JOURNAL OF PHARMA INSIGHTS AND RESEARCH ISSN NO. 3048-5428

REVIEW ARTICLE

# A Review on Mechanisms, Drug Loading, and Clinical Applications of Bioengineered Exosomes as Therapeutic Nanocarriers

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Publication history: Received on 10th December 2025; Revised on 21st January 2026; Accepted on 23rd January 2026

Article DOI: 10.69613/wzs20z59

**Abstract**: Natural intercellular communication relies heavily on exosomes, a subset of endosome-derived extracellular vesicles ranging from 30 to 150 nm in diameter. These biogenic nanovesicles facilitate the transfer of functional proteins, lipids, and diverse RNA species between distant cell populations, maintaining physiological homeostasis and influencing pathological progression. The inherent biological origin of exosomes confers superior advantages over traditional synthetic delivery systems, specifically regarding biocompatibility, extended systemic circulation, and minimal immunogenic profiles. Their intrinsic ability to bypass formidable biological barriers, such as the blood-brain barrier, positions them as ideal candidates for treating complex neurological and oncological conditions. Advanced engineering techniques now allow for the precise modification of exosomal surfaces and the encapsulation of diverse therapeutic cargoes, including small-molecule drugs and nucleic acids. Despite these promising attributes, the transition from laboratory-scale research to clinical application faces significant hurdles related to standardized isolation protocols, large-scale manufacturing scalability, and stringent regulatory requirements. Optimization of loading efficiencies and the development of high-yield production cell lines remain critical areas of focus. Combining biotechnology with nanotechnology offers the potential to refine these vesicles into highly targeted, personalized therapeutic agents. Resolving current technical limitations will facilitate the integration of exosome-mediated delivery into routine clinical practice, representing a transformative shift in precision medicine and targeted therapeutics.

**Keywords:** Extracellular vesicles; Nanomedicine; Targeted drug delivery; Biogenesis; Bioengineering.

## 1. Introduction

The evolution of pharmacology necessitates the development of sophisticated delivery platforms capable of site-specific action while minimizing off-target toxicity [1]. Conventional systemic administration often results in suboptimal therapeutic indices due to rapid renal clearance, enzymatic degradation, and non-specific distribution in healthy tissues [2]. While synthetic lipid nanoparticles and polymer-based carriers have addressed some of these issues, they frequently encounter challenges such as accelerated blood clearance and potential immunogenicity upon repeated administration [3]. In response to these limitations, biologically derived nanovesicles, specifically exosomes, have emerged as a promising alternative for the transport of therapeutic moieties [4].

Exosomes are secreted by virtually all cell types and function as specialized mediators of paracrine and endocrine signaling [5]. Their structural integrity, characterized by a robust lipid bilayer, protects sensitive internal cargo from the harsh extracellular environment [6]. Unlike many synthetic counterparts, exosomes possess a repertoire of surface proteins that facilitate natural targeting and cellular uptake through specific ligand-receptor interactions [7]. This innate targeting capability, combined with the potential for surface functionalization, allows for the delivery of potent drugs directly to diseased cells [8].

Recent advancements in molecular biology have expanded the utility of exosomes from simple biomarkers to active therapeutic vehicles [9]. The capacity to load these vesicles with chemotherapeutic agents, microRNAs, or CRISPR/Cas9 components enables the treatment of previously intractable diseases at the genetic and molecular levels [10]. As the demand for personalized medicine grows, the use of patient-derived or cell-specific exosomes offers a path toward highly tailored interventions that align with the biological profile of the recipient [11].

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## 2. Biogenesis and Structural Architecture

The utility of exosomes as delivery vehicles is fundamentally rooted in their complex biological origin and unique structural configuration. Their formation is a highly regulated process that distinguishes them from other extracellular vesicles, such as microvesicles or apoptotic bodies.

