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USE OF EXOSOMES AS NOVEL DRUG DELIVERY VEHICLES IN ONCOLOGY

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Abstract: Cancer remains a major global health challenge due to limitations of conventional chemotherapy, including systemic toxicity, poor target specificity, and drug resistance. Exosomes—cell-derived nano-vesicles involved in intercellular communication—have emerged as promising natural carriers for targeted delivery of therapeutic agents. This review summarises exosome biogenesis, isolation strategies, drug loading techniques, applications in cancer therapy, challenges, and prospects for clinical translation.

Keywords: Exosomes; Drug delivery systems; Cancer therapy; Targeted drug delivery; Nanovesicles; Oncology

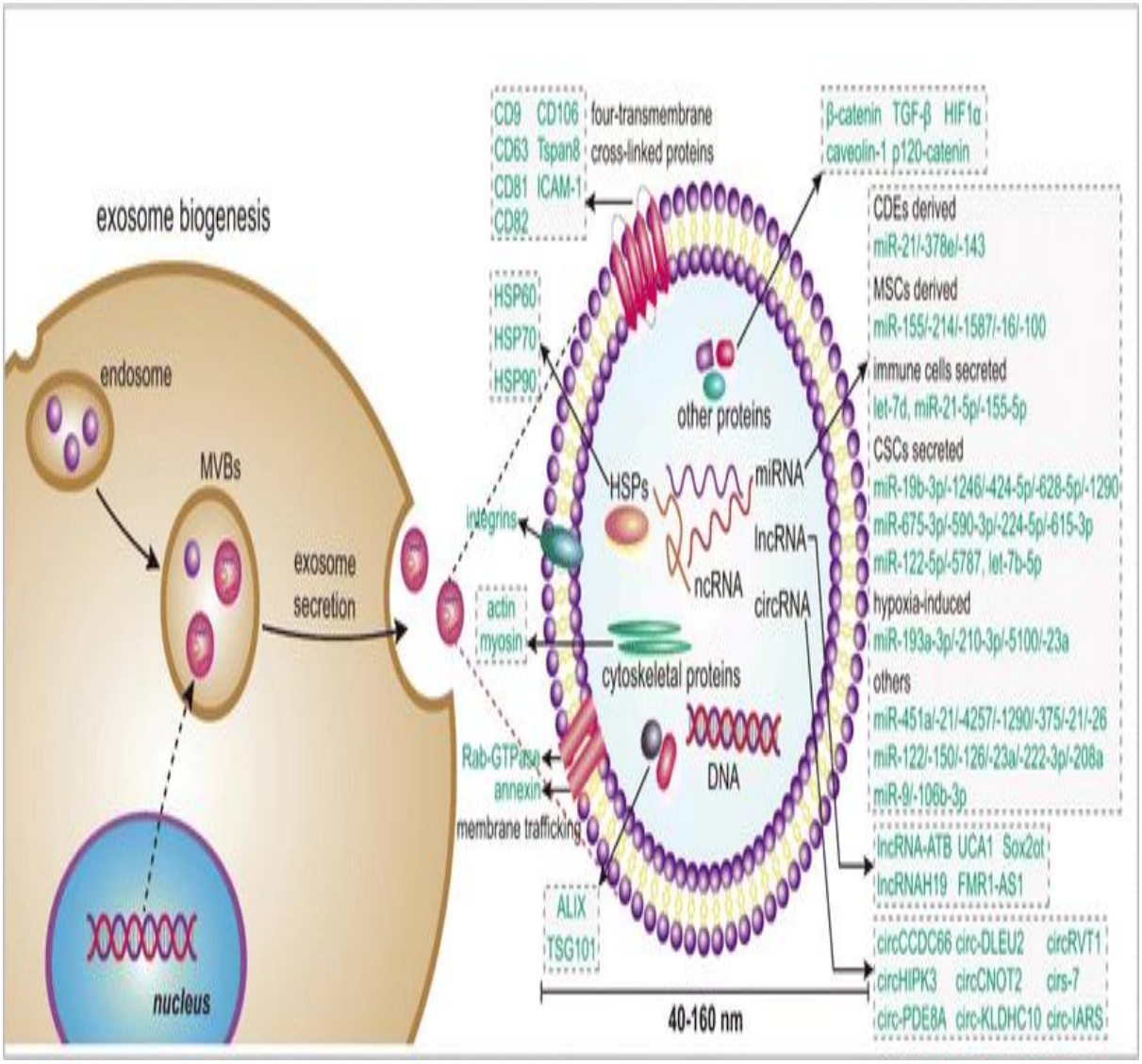
INTRODUCTION

Cancer therapy has evolved significantly, yet effective and targeted delivery of anticancer agents remains a hurdle. Traditional drug delivery systems often exhibit suboptimal biodistribution, nonspecific toxicity, and low therapeutic index. Nanotechnology has offered solutions such as liposomes, polymeric nanoparticles, and lipid carriers, but safety and immune clearance issues persist.

Exosomes are natural extracellular vesicles (~30–150 nm) secreted by most cell types. They mediate intercellular communication through the transfer of nucleic acids, proteins, and lipids. Their **intrinsic stability**, **biocompatibility**, and **ability to cross biological barriers** make them promising platforms for drug delivery—particularly in oncology.

EXOSOME BIOGENESIS

Exosomes are associated with cellular signalling. Exosome biogenesis occurs dependently or independently of the endosomal sorting complex Fig



1: Biogenesis Exosome

required for transport (ESCRT). The ESCRT complexes, which include ESCRT0 to III along with proteins like vacuolar protein sorting 4 (VPS4), are primarily involved in regulating exosome biogenesis. Other ubiquitinated proteins are recognised, and sorting is initiated by ESCRT-0, while ESCRT-1 and ESCRT-2 are responsible for the induction of membrane deformation and cargo processing.The