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Review Article

Extracellular Vehicles (EVS) as Next-Generation Nanomedicine in Cancer Therapy: A Review of Drug Delivery Approaches.

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ABSTRACT

Extracellular vesicles (EVs) are increasingly recognized as fundamental mediators of intercellular communication, a process conserved across all kingdoms of life. Compelling evidence demonstrates their involvement in diverse physiological and pathological contexts, including cellular homeostasis, infection propagation, cancer progression, and cardiovascular disease. Their natural ability to transport bioactive molecules provides several advantages over conventional synthetic carriers, such as enhanced biocompatibility, reduced immunogenicity, and intrinsic targeting capacity, positioning EVs as promising candidates for next-generation drug delivery systems. However, despite significant advances in preclinical research, the clinical translation of EV-based therapeutics remains challenging. Key barriers include the development of efficient and reproducible cargo-loading methods, rigorous characterization of heterogeneous vesicle populations, and scalable manufacturing processes that meet regulatory standards. Addressing these issues is critical to unlock the full therapeutic potential of EVs. This review highlights the distinctive biological features of extracellular vesicles (EVs) and outlines key considerations for their development as drug delivery systems. Special attention is given to novel approaches for therapeutic cargo loading, advanced methods for vesicle characterization, and strategies for scalable production. In cancer therapy, EVs have gained prominence due to their ability to transport bioactive molecules, influence the tumor microenvironment, and deliver anticancer agents with high specificity. Their natural biocompatibility, reduced immunogenicity, and capacity to cross biological barriers provide clear advantages over synthetic carriers, positioning them as promising tools for precision oncology. However, challenges such as vesicle heterogeneity, lack of standardized isolation protocols, and regulatory complexities continue to hinder clinical translation. To contextualize their potential, this review compares EV-based systems with established liposomal technologies, highlighting both strengths and limitations. By synthesizing current

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evidence and identifying future directions, we aim to provide a roadmap for advancing EV-based drug delivery platforms, particularly in cancer treatment, and accelerating their transition into effective clinical applications.

INTRODUCTION

Over the past decade, significant progress has been made in advancing our understanding of the diversity, structural characteristics, and biological functions of extracellular vesicles (EVs) in both physiological and pathological contexts. The International Society for Extracellular Vesicles (ISEV) defines EVs as a collective term for cell-derived particles that are enclosed by a lipid bilayer, lack the ability to replicate, and do not contain a functional nucleus[1]. Extracellular vesicle (EV) secretion is widely recognized as an evolutionarily conserved phenomenon observed across virtually all kingdoms of life. In the context of fundamental biology, the study of EVs has centered on advancing our understanding of their mechanisms of biogenesis, release, and subsequent interactions with recipient cells. These investigations extend to the molecular and cellular responses triggered by EV uptake, including alterations in gene expression and phenotype, as well as the diverse pathways through which EVs facilitate intercellular communication. Such insights have positioned EVs as critical mediators of biological signalling, underscoring their relevance in both normal physiology and disease progression. Multiple subtypes of EVs have been described, including exosomes, ectosomes, microvesicles, membrane-derived vesicles, and apoptotic bodies. Each subtype is thought to arise through distinct biogenetic pathways, reflecting the complexity of vesicle formation and release. EVs have been successfully isolated from a wide range of biological sources, spanning mammalian and prokaryotic cell cultures, blood plasma, bovine milk, and even plant tissues. This diversity highlights their ubiquity and functional versatility. However, because the precise cellular origin of many EV populations cannot be definitively determined, rigorous characterization remains essential to distinguish their properties and biological roles. Comprehensive profiling of EVs—encompassing their molecular cargo, surface markers, and functional attributes—provides the foundation for understanding their potential as natural carriers in therapeutic applications, particularly in the field of nanomedicine and cancer therapy[2]. In recent years, extracellular vesicles (EVs) have gained considerable attention as innovative drug delivery platforms in oncology owing to their distinctive biological advantages. Unlike synthetic nanocarriers, EVs demonstrate low toxicity, high biocompatibility, and minimal immunogenicity, thereby reducing the likelihood of adverse immune responses. Furthermore, their natural capacity for selective cellular targeting enhances therapeutic precision, which is particularly valuable in cancer treatment where specificity is essential. Collectively, these attributes establish EVs as versatile and effective carriers capable of addressing many of the limitations associated with conventional delivery systems. EVs can be engineered to encapsulate a wide range of therapeutic agents, including chemotherapeutic drugs, nucleic acids, and proteins. Notably, vesicles derived from different cellular sources—such as macrophages, dendritic cells, and red blood cells—have demonstrated enhanced anti-cancer efficacy compared to traditional formulations. For example, macrophage-derived EVs exploit their inherent tumour-targeting ability, dendritic cell-derived EVs can simultaneously stimulate anti-tumour immune responses, and red blood cell-derived EVs offer abundant availability with reduced immunogenicity, making them suitable for scalable therapeutic applications. These examples highlight the adaptability of EVs, which can be