### 2.1 Biogenesis and Secretion

Exosomal biogenesis initiates within the endosomal system rather than through direct budding from the plasma membrane [12]. The process begins with the inward invagination of the late endosomal membrane, leading to the formation of intraluminal vesicles (ILVs) within large, membrane-bound structures known as multivesicular bodies (MVBs) [13].

#### 2.1.1 ESCRT-Dependent Mechanism

A primary pathway for ILV formation involves the Endosomal Sorting Complex Required for Transport (ESCRT) machinery [14]. This system consists of four complexes (ESCRT-0, -I, -II, and -III) that act sequentially to recognize ubiquitinated proteins, deform the endosomal membrane, and facilitate the scission of the vesicles into the MVB lumen [15]. ESCRT-0 and -I are responsible for cargo clustering, while ESCRT-III drives the final membrane fission process [16].

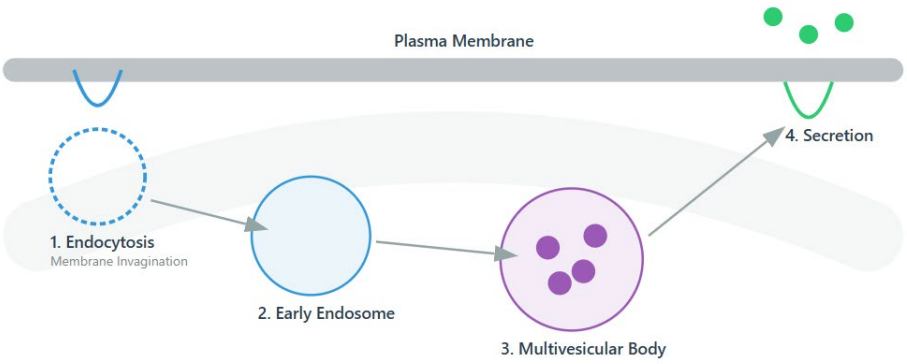


**Figure 1. Intracellular Pathway of Exosome Biogenesis and Secretion**

#### 2.1.2 ESCRT-Independent Mechanism

Evidence also supports ESCRT-independent pathways, which often involve the action of neutral sphingomyelinase and the subsequent generation of ceramide [17]. Ceramide-rich lipid microdomains promote spontaneous membrane curvature, facilitating the formation of ILVs without the requirement for the full ESCRT protein machinery [18]. Additionally, tetraspanins such as CD63, CD81, and CD9 play a vital role in organizing these membrane domains and sorting specific cargo into the nascent vesicles [19].

### 2.2 Molecular Composition and Cargo Specification

The structural framework of an exosome consists of a lipid bilayer enriched with cholesterol, sphingomyelin, and phosphatidylserine, which contributes to its exceptional mechanical stability [20]. This membrane encapsulates a diverse array of bioactive molecules that reflect the physiological state of the parent cell.

#### 2.2.1 Proteomic and Lipidomic Profile

Exosomes contain a core set of proteins, including Annexins and Rabs involved in membrane fusion, as well as Alix and TSG101 related to their endosomal origin [21]. The lipid composition is not a mere reflection of the plasma membrane; rather, it is specifically enriched in saturated fatty acids that enhance the vesicle's resistance to detergents and physical stress [22].

#### 2.2.2 Nucleic Acid Encapsulation

One of the most significant features of exosomes is their role in horizontal gene transfer. They carry a variety of RNA species, including messenger RNA (mRNA), microRNA (miRNA), and other non-coding RNAs [23]. These molecules remain functional

upon delivery to recipient cells, where they can modulate gene expression and alter cellular phenotypes [24]. This natural ability to transport genetic information serves as the biological blueprint for utilizing exosomes in gene therapy applications [25].

