Liposomal Gene Delivery Systems: Advances, Mechanisms, and Therapeutic Applications

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ABSTRACT

Liposomal gene delivery systems have emerged as a transformative platform in the field of gene therapy, offering a promising alternative to viral vectors for the safe and effective transport of genetic material into target cells. As non-viral carriers, liposomes possess several advantages, including biocompatibility, biodegradability, low immunogenicity, and the ability to encapsulate a wide variety of nucleic acids such as plasmid DNA, mRNA, siRNA, and CRISPR/Cas9 components. This review provides a comprehensive overview of liposomal gene delivery systems, focusing on their structural design, classification, mechanisms of cellular uptake, and various preparation methods. The mechanisms of gene transfer—encompassing encapsulation, cellular internalization, endosomal escape, and gene expression—are critically discussed, highlighting the parameters that influence delivery efficiency. Moreover, strategies to enhance transfection, including surface modifications, PEGylation, and incorporation of targeting ligands, are explored. The review also addresses therapeutic applications across oncology, genetic disorders, vaccination, neurology, and cardiovascular diseases, underscoring

the clinical potential of liposomal platforms. Despite their promise, challenges such as low transfection efficiency, endosomal entrapment, and manufacturing scalability persist. Recent advances in lipid nanoparticle technology, AI-driven formulation optimization, and personalized medicine approaches are shaping the next generation of liposomal vectors. Finally, current clinical trials and regulatory considerations are reviewed, providing insight into the translational landscape of these systems. This synthesis highlights the pivotal role of liposomal gene delivery in advancing gene-based therapeutics and outlines the innovations driving their future clinical success.

Keywords: Liposomal gene delivery, non-viral vectors, targeted liposomes, gene therapy, endosomal escape, personalized medicine.

1. INTRODUCTION

Gene therapy represents a transformative approach in the management and potential cure of a broad spectrum of diseases, ranging from genetic disorders and cancers to infectious and neurodegenerative diseases. By enabling the introduction, deletion, or correction of specific genetic material within a patient's cells, gene therapy seeks to address the root causes of disease rather than merely alleviating symptoms. The promise of gene therapy is increasingly evident in recent clinical successes and FDA-approved products, such as onasemnogene abeparvovec for spinal muscular atrophy and voretigene neparvovec for inherited retinal dystrophy [1,2]. These developments have catalyzed interest in the development of more efficient and safe gene delivery systems.Gene delivery is central to the success of gene therapy, as therapeutic nucleic acids such as DNA, messenger RNA (mRNA), small interfering RNA (siRNA), and CRISPR/Cas9 systems must be efficiently transported into target cells and reach their intracellular sites of action. Broadly, gene delivery systems can be categorized into viral and non-viral vectors. Viral vectors, including adenoviruses, lentiviruses, and adeno-associated viruses (AAVs), offer high transfection efficiencies and durable gene expression. However, they are often associated with significant drawbacks, such as immunogenicity, mutagenesis risk, limited payload capacity, and complex manufacturing processes [3,4].In contrast, non-viral vectors, especially lipid-based systems such as liposomes, have emerged as attractive alternatives due to their low immunogenicity, biocompatibility, structural versatility, and

potential for repeated administration [5]. Liposomes are spherical vesicles composed of one or more phospholipid bilayers surrounding an aqueous core, capable of encapsulating both hydrophilic and hydrophobic therapeutic molecules. Their ability to encapsulate nucleic acids and protect them from enzymatic degradation in the bloodstream makes them particularly valuable in gene delivery applications [6]. Moreover, liposomal formulations can be engineered for targeted delivery, controlled release, and enhanced cellular uptake, expanding their utility across a variety of therapeutic areas.The recent success of lipid nanoparticle-based mRNA vaccines against SARS-CoV-2 (e.g., Pfizer-BioNTech and Moderna vaccines) has further validated lipid-based delivery technologies, invigorating research into next-generation liposomal systems for gene therapy [7].This review aims to provide a comprehensive overview of liposomal gene delivery systems, focusing on their structural features, mechanisms of nucleic acid delivery, formulation strategies, therapeutic applications, and the challenges associated with their clinical translation. By highlighting recent advances and ongoing developments, this review seeks to elucidate the role of liposomes as a cornerstone in the future of gene therapy.

2. LIPOSOME BASICS AND CLASSIFICATION

Liposomes are self-assembled, spherical vesicles consisting of one or more phospholipid bilayers surrounding an aqueous core. Their structural similarity to biological membranes makes them ideal carriers for both hydrophilic and lipophilic drugs, including nucleic acids for gene delivery applications. First described in the 1960s, liposomes have evolved from simple lipid vesicles to highly engineered systems capable of targeted, efficient, and safe delivery of genetic material [8].

2.1 Structure and Composition of Liposomes

Liposomes are primarily composed of natural or synthetic phospholipids and cholesterol. The amphiphilic nature of phospholipids—hydrophilic heads and hydrophobic tails—facilitates the spontaneous formation of bilayer membranes in aqueous environments. The hydrophilic core allows for encapsulation of water-soluble compounds such as DNA, RNA, or oligonucleotides, while the lipid bilayer can incorporate hydrophobic molecules, providing dual-loading capacity [9].Cholesterol is often included in formulations to enhance membrane rigidity, reduce permeability, and improve the stability of liposomes under physiological conditions. The physicochemical characteristics of liposomes—including lipid composition, charge, size, and

surface modifications—can be tailored to influence circulation time, biodistribution, and cellular uptake [10].

2.2 Types of Liposomes

Based on composition and functional modifications, liposomes can be broadly categorized into the following types:

2.2.1 Conventional Liposomes

Conventional liposomes are composed of neutral or anionic phospholipids and cholesterol. These liposomes are relatively simple in structure but face rapid clearance from systemic circulation due to uptake by the mononuclear phagocyte system (MPS), limiting their utility in targeted or sustained gene delivery [11].