tailored to meet diverse therapeutic requirements. Taken together, the combination of safety, biocompatibility, and intrinsic targeting potential underscores the promise of EVs as next-generation nanomedicine platforms. Their ability to integrate seamlessly into biological systems while delivering potent therapeutic cargo provides a strong foundation for advancing cancer therapy toward clinical application[3,4]. Selecting the most suitable cellular source for extracellular vesicle (EV) isolation is a critical step in advancing drug delivery research. The biological origin of EVs directly influences their molecular composition, functional properties, and therapeutic potential, making this choice central to the design of effective delivery systems. Among the various cell types explored, mesenchymal stem cells (MSCs) have emerged as one of the most widely utilized sources for generating EVs. EVs derived from MSCs (MSC-EVs) are particularly valued because of their regenerative capacity and immunomodulatory effects, which provide unique advantages in therapeutic applications. MSC-EVs have demonstrated the ability to modulate immune responses, promote tissue repair, and enhance cellular communication, thereby offering a dual benefit as both delivery vehicles and biologically active agents. Their natural compatibility with human physiology reduces the risk of toxicity and immunogenicity, while their capacity to carry diverse bioactive molecules—including proteins, lipids, and nucleic acids—makes them versatile platforms for drug encapsulation. Furthermore, MSC-EVs exhibit inherent homing abilities toward sites of injury or inflammation, which can be utilized to achieve targeted delivery in cancer therapy and other pathological conditions. Consequently, the use of MSC-derived EVs represents a promising strategy for developing next-generation nanomedicine platforms. Their combination of safety, regenerative potential, and immunomodulatory activity underscores their relevance as a foundation for innovative drug delivery approaches in oncology and beyond[5-8]. Pascucci and colleagues demonstrated that mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) loaded with paclitaxel were capable of suppressing the proliferation of pancreatic cancer cells, highlighting their potential as effective drug delivery vehicles. This finding underscores the therapeutic promise of MSC-EVs, which can encapsulate and transport chemotherapeutic agents while maintaining biocompatibility and reducing systemic toxicity. By harnessing the natural properties of EVs, such approaches may offer a more targeted and efficient means of delivering anti-cancer drugs compared to conventional formulations. Despite these encouraging results, the clinical application of mesenchymal stem cells themselves has been constrained by concerns regarding their potential tumorigenicity. MSCs, while possessing regenerative and immunomodulatory properties, have been reported in some contexts to contribute to tumor progression or create a microenvironment favorable to cancer cell survival. This duality has prompted researchers to explore MSC-EVs as a safer alternative, since EVs retain many of the beneficial biological functions of their parent cells without the same risks associated with direct MSC transplantation. Consequently, MSC-EVs represent a promising next-generation therapeutic platform, combining the advantages of stem cell biology with the safety profile of acellular delivery systems. Their ability to carry potent drugs such as paclitaxel while minimizing oncogenic risks positions them as an attractive candidate for future cancer therapies[9]. Recent studies suggest that extracellular vesicles (EVs) derived from bovine milk hold considerable promise as innovative drug delivery systems. Their natural origin, biocompatibility, and ability to encapsulate bioactive molecules make them attractive candidates for therapeutic applications. Unlike