**Table 1. Molecular Markers and Proteins in Exosomal Biogenesis**

| Category | Specific Markers | Biological Function in Exosomes |
|---|---|---|
| Tetraspanins | CD9, CD63, CD81 | Cargo sorting and membrane organization |
| ESCRT Proteins | TSG101, Alix | Vesicle budding and membrane scission |
| Membrane Transport | Rab27a, Rab27b | Intracellular trafficking and secretion |
| Fusion Proteins | Annexins, Flotillin | Docking and fusion with recipient cells |
| Heat Shock Proteins | HSP70, HSP90 | Protein folding and stress response markers |
| Lipid Microdomains | Cholesterol, Ceramide | Structural rigidity and ESCRT-independent budding |

## 3. Advantages of Exosome-Mediated Delivery

Exosomes possess intrinsic properties that address many of the pharmacological limitations associated with synthetic nanocarriers. Their biological nature provides a unique interface for interaction with systemic environments and target cell populations.

### 3.1. Biocompatibility and Reduced Immunogenicity

The most significant attribute of exosome-based systems is their high degree of biocompatibility [26]. Since these vesicles are products of endogenous cellular processes, they are often recognized as self-components by the host's immune system, particularly when derived from autologous sources [27]. Unlike PEGylated liposomes, which can trigger the production of anti-PEG antibodies and lead to accelerated blood clearance (ABC), exosomes typically exhibit minimal immunogenicity [28]. This allows for repeated dosing schedules without compromising the stability of the carrier or inducing systemic inflammatory responses [29].

**Table 2. Comparative Analysis of Exosomes and Synthetic Nanoparticles**

| Feature | Exosomes (Biogenic) | Liposomes (Synthetic) | Polymeric NPs (Synthetic) |
|---|---|---|---|
| Origin | Endogenous (Cell-derived) | Synthetic lipids | Synthetic polymers |
| Biocompatibility | Exceptional; recognized as "self" | High, but can trigger ABC | Variable; potential for toxicity |
| Immunogenicity | Minimal (especially autologous) | Low; potential for anti-PEG antibodies | Can be high depending on polymer |
| Targeting | Innate tropism; surface proteins | Requires complex functionalization | Requires surface modification |
| Stability | High due to unique lipid ratio | Moderate; prone to leakage | High, but degradation varies |
| BBB Permeability | High (natural transcytosis) | Limited without specific ligands | Generally poor |

### 3.2. Enhanced Stability and Circulation Kinetics

The unique lipid composition of the exosomal membrane, characterized by high concentrations of cholesterol and sphingolipids, confers significant structural rigidity [30]. This physical robustness protects encapsulated cargo, such as delicate mRNA or siRNA sequences, from degradation by circulating nucleases and proteases [31]. The presence of specific surface proteins, such as CD47 (a "don't eat me" signal), allows exosomes to evade phagocytosis by the mononuclear phagocyte system (MPS), thereby extending their half-life in systemic circulation [32].

### 3.3. Natural Tropism and Barrier Permeability

Exosomes exhibit an inherent capacity to traverse complex biological barriers that are largely impermeable to conventional drugs.

#### *3.3.1. Blood-Brain Barrier (BBB) Penetration*

One of the most profound capabilities of exosomes is their ability to cross the blood-brain barrier [33]. This is facilitated by their small size and the presence of specific surface proteins that engage in transcytosis or membrane fusion with the brain microvascular endothelial cells [34]. This natural permeability positions exosomes as superior vehicles for delivering therapeutics to the central nervous system (CNS) without the need for invasive administration routes [35].

*3.3.2. Cell-Type Specificity*

Exosomes often demonstrate a degree of natural tropism toward specific organs or cell types, influenced by the integrin profiles of their parent cells [36]. For example, exosomes derived from certain immune cells naturally home to sites of inflammation, while those derived from mesenchymal stem cells (MSCs) show affinity for damaged tissues [37]. This pre-existing targeting can be further refined through surface engineering to achieve high-precision drug delivery [38].

## 4. Strategic Methodologies for Cargo Loading

The successful application of exosomes in therapy depends on the efficiency with which therapeutic agents are encapsulated. Loading strategies are generally categorized into exogenous (post-isolation) and endogenous (pre-isolation) methods.