2.2.2 Cationic Liposomes

Cationic liposomes are formulated using positively charged lipids such as DOTAP (1,2-dioleoyl-3-trimethylammonium-propane) or DOTMA (N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium). These liposomes can electrostatically interact with negatively charged nucleic acids, forming stable lipoplexes that facilitate cellular uptake and endosomal escape [12]. Due to their strong gene-condensing ability, cationic liposomes are widely used in non-viral gene delivery applications. However, their clinical translation is limited by toxicity, immune activation, and nonspecific biodistribution.

2.2.3 PEGylated (Stealth) Liposomes

PEGylation involves the covalent attachment of polyethylene glycol (PEG) chains to the liposome surface. This modification creates a hydrophilic “stealth” barrier that reduces recognition by the immune system, prolongs circulation time, and enhances passive accumulation in tumor tissues via the enhanced permeability and retention (EPR) effect [13]. PEGylated liposomes have been successfully used in several FDA-approved drug products (e.g., Doxil), and they are increasingly being explored for gene delivery.

2.2.4 Targeted Liposomes

To improve specificity, liposomes can be functionalized with ligands such as antibodies, peptides, aptamers, or small molecules that bind to overexpressed receptors on target cells. These **targeted liposomes** enhance gene delivery efficiency by promoting receptor-mediated endocytosis and minimizing off-target effects [14]. This approach is especially relevant in cancer gene therapy, where tumor-specific antigens can be exploited for selective delivery.

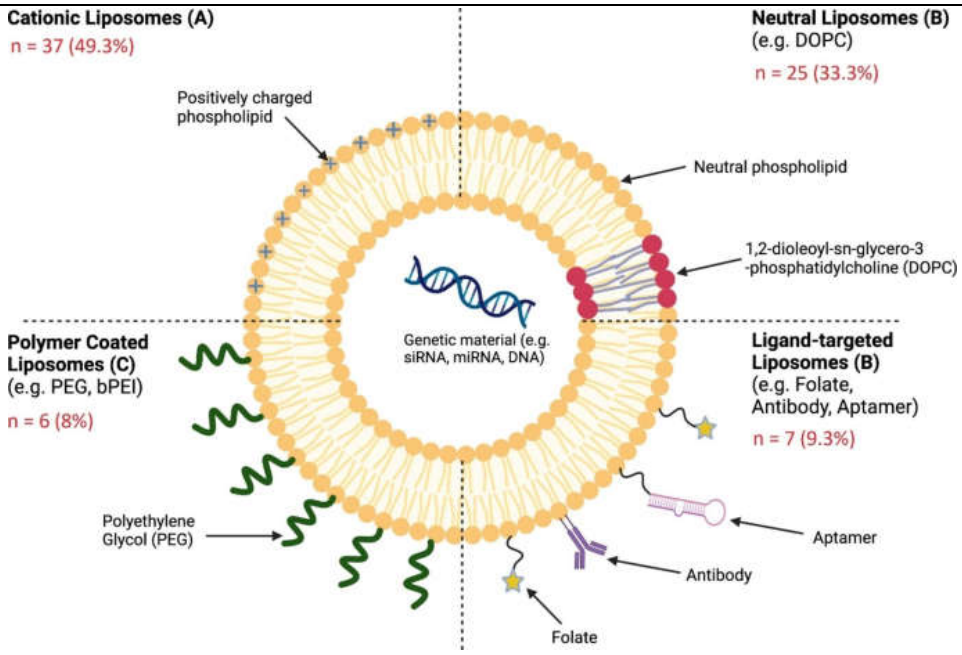

Fig.1. Liposomal delivery of gene therapy for ovarian cancer

2.3 Physical Characteristics Influencing Gene Delivery

Several physical parameters of liposomes critically influence their performance as gene delivery vehicles. One of the most significant factors i**s size.** Liposomes generally range from 50 nanometers to several micrometers in diameter; however, for systemic gene delivery applications, nanosized liposomes (<200 nm) are preferred. These smaller particles demonstrate enhanced tissue penetration and are less likely to be cleared by the mononuclear phagocyte system (MPS), improving their circulation time and targeting potential [15].

Another important characteristic is the surface charge, often expressed as zeta potential. The charge on the liposomal surface plays a key role in nucleic acid complexation and interaction with cell membranes. Cationic liposomes, due to their positive charge, effectively bind to

negatively charged DNA or RNA and promote cellular uptake through electrostatic interactions. In contrast, neutral or slightly anionic liposomes tend to exhibit better biocompatibility and lower toxicity but often at the expense of reduced transfection efficiency.

Lamellarity, or the number of lipid bilayers surrounding the aqueous core, also influences gene delivery performance. Liposomes can be unilamellar, with a single lipid bilayer, or multilamellar, consisting of multiple concentric bilayers. Among the unilamellar types, small unilamellar vesicles (SUVs) and large unilamellar vesicles (LUVs) are frequently employed in gene delivery. SUVs are typically associated with higher cellular uptake due to their smaller size, though they may exhibit lower encapsulation efficiency when compared to LUVs, which offer greater capacity for nucleic acid loading and sustained release profiles [16].

3. MECHANISM OF LIPOSOMAL GENE DELIVERY

The efficiency of liposomal gene delivery systems relies heavily on a sequence of biological events that begin with the encapsulation of nucleic acids and culminate in the successful expression of therapeutic genes in the target cells. This process involves multiple coordinated steps: loading of genetic material into the liposomes, interaction with cellular membranes, internalization through specific uptake mechanisms, escape from endosomal compartments, and finally the nuclear entry and expression of the delivered gene. Optimizing each of these steps is crucial for enhancing the overall transfection efficiency and therapeutic outcomes.

