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ENGINEERING EXOSOMES FOR ENHANCED DRUG DELIVERY

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ABSTRACT

Exosomes, cell-secreted extracellular vesicles, are promising drug delivery tools due to biocompatibility, low immunogenicity, and innate ability to cross biological barriers. This review examines their therapeutic potential, detailing biogenesis via endosomal multivesicular body formation. Engineering strategies including encapsulation of therapeutic agents (e.g., CRISPR components, anticancer drugs), and surface modifications, such as ligand conjugation and membrane functionalization, enhance targeting and delivery efficiency. Their applications span diverse fields like oncology, neurodegeneration, and tissue regeneration, leveraging their cell-specific delivery and immune modulation. Clinical trials highlight progress in cancer and inflammatory diseases, yet challenges in manufacturing standardization, scalability, and regulatory hurdles remain. Exosome-based therapies offer transformative potential for precision medicine but require solutions to current limitations for successful clinical translation.

KEYWORDS: Multivesicular, Regenerative medicine, Nanocarriers, Surface modification, Electroporation, Microfluidics, Sonication, Extrusion, Immunogenicity, Targeting ligands.

INTRODUCTION

Exosomes, which represent a specific type of extracellular vesicles (EVs) with diameters ranging from 30 to 150 nm, have emerged as pivotal agents in facilitating intercellular communication and driving therapeutic advancements.[1] These lipid-bilayer-enclosed vesicles originate from the endosomal system and are secreted by nearly every cell type. They transport a diverse cargo composed of proteins, lipids, and various nucleic acids,

including mRNA, miRNA, and non-coding RNAs. Importantly, this cargo mirrors the physiological or pathological conditions of their originating or parent cells, thus providing valuable insights into cellular states and potential therapeutic applications.[2] Once being considered as waste products of cells, exosomes are currently seen as vital contributors to biological processes, which enable cells to send messages to one another by transporting bioactive molecules from one tissue or organ to the other. Their intrinsic capacity to move across the blood-brain barrier and their natural ability to target cells of certain types make them perfectly suited for drug delivery applications.[3]

The potential of drug delivery to targets of conventional pharmaceuticals such as synthetic nanoparticles, liposomes, and polymeric carriers has generated interest for exosome-based therapies.[4] The classical manner of delivery often faces challenges like quick clearance by the immune system, which may cause off-target toxicity and ineffective cellular uptake. On the other hand, the fact that exosomes exist naturally in the body makes them low immunogenic, high biocompatibility and have in-built targeting properties due to surface markers like tetraspanins (CD9, CD63, CD81) and integrins.[5] The latest innovations in bioengineering have optimised their functionality even further by allowing precise cargo loading (such as chemotherapeutics, siRNA, or CRISPR-Cas9 components) and surface modifications to facilitate better tissue-specific targeting. For example, some bioengineered exosomes loaded with targeting ligands (e.g., folate, RGD peptides) have shown the most amplified transport to cancer cells, whereas anti-inflammatory miRNAs containing exhibit potential in treating neurodegenerative diseases.[6,7]

Despite their promise, translating exosome-based therapies into clinical practice faces hurdles. Key challenges include the lack of standardized methods for large-scale production, heterogeneity in vesicle size and content and regulatory ambiguities.[8] Moreover, isolating high-purity exosomes from complex biological fluids remains technically demanding, with current methods like ultracentrifugation and size-exclusion chromatography often compromising yield or scalability.[8] Recent innovations, such as micro fluidic isolation and exosome-mimetic synthetic vesicles, aim to address these gaps but require further validation.[9]

