake, exosomal cargo may influence gene expression,

signaling pathways, metabolism, immune responses, and cellular phenotype.

Importantly, the terminology surrounding extracellular vesicles has evolved. Current International Society for Extracellular Vesicles (ISEV) recommendations emphasize appropriate terminology and rigorous characterization because currently available isolation procedures cannot always prove that an isolated population consists exclusively of endosome-derived exosomes. MISEV2023 provides recommendations for extracellular-vesicle collection, separation, characterization, functional studies, and reporting.

Exosomes have attracted particular attention as drug-delivery carriers because their lipid bilayer can protect biological cargo, while their surface molecules may facilitate interaction with specific recipient cells. Natural or engineered exosomes have been investigated for delivery of small-molecule drugs, proteins, miRNAs, siRNAs, mRNAs, and other therapeutic agents. The present review discusses exosome biology, composition, isolation and characterization techniques, advantages, pharmaceutical applications, challenges, and future prospects.

Biogenesis of Exosomes

Exosome formation is closely associated with the endosomal system. The process begins with the internalization of plasma-membrane components and extracellular molecules through endocytosis. Early endosomes subsequently mature into late endosomal compartments. During this process, portions of the endosomal membrane undergo inward budding, resulting in the formation of intraluminal vesicles (ILVs). An endosome containing multiple ILVs is known as a multivesicular body (MVB). MVBs have several possible intracellular destinations. They may fuse with lysosomes, resulting in degradation of their contents, or they may fuse with the plasma membrane. When an MVB fuses with the plasma membrane, the ILVs are released into the extracellular environment and are commonly referred to as exosomes.

The endosomal sorting complex required for transport (ESCRT) machinery plays an important role in the formation and cargo sorting of many extracellular vesicles. However, ESCRT-independent mechanisms also contribute to exosome biogenesis. Tetraspanins, lipids, ALIX, TSG101, and other molecular components have been associated with exosome formation and cargo sorting. The biological composition of exosomes depends strongly on their cell of origin and the physiological or pathological environment. Therefore, exosomes isolated from different cell types may have substantially different properties and biological effects.

Structure and Composition of Exosomes

Exosomes consist primarily of a lipid bilayer surrounding an aqueous lumen. The membrane contains cholesterol, phospholipids, sphingolipids, membrane proteins, adhesion molecules, receptors, and tetraspanins. The internal compartment contains a complex mixture of proteins, nucleic acids, metabolites, and other cellular components.

Proteins

Exosomes contain a variety of membrane-associated and intraluminal proteins. Commonly investigated exosomal-associated proteins include tetraspanins such as CD9, CD63, and CD81, as well as proteins associated with endosomal trafficking such as ALIX and TSG101. Heat-shock proteins and other proteins may also be present.

However, the detection of individual markers should not by itself be regarded as definitive proof of exosome identity. Characterization should involve complementary approaches addressing vesicle-associated proteins, particle characteristics, contaminants, and functional properties.

Lipids

The lipid composition of exosomes contributes to membrane stability, vesicle formation, cellular interaction, and uptake. Cholesterol, phospholipids, sphingolipids, and other lipid species may be present.

Nucleic Acids

Exosomes can contain several types of nucleic acids, including:

- mRNA
- miRNA
- long non-coding RNA
- circular RNA
- DNA fragments
- other regulatory RNA molecules

The transfer of nucleic acids is particularly important because exosomes may modify gene expression and cellular functions in recipient cells. Their natural ability to protect RNA molecules from extracellular degradation has made them attractive candidates for RNA delivery.

Sources of Exosomes

Exosomes can be obtained from a wide variety of biological sources. Common sources include cultured mammalian cells and biological fluids.

Important sources include:

1. Mesenchymal stem/stromal cells
2. Dendritic cells
3. Immune cells
4. Cancer cells
5. Epithelial cells

6. Endothelial cells
7. Fibroblasts
8. Blood and plasma
9. Serum
10. Urine
11. Saliva
12. Breast milk
13. Cerebrospinal fluid

The source of exosomes is an important determinant of their composition and function. For therapeutic applications, donor-cell selection is therefore a critical consideration. Stem-cell-derived extracellular vesicles have received considerable attention because of their reported immunomodulatory and regenerative properties, whereas tumor-derived vesicles may have disease-promoting activities and require careful evaluation.