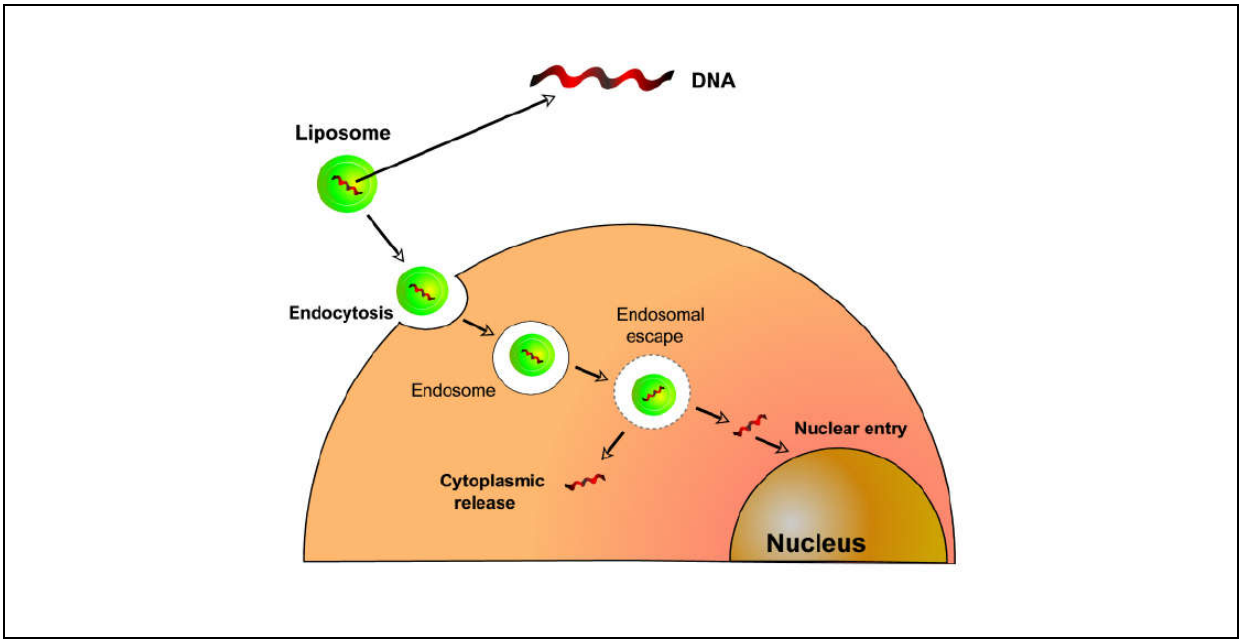

Fig.2. Schematic representation of endocytosis as a main mechanism of liposomal gene delivery into cells

3.1 Encapsulation of DNA/RNA in Liposomes

Liposomal encapsulation involves the integration of nucleic acids (such as plasmid DNA, siRNA, or mRNA) within the aqueous core or surface of the vesicle. Hydrophilic nucleic acids are typically entrapped in the internal aqueous phase, while electrostatic interactions are used in cationic liposomes to complex with the negatively charged phosphate backbone of DNA or RNA, forming lipoplexes [17]. Various methods such as thin-film hydration, ethanol injection, or microfluidic mixing are employed to ensure high encapsulation efficiency and structural integrity of the nucleic acids during formulation [18].

3.2 Interaction with the Cell Membrane

Once administered, liposomes reach their target tissues and come into contact with cellular membranes. Surface properties—particularly lipid composition**,** surface charge**,** and **presence** of targeting ligands influence the strength and specificity of liposome-cell interactions. Cationic liposomes**,** due to their positive charge, interact efficiently with the negatively charged components of the cell membrane, facilitating close apposition and uptake [19]. Targeted liposomes, conjugated with ligands such as antibodies or peptides, enhance cellular specificity via receptor-mediated interactions [20].

3.3 Internalization Pathways: Endocytosis and Fusion

Cellular uptake of liposomes predominantly occurs through endocytosis**,** including clathrin-mediated, caveolin-mediated, and macropinocytosis pathways. The specific mechanism depends on liposome size, surface charge, and receptor-ligand interactions [21]. Once inside the cell, liposomes are typically sequestered in endosomal compartments**.**

In some cases, liposomal fusion with the plasma membrane or endosomal membrane can directly release genetic material into the cytosol. Fusogenic lipids such as DOPE (dioleoylphosphatidylethanolamine) are often incorporated into liposomal formulations to enhance membrane destabilization and promote fusion [22].

3.4 Endosomal Escape and Nuclear Delivery

A major barrier to successful gene delivery is endosomal entrapment**,** where nucleic acids are degraded in lysosomes if not efficiently released. Liposomes are often engineered with pH-sensitive or fusogenic components that trigger membrane disruption in the acidic endosomal environment, thereby enabling endosomal escape [23].

For gene delivery involving plasmid DNA**,** nuclear entry is essential. This step is typically dependent on cell division**,** where the nuclear envelope temporarily disassembles, allowing plasmid DNA access to the nucleus. In contrast, **mRNA** and **siRNA** function in the cytoplasm and do not require nuclear localization, simplifying delivery for transient gene expression applications [24].

3.5 Expression of the Delivered Gene

Following successful delivery into the nucleus (in the case of DNA), the therapeutic gene undergoes transcription into mRNA**,** followed by translation into protein**.** The extent and duration of gene expression are influenced by promoter strength, epigenetic factors, and intracellular stability of the genetic material. For mRNA-based delivery**,** the gene expression occurs directly in the cytoplasm, enabling rapid and transient protein synthesis, which is ideal for applications like vaccination and short-term protein replacement therapy [25].

Optimizing liposomal formulations to enhance encapsulation, cellular uptake, endosomal escape, and nuclear delivery is central to improving gene delivery outcomes and therapeutic efficacy.

4. FORMULATION AND PREPARATION METHODS

The method of liposome preparation plays a critical role in determining its size, lamellarity, surface charge, encapsulation efficiency**,** and stability all of which are essential for its effectiveness as a gene delivery vector. Different techniques have been developed over time to formulate liposomes suitable for nucleic acid delivery, ranging from traditional batch methods to advanced nanofabrication strategies. Here, we discuss the most widely used formulation methods for liposomal gene delivery.