### 4.1. Exogenous (Passive and Active) Loading

Exogenous loading involves the incorporation of drugs into pre-isolated exosomes. This can be achieved through various physical and chemical perturbations.

*4.1.1. Passive Incubation*

The simplest approach involves the co-incubation of purified exosomes with a concentrated drug solution [39]. While this method maintains the structural integrity of the vesicles, it is primarily effective for hydrophobic molecules that can passively diffuse across the lipid bilayer [40]. The loading efficiency for hydrophilic drugs and larger macromolecules is typically low, necessitating the use of active techniques [41].

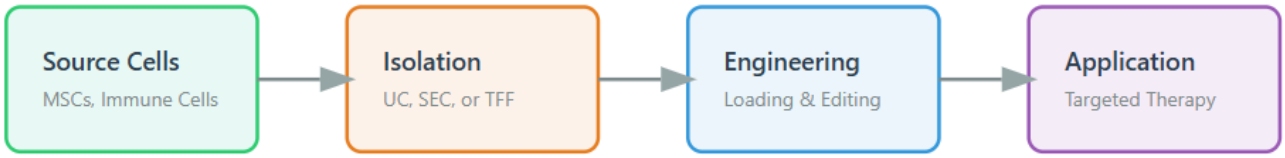


**Figure 2. Process for Exosome-Based Drug Delivery**

*4.1.2. Physical and Chemical Active Loading*

To improve encapsulation rates, active methods temporarily disrupt the exosomal membrane.

Electroporation: High-voltage electrical pulses create transient pores in the membrane, allowing nucleic acids or large proteins to enter [42]. Precise calibration is required to prevent vesicle aggregation or degradation.

**Table 3. Exosomal Cargo Loading Methods**

| Method | Mechanism of Action | Loading Efficiency | Structural Impact on Exosome | Typical Cargo |
|---|---|---|---|---|
| Passive Incubation | Diffusion along concentration gradient | Low | Negligible; maintains integrity | Hydrophobic small molecules |
| Electroporation | Transient pore formation via electric field | High | Moderate; risk of RNA aggregation | siRNA, miRNA, pDNA |
| Sonication | Mechanical membrane disruption | Very High | Significant; may alter size/shape | Proteins, hydrophilic drugs |
| Freeze-Thaw | Membrane fusion during phase change | Moderate | High; results in heterogeneity | Small molecules, proteins |
| Extrusion | Forcing through polycarbonate pores | High | High; intensive restructuring | Large macromolecules |

Sonication: Ultrasonic energy is used to disturb the membrane, facilitating the entry of drugs [43]. Although highly efficient, sonication can significantly alter the physical properties of the exosome.

Freeze-Thaw Cycles: Repeated cycles of freezing and thawing promote membrane fusion and drug entrapment, though this method often results in a heterogeneous population of vesicles [44].

### 4.2. Endogenous Loading via Cellular Engineering

Endogenous loading utilizes the internal machinery of the donor cell to package therapeutic cargo into exosomes during their biogenesis.

#### *4.2.1. Genetic Modification of Donor Cells*

By transfecting donor cells with specific plasmids or viral vectors, the cells can be induced to overexpress a target protein or RNA [45]. These molecules are then naturally sorted into the ILVs and subsequently secreted within the exosomes [46]. This method ensures that the cargo is protected within the vesicle and maintains its functional conformation [47].