ESCRT-3 forms spiral-shaped bundles to drive vascular scission and budding with the help of complexes like the C-terminal residues of the human CHMP4 proteins (CHMP4). The VPS4 recycles the ESCRT-3 after the vascular scission. This synchronous process regulates the intraluminal vesicle formation and, hence, facilitates cellular communication. The ESCRT-independent mechanisms provide diverse paths for the formation of exosomes, one of which is in the form of lipid components like ceramide and lipid rafts. The accumulation of ceramide initiates budding of exosomes after fusion of multivesicular bodies (MVBs) with the plasma membrane. In the ESCRT independent pathway,tetraspanin proteins havea significant role in exosomebiogenesis. However, evidence of crosstalk between the two pathways has been observed. For example, the CHMP4C component of the ESCRT-III has interactions with the lipid rafts that are associated with proteins like syntenin.Another example is the involvement of syndecan-syntenin-ALIX, which is responsible for the release of exosomes and indicates connections between the components of the ESCRT machinery. A dysregulation in the ESCRT-dependent or independent pathway can lead to aberrant production of exosomes. This can establish a pro-tumorigenic microenvironment. Hence, understanding this crosstalk could pave the way for further development of therapeutics, which may offer avenues for the modulation of the vesicular cargo. During cancer development, exosome secretion depends on the low pH of the tumour microenvironment (TME) and ESCRT-independent pathways.

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BIOGENESIS AND CHARACTERISTICS OF EXOSOMES

Exosomes are generated via the endosomal pathway:

- Endocytosis forms early endosomes.
- Intraluminal vesicles (ILVs) develop inside multivesicular bodies (MVBs).
- Fusion of MVBs with the plasma membrane releases exosomes into circulation.

Exosomes contain:

- **Surface markers** (CD9, CD63, CD81)
- **Proteins, lipids, and RNAs** reflective of the parent cell

Their **nano-size** allows uptake by tumour cells through endocytosis, fusion, or receptor-mediated mechanisms.