4.1 Thin-Film Hydration Method

The thin-film hydration method**,** also known as the Bangham method, is one of the oldest and most commonly used techniques for liposome preparation. In this process, a mixture of lipids is dissolved in an organic solvent such as chloroform or methanol. The solvent is then evaporated under reduced pressure using a rotary evaporator, leaving behind a thin lipid film on the wall of a round-bottom flask. This film is subsequently hydrated with an aqueous solution containing the nucleic acid payload, resulting in the formation of multilamellar vesicles (MLVs) [26].

To improve encapsulation efficiency and produce smaller vesicles, the MLVs are often subjected to sonication **or** extrusion to generate small unilamellar vesicles (SUVs) or **large** unilamellar vesicles (LUVs)**.** Although simple and widely applicable, this method may suffer from low encapsulation efficiency for hydrophilic nucleic acids and batch-to-batch variability [27].

4.2 Reverse-Phase Evaporation Method

The reverse-phase evaporation method offers a higher encapsulation efficiency for hydrophilic drugs and nucleic acids compared to the thin-film method. Here, lipids are first dissolved in an organic solvent and mixed with an aqueous solution containing the nucleic acid. The mixture is then emulsified using sonication to form a water-in-oil (w/o) emulsion. Upon removal of the organic solvent under reduced pressure, the emulsion collapses to form liposomes [28].This technique produces large unilamellar vesicles and is particularly useful for encapsulating large nucleic acid molecules like plasmid DNA or siRNA. However, residual solvent toxicity and mechanical stress during emulsification can potentially degrade sensitive biomolecules [29].

4.3 Ethanol Injection Method

In the **ethanol injection method**, lipids dissolved in ethanol are rapidly injected into an aqueous phase containing the nucleic acid under vigorous stirring. The sudden change in solvent polarity induces the self-assembly of lipid molecules into vesicles [30]. This method is advantageous due to its simplicity, scalability, and ability to produce small liposomes without the need for post-processing steps like sonication or extrusion. However, it typically yields unilamellar liposomes with low encapsulation efficiency for hydrophilic nucleic acids, since

the process lacks a strong nucleic acid entrapment step. Also, complete removal of ethanol is essential to avoid toxicity in biological systems [31].

4.4 Detergent Removal Method

The detergent removal method involves solubilizing lipids and nucleic acids in the presence of a detergent such as sodium cholate. Once mixed, the detergent is gradually removed by dialysis, gel filtration, or adsorption onto polystyrene beads, prompting the self-assembly of liposomes [32].

This method allows for precise control over liposome composition and is suitable for incorporating proteins or ligands on the liposomal surface. However, detergent residues must be completely removed to avoid cytotoxicity, and the process can be time-consuming [33].

4.5 Microfluidics and Novel Nanofabrication Techniques

Recent advancements in microfluidic technology have enabled the controlled and reproducible production of liposomes at nanoscale dimensions. In microfluidic mixing**,** lipid solutions in organic solvents and aqueous nucleic acid solutions are combined in microchannels, allowing rapid and uniform mixing under laminar flow conditions. This leads to the spontaneous formation of liposomes with tight control over size and polydispersity index (PDI) [34].These systems offer significant advantages over traditional methods, including high reproducibility, scalability, and minimal use of toxic solvents. Moreover, nanofabrication techniques like microfluidic hydrodynamic focusing**,** flash nanoprecipitation, and 3D printing-based assembly are being explored to develop next-generation liposomal platforms for gene delivery [35].

5. ADVANTAGES OF LIPOSOMAL GENE DELIVERY

Liposomal systems have emerged as one of the most promising non-viral vectors for gene delivery due to their unique physicochemical and biological characteristics. Their ability to encapsulate, protect, and deliver a wide range of nucleic acids—combined with excellent safety profiles and engineering versatility—offers several key advantages over both viral vectors and other non-viral systems.

5.1 Biocompatibility and Biodegradability

Liposomes are primarily composed of naturally occurring or synthetic phospholipids that closely mimic the structure of biological membranes. This similarity confers excellent biocompatibility, allowing liposomes to be well-tolerated when administered in vivo. Moreover, liposomes are biodegradable, breaking down into non-toxic metabolites such as fatty acids and glycerol, which are readily processed by normal metabolic pathways [36].

This inherent biocompatibility reduces the risk of long-term toxicity and supports repeated administration, which is critical for chronic disease therapies requiring multiple dosing regimens [37].

5.2 Low Immunogenicity

Unlike viral vectors, liposomes are non-immunogenic or exhibit minimal immunostimulation, particularly when formulated with neutral or PEGylated lipids. This property minimizes the risk of immune-mediated adverse events, such as inflammation or cytokine storms, and avoids the development of neutralizing antibodies that commonly compromise the efficacy of viral gene therapies [38]. PEGylation (surface modification with polyethylene glycol) further reduces recognition by the immune system and enhances circulatory half-life, thereby improving delivery to target tissues [39].

5.3 Protection of Nucleic Acids from Degradation

Nucleic acids such as plasmid DNA, siRNA, or mRNA are susceptible to enzymatic degradation by nucleases present in blood and extracellular environments. Liposomal encapsulation provides a physical barrier that shields genetic material from these enzymes, thereby enhancing stability and bioavailability [40].Cationic liposomes form electrostatic complexes (lipoplexes) with nucleic acids, further stabilizing them and improving cellular uptake. This protective effect is especially crucial for systemic gene delivery applications, where exposure to blood components is inevitable [41].

5.4 Customizable Targeting and Controlled Release

A significant advantage of liposomal systems is their customizability. Liposomes can be engineered with a variety of surface ligands—such as antibodies, peptides, or aptamers—that

enable targeted delivery to specific cell types or tissues via receptor-mediated endocytosis [42]. Additionally, liposomes can be designed for stimuli-responsive behavior**,** releasing their genetic payload in response to environmental triggers such **as** pH**,** temperature**, or** enzymes**.** This enables spatial and temporal control over gene release, which is particularly valuable for cancer gene therapy, where site-specific delivery is essential to minimize off-target effects [43].