Methods of Isolation of Exosomes

Isolation represents one of the most important stages in exosome research. An ideal isolation method should provide high recovery, high purity, and reproducibility, preservation of vesicle integrity, low contamination, reasonable cost, and scalability. However, no currently available technique fulfills all these requirements simultaneously.

Differential Ultracentrifugation

Differential ultracentrifugation is among the most commonly used approaches for extracellular-vesicle isolation. The method is based on sequential centrifugation at increasing centrifugal forces.

Initially, low-speed centrifugation removes intact cells and large debris. Subsequent centrifugation removes larger vesicles and cellular components. Finally, high-speed ultracentrifugation is used to collect the small-vesicle fraction.

The pellet may be washed in a suitable buffer and centrifuged again to reduce soluble contaminants.

Advantages:

- Widely established
- Suitable for relatively large sample volumes
- Does not require expensive commercial isolation kits
- Useful for research-scale preparation

Limitations:

- Time-consuming
- Requires an ultracentrifuge
- Possible vesicle aggregation
- Potential damage caused by high centrifugal forces
- Protein and other vesicle contamination may occur
- Reproducibility can depend on protocol conditions

Density-Gradient Ultracentrifugation

Density-gradient centrifugation separates vesicles according to their buoyant density. Sucrose or iodixanol gradients can be employed. Vesicles migrate toward the region corresponding to their density and can subsequently be collected.

This technique generally improves purity compared with differential ultracentrifugation alone.

Advantages:

- High purity
- Efficient removal of some protein contaminants
- Useful for mechanistic and molecular studies

Limitations:

- Labor-intensive
- Requires specialized equipment
- Relatively low throughput
- Longer processing time

Size-Exclusion Chromatography

Size-exclusion chromatography (SEC) separates particles based mainly on their hydrodynamic size. Exosome-rich fractions can be separated from smaller soluble proteins and molecules using porous chromatographic matrices.

SEC is increasingly attractive because it is relatively gentle and can preserve vesicle structure and function. It can also be combined with other purification approaches.

Advantages:

- Relatively rapid
- Gentle on vesicles
- Good removal of soluble proteins
- Suitable for downstream functional studies

Limitations:

- Limited column capacity
- May require concentration of dilute samples
- Similar-sized contaminants may remain

Ultrafiltration

Ultrafiltration employs membranes with defined molecular-weight or size cutoffs. Exosomes are retained while smaller molecules pass through the membrane.

Advantages:

- Rapid
- Relatively simple
- Does not require ultracentrifugation
- Suitable for concentration of extracellular-vesicle preparations

Limitations:

- Membrane clogging
- Vesicle adsorption and loss

- Potential deformation of vesicles
- Purity may be inadequate when used alone

Polymer-Based Precipitation: Polymer-based methods commonly use polyethylene glycol (PEG) or commercially available precipitation reagents. These approaches reduce vesicle solubility and permit recovery through relatively simple centrifugation.

Advantages:

- Easy to perform
- Inexpensive compared with some specialized methods
- No ultracentrifuge required
- Suitable for processing multiple samples

Limitations:

- Co-precipitation of proteins and other molecules
- Relatively low purity
- Residual polymer may interfere with downstream experiments
- Additional purification may be necessary

Immunoaffinity Isolation: Immunoaffinity isolation uses antibodies directed against selected surface molecules. Antibodies may be immobilized on magnetic beads or other solid surfaces to selectively capture extracellular vesicles expressing the target marker.

Advantages:

- High specificity

- Useful for selective subpopulation isolation
- Suitable for biomarker studies
- Can work with relatively small sample volumes

Limitations:

- Expensive
- Dependent on the selected surface marker
- May recover only a subset of vesicles
- Antibody binding can influence downstream applications

Microfluidic Isolation

Microfluidic platforms use microscale devices to isolate extracellular vesicles through mechanisms such as size-based separation, immunocapture, acoustic separation, and other physical or biochemical properties.

Microfluidic technologies have potential applications in rapid diagnostics and point-of-care testing.