Biogenesis of Exosomes: Formation and Mechanisms

Exosomes are produced within the endosomal compartment as nanoscale extracellular vesicles (30-150 nm) required for cellular communication.[5] Their biogenesis begins at early

endosomes, which later become multivesicular bodies (MVBs). As this maturation occurs, intraluminal vesicles (ILVs) bud inward and capture lipids, proteins, and nucleic acids, inclusively mRNA and miRNA, from the cytosol. This process is coordinated by two primary mechanisms: the ESCRT-dependent pathway and the ESCRT-independent pathway.[2] The ESCRT machinery (endosomal sorting complex required for transport), which consists of ubiquitin tagged proteins and RNA, selectively acts in ILVs using complex proteins like ESCRT-0, ESCRT I, ESCRT II, and ESCRT-III together with helper proteins like VTA-1, ALIX, and VPS4.[1,5] On the other hand, ESCRT-independent pathways involve tetraspanins (for example, CD63 and CD81) and lipid-involved sorting like ceramide build-up.[7] Neutral sphingomyelinase 2 (nSMase2) synthesizes ceramide, which causes spontaneous membrane curvature, making vesicle budding easier. The molecular makeup ofILVs reflects their cellular origin, such as tumor-derived exosomes which frequently have tumorigenic proteins like EGFR and MET, and metastatic miRNAs, while exosomes from mesenchymal stem cells (MSCs) are rich in regenerative proteins, such as VEGF and TGF-β[1,5]

Once MVBs are formed, they are transported to the plasma membrane with the assistance of cytoskeletal components, including actin filaments and microtubules, as well as Rab GTPases like Rab27a/b and Rab35.[2] The fusion of MVBs with the plasma membrane, regulated by SNARE proteins such as VAMP7 and Syntaxin-1A, results in the release of intraluminal vesicles (ILVs) into the extracellular space as “exosomes”.[5] Cellular stress signals, such as hypoxia or inflammation, as well as oncogenic activation, can significantly increase exosome secretion. This heightened production often correlates with disease states, making affected cells prolific sources of exosomes.[3] Following their release, exosomes engage with target cells via three key uptake pathways: **endocytosis**, **direct membrane fusion**, and **receptor-mediated interactions.**[7] Exosomes are typically internalized through clathrin- or caveolin-dependent endocytosis, a process aided by surface integrins such as α6β4 and αvβ5 that bind to extracellular matrix components.[1,7] Their lipid exosomes to merge directly with the host cell’s membrane, facilitating cytoplasmic delivery of their cargo.[2] Additionally, receptor-ligand binding—such as interactions between exosomaltetrasp-rich composition, particularly high levels of cholesterol and phosphatidylserine, allows anins (e.g., CD9, CD81) or heparan sulfate proteoglycans (HSPGs) and receptors like TIM-1 or Siglecs on recipient cells—promotes cellular uptake and initiates downstream signaling pathways. Exosomes exhibit inherent **tissue specificity** governed by their surface markers.[6] For instance, those displaying integrin α6β4 preferentially accumulate in laminin-abundant environments like the lungs,

whereas exosomes carrying neural cadherin target neuronal cells.[3] This natural targeting ability, rooted in their molecular architecture, is harnessed in developing precision-engineered drug delivery platforms. By mimicking these tropic properties, researchers optimize exosomes to navigate biological systems with remarkable accuracy, enhancing therapeutic outcomes.

The molecular architecture of exosomes is central to their structural stability and functional adaptability. Their lipid bilayer, enriched with cholesterol, sphingomyelin, and ceramide, provides mechanical strength and resilience.[6] Surface proteins, includingtetraspanins (CD9, CD63, CD81), adhesion molecules (e.g., integrins, ICAM-1), and immune-modulating proteins (MHC-I/II), play a crucial role in mediating interactions with target cells.[5] Inside the lumen, exosomes house a diverse array of cargo, such as nucleic acids (miR-21, miR-155), mRNAs, non-coding RNAs, heat shock proteins (HSP70, HSP90), and enzymes like GAPDH.[5] This unique composition not only safeguards the cargo from degradation during transport but also allows exosomes to bypass immune surveillance and traverse biological barriers—capabilities that synthetic nanoparticles often lack.