5.5 Scalable Production for Clinical Use

Unlike viral vectors that require complex cell-based manufacturing processes, liposomes can be synthesized using relatively simple, reproducible, and scalable methods**, such as** microfluidics**,** thin-film hydration**, or** ethanol injection [44].

This scalability supports good manufacturing practice (GMP) production and facilitates cost-effective transition from lab-scale formulations to clinical-grade therapeutics. The success of lipid nanoparticle (LNP)-based mRNA vaccines during the COVID-19 pandemic (e.g., Pfizer-BioNTech and Moderna) demonstrated the feasibility of large-scale production and rapid deployment of liposomal nucleic acid delivery systems [45].

Fig.3. Advantages of Liposomes

6. CHALLENGES AND LIMITATIONS

Despite their numerous advantages, liposomal gene delivery systems face several technical, biological, and manufacturing challenges that limit their widespread clinical application. While

liposomes are safer and more versatile than viral vectors, their overall efficiency and consistency in therapeutic gene expression often fall short. Addressing these limitations is essential for improving clinical outcomes and enabling broader adoption of liposomal gene therapies.

6.1 Stability and Shelf-Life Issues

One of the primary concerns with liposomal formulations is their physical and chemical instability during storage. Liposomes are prone to aggregation, fusion, and leakage of encapsulated contents over time, especially when stored at non-ideal temperatures or in aqueous environments. Degradation of phospholipids via hydrolysis and oxidation can compromise membrane integrity and reduce gene delivery efficiency [46]. Stability can be improved by lyophilization (freeze-drying), but this requires cryoprotectants and careful optimization to preserve the vesicle structure. Additionally, the presence of cationic lipids, which are commonly used for complexing nucleic acids, may further reduce shelf-life due to their susceptibility to oxidation and aggregation [47].

6.2 Low Transfection Efficiency Compared to Viral Vectors

While liposomes avoid many of the safety concerns associated with viral vectors, they generally exhibit lower transfection efficiency, particularly in non-dividing or hard-to-transfect cells such as neurons and primary cells [48]. This is mainly due to suboptimal cellular uptake, limited endosomal escape, and inefficient nuclear entry of the delivered nucleic acids.In contrast, viral vectors have evolved natural mechanisms for gene integration and nuclear trafficking, giving them superior transduction performance. As a result, liposomal vectors often require higher doses **or** multiple administrations, which may increase cost and potential toxicity [49].

6.3 Endosomal Entrapment

A critical intracellular barrier in liposomal gene delivery is endosomal entrapment. After cellular uptake via endocytosis, many liposomes are sequestered in **endosomes** and eventually trafficked to lysosomes, where their contents are degraded. Failure to escape this compartment significantly reduces gene transfer efficiency [50].To overcome this, liposomes can be formulated with fusogenic lipids (e.g., DOPE), pH-sensitive components, or endosomolytic

peptides that trigger membrane destabilization in the acidic endosomal environment. However, balancing membrane disruption with biocompatibility remains a formulation challenge [51].

6.4 Immune System Clearance

Another limitation is the rapid clearance of liposomes by the mononuclear phagocyte system (MPS), particularly when administered intravenously. Liposomes are recognized as foreign particles by macrophages in the liver and spleen, leading to their opsonization and phagocytosis, which diminishes their circulation time and reduces therapeutic efficacy [52].PEGylation can prolong circulation by creating a hydrophilic barrier, but repeated exposure may result in anti-PEG antibodies and accelerated blood clearance (ABC) phenomenon, limiting its long-term use [53]. Additionally, the use of cationic lipids, though beneficial for gene binding, can stimulate inflammatory responses and complement activation, contributing to dose-limiting toxicities [54].

6.5 Scale-Up and Reproducibility Concerns

The transition from laboratory-scale to large-scale, GMP-compliant production remains a major hurdle for liposomal gene delivery systems. Conventional batch methods such as thin-film hydration are labor-intensive and prone to batch-to-batch variability, making them unsuitable for industrial-scale manufacturing [55]. While microfluidics and continuous-flow processes offer promising solutions for scalable and reproducible liposome production, they require specialized equipment and may not yet be widely accessible. Furthermore, ensuring uniformity in liposome size, charge, and nucleic acid loading is critical to achieve consistent pharmacokinetics and therapeutic outcomes in clinical settings.

7. STRATEGIES TO ENHANCE GENE DELIVERY EFFICIENCY

Despite the inherent advantages of liposomal systems, their gene transfection efficiency remains a significant barrier, particularly in comparison to viral vectors. To overcome this, researchers have employed a range of rational design strategies to optimize liposomal formulations and enhance the intracellular delivery of nucleic acids. These strategies include surface functionalization, membrane composition adjustments, co-delivery of auxiliary agents, and fine-tuning of physicochemical parameters.

7.1 Surface Modification with Ligands (e.g., Antibodies, Peptides)

Functionalizing the surface of liposomes with targeting ligands improves cell specificity and facilitates receptor-mediated endocytosis, thereby increasing transfection efficiency and minimizing off-target effects. Common ligands include monoclonal antibodies, peptides (e.g., RGD or transferrin), aptamers, and folic acid, which bind to overexpressed receptors on tumor or diseased cells [56].For instance, folate-conjugated liposomes have been used to target folate receptor-expressing cancer cells, showing significantly enhanced uptake and gene expression in vitro and in vivo [57]. Such ligand-liposome conjugates allow for precise tissue targeting and improve the therapeutic index of gene-based treatments.

7.2 PEGylation for Stealth Properties

Polyethylene glycol (PEG) is commonly grafted onto the liposome surface to impart “stealth” characteristics, reducing recognition and clearance by the mononuclear phagocyte system (MPS). PEGylation improves circulation time, enhances passive targeting via the enhanced permeability and retention (EPR) effect, and supports repeat dosing by minimizing immunogenicity [58].However, excessive PEGylation may hinder cellular uptake and endosomal escape, leading to the "PEG dilemma." To address this, cleavable PEG linkers that detach in response to environmental stimuli (e.g., pH or enzymes) have been developed to optimize both stealth and delivery efficiency [59].