Advantages:

- Small sample requirement
- Rapid processing
- Potential for automation
- High specificity
- Potential integration with diagnostic platforms

Limitations:

- Specialized equipment
- Higher development costs
- Limited large-scale production
- Need for further standardization

Table 1. Comparison of Exosome Isolation Methods

Method	Purity	Yield	Time	Major advantage	Major limitation
Differential ultracentrifugation	Moderate	High	Long	Established method	Aggregation/contamination
Density-gradient centrifugation	High	Moderate	Long	High purity	Labor-intensive
Size-exclusion chromatography	High	Moderate	Moderate	Gentle separation	Limited capacity
Ultrafiltration	Moderate	Moderate–high	Short	Rapid concentration	Membrane loss
Polymer precipitation	Low–moderate	High	Short	Simple and inexpensive	Contamination
Immunoaffinity	High/selective	Moderate	Short	High specificity	Expensive
Microfluidics	High/selective	Variable	Short	Rapid and miniaturized	Limited scalability

The selection of an isolation method should therefore be determined by the intended application, required purity, sample volume, available equipment, and downstream analysis.

Characterization of Exosomes: Isolation alone is insufficient to establish the identity and quality of an

extracellular-vesicle preparation. Characterization should evaluate physical properties, concentration, morphology, molecular markers, and contaminants. MISEV2023 provides recommendations for rigorous extracellular-vesicle characterization and reporting.

Nanoparticle Tracking Analysis: Nanoparticle tracking analysis (NTA) estimates particle concentration and size distribution by analyzing the Brownian motion of individual particles.

Dynamic Light Scattering

Dynamic light scattering (DLS) provides information about the hydrodynamic diameter and size distribution of particles in suspension.

Electron Microscopy

Transmission electron microscopy and related imaging methods can provide information about vesicle morphology and size.

Western Blotting

Western blotting can be used to assess selected vesicle-associated proteins such as CD9, CD63, CD81, ALIX, and TSG101, along with appropriate negative or contaminant-associated markers.

Flow Cytometry

Flow cytometry can be used for surface-marker analysis, although the small size of extracellular vesicles presents technical challenges and appropriate specialized methods may be required.

A combination of complementary techniques is preferable to relying on a single analytical method.

Advantages of Exosomes

Exosomes have several properties that make them attractive as therapeutic and diagnostic platforms.

High Biocompatibility

Because exosomes are naturally produced by cells, they can exhibit favorable biological compatibility. This property is particularly attractive when compared with some synthetic nanocarriers.

Low or Relatively Limited Immunogenicity

Many exosome preparations can demonstrate relatively low immunogenicity, although immune effects depend on the source, dose, manufacturing process, and biological context. Therefore, low immunogenicity should not be interpreted as universal immunological invisibility.

Efficient Cellular Uptake

Exosomes naturally participate in intercellular communication and can be taken up by recipient cells through multiple pathways. This property supports their investigation as intracellular delivery systems.

Protection of Therapeutic Cargo

The lipid bilayer provides physical protection to nucleic acids and other molecules enclosed within vesicles. This is particularly valuable for unstable therapeutic RNA molecules.

Ability to Carry Multiple Types of Cargo

Exosomes can naturally transport proteins, lipids, mRNA, miRNA, and other nucleic acids. Engineered systems can also be designed to carry therapeutic small molecules and biological agents.

Potential Targeting Ability

Surface proteins and other molecules inherited from the parent cell can influence exosome interactions with recipient tissues. In addition, exosome surfaces can be engineered to improve targeting.

Potential to Cross Biological Barriers

Exosomes have been investigated for delivery across physiological barriers, including the blood–brain barrier, making them attractive candidates for neurological drug delivery.

Potential for Personalized Medicine

Because exosomal cargo can reflect the state of the originating cells, exosomes have potential applications in individualized diagnosis and therapy.

Versatility

Exosome-based systems are being investigated for the delivery of chemotherapeutic agents, proteins, RNA, gene-editing components, and other therapeutic substances.

Exosomes as Drug Delivery Systems

Exosomes are increasingly being investigated as natural nanocarriers. Their membrane structure and biological functions can potentially improve the delivery of therapeutic molecules to target cells.

Therapeutic cargo may be incorporated into exosomes through endogenous or exogenous loading approaches.

Passive Loading

Passive loading involves incubation of exosomes with a drug or active molecule under appropriate conditions. This method is relatively simple but may result in limited loading efficiency.

Electroporation

Electroporation uses short electrical pulses to temporarily alter membrane permeability and facilitate incorporation of nucleic acids or other molecules.

Sonication

Sonication applies mechanical energy to temporarily disturb the vesicle membrane and promote cargo incorporation.