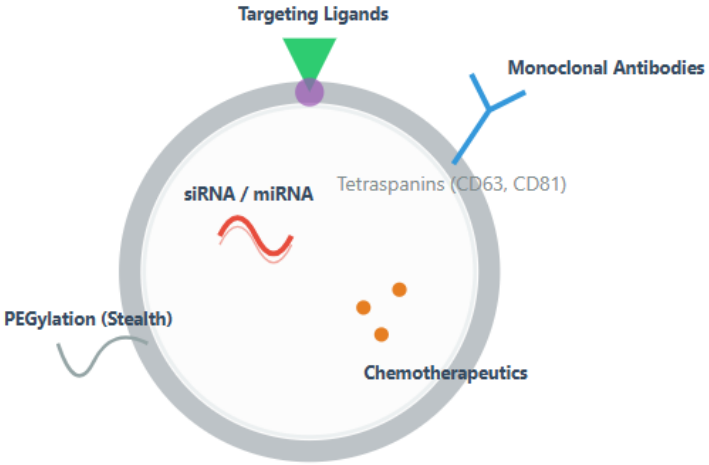


**Figure 3. Structural Anatomy and Surface Engineering of a Therapeutic Exosome**

#### *4.2.2. Priming and Metabolic Labeling*

Exposing donor cells to specific environmental stressors or chemical agents (priming) can alter the content of the secreted exosomes [48]. Similarly, metabolic labeling involves providing cells with modified precursors that are incorporated into exosomal lipids or proteins, enabling the attachment of therapeutic ligands via "click chemistry" after secretion [49]. This integrative approach allows for the creation of standardized "designer" exosomes with high therapeutic potency [50].

## 5. Therapeutic Applications of Engineered Exosomes

The versatile nature of exosomes allows for their application across a broad spectrum of pathological conditions. Their ability to carry multi-modal cargo including small molecules, proteins, and nucleic acids enables a combinatorial approach to therapy.

### 5.1. Precision Oncology

In cancer therapy, the primary objective is to maximize the concentration of cytotoxic agents within the tumor microenvironment while sparing systemic tissues [51]. Exosomes are uniquely suited for this due to their ability to exploit the enhanced permeability and retention (EPR) effect and their capacity for ligand-mediated targeting [52].

*5.1.1. Delivery of Chemotherapeutic Agents*

Loading conventional drugs like doxorubicin or paclitaxel into exosomes significantly alters their pharmacokinetic profile [53]. Exosomal encapsulation has been shown to reduce the cardiotoxicity associated with free doxorubicin while enhancing its anti-tumor efficacy in murine models [54]. Exosomes derived from specific cell types, such as mesenchymal stem cells, exhibit a natural affinity for tumor stroma, facilitating deep tissue penetration [55].

**Table 4. Exosome-Mediated Therapeutics in Disease Models**

| Disease Category | Target Cell/Tissue | Therapeutic Cargo | Mechanism of Action |
|---|---|---|---|
| Oncology | Pancreatic Cancer (KRAS) | siRNA (G12D) | Silencing of oncogenic signaling |
| Oncology | Glioblastoma | Doxorubicin / Paclitaxel | Targeted cytotoxicity via EPR/Ligands |
| Neurology | Alzheimer's Disease | BACE1 siRNA | Reduction of amyloid-β plaque formation |
| Neurology | Parkinson's Disease | Catalase / Dopamine | Mitigation of oxidative stress |
| Immunology | Autoimmune Disorders | miRNA-146b | Suppression of pro-inflammatory cytokines |
| Infectious Disease | Viral Pathogens | Viral spike proteins | Antigen presentation to T-cells (Vaccine) |