METHODS OF ISOLATION AND CHARACTERISATION OF EXOSOMES

High-purity exosomes are essential for their effective application as therapeutic drug delivery vehicles in oncology, as impurities or heterogeneous vesicle populations can affect safety, targeting efficiency, and reproducibility. Consequently, reliable isolation and characterisation techniques are crucial for ensuring the quality, functionality, and clinical translational potential of exosome-based formulations.

Ultracentrifugation is the most widely used and traditionally accepted method for isolating exosomes, often regarded as the gold standard. This technique involves sequential centrifugation steps at increasing speeds to remove cells, cell debris, and larger vesicles, followed by high-speed centrifugation to pellet exosomes. Although ultracentrifugation provides relatively pure exosome preparations, it is time-consuming, requires expensive equipment, and may cause vesicle aggregation or structural damage due to high centrifugal forces.

Size exclusion chromatography (SEC) separates exosomes based on particle size by passing the sample through a porous matrix. Larger vesicles elute earlier, while smaller proteins and contaminants are retained within the pores. SEC preserves the structural integrity and biological activity of exosomes and offers better reproducibility than ultracentrifugation. However, its major limitations include lower yield and the need for additional concentration steps, which may hinder large-scale production.

Immunoaffinity capture techniques utilize antibodies directed against specific exosomal surface markers such as CD63, CD9, or CD81. This method allows highly selective isolation of exosomes from complex biological fluids and is particularly useful for targeting tumor-derived exosomes. Despite its high specificity, immunoaffinity capture is costly, limited in scalability, and may isolate only specific subpopulations of exosomes rather than the entire vesicle pool.

Ultrafiltration employs membrane filters with defined pore sizes to separate exosomes based on size. This method is relatively rapid, cost-effective, and suitable for processing larger sample volumes. However, membrane clogging, vesicle deformation, and co-isolation of similarly sized contaminants can compromise purity and consistency.

Polymer-based precipitation methods use hydrophilic polymers such as polyethylene glycol (PEG) to reduce exosome solubility, resulting in their precipitation at low centrifugal forces. These methods are simple, scalable, and compatible with clinical workflows, but they often co-precipitate proteins and other extracellular vesicles, leading to reduced purity and potential interference in downstream applications.

For confirmation and quality assessment, multiple **characterization techniques** are employed. **Transmission Electron Microscopy (TEM)** provides direct visualization of exosome morphology, size, and membrane structure, typically revealing their characteristic cup-shaped appearance. **Nanoparticle Tracking Analysis (NTA)** is used to determine particle size distribution and concentration by analyzing Brownian motion of vesicles in suspension. **Western blotting** is commonly employed to detect specific exosomal protein markers such as CD63, CD9, CD81, TSG101, and Alix, confirming vesicle identity. **Flow cytometry**, often combined with bead-based assays, enables surface marker analysis and quantitative evaluation of exosome populations.

Despite technological advancements, several challenges remain in exosome isolation and characterization. **Scalability** is a major limitation, particularly for clinical and commercial production, as many methods are labor-intensive and low-throughput. **Purity** remains difficult to achieve due to overlapping sizes and densities of extracellular vesicles and protein aggregates. Additionally, **yield consistency** varies significantly depending on the isolation method, biological source, and processing conditions, posing challenges for standardization and regulatory approval. Addressing these issues is critical for advancing exosome-based drug delivery systems toward clinical translation.

STRATEGIES FOR DRUG LOADING INTO EXOSOMES

Exosomes possess the unique ability to transport a wide range of therapeutic cargos, including small-molecule drugs, proteins, peptides, and nucleic acids, making them highly versatile drug delivery vehicles in oncology. The efficiency and stability of drug loading significantly influence the therapeutic performance of exosome-based formulations. Broadly, drug loading strategies are classified into **passive loading** and **active loading** methods, each with distinct advantages and limitations.

Passive Loading

Passive loading is the simplest and most commonly employed approach for incorporating drugs into exosomes. This method involves **incubating isolated exosomes with the drug of interest**, allowing the therapeutic molecules to diffuse across the lipid bilayer of the exosomal membrane. Passive loading relies primarily on concentration gradients and hydrophobic interactions between the drug and the exosomal membrane.