Extrusion

Extrusion forces vesicles through membranes with defined pore sizes. It can alter vesicle structure and

facilitate incorporation of certain therapeutic compounds.

Freeze–Thaw Method

Repeated freezing and thawing can temporarily disrupt membranes and permit incorporation of selected cargoes. However, excessive freeze–thaw cycles may affect vesicle integrity.

Endogenous Loading

In endogenous loading, donor cells are exposed to the therapeutic molecule or genetically modified so that the desired cargo becomes incorporated during exosome formation. This approach may be useful for loading specific RNAs or proteins. The optimal loading strategy depends on cargo characteristics, desired loading efficiency, vesicle integrity, and intended therapeutic application.

Pharmaceutical and Biomedical Applications

Cancer Therapy

Cancer represents one of the most extensively investigated applications of exosome-based drug delivery. Exosomes can potentially transport anticancer drugs, RNA molecules, proteins, and other therapeutic agents. Engineered exosomes can be designed for tumor targeting through modification of their surface molecules. Preclinical research has investigated exosome-mediated delivery of chemotherapeutics and nucleic acids to tumor cells. However, tumor-derived exosomes can also contribute to tumor progression, immune suppression, metastasis, and drug resistance. Therefore, therapeutic applications generally require careful selection of exosome source.

Neurological Disorders

The ability of certain extracellular vesicles to interact with biological barriers has encouraged investigation in neurological disorders. Potential applications include delivery of RNA, proteins, and small molecules in conditions such as neurodegenerative diseases, brain tumors, and other neurological disorders.

Cardiovascular Diseases: Stem-cell-derived extracellular vesicles have been investigated for their potential role in cardiovascular repair. Exosomal cargo may influence angiogenesis, inflammation, apoptosis, and tissue regeneration.

Regenerative Medicine: Exosomes derived from stem/stromal cells have attracted attention in tissue repair. They may influence cell proliferation, angiogenesis, immune modulation, and extracellular matrix remodeling.

Potential areas include:

- Wound healing
- Bone regeneration
- Cartilage repair
- Cardiac repair
- Skin regeneration
- Neural regeneration

Gene and RNA Delivery

RNA molecules such as miRNA and siRNA are promising therapeutic agents but can suffer from instability and poor cellular uptake. Exosomes can provide a protective biological environment for these molecules and facilitate intracellular delivery.

Diagnostic Applications

Exosomes are present in several biological fluids and contain molecular information derived from their cells of origin. Consequently, they have been investigated as minimally invasive biomarkers. Potential applications include:

- Cancer diagnosis
- Disease monitoring
- Treatment-response assessment
- Cardiovascular disease
- Neurological disorders
- Inflammatory diseases
- Infectious diseases

Exosome-based liquid biopsy approaches are particularly attractive because samples such as blood or urine can be obtained relatively easily.

Vaccines and Immunotherapy

Exosomes may carry antigenic proteins and immune-modulating molecules.

Their ability to interact with immune cells has stimulated investigation of exosome-based vaccines and immunotherapeutic approaches.

Advantages of Exosome-Based Drug Delivery over Conventional Systems

Exosome-based delivery systems may provide several advantages over conventional drug-delivery approaches.

Table 2.

Feature	Exosomes	Conventional synthetic nanoparticles
Biological origin	Natural	Usually synthetic
Biocompatibility	Generally favorable	Depends on material
Cargo protection	High potential	Variable
RNA delivery	Highly promising	Often requires specialized formulation
Cellular uptake	Natural biological interactions	Depends on formulation

Surface engineering	Possible	Possible
Targeting	Natural and engineered	Usually engineered
Manufacturing	Complex	More established
Standardization	Still developing	Generally better established

Although exosomes offer important biological advantages, synthetic nanoparticles currently have significant manufacturing and regulatory advantages. Therefore, exosomes should not be considered universally superior; their usefulness depends on the therapeutic objective.

Limitations and Challenges

Despite their considerable promise, multiple barriers remain before exosome-based therapeutics can become widely established.

Lack of Standardized Isolation

Different isolation techniques can produce preparations with different levels of purity and recovery. This complicates comparison among studies.

Heterogeneity

Exosomes and other extracellular vesicles are heterogeneous in size, molecular composition, origin, and biological activity. The term “exosome” can therefore encompass biologically diverse populations when rigorous separation and characterization are not performed.