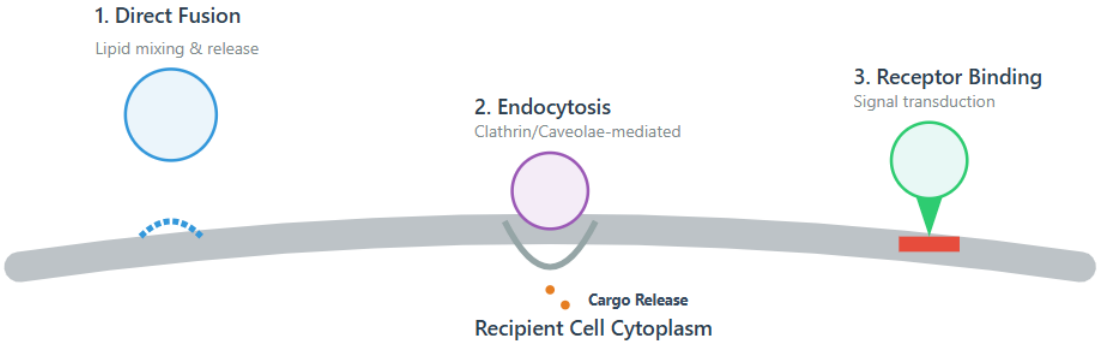


**Figure 4. Mechanisms of Exosome-Mediated Cargo Delivery into Recipient Cells**

*5.1.2. RNA Interference and Gene Silencing*

Exosomes serve as highly efficient vehicles for the delivery of small interfering RNA (siRNA) and microRNA (miRNA) to silence oncogenes [56]. For instance, exosomes engineered to carry siRNA against KRAS G12D a common mutation in pancreatic cancer have demonstrated superior suppressive effects compared to synthetic liposomes [57]. The protection offered by the exosomal lipid bilayer ensures that the nucleic acid cargo remains intact during systemic transit [58].

**5.2. Neurotherapeutics and CNS Disorders**

The treatment of neurological disorders is often limited by the inability of most therapeutic agents to cross the blood-brain barrier (BBB). Exosomes represent a natural solution to this physiological constraint [59].

*5.2.1. Neurodegenerative Diseases*

In Alzheimer's and Parkinson's disease research, exosomes are being utilized to deliver siRNA targeting BACE1 or alpha-synuclein, respectively [60]. By utilizing brain-targeting peptides, such as the rabies virus glycoprotein (RVG), on the exosomal surface, researchers have achieved highly specific delivery to neurons and microglia [61]. This targeted approach reduces the dosage required and minimizes potential peripheral side effects [62].

*5.2.2. Brain Tumor Intervention*

Glial tumors, particularly glioblastoma multiforme, are notoriously difficult to treat due to their infiltrative nature and the protective presence of the BBB [63]. Exosomes loaded with antisense oligonucleotides or prodrug-converting enzymes have shown the ability to migrate through the brain parenchyma to reach these malignant foci, offering a potential breakthrough in neuro-oncology [64].

### 5.3. Immunotherapy and Vaccine Development

Exosomes derived from immune cells, such as dendritic cells (dexosomes), possess intrinsic immunostimulatory properties [65]. These vesicles carry MHC-I and MHC-II complexes, allowing them to present antigens directly to T-cells [66]. This capability is being harnessed to develop cell-free vaccines for both infectious diseases and cancer, providing a safer alternative to live-attenuated or whole-cell vaccines [67].

## 6. Technical Obstacles and Translational Challenges

Despite the robust therapeutic potential of exosomes, several critical bottlenecks must be addressed before they can be successfully integrated into standard clinical workflows.

### 6.1. Standardization of Isolation and Purification

There is currently no universally accepted "gold standard" for exosome isolation [68]. Methods such as ultracentrifugation are time-consuming and often result in low yields with significant protein contamination [69]. Alternative techniques like size-exclusion chromatography (SEC) and tangential flow filtration (TFF) offer better scalability but require rigorous optimization to ensure the removal of non-exosomal extracellular vesicles and lipoproteins [70].

### 6.2. Scalability and Manufacturing (GMP Compliance)

Moving from laboratory-scale production to industrial-scale manufacturing requires the development of bioreactor-based systems capable of producing kilogram-quantities of standardized exosomes [71]. Maintaining batch-to-batch consistency in terms of size distribution, surface markers, and cargo concentration is a formidable challenge [72]. Ensuring that the production process adheres to Good Manufacturing Practice (GMP) standards is essential for regulatory approval [73].