This approach is particularly suitable for **small, hydrophobic drugs**, such as paclitaxel and curcumin, which can readily partition into the lipid bilayer. Passive loading is advantageous due to its simplicity, minimal processing steps, and preservation of exosome structure and biological function. However, the major limitation of this method is its **low loading efficiency**, especially for hydrophilic drugs and large biomolecules. Additionally, drug encapsulation levels are difficult to control, which may result in batch-to-batch variability.

Active Loading

Active loading techniques are employed to enhance drug encapsulation efficiency by temporarily disrupting the exosomal membrane, thereby facilitating the entry of therapeutic cargo into the vesicle interior. These methods are particularly useful for loading **hydrophilic drugs, proteins, and nucleic acids**, which cannot efficiently penetrate the lipid membrane through passive diffusion.

Electroporation involves the application of an electrical field to create transient pores in the exosomal membrane, allowing charged molecules such as siRNA or miRNA to enter the vesicles. While electroporation significantly improves loading efficiency, it may cause vesicle aggregation or degradation of nucleic acids if not carefully optimised.

Sonication uses ultrasonic waves to temporarily deform the exosomal membrane, enabling drug diffusion into the vesicles. This method provides higher loading capacity and sustained drug release but may alter membrane integrity and affect surface proteins critical for targeting.

Freeze–thaw cycles involve repeated freezing and thawing of exosome–drug mixtures, leading to temporary membrane disruption and cargo incorporation. Although this technique is simple and cost-effective, repeated cycles can cause vesicle fusion, aggregation, and structural instability.

Extrusion forces exosome–drug mixtures through membranes with defined pore sizes, facilitating uniform drug encapsulation. This method allows higher drug loading and homogeneity but may modify the natural structure and surface characteristics of exosomes.

An alternative and increasingly preferred strategy is **transfection of parental cells**, wherein donor cells are genetically or chemically engineered to express or uptake therapeutic cargo. These cells naturally package the cargo into exosomes during biogenesis, producing drug-loaded exosomes with preserved membrane integrity and biological functionality. Although this approach offers superior stability and targeting potential, it is time-consuming and poses challenges related to scalability and regulatory compliance.

Overall, while **active loading methods** significantly improve drug encapsulation efficiency compared to passive techniques, they may compromise membrane integrity and biological activity if not carefully optimised. Therefore, selecting an appropriate loading strategy requires balancing loading efficiency, structural preservation, therapeutic payload, and intended clinical application.

EXOSOME-MEDIATED DELIVERY IN CANCER THERAPY

Exosomes play a dual role in oncology, functioning not only as natural mediators of intercellular communication but also as highly efficient delivery carriers for therapeutic agents. Their intrinsic ability to interact with tumor cells, evade immune clearance, and transport bioactive molecules makes them an attractive platform for cancer therapy. Exosome-mediated delivery has demonstrated significant potential across multiple therapeutic modalities, including chemotherapy, gene therapy, immunotherapy, and strategies to overcome drug resistance.

Delivery of Chemotherapeutic Agents

Exosomes have been extensively investigated as carriers for conventional chemotherapeutic drugs due to their ability to enhance drug solubility, stability, and targeted delivery. Studies have demonstrated that anticancer drugs such as **paclitaxel, doxorubicin, and curcumin** can be efficiently loaded into exosomes and delivered to tumor tissues. Exosome-encapsulated chemotherapeutics exhibit **improved tumor targeting** through enhanced cellular uptake mediated by membrane fusion or receptor-mediated endocytosis. This targeted delivery minimizes drug exposure to healthy tissues, thereby reducing systemic toxicity.

Furthermore, exosomal delivery enhances the **therapeutic index** of chemotherapeutic agents by increasing intracellular drug concentration within cancer cells while lowering required doses. For example, paclitaxel-loaded exosomes have shown superior cytotoxicity against drug-resistant cancer cells compared to free drug formulations, highlighting their ability to bypass biological barriers and improve therapeutic outcomes.