synthetic nanocarriers, milk-derived EVs are abundant, relatively easy to isolate, and can be administered orally, offering a practical and cost-effective approach to drug delivery. One of the most compelling features of bovine milk EVs is their stability in the gastrointestinal tract, which enables them to protect therapeutic cargo from enzymatic degradation and facilitate absorption across biological barriers. This property enhances their potential for delivering drugs, nucleic acids, or proteins in a non-invasive manner. Furthermore, their low immunogenicity reduces the risk of adverse immune responses, while their natural targeting ability allows for more precise delivery to specific tissues or cells. Taken together, these characteristics highlight bovine milk-derived EVs as a versatile and sustainable platform for drug delivery research. Their accessibility, safety profile, and functional adaptability position them as a promising alternative to conventional nanocarriers, particularly in the development of novel strategies for cancer therapy and other chronic diseases[10]. Bovine milk provides an abundant source of extracellular vesicles (EVs), which can be efficiently isolated for therapeutic use. Preclinical studies in mouse models have shown that bovine milk-derived EVs are well tolerated, exhibiting no cytotoxicity or allergic reactions, thereby underscoring their safety and potential as drug delivery vehicles[11]. Bovine milk-derived extracellular vesicles (EVs) exhibit notable stability and low immunogenicity within the intestinal environment, making them promising carriers for chemotherapeutic agents. Experimental studies have shown that these vesicles can be effectively loaded with drugs such as paclitaxel, docetaxel, and doxorubicin, resulting in enhanced bioavailability and improved therapeutic efficacy in both in vitro and in vivo cancer models. Their natural abundance, biocompatibility, and favorable safety profile further strengthen their potential as cost-efficient and effective drug delivery systems. Collectively, drug-loaded milk EVs represent a compelling strategy for targeted cancer therapy and next-generation nanomedicine development[12-17].

LITERATURE REVIEW:

The literature on EV-based drug delivery systems in cancer therapy has expanded dramatically over the past decade, with research spanning fundamental biology, engineering strategies, and translational applications. Multiple comprehensive reviews have synthesized the state of knowledge, identifying EVs as natural nanocarriers with distinct advantages over synthetic alternatives. The literature consistently emphasizes three key themes: the biological properties that make EVs attractive delivery vehicles, the diversity of therapeutic cargos that can be loaded, and the engineering strategies available to enhance targeting and efficacy. Research has documented the successful delivery of small-molecule chemotherapeutics, RNA therapeutics, and proteins using EVs, with studies demonstrating improved tumour targeting and reduced systemic toxicity compared to conventional formulations. The literature also reveals ongoing efforts to address practical challenges including low yield from natural sources, variable EV characteristics depending on cell origin, and the need for scalable, reproducible manufacturing processes. Recent work has begun to address these limitations through the development of novel isolation methods, loading techniques, and surface functionalization strategies.

Title of article	Author	Contribution
Extracellular vesicles as an emerging drug delivery system for cancer treatment.	Zheng-gang Wang, et al.	Wang and colleagues provide a comprehensive review of EV-based drug delivery, categorizing therapeutic cargos such as small molecules, nucleic acids, and proteins, while critically evaluating loading strategies and their advantages, offering essential context for technical method selection[18].
Extracellular vesicles: emerging anti-cancer drugs and advanced functionalization platforms.	M. Wu, et al.	Wu and colleagues review strategies for functionalizing extracellular vesicles (EVs) into targeted therapeutics. They highlight methods for protein and peptide loading, surface engineering to improve specificity, and the dual role of EVs as anti-cancer agents and delivery platforms. This work emphasizes advanced engineering approaches that enhance EV therapeutic potential[19].
Engineered exosomes for tumour-targeted drug delivery: A focus on genetic and chemical functionalization.	Ali Akbari, Fereshteh Nazari-Khanamiri, Mahdi Ahmadi, Maryam Shoaran, Jafar Rezaie	Akbari and colleagues review exosome engineering strategies for enhanced tumor targeting, detailing genetic and chemical functionalization methods, cargo loading techniques, and preclinical examples, while addressing clinical translation challenges such as safety, reproducibility, and scalable manufacturing[20].