Low Production Yield

Many therapeutic applications require large quantities of vesicles. Obtaining consistent, high-yield preparations from suitable donor cells remains challenging.

Cargo Loading Efficiency

Although exosomes can carry different therapeutic molecules, loading efficiency can vary substantially according to the molecule and loading technique.

Storage Stability: Long-term storage can influence particle integrity, aggregation, biological activity, and cargo stability. Appropriate formulation and storage conditions therefore require systematic investigation. Current literature identifies stability and shelf-life as important translational gaps.

Biodistribution and Clearance

After administration, exosomes may be rapidly removed from circulation or accumulate in organs other than the intended target. Understanding their pharmacokinetics and biodistribution is essential.

Safety

The biological activity of exosomes depends on their source. Exosomes derived from inappropriate or diseased cells may contain undesirable molecules. Potential immunological, pro-inflammatory, pro-

thrombotic, or tumor-promoting effects therefore need to be evaluated.

Scale-Up: Laboratory-scale preparation does not automatically translate into industrial manufacturing. Large-scale production must maintain consistent quality, potency, purity, and safety.

Regulatory Challenges

Exosome-based products may have complex regulatory classifications depending on their source, manufacturing process, intended use, and degree of engineering. Standardized quality-control criteria will be important for regulatory approval.

Future Perspectives

The future development of exosome technology is likely to involve a combination of biological engineering, pharmaceutical technology, analytical science, and personalized medicine.

Engineered Exosomes

Genetic and biochemical engineering can potentially modify exosomes to enhance targeting, increase therapeutic loading, improve circulation, or control biological activity. Engineered exosomes are being explored in cancer, neurological, cardiovascular, infectious, and immune-related applications.

Targeted Delivery

Surface engineering may allow exosomes to display peptides, antibodies, proteins, or other targeting ligands. Such modifications could improve selective delivery to tumors or diseased tissues.

Hybrid Nanocarriers

Combining exosomes with synthetic liposomes or other nanomaterials may help overcome limitations of both natural and synthetic delivery systems. Such hybrid systems are an emerging area of research.

Artificial or Cell-Free Exosome Manufacturing

Future manufacturing strategies may use controlled cellular systems or cell-free technologies to produce more homogeneous extracellular-vesicle preparations.

Artificial Intelligence and Omics

Proteomics, transcriptomics, lipidomics, metabolomics, and artificial-intelligence-based analysis may facilitate identification of exosomal biomarkers and optimization of therapeutic cargo.

Personalized Exosome Therapy

Patient-specific exosomal profiles could potentially guide disease diagnosis, treatment selection, and monitoring. This could contribute to precision medicine.

Improved Regulatory Standardization

Future progress will require standardized definitions, manufacturing procedures, potency assays, characterization criteria, stability studies, and safety testing. MISEV2023 represents an important framework for improving rigor and consistency in extracellular-vesicle research.

Conclusion

Exosomes have emerged as promising biological nanocarriers with potential applications in pharmaceutical sciences, diagnostics, drug delivery, gene therapy, regenerative medicine, and personalized healthcare. Their natural origin, nanoscale size, lipid-bilayer structure, ability to transport diverse biological molecules, potential for cellular uptake, and opportunities for surface engineering provide several advantages over some conventional delivery systems.

A wide variety of isolation techniques have been developed, including differential ultracentrifugation, density-gradient centrifugation, size-exclusion chromatography, ultrafiltration, polymer-based precipitation, immunoaffinity capture, and microfluidic technologies. However, each method has limitations with respect to purity, yield, time, cost, scalability, and preservation of biological activity. Consequently, the isolation strategy should be selected according to the intended application and supported by appropriate characterization.

Exosomes are particularly promising for delivery of nucleic acids, proteins, small-molecule drugs, and other therapeutic agents. Their potential applications extend across cancer therapy, neurological disorders, cardiovascular diseases, tissue regeneration, inflammatory diseases, gene therapy, and diagnostic biomarker development. Nevertheless, the field continues to face major challenges related to heterogeneity, source selection, purification, cargo loading, storage stability, pharmacokinetics, large-scale manufacturing, safety, and regulatory approval.