Exosomes in Gene Therapy

Gene therapy represents a promising approach for cancer treatment, but its clinical application is limited by instability of nucleic acids and inefficient delivery systems. Exosomes offer a protective and biocompatible platform for the delivery of genetic materials such as **small interfering RNA (siRNA), microRNA (miRNA), and CRISPR/Cas9 gene-editing components**. Their lipid bilayer protects nucleic acids from enzymatic degradation and facilitates efficient cellular internalization.

Notably, **siRNA-loaded exosomes targeting KRAS oncogene mutations** have demonstrated effective gene silencing in pancreatic cancer models, resulting in inhibited tumor growth. Similarly, delivery of **miR-21 inhibitors via exosomes** has been shown to reverse chemoresistance by modulating oncogenic signaling pathways. These findings underscore the potential of exosomes as precision tools for gene-based cancer therapy.

Exosome-Based Immunotherapy

Exosomes also play a pivotal role in cancer immunotherapy by modulating immune responses. **Dendritic cell-derived exosomes** and genetically engineered exosomes expressing tumor-associated antigens can act as potent immune stimulators. These exosomes facilitate **antigen presentation to T-cells**, leading to activation of cytotoxic T lymphocytes and enhanced antitumor immune responses.

Additionally, exosomes can carry immunostimulatory molecules such as cytokines or co-stimulatory ligands, further amplifying immune activation. Their ability to cross biological barriers and interact with immune cells positions exosomes as promising candidates for cancer vaccines and immune-based therapies.

Overcoming Drug Resistance

Multidrug resistance remains a significant challenge in cancer chemotherapy. Exosome-mediated delivery offers innovative strategies to overcome resistance mechanisms. Exosomes can transport therapeutic agents that **suppress drug efflux pumps** such as P-glycoprotein or modulate intracellular signaling pathways responsible for resistance development. By altering pathways associated with apoptosis, cell survival, and drug metabolism, exosome-based formulations can restore chemosensitivity in resistant cancer cells.

Furthermore, exosomes can deliver gene-silencing molecules or regulatory RNAs that downregulate resistance-related genes, providing a multifaceted approach to counteract tumor adaptability. This ability to reverse resistance highlights the transformative potential of exosomes in improving long-term cancer treatment efficacy.

Overall, exosome-mediated delivery systems represent a versatile and powerful strategy in cancer therapy, offering improved targeting, reduced toxicity, and enhanced therapeutic effectiveness across multiple treatment modalities.

TUMOR TARGETING AND BIODISTRIBUTION OF EXOSOMES

One of the most advantageous features of exosomes as drug delivery vehicles is their **intrinsic tumour-homing ability**, which arises from the presence of specific surface molecules inherited from their parental cells. These surface proteins, lipids, and adhesion molecules enable exosomes to preferentially interact with tumour tissues and the tumour microenvironment, thereby enhancing site-specific drug accumulation. This natural targeting capability contributes to favourable**biodistribution profiles**, prolonged circulation time, and reduced uptake by the reticuloendothelial system compared to many synthetic nanocarriers.

To further enhance tumour specificity and therapeutic efficiency, various **engineering strategies** have been developed to modify the exosomal surface. **Surface modification with targeting ligands**, such as peptides, antibodies, or antibody fragments, allows exosomes to selectively bind to overexpressed receptors on cancer cells. For instance, peptides targeting integrins or antibodies against tumour-associated receptors like HER2 or EGFR can significantly improve cellular uptake and intracellular drug delivery. This receptor-mediated targeting minimises nonspecific distribution and reduces systemic toxicity.

Click chemistry-based conjugation is an advanced and highly efficient technique used to attach targeting moieties to the exosomal membrane without compromising vesicle integrity. This bioorthogonal approach enables precise and stable ligand attachment under mild conditions, preserving the biological functionality of exosomes. Click chemistry offers flexibility in surface engineering and ensures reproducibility, making it particularly attractive for scalable and clinical applications.