7.3 Use of Helper Lipids and Fusogenic Agents

Incorporation of helper lipids such as DOPE (dioleoylphosphatidylethanolamine) and cholesterol improves membrane fluidity, fusion capability, and structural stability. DOPE is particularly notable for its fusogenic properties, which promote endosomal escape through pH-dependent membrane destabilization [60].These helper lipids modulate liposome bilayer characteristics, facilitating lipid mixing with endosomal membranes and enabling efficient release of nucleic acids into the cytosol, a critical step for successful gene expression.

7.4 Co-delivery of Endosomal Escape Enhancers

To overcome endosomal entrapment, liposomes can co-deliver endosomolytic agents such as chloroquine, cationic polymers (e.g., polyethylenimine, PEI), or membrane-disruptive peptides. These agents disrupt endosomal membranes through osmotic swelling, proton sponge

effects, or direct lysis, enabling the escape of nucleic acids into the cytoplasm before lysosomal degradation occurs [61].Such co-formulation strategies significantly improve gene expression, especially for plasmid DNA and siRNA therapies, which require cytosolic or nuclear access for activity.

7.5 Optimization of Charge Ratio and Particle Size

The charge ratio between cationic lipids and anionic nucleic acids (often referred to as the N/P ratio) is a key determinant of lipoplex stability, binding affinity, and transfection efficiency. A higher positive surface charge generally enhances cellular uptake, but may also increase cytotoxicity and serum protein binding [62]. Therefore, a balanced N/P ratio must be selected based on the application and target cell type.Particle size also plays a crucial role: liposomes sized 100–200 nm exhibit favorable biodistribution, enhanced cell uptake via endocytosis, and preferential accumulation in tumor tissue through the EPR effect [63]. Microfluidic and nanoprecipitation techniques are now commonly employed to fine-tune these parameters for optimal in vivo performance.

8. THERAPEUTIC APPLICATIONS

Liposomal gene delivery systems have gained increasing attention for their therapeutic potential across a variety of clinical domains, owing to their biocompatibility, customizability, and ability to safely encapsulate and deliver nucleic acids. These systems have been widely explored in oncology, genetic disorders, vaccinology, neurology, and cardiovascular medicine, among others. Their role is especially prominent in diseases where targeted, efficient, and non-immunogenic gene modulation is critical.

8.1 Cancer Gene Therapy: Delivery of Tumor Suppressor Genes, siRNA, etc.

Cancer remains a primary target for gene therapy, with liposomal platforms extensively studied for delivering tumor suppressor genes, oncogene-silencing siRNAs, and pro-apoptotic agents. Liposomal systems allow for targeted delivery to tumors via passive (EPR effect) and active targeting (ligand modification), minimizing damage to healthy tissues.For example, cationic liposomes have been used to deliver p53 tumor suppressor genes in lung and breast cancer models, resulting in cell cycle arrest and apoptosis [64]. Similarly, liposomes loaded with siRNA against VEGF or BCL-2 have been shown to suppress angiogenesis and promote tumor

regression in vivo [65].FDA-approved products like Onpattro® (patisiran)**,** although based on lipid nanoparticles (LNPs), represent the clinical translation of liposomal technology for RNA interference-based therapies in rare cancers and beyond [66].

8.2 Genetic Disorders: Cystic Fibrosis, Hemophilia, etc.

Monogenic disorders, caused by mutations in a single gene, are ideal candidates for gene therapy. Liposomes offer a safer alternative to viral vectors for such applications, especially in conditions where repeated dosing is required.In cystic fibrosis**,** liposomal formulations have been used to deliver CFTR-encoding plasmids to airway epithelial cells via inhalation. Although early clinical trials showed modest gene expression, improved liposome formulations continue to be evaluated to enhance mucosal penetration and transfection efficiency [67].In hemophilia**,** studies using liposomes to deliver factor VIII or IX genes have demonstrated partial correction of the coagulation defect in animal models, with reduced immunogenicity and good tolerability [68].

8.3 Vaccination: DNA/RNA Vaccines (e.g., COVID-19)

Liposomal and lipid nanoparticle-based platforms have revolutionized vaccine development, particularly in the realm of nucleic acid vaccines. The most prominent demonstration of their potential is the success of mRNA vaccines against SARS-CoV-2, specifically Pfizer-BioNTech's BNT162b2 and Moderna's mRNA-1273, both of which employ lipid-based delivery systems for intramuscular mRNA administration [69].

These vaccines have exemplified several key advantages of liposomal carriers. They effectively protect mRNA from enzymatic degradation, ensuring stability until cellular uptake occurs. Once inside the cell, the liposomal structure facilitates endosomal escape, allowing the mRNA to reach the cytoplasm where translation into the target antigen takes place. This process triggers robust humoral and cellular immune responses, contributing to the vaccines' high efficacy. Furthermore, the platform's modularity and simplicity have allowed these vaccines to be rapidly developed and manufactured at scale, a critical factor during the COVID-19 pandemic.

Encouraged by this success, researchers are now extending lipid-based nucleic acid vaccine technology to a broader range of applications, including influenza, Zika virus, HIV, and even

cancer immunotherapy, highlighting its versatility and transformative potential in modern vaccinology [70].

8.4 Neurological Diseases: Delivery Across Blood-Brain Barrier

One of the most challenging areas of drug and gene delivery is targeting the central nervous system (CNS) due to the blood-brain barrier (BBB). Liposomes can be modified with transferrin, glucose, or ApoE ligands to facilitate receptor-mediated transcytosis across the BBB [71].

In diseases such as Parkinson's, Alzheimer's, and glioblastoma, liposomal gene delivery has been explored for delivering siRNA, miRNA, or neuroprotective genes, with some success in enhancing neuronal uptake and reducing neuroinflammation [72].