AIM & OBJECTIVES:

The main aim of EV-based drug delivery research in cancer therapy is to develop clinically viable therapeutic platforms that leverage the natural properties of EVs to achieve superior tumour targeting, reduced systemic toxicity, and improved therapeutic outcomes compared to conventional chemotherapy and synthetic nanocarrier systems. This aim encompasses both fundamental research to understand and optimize EV properties and translational research to address manufacturing, regulatory, and clinical implementation challenges.

OBJECTIVE 1: Improve Encapsulation Efficiency and Cargo Stability.

Achieve therapeutically relevant drug loading within EVs while maintaining cargo stability during storage, circulation, and delivery to target cells. This requires optimization of loading methods to maximize encapsulation efficiency for diverse cargo types including small molecules, nucleic acids, and proteins, while preserving both EV integrity and cargo bioactivity[19].

OBJECTIVE 2: Enhance Tumour Targeting and Specificity.

Develop surface engineering strategies to improve EV accumulation in tumour tissue and uptake by cancer cells while minimizing off-target distribution. This includes both exploitation of intrinsic targeting properties and implementation of active targeting through ligand attachment or genetic modification to display tumour-specific binding moieties[21].

OBJECTIVE 3: Develop Scalable and Reproducible Manufacturing.

Establish production workflows that can generate clinically relevant quantities of EVs with consistent characteristics, addressing current limitations in yield, purity, and batch-to-batch variability. This objective is critical for translating EV therapeutics from research settings to clinical applications[21,22].

OBJECTIVE 4: Demonstrate In-Vivo Efficacy and Safety.

Validate antitumor activity and safety profiles in relevant preclinical models, establishing proof-of-concept for therapeutic benefit and identifying potential toxicities or limitations that must be addressed before clinical testing[22,23].

RATIONALE:

The rationale for developing EV-based drug delivery systems in cancer therapy rests on several compelling advantages that EVs possess over synthetic nanocarriers and conventional drug formulations. These advantages stem from the biological origin and natural functions of EVs, which have evolved to mediate intercellular communication and transfer bioactive molecules between cells.

1) Biocompatibility and Low Immunogenicity:

EVs exhibit significantly lower toxicity and immunogenicity compared to many synthetic nanocarriers, making them naturally suited for therapeutic applications. Their membrane composition, derived from cellular membranes, is recognized as "self" by the immune system, reducing the risk of immune-mediated clearance and adverse reactions. This biocompatibility advantage is particularly important for cancer therapy, where repeated dosing may be required and where synthetic nanoparticles often trigger immune responses that limit efficacy and cause toxicity[18].

2) Intrinsic Targeting Capabilities:

Native EVs possess surface proteins and lipids that can promote tissue-specific uptake, providing a foundation for natural targeting that can be further enhanced through engineering. This intrinsic targeting capability reduces the need for extensive synthetic modification and provides a starting point for developing tumor-specific delivery systems. The biological origin of these targeting properties means they are less likely to be recognized as foreign and cleared by the immune system[23].

3) Ability to Cross Biological Barriers:

EVs have demonstrated the ability to cross certain biological barriers that limit the distribution of synthetic nanoparticles and free drugs. This property has been exploited in engineered approaches to target difficult-to-reach sites, including the brain, where the blood-brain barrier typically prevents drug delivery. For cancer therapy, this capability is valuable for treating metastatic disease and tumours in sanctuary sites[20].