**Table 5. Challenges and Mitigation Techniques in Clinical Applications**

| Translational Hurdle | Challenge | Potential Mitigation Methods |
|---|---|---|
| Isolation Purity | Contamination with lipoproteins/protein aggregates | Use of TFF combined with Size-Exclusion Chromatography |
| Scale-up | Low yield from 2D culture systems | Utilization of 3D stirred-tank bioreactors |
| Characterization | Heterogeneity in size and cargo | Implementation of single-vesicle flow cytometry |
| Potency Assays | Defining quantitative biological activity | Development of standardized *in vitro* reporter assays |
| Storage | Loss of function during freeze-thaw | Lyophilization using cryoprotectants (e.g., Trehalose) |
| Regulatory | Lack of specific FDA/EMA guidelines | Early alignment with GMP/CMC requirements |

### 6.3. Quality Control and Characterization

Characterizing the complex molecular landscape of engineered exosomes is technically demanding [74]. Advanced analytical tools, such as nanoparticle tracking analysis (NTA), cryo-electron microscopy, and high-sensitivity flow cytometry, are required to verify the identity and purity of the vesicles [75]. Defining the potency of an exosome-based drug the quantitative measure of its biological activity remains one of the most difficult hurdles for clinical validation [76].

### 6.4. Storage and Long-Term Stability

Exosomes are sensitive to environmental conditions; their structural and functional integrity can be compromised by fluctuations in temperature or pH [77]. While lyophilization (freeze-drying) is being explored as a method for long-term storage, the impact of the dehydration-rehydration process on the vesicle membrane and its cargo needs further investigation to ensure shelf-life stability [78]

## 7. Future Perspectives

The trajectory of exosome-based therapeutics is increasingly defined by the integration of interdisciplinary technologies. As the biological understanding of these vesicles matures, the focus is shifting from simple isolation to sophisticated bioengineering and "smart" delivery systems.

### 7.1. Artificial Intelligence and Computational Modeling

The application of machine learning (ML) algorithms is set to revolutionize the identification of optimal exosomal subpopulations for drug delivery [79]. Predictive modeling can assist in determining the best parent cell lines and surface modification strategies based on the target tissue's proteomic profile [80]. AI-driven analysis of exosomal cargo can lead to the discovery of novel biomarkers, enabling a dual-function "theranostic" approach where the same vesicle provides both diagnostic information and therapeutic intervention [81].

### 7.2. Hybrid Nanostructures and Biomaterials

Recent efforts have explored the creation of "synthetic exosomes" or hybrid vesicles by fusing natural exosomal membranes with synthetic lipid nanoparticles [82]. These hybrids combine the high loading capacity and structural controllability of synthetic systems with the biocompatibility and targeting features of natural exosomes [83]. Additionally, incorporating exosomes into hydrogels or other biocompatible scaffolds allows for sustained, localized release of therapeutic agents, which is particularly beneficial for wound healing and bone regeneration [84].

### 7.3. Integration with Personalized Medicine

The future of healthcare lies in tailoring interventions to the individual [85]. Using a patient's own cells (autologous sources) to generate therapeutic exosomes eliminates the risk of immune rejection and allows for a truly personalized treatment regimen [86]. Advances in microfluidic technologies may soon enable "point-of-care" exosome isolation and loading, making personalized nanomedicine a clinical reality [87].

## 8. Conclusion

Exosome-based drug delivery systems represent a paradigm shift in the field of nanomedicine, offering a biologically sophisticated alternative to traditional synthetic carriers. Their unique structural architecture and innate physiological roles provide a foundation for highly targeted, stable, and biocompatible therapeutic delivery. By overcoming the limitations of conventional systemic administration, these vesicles hold the potential to drastically improve treatment outcomes for oncology, neurology, and beyond. However, realizing this potential requires a concerted global effort to standardize isolation protocols, scale up production under GMP conditions, and establish rigorous regulatory frameworks. As technical hurdles are systematically addressed, engineered exosomes are poised to become a cornerstone of next-generation precision therapeutics, transforming the management of complex and previously untreatable diseases.

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