8.5 Cardiovascular and Inflammatory Conditions

Gene therapy for cardiovascular diseases such as atherosclerosis, myocardial infarction, and **hypertension** has seen promising developments with liposomal vectors. These systems can be engineered to deliver anti-inflammatory cytokines, angiogenic factors (e.g., VEGF), or siRNAs targeting adhesion molecules to endothelial cells and ischemic tissues [73].Similarly, in inflammatory diseases like rheumatoid arthritis and inflammatory bowel disease, liposomes have been used to deliver anti-TNFα siRNA and other immunoregulatory nucleic acids to inflamed tissues, showing reduced systemic side effects and improved local efficacy [74].

9. CLINICAL TRIALS AND REGULATORY STATUS

Liposomal gene delivery systems have evolved from conceptual and preclinical investigations to translational and clinical-stage therapies, largely driven by their superior safety profile and flexibility compared to viral vectors. Over the past two decades, numerous liposome-based gene therapies have entered clinical trials, targeting a wide range of conditions including cancers, monogenic disorders, and infectious diseases. The regulatory pathway for such therapies requires comprehensive safety assessments, pharmacokinetic profiling, and strict GMP manufacturing compliance.

9.1 Summary of Ongoing or Completed Clinical Trials

Several liposomal formulations have progressed into clinical testing for gene delivery applications, demonstrating both the promise and challenges of this technology. One of the earliest examples is Allovectin-7®, developed by Vical, which consisted of a liposome-DNA complex carrying the HLA-B7 gene and β2-microglobulin. It was designed to stimulate immune responses in melanoma patients and advanced to Phase III clinical trials. However, despite initial promise, the formulation failed to meet its primary efficacy endpoints and was ultimately discontinued [75].Another notable formulation is GL67A, a cationic liposome complexed with plasmid DNA encoding the CFTR gene, which was evaluated for cystic fibrosis treatment. Conducted by the UK Cystic Fibrosis Gene Therapy Consortium, a Phase IIb clinical trial demonstrated a modest but statistically significant stabilization in lung function. This study marked the first instance of a long-term clinical benefit from a non-viral gene therapy in cystic fibrosis patients [76].A more recent and highly successful example is Patisiran (Onpattro®), a therapeutic based on lipid nanoparticle technology, often grouped under advanced liposomal systems. Approved by the FDA in 2018, it became the first siRNA-based drug to reach the market, offering a treatment for hereditary transthyretin-mediated amyloidosis (hATTR) and establishing a key milestone in lipid-based gene delivery [77].

Furthermore, the global success of mRNA vaccines, such as Pfizer-BioNTech’s BNT162b2 and Moderna’s mRNA-1273, which also utilize lipid nanoparticle platforms, has showcased the capability of liposomal delivery systems to support the rapid, scalable, and safe administration of nucleic acids. These vaccines gained emergency use authorization and subsequent full regulatory approval, especially in the context of the COVID-19 pandemic, underscoring their practical applicability and transformative potential [78].Collectively, these clinical examples highlight that liposomal gene delivery systems, especially those employing cationic and PEGylated lipids, can be successfully translated into clinical use when carefully formulated and rigorously evaluated.

9.2 Regulatory Considerations for Gene Delivery Systems

Regulatory agencies such as the U.S. Food and Drug Administration (FDA), the European Medicines Agency (EMA), and Japan's Pharmaceuticals and Medical Devices Agency (PMDA) classify gene therapies, including those utilizing liposomal vectors, under the category of Advanced Therapy Medicinal Products (ATMPs). These therapies are evaluated

through comprehensive and stringent regulatory frameworks that encompass assessments of biodistribution, toxicology, immunogenicity, pharmacokinetics, and efficacy in relevant disease models.Compared to traditional small-molecule drugs, liposomal gene therapies present unique regulatory challenges. These include complex manufacturing processes, formulation variability, and issues with lot-to-lot consistency, all of which can affect product safety and efficacy [79]. To meet regulatory approval, developers must provide robust data demonstrating product stability over its intended shelf life, reproducibility of batch production, and full compliance with Good Manufacturing Practice (GMP) standards, particularly for the production of critical components such as lipid excipients and plasmid DNA or mRNA payloads [80].

Furthermore, international regulatory guidance documents such as ICH Q5A-E and directives from the FDA's Center for Biologics Evaluation and Research (CBER) offer a structural framework specifically tailored to non-viral gene delivery vectors, including liposomes. These guidelines mandate detailed evaluations of long-term gene expression, vector biodistribution, and potential off-target effects, ensuring that liposomal gene therapies meet the necessary safety and efficacy standards before entering clinical use [81].

9.3 Safety Assessments and Pharmacokinetics

Safety and pharmacokinetic profiling are critical components of the regulatory evaluation process for liposomal gene delivery systems. One major safety consideration is immunogenicity, as cationic liposomes have been shown to trigger complement activation-related pseudoallergy (CARPA) and provoke cytokine responses. To mitigate this, PEGylation is often employed to reduce immune recognition; however, this strategy is not without its limitations, as anti-PEG antibodies may develop with repeated administration, potentially compromising long-term efficacy and safety [82].

In terms of toxicity, preclinical studies have demonstrated that certain cationic lipids, especially when administered at high doses, can induce hepatic and renal toxicity. This highlights the importance of selecting biodegradable lipid components and carefully optimizing dosage regimens to maintain a safe therapeutic index [83].

The pharmacokinetic behavior of liposomal formulations is strongly influenced by their size and surface charge, which dictate their clearance patterns. Most liposomes are primarily cleared

by the reticuloendothelial system (RES), particularly in the liver and spleen. To enhance systemic circulation time, PEGylation is commonly utilized; however, while it extends half-life, it may also reduce cellular uptake and transfection efficiency. To overcome this trade-off, stimuli-responsive or cleavable PEG systems are being developed, allowing for extended circulation in the bloodstream followed by efficient uptake and gene delivery once the liposomes reach their target tissues [84].Overall, significant regulatory progress has been achieved in the field, largely propelled by the success of lipid nanoparticle platforms in vaccine development and RNA interference (RNAi) therapies. Nonetheless, continued clinical validation, as well as ongoing refinement of safety profiles and pharmacokinetic properties, will be essential to support future approvals and widespread clinical adoption of liposomal gene therapies.