4) Translational Considerations and Challenges:

Despite these advantages, the rationale for EV-based delivery must be balanced against practical challenges including low yield from natural sources, variability depending on cell origin, and standardization hurdles. These limitations must be addressed through improved manufacturing processes and quality control measures to realize the full potential of EV therapeutics[21].

PHASES:

A systematic plan of work for developing EV-based drug delivery systems in cancer therapy must address both technical optimization and translational validation. The following plan synthesizes priorities identified across the literature and provides an actionable framework for advancing EV therapeutics from concept to clinical testing.

PHASE 1: Optimization of Loading Methods (Months 1-6)

Conduct comparative evaluations of loading techniques including passive incubation, electroporation, sonication, and high-pressure homogenization, to identify optimal methods for specific cargo types. Quantify loading efficiency, cargo retention, and functional delivery in vitro using standardized assays. Establish protocols that balance loading efficiency with preservation of EV integrity and cargo bioactivity[20].

PHASE 2: Surface Functionalization Development (Months 4-9)

Implement parallel genetic and chemical surface engineering workflows to display tumour-targeting ligands on EV surfaces. Genetic approaches will involve transfection of producer cells to express targeting moieties fused to exosomal membrane proteins, while chemical approaches will employ post-isolation conjugation strategies. Evaluate targeting efficiency using tumour cell lines and compare specificity of different functionalization strategies[20].

PHASE 3: Characterization and Quality Control (Months 7-12)

Establish comprehensive characterization protocols including size distribution analysis, surface marker profiling, cargo quantification, and functional assays. Develop quality control criteria for batch release and implement standardized methods to assess batch-to-batch consistency. This phase is essential for addressing the reproducibility challenges that currently limit clinical translation[21].

PHASE 4: Scalable Manufacturing Development (Months 10-15)

Optimize production workflows to generate clinically relevant quantities of EVs with consistent characteristics. Evaluate scalable isolation methods including tangential flow filtration and chromatography-based approaches. Implement high-pressure homogenization or other scalable loading techniques that can be performed under GMP-compatible conditions. Address yield limitations through optimization of producer cell culture conditions and EV secretion[22].

PHASE 5: Preclinical Efficacy Studies (Months 13-20)

Conduct in vivo efficacy studies in relevant tumour models, comparing EV-formulated drugs to conventional formulations and free drugs. Assess tumour targeting through biodistribution studies, evaluate antitumor activity through tumour growth inhibition and survival endpoints, and characterize pharmacokinetics and pharmacodynamics. Use these studies to identify lead formulations for further development[22].

PHASE 6: Safety and Toxicology Assessment (Months 18-24)

Perform comprehensive safety evaluation including acute and repeat-dose toxicity studies, immunogenicity assessment, and evaluation of potential off-target effects. Characterize the safety profile of both empty EVs and drug-loaded EVs to distinguish cargo-related toxicity from carrier-related effects. This phase provides essential data for regulatory submissions and clinical trial design[21].

PHASE 7: Regulatory Strategy and Clinical Translation Planning (Months 22-30)

Develop a regulatory strategy in consultation with regulatory authorities, addressing the classification of EV products, manufacturing

controls, and the clinical development pathway. Prepare investigational new drug (IND) application materials including chemistry, manufacturing, and controls (CMC) documentation, preclinical safety data, and clinical protocol. Establish partnerships for clinical trial execution[21].

DRUGS & EXCIPIENTS:

EV-based drug delivery systems have been developed for a diverse range of therapeutic agents, with the choice of cargo and excipients depending on the therapeutic goal, target tumour type, and desired mechanism of action. The literature documents successful encapsulation of three major cargo categories: small-molecule chemotherapeutics, nucleic acid therapeutics, and protein-based therapeutics.