Engineering Strategies for Enhanced Exosome Functionality

To further refine exosome performance, advanced engineering techniques are employed to tailor their biological and physicochemical properties.[6] These strategies include surface ligand conjugation, membrane hybridization, and stimuli-responsive modifications, each designed to address specific challenges in drug delivery.[4,9]

Encapsulation of Therapeutic Agents

Exosomes serve as natural nanocarriers capable of encapsulating diverse therapeutic payloads within their aqueous lumen or lipid membranes.[10] This protective encapsulation maintains drug stability during circulation while preventing premature degradation.[4,6] This process varies significantly based on the physicochemical properties of the cargo, with different techniques optimized for small molecules, nucleic acids, or proteins.

1. Passive Diffusion

The lipid-rich membrane of exosomes facilitates spontaneous incorporation of hydrophobic compounds through passive diffusion. Small molecule drugs with lipophilic characteristics, including paclitaxel (an anticancer agent) and curcumin (an anti-inflammatory compound), readily partition into exosomal membranes when incubated together.[6] For example

doxorubicin, where passive loading enhanced its therapeutic index by improving tumor-specific delivery while reducing systemic toxicity in preclinical cancer models.[7,11] However, loading efficiencies typically remain below 20%, and it proves ineffective for water-soluble compounds or macromolecular drugs.

2. Electroporation-Based Loading

Electroporation employs controlled electrical pulses to temporarily permeabilize exosomal membranes, facilitating the entry of hydrophilic therapeutic agents such as nucleic acids (siRNA, mRNA) and proteins.[6] This technique gained prominence when Alvarez-Erviti's team (2011) successfully delivered neuron-targeting siRNA across the blood-brain barrier using electroporated exosomes.[12] While effective, the method requires precise optimization of pulse parameters (voltage, duration, and number) to balance cargo loading with vesicle integrity. Suboptimal conditions may cause irreversible membrane damage, resulting in exosome aggregation and reduced biological activity.

3. Mechanical Loading Techniques

Physical disruption methods offer alternative approaches for drug encapsulation: **Sonication** utilizes Ultrasound waves to create transient openings in exosomal membranes, demonstrated by 3-fold enhanced cisplatin loading compared to passive methods.[13] The shear forces must be carefully controlled to prevent permanent structural damage.[10] While **Extrusion** involves forcing exosomes through nano-porous membranes, enables uniform drug distribution, as shown in Parkinson's models using catalase-loaded exosomes yielding uniform vesicles with high catalytic activity.[10,13] However, the mechanical stress may alter surface protein composition, potentially affecting natural tropism.

4. Membrane Permeabilization Strategies

Freeze-thaw cyclinginvolves Phase transitions during repeated freezing/thawing enable membrane incorporation of lipophilic compounds like curcumin. While **Saponin-assisted loading** includes plant-derived surfactant selectively binds membrane cholesterol to create temporary pores, saponina membrane-permeabilizing agent, enhances loading of hydrophilic drugs (e.g., siRNA) without permanent damage achieving remarkable 50% loading efficiency for 5-fluorouracil in colorectal cancer models.[10] However, residual saponin requires thorough removal due to potential cytotoxicity at concentrations >0.01%.

I. Surface Engineering of Exosomes for Targeted Drug Delivery

Exosome surface modification represents a powerful strategy to enhance their therapeutic potential by enabling precise targeting and controlled drug release.[14] These engineered nanovesicles can be tailored through various surface functionalization approaches to display specific ligands, antibodies, or responsive molecules while maintaining their inherent biological properties.[8,14]

1. Covalent Surface Functionalization

Chemical conjugation techniques allow the formation of stable chemical bonds between exogenous molecules and functional groups naturally present on exosomal membrane proteins or lipids, including the use of NHS-PEG4-Maleimide crosslinkers, click chemistry approaches, and sulfo-SMCC-mediated couplings.[15,16] These methods have enabled significant advances, such as the pH-sensitive conjugation of chemotherapeutic agents like methotrexate for tumor-specific release and the attachment of RGD peptides to target αvβ3 integrin-rich cancer cells.[4,7] However, Critical considerations for chemical modification include: Preserving native exosome surface markers essential for biological function, Maintaining membrane integrity during conjugation reactions, Optimizing ligand density to balance targeting efficacy with natural biodistribution.[6]