Another promising strategy involves **aptamer decoration**, where short, single-stranded nucleic acid sequences with high affinity for specific cancer biomarkers are conjugated to the exosome surface. Aptamers provide high specificity, low immunogenicity, and excellent stability, enabling selective recognition of tumour cells. Aptamer-modified exosomes have demonstrated enhanced tumor accumulation and efficient drug internalization, especially in cancers with well-defined molecular targets.

Collectively, these surface engineering approaches significantly enhance **tumour targeting and biodistribution** of exosome-based delivery systems. By increasing tumour specificity and minimising off-target interactions, engineered exosomes improve

therapeutic efficacy while reducing adverse effects, thereby reinforcing their potential as next-generation drug delivery platforms in oncology.

ADVANTAGES OVER SYNTHETIC NANOCARRIERS

Feature	Exosomes	Synthetic Nanoparticles
Biocompatibility	High	Moderate to Low
Immunogenicity	Low	Variable
Targeting ability	Intrinsic	Requires surface design
Stability in circulation	Excellent	Varies
Membrane fusion delivery	Yes	Often limited

CHALLENGES AND LIMITATIONS OF EXOSOME-BASED DRUG DELIVERY SYSTEMS

Despite the significant promise of exosomes as novel drug delivery vehicles in oncology, several **technical, biological, and regulatory challenges** must be addressed before their widespread clinical application can be realized. These limitations currently hinder large-scale production, standardization, and regulatory approval of exosome-based therapeutics.

One of the major challenges is **scale-up production**. Most exosome isolation and loading techniques are optimized for laboratory-scale research and are not readily adaptable to industrial or clinical manufacturing. Large-scale production requires consistent yields, cost-effective processes, and maintenance of exosome integrity and functionality. Achieving reproducible exosome production from biological sources while meeting good manufacturing practice (GMP) standards remains a significant hurdle.

Isolation and standardization present additional challenges, as exosomes are heterogeneous in nature and overlap in size and density with other extracellular vesicles and protein aggregates. Variability in isolation techniques leads to inconsistencies in purity, yield, and biological activity. The lack of universally accepted protocols and standardized quality control parameters complicates comparison across studies and impedes regulatory approval.

Another critical limitation is **cargo loading efficiency**. While various passive and active loading methods have been developed, achieving high and reproducible drug encapsulation without compromising exosomal membrane integrity remains difficult. Inefficient loading may necessitate higher dosing, which can affect safety and cost-effectiveness. Moreover, certain loading techniques can alter surface proteins responsible for targeting, potentially reducing therapeutic efficacy.
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Concerns regarding **immunogenicity and safety**, particularly with repeated administration, also require careful evaluation. Although exosomes are generally considered biocompatible and less immunogenic than synthetic nanocarriers, their biological origin may elicit immune responses depending on the source, cargo, and route of administration. Long-term safety data are limited, and the risk of unintended biological effects, such as immune modulation or off-target gene regulation, must be thoroughly investigated.

Finally, **regulatory pathways** for exosome-based therapeutics remain unclear. Exosomes fall at the intersection of biologics, cell-derived products, and nanomedicines, creating ambiguity in regulatory classification. The absence of clear guidelines for characterization, quality control, and clinical evaluation poses challenges for regulatory approval. Establishing robust regulatory frameworks and standardized evaluation criteria is essential to facilitate clinical translation.

Overall, successful clinical implementation of exosome-based drug delivery systems will require coordinated efforts to overcome these **technical, manufacturing, safety, and regulatory gaps**. Addressing these challenges will be crucial for translating the promising potential of exosomes into safe and effective cancer therapies.