1) SMALL-MOLECULE CHEMOTHERAPEUTICS:

Paclitaxel has emerged as a model compound for EV encapsulation, with multiple studies demonstrating successful loading and enhanced antitumor activity. Paclitaxel-loaded EVs have shown improved tumour targeting and reduced systemic toxicity compared to conventional formulations. The hydrophobic nature of paclitaxel facilitates incorporation into EV membranes, making it an attractive candidate for EV delivery[22]. Other chemotherapeutics including doxorubicin and curcumin have also been successfully loaded into EVs, with studies demonstrating maintained or enhanced cytotoxicity against cancer cells while reducing off-target effects[18].

2) NUCLEIC ACID THERAPEUTICS:

MicroRNAs (miRNAs) represent a major class of EV cargo, with both tumour-suppressor miRNAs and immune-modulating miRNAs being delivered to cancer cells. EVs naturally carry miRNAs as part of their native cargo, making them particularly well-suited for miRNA delivery. Therapeutic applications include restoration of tumour-suppressor function and modulation of the tumour microenvironment[18,24]. Small interfering RNAs (siRNAs) have been loaded into EVs for gene silencing applications, targeting oncogenes and genes involved in drug resistance. The protective environment within EVs shields siRNAs from degradation by serum nucleases, improving their stability and bioavailability[24].

3) PROTEIN AND PEPTIDE THERAPEUTICS:

Tumour-suppressor proteins and therapeutic peptides have been incorporated into EVs through various loading strategies. These protein cargos can exert direct anticancer effects or modulate signalling pathways in target cells. The ability of EVs to deliver functional proteins represents a significant advantage over many synthetic delivery systems that can denature or inactivate protein cargo[19].

4) EXCIPIENTS AND MEMBRANE MODIFIERS:

DSPE-PEG (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-polyethene glycol) conjugates have been employed to PEGylate EVs and serve as anchors for targeting moieties. PEGylation can improve EV circulation time and provide a platform for attaching targeting ligands. DSPE-PEG has been successfully incorporated into EVs using scalable methods including high-pressure homogenization[22]. Targeting Ligands such as cyclo(Arg-Gly-Asp-d-Phe-Lys) (cRGD) have been conjugated to DSPE-PEG and incorporated into EVs to enhance tumour targeting. cRGD targets integrin receptors that are

overexpressed on tumour cells and tumour vasculature, improving EV accumulation in tumours[22]. Hybrid Strategies involving the fusion of exosomes with liposomes or other nanocarriers have been explored to boost loading capacity and stability while maintaining the biological advantages of EVs[20]].

METHOD OF PREPARATION:

The preparation of EV-based drug delivery systems involves two critical steps: isolation of EVs from producer cells and loading of therapeutic cargo. The choice of methods significantly impacts EV yield, purity, cargo loading efficiency, and functional properties, with important implications for both research applications and clinical translation.

1) EV ISOLATION METHODS:

Ultracentrifugation remains the most widely used method for EV isolation in research settings. This technique involves sequential centrifugation steps at increasing speeds to pellet EVs based on their size and density. While ultracentrifugation is accessible for research laboratories and can process large volumes, it suffers from low throughput, long processing times, and potential co-purification of protein aggregates and other contaminants. The high centrifugal forces may also affect EV integrity[25]. Size-Exclusion Chromatography (SEC) separates EVs from soluble proteins and other contaminants based on size, providing better purity and gentler separation conditions that preserve EV functionality. SEC is particularly valuable for functional studies where EV integrity is critical. However, SEC typically yields lower EV concentrations than ultracentrifugation unless additional concentration steps are employed[21,25]. Tangential Flow Filtration (TFF) and other membrane-based methods offer scalability advantages and are increasingly used for larger-scale EV production. These methods can process large volumes efficiently and are more amenable to GMP manufacturing than ultracentrifugation[21].