Future research should focus on developing standardized, reproducible, scalable, and economically feasible production systems. Improved analytical characterization, targeted engineering, hybrid delivery platforms, artificial intelligence-assisted cargo selection, and personalized approaches may substantially increase the therapeutic value of exosomes.

Overall, exosomes represent an important and rapidly developing area of nanomedicine. Their

ability to combine the biological functions of naturally occurring extracellular vesicles with the principles of modern drug-delivery technology makes them promising candidates for the development of next-generation therapeutic and diagnostic platforms. However, rigorous scientific validation and standardized manufacturing will be essential before their full clinical potential can be realized.

References

1. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles*. 2018;7(1):1535750.
2. Welsh JA, Goberdhan DCI, O'Driscoll L, Buzás EI, Blenkiron C, Bussolati B, et al. Minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches. *J Extracell Vesicles*. 2024;13(2).
3. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science*. 2020;367(6478).
4. Pegtel DM, Gould SJ. Exosomes. *Annu Rev Biochem*. 2019;88:487-514.
5. van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. *Nat Rev Mol Cell Biol*. 2018;19:213-228.
6. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. *J Cell Biol*. 2013;200(4):373-383.
7. Yáñez-Mó M, Siljander PRM, Andreu Z, Zavec AB, Borràs FE, Buzas EI, et al. Biological properties of extracellular vesicles and their physiological functions. *J Extracell Vesicles*. 2015;4:27066.
8. Colombo M, Raposo G, Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu Rev Cell Dev Biol*. 2014;30:255-289.
9. Théry C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. *Nat Rev Immunol*. 2002;2:569-579.
10. Xu R, Rai A, Chen M, Suwakulsiri W, Greening DW, Simpson RJ. Extracellular vesicles in cancer—implications for future improvements in cancer care. *Nat Rev Clin Oncol*. 2018;15:617-638.
11. Wortzel I, Dror S, Kenific CM, Lyden D. Exosome-mediated metastasis: communication from a distance. *Dev Cell*. 2019;49(3):347-360.
12. György B, Szabó TG, Pászti EA, Pál Z, Misják P, Aradi B, et al. Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. *Cell Mol Life Sci*. 2011;68:2667-2688.

13. El Andaloussi S, Mäger I, Breakefield XO, Wood MJA. Extracellular vesicles: biology and emerging therapeutic opportunities. *Nat Rev Drug Discov.* 2013;12:347-357.
14. Vader P, Mol EA, Pasterkamp G, Schiffelers RM. Extracellular vesicles for drug delivery. *Adv Drug Deliv Rev.* 2016;106:148-156.
15. Ha D, Yang N, Nadithe V. Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges. *Acta Pharm Sin B.* 2016;6(4):287-296.
16. Batrakova EV, Kim MS. Using exosomes, naturally-equipped nanocarriers, for drug delivery. *J Control Release.* 2015;219:396-405.
17. Kamerkar S, LeBleu VS, Sugimoto H, Yang S, Ruivo CF, Melo SA, et al. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. *Nature.* 2017;546:498-503.
18. Kalluri R. The biology and function of exosomes in cancer. *J Clin Invest.* 2016;126(4):1208-1215.
19. O'Brien K, Breyne K, Ughetto S, Laurent LC, Breakefield XO. RNA delivery by extracellular vesicles in mammalian cells and its applications. *Nat Rev Mol Cell Biol.* 2020;21:585-606.
20. Zhang Y, Liu Q, Zhang X, Huang H, Tang S, Chai Y, et al. Recent advances in exosome-mediated nucleic acid delivery for cancer therapy. *J Nanobiotechnology.* 2020;18:10.
21. Li P, Kaslan M, Lee SH, Yao J, Gao Z. Progress in exosome isolation techniques. *Theranostics.* 2017;7(3):789-804.
22. Théry C, Amigorena S, Raposo G, Clayton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Curr Protoc Cell Biol.* 2006;30:3.22.1-3.22.29.
23. Momen-Heravi F. Isolation of extracellular vesicles by ultracentrifugation. *Methods Mol Biol.* 2017;1660:25-32.
24. Doyle LM, Wang MZ. Overview of extracellular vesicles, their origin, composition, purpose, and methods for exosome isolation and analysis. *Cells.* 2019;8(7):727.
25. Ludwig AK, Giebel B. Exosomes: small vesicles participating in intercellular communication. *Int J Biochem Cell Biol.* 2012;44(1):11-15.