CLINICAL APPLICATIONS AND TRIALS OF EXOSOME-BASED THERAPIES

Exosome-based drug delivery systems have progressed from preclinical research to **early-phase clinical evaluation**, highlighting their potential for translation into clinical oncology practice. Several investigational therapies utilizing exosomes as carriers for therapeutic agents have entered Phase I and early Phase II clinical trials, primarily focusing on safety, tolerability, and feasibility.
One major area of clinical investigation involves the **exosome-mediated delivery of siRNA and chemotherapeutic agents**. In these studies, exosomes are engineered to transport nucleic acids or cytotoxic drugs directly to tumor cells, aiming to achieve targeted gene silencing or enhanced cytotoxic effects with reduced systemic toxicity. Early clinical findings suggest that exosome-based delivery systems are **well tolerated**, exhibit favorable pharmacokinetic profiles, and demonstrate the ability to protect therapeutic cargo from degradation in circulation. These attributes are particularly advantageous for nucleic acid–based therapies, which traditionally suffer from instability and poor cellular uptake.
Another promising clinical application is the development of **immune-stimulating exosomes**, particularly those derived from

dendritic cells or genetically modified to express tumor-associated antigens. Such exosomes function as cancer vaccines by promoting antigen presentation and activating cytotoxic T lymphocyte responses. Initial clinical studies have demonstrated that these exosome-based immunotherapies are **safe and capable of eliciting immune responses**, supporting their feasibility as novel immunotherapeutic platforms.

Despite these encouraging outcomes, current clinical trials remain largely exploratory and are primarily designed to assess safety rather than therapeutic efficacy. While preliminary data indicate biological activity and acceptable safety profiles, **robust evidence of clinical efficacy**, such as significant tumor regression or improved survival outcomes, is still limited. Larger, well-controlled clinical trials with standardized exosome formulations and long-term follow-up are necessary to validate their therapeutic benefit.

Overall, early clinical experiences confirm that exosome-based therapies are **safe and feasible**, reinforcing their potential as innovative drug delivery systems in oncology. However, comprehensive clinical validation is required before these technologies can be integrated into routine cancer treatment protocols.

FUTURE PERSPECTIVES OF EXOSOME-BASED DRUG DELIVERY IN ONCOLOGY

The future of exosome-based drug delivery systems in oncology is highly promising, with ongoing research focused on overcoming current limitations and expanding their therapeutic potential. Several innovative strategies are being explored to enhance the clinical applicability, efficacy, and scalability of exosome-based therapies.

One of the most exciting future directions is the development of **personalized exosome therapy derived from patient-specific cells**. By isolating and engineering exosomes from a patient's own cells, it may be possible to create highly individualized treatment systems with minimal immunogenicity and improved targeting efficiency. Personalized exosomes can be tailored to carry specific drugs or genetic material based on the patient's tumor profile, aligning well with the principles of precision and personalized medicine.

Another emerging approach involves **hybrid exosome–synthetic systems**, often referred to as **exosome mimetics**. These systems combine the biological advantages of natural exosomes—such as biocompatibility and targeting ability—with the controllable properties of synthetic nanocarriers. Exosome mimetics offer improved reproducibility, higher drug loading capacity, and easier large-scale manufacturing while retaining key functional characteristics of native exosomes.

The **integration of exosome technology with immunotherapy and gene-editing platforms** represents a transformative direction in cancer treatment. Exosomes are being investigated as carriers for immune-modulating agents and gene-editing tools such as CRISPR/Cas9, enabling precise modification of oncogenes or immune checkpoints. Such combinational approaches have the potential to produce synergistic therapeutic effects, enhancing antitumor immunity and long-term treatment outcomes.

Additionally, the advancement of **automated and large-scale production platforms** is critical for the successful clinical translation of exosome-based therapies. Innovations in bioreactor systems, microfluidic technologies, and standardized purification processes are expected to improve yield consistency, reduce costs, and ensure compliance with regulatory standards. Automation will play a vital role in enabling reproducible, GMP-compliant exosome manufacturing suitable for commercial and clinical use.

Overall, continued technological innovation and interdisciplinary collaboration will be essential to fully realize the potential of exosomes as next-generation drug delivery systems in oncology.

CONCLUSION

Exosomes represent a promising frontier in oncology drug delivery due to their natural compatibility, targeting capabilities, and ability to carry diverse therapeutics. Continued research in scalable production, cargo optimization, and regulatory pathways is crucial for clinical translation.

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