2) CARGO LOADING METHODS:

Passive Incubation involves mixing EVs with therapeutic cargo under conditions that promote cargo association with or incorporation into EVs. This simple approach preserves EV integrity and is suitable for hydrophobic drugs that can partition into EV membranes. However, passive loading typically yields low encapsulation efficiency for hydrophilic cargos and may result in surface association rather than true encapsulation[18,25]. Electroporation applies brief electrical pulses to create transient pores in EV membranes, facilitating entry of nucleic acids and other charged molecules. Electroporation can achieve higher loading efficiency than passive methods, particularly for siRNA and other nucleic acids. However, the technique can cause RNA aggregation, variable efficiency depending on cargo and EV characteristics, and potential damage to EV membranes[25]. Sonication and Extrusion use mechanical disruption to permeabilize EV membranes and improve drug loading. Sonication applies ultrasonic energy to create transient membrane disruptions, while extrusion forces EVs through membranes with defined pore sizes. Both methods can increase loading efficiency but may alter EV surface markers and functionality, potentially affecting targeting and uptake properties[25]. High-Pressure Homogenization (HPH) represents a scalable approach that simultaneously mixes EVs, drug, and membrane modifiers (such as DSPE-PEG) to produce loaded and functionalized EVs in a single step. HPH has been demonstrated for paclitaxel encapsulation in milk-derived EVs, producing PEG-modified, ligand-functionalized EVs with

improved in vivo antitumor efficacy. This method offers significant advantages for translation due to its scalability and ability to perform multiple processing steps simultaneously[22].

3) METHOD SELECTION CONSIDERATIONS:

The choice of isolation and loading methods involves trade-offs between throughput, purity, loading efficiency, and preservation of EV functionality. Passive incubation preserves EV integrity but yields lower encapsulation efficiency, while electroporation and sonication increase loading but risk cargo aggregation or membrane alteration. For translational applications, scalable methods like HPH that can be performed under controlled manufacturing conditions are increasingly preferred despite requiring process optimization[22,25].

CONCLUSION

Extracellular vesicle-based drug delivery systems represent a promising frontier in cancer therapy, offering unique advantages over conventional chemotherapy and synthetic nanocarrier systems. The papers reviewed in this report collectively demonstrate that EVs possess intrinsic properties—including biocompatibility, low immunogenicity, natural targeting capabilities, and the ability to cross biological barriers—that make them attractive platforms for delivering diverse therapeutic cargos to tumours. The literature documents successful encapsulation of small-molecule chemotherapeutics, nucleic acid therapeutics, and proteins within EVs, with multiple studies showing improved tumour targeting and reduced systemic toxicity compared to conventional formulations. Engineering strategies including surface functionalization with targeting ligands and optimization of loading methods have further enhanced the therapeutic potential of EV-based delivery systems. Notably, the development of scalable preparation methods such as high-pressure homogenization addresses one of the key translational challenges facing the field. Despite these advances, significant challenges remain before EV therapeutics can achieve widespread clinical implementation. Standardization of production methods, optimization of loading efficiency, validation of batch-to-batch consistency, and comprehensive safety assessment in relevant preclinical models are all essential prerequisites for clinical translation. The field must also address regulatory considerations specific to biologically-derived nanocarriers and establish manufacturing processes compatible with good manufacturing practice (GMP) requirements. The research objectives identified across the reviewed papers—improving encapsulation efficiency, enhancing tumour targeting, developing scalable manufacturing, and demonstrating in vivo efficacy and safety—provide a clear roadmap for advancing EV therapeutics from bench to bedside. The plan of work outlined in this report synthesizes these objectives into an actionable framework that addresses both technical optimization and translational validation. Looking forward, the continued development of EV-based drug delivery systems will require multidisciplinary collaboration spanning cell biology, pharmaceutical sciences, engineering, and clinical medicine. Success will depend on systematic optimization of each component of the EV therapeutic platform—from producer cell selection and culture conditions through isolation, loading, functionalization, and quality control—while maintaining focus on the ultimate goal of improving outcomes for cancer patients. The convergence of improved understanding of EV biology, advanced engineering techniques, and scalable manufacturing methods positions the field for significant progress in the coming years. As the

challenges of standardization and scale-up are addressed, EV-based drug delivery systems have the potential to become a major modality in the oncology therapeutic arsenal, offering patients more effective and better-tolerated treatment options.

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