2. Genetic Modification of Donor Cells

exosome surface functionalization involves genetically engineering parent cells to produce modified exosomes with desired targeting properties. This approach relies on transfecting donor cells to express chimeric proteins that become incorporated into exosomal membranes during vesicle biogenesis. A seminal study by Alvarez-Erviti and colleagues (2011) demonstrated this concept by fusing the neuronal-targeting RVG peptide to the exosomal membrane protein Lamp2b in dendritic cells, resulting in exosomes capable of efficiently delivering siRNA across the blood-brain barrier.[12] Similar approaches have shown promise in cancer therapeutics, where HER2-specific affibodies displayed on exosome surfaces enhanced trastuzumab delivery to breast tumors.[3,4] While this method enables precise control over exosome surface composition, it faces challenges related to the complex and time-consuming nature of genetic manipulation, along with potentially lowyields of engineered exosomes, which may hinder large-scale clinical production.

3. Membrane Integration via Hydrophobic Anchoring

This exosome modification involves the hydrophobic insertion of therapeutic compounds into the exosomal lipid bilayer. This technique utilizes molecules conjugated to lipophilic moieties such as cholesterol or dioleoylphosphatidylethanolamine (DOPE), which spontaneously integrate into the exosome membrane.[17] For instance, cholesterol-conjugated paclitaxel has been successfully incorporated into exosome surfaces, resulting in improved drug solubility and prolonged release kinetics compared to conventional formulations. However, this approach is limited by the possibility of premature drug detachment during systemic circulation, which could compromise therapeutic effectiveness.[17] The stability of membrane-inserted compounds depends on both the strength of hydrophobic interactions and the dynamic nature of exosome membranes in biological environment.

Breakthrough Applications of Engineered Exosomes in Therapeutics

The development of exosome-based drug delivery systems has demonstrated remarkable success across various medical applications. Several notable examples highlight their therapeutic potential

1. Enhanced Chemotherapy Delivery

Doxorubicin-loaded exosomes exhibited significantly greater tumor accumulation (5-fold increase) than conventional administration in melanoma models. A 2020 clinical study revealed that exosomal doxorubicin minimized cardiotoxic side effects while preserving anticancer activity in breast cancer treatment.[11]

2. Nucleic Acid Therapeutics

Neuron-targeted exosomes successfully delivered siRNA across the blood-brain barrier, achieving 60% reduction in BACE1 protein levels for Alzheimer's management.[12] miRNA-21 inhibitor-loaded exosomes effectively silenced oncogenic pathways in ovarian cancer.[1,3]

3. Natural Compound Delivery

Curcumin encapsulated in exosomes demonstrated enhanced anti-inflammatory properties in colitis models compared to standard formulations[14,18] Research in 2021 established that exosomal curcumin accelerated diabetic wound healing through oxidative stress modulation.[18]

4. Gene Editing Applications

CRISPR-Cas9 plasmid-loaded exosomes achieved efficient gene modification in cellular models, opening new possibilities for genetic disorder interventions.[19]

5. Antiviral Therapeutics

Mesenchymal stem cell (MSC)-derived exosomes have emerged as potent antiviral agents against SARS-CoV-2, leveraging their intrinsic immunomodulatory and regenerative properties.[20,21]

CONCLUSION

Engineered exosomes represent a transformative frontier in precision drug delivery, leveraging their innate biocompatibility and targeting ability to overcome limitations of conventional systems.[1,8] Advanced modifications—cargo encapsulation, surface engineering, genetic modification—enable tailored therapies in oncology, gene editing, and antivirals.[8] Overcoming scalable production and standardization challenges via interdisciplinary innovation promises to unlock their potential, ushering in an era of personalized, minimally invasive medicine.

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