10. FUTURE PERSPECTIVES AND EMERGING TRENDS

The landscape of gene delivery is rapidly evolving with innovations that promise to enhance the safety, specificity, and efficiency of liposomal gene delivery systems. Future directions include the refinement of lipid nanoparticle (LNP) technologies**,** incorporation of CRISPR/Cas9 gene editing**,** adoption of personalized medicine paradigms**,** and the application of artificial intelligence (AI) in formulation design. These trends aim to bridge the gap between experimental efficacy and clinical utility, enabling next-generation therapies for complex diseases.

10.1 Lipid Nanoparticles (LNPs) vs. Traditional Liposomes

While traditional liposomes have laid the foundation for non-viral gene delivery, lipid nanoparticles (LNPs) represent a major advancement in the field. Unlike multilamellar liposomes, LNPs are typically composed of ionizable lipids**,** cholesterol**,** helper phospholipids**,** and PEGylated lipids**,** forming stable nanoparticles ideal for nucleic acid encapsulation and systemic delivery [85].LNPs have demonstrated superior performance in endosomal escape**,** nucleic acid protection**,** and hepatic targeting**.** This was most notably proven by mRNA vaccines for COVID-19 (Pfizer-BioNTech and Moderna), which utilized LNP platforms to deliver mRNA safely and effectively [86]. Future LNPs may evolve to include stimuli-

responsive components, organelle-specific targeting, and multifunctional payloads, potentially outperforming traditional liposomes in gene therapy applications.

10.2 CRISPR/Cas9 Delivery Using Liposomal Systems

The gene-editing revolution sparked by CRISPR/Cas9 technology has created a demand for non-viral vectors capable of delivering Cas9 proteins, mRNA, or plasmids alongside guide RNAs. Liposomal systems are well-suited for this role due to their ability to co-encapsulate multiple components, their low immunogenicity, and modular surface design.Recent studies have shown successful delivery of Cas9-sgRNA complexes using cationic liposomes and LNPs, achieving in vivo gene editing in models of genetic liver disease and cancer [87]. The ability to transiently express Cas9 reduces the risk of off-target effects compared to viral delivery. In addition, the use of tissue-specific ligands and pH-sensitive lipids may enable precise control over gene editing at the cellular level [88].

10.3 Personalized and Precision Medicine Approaches

As genomic sequencing becomes more accessible, personalized gene therapies tailored to individual mutations, disease phenotypes, or patient-specific biomarkers are emerging. Liposomal vectors offer an adaptable platform for such applications due to their modifiability in cargo and targeting.For instance, cancer patients with unique tumor-specific mutations can receive liposome-encapsulated customized mRNA vaccines **or** siRNA cocktails designed to silence oncogenes. Similarly, rare genetic disorders with diverse genotypic presentations may benefit from patient-specific plasmid or mRNA formulations, enabling personalized gene correction [89].Liposomal systems also enable repeat dosing, an essential feature in chronic or progressive diseases, supporting long-term and adaptable therapeutic regimens without eliciting strong immune responses.

10.4 AI-Driven Liposome Design and Optimization

Artificial intelligence (AI) and machine learning (ML) are increasingly revolutionizing the drug development landscape, with growing applications in the design and optimization of liposomal gene delivery systems. By processing and analyzing large datasets that include lipid compositions, delivery outcomes, and various biophysical parameters, AI models are now capable of predicting optimal liposomal formulations tailored for specific nucleic acid cargos

and target tissues [90].Advanced deep learning algorithms are being developed to further refine this process. These models can predict transfection efficiency based on the structural and electrostatic characteristics of the lipids, optimize liposome size and colloidal stability, simulate biodistribution patterns and drug release kinetics, and even design custom ligand-lipid conjugates that enable precision targeting of specific cell types or tissues [91].The integration of AI into liposomal gene delivery research offers the potential to significantly accelerate the development timeline. It minimizes the need for time-consuming and costly trial-and-error experimentation, streamlining the transition from formulation development to preclinical testing and eventually clinical application. As AI-driven design tools continue to evolve, they are poised to play a critical role in the advancement of next-generation liposomal gene therapies, facilitating their translation from the laboratory to real-world clinical settings.

CONCLUSION

Liposomal gene delivery systems represent a transformative advancement in non-viral gene therapy, offering a unique combination of biocompatibility, structural flexibility, and customizable functionality. This review has highlighted the structural fundamentals of liposomes, their mechanism of gene delivery, and their potential in delivering diverse nucleic acid cargos, including plasmid DNA, siRNA, mRNA, and CRISPR/Cas9 components. Advances in formulation techniques—from traditional thin-film hydration to microfluidics—have enabled the development of more precise and scalable liposomal systems. Liposomal carriers not only protect genetic material from enzymatic degradation but also facilitate targeted delivery and controlled intracellular release, significantly enhancing transfection efficiency and therapeutic outcomes.

Despite these advantages, several challenges remain, including limited transfection efficiency compared to viral vectors, endosomal entrapment, immune clearance, and scale-up difficulties. However, current strategies such as PEGylation, ligand-mediated targeting, and the use of fusogenic or pH-sensitive lipids have shown considerable promise in overcoming these barriers. The emergence of lipid nanoparticle (LNP) platforms, particularly in mRNA vaccine success, has further validated the clinical relevance of lipid-based vectors.

Looking ahead, integration of liposomal systems with cutting-edge technologies like CRISPR gene editing, personalized medicine, and artificial intelligence-driven design is expected to revolutionize the landscape of gene delivery. Continued progress in clinical trials and

regulatory standardization will be essential for transitioning liposomal gene therapies from experimental innovation to mainstream clinical practice. Overall, liposomes are poised to play a foundational role in the future of safe, efficient, and targeted gene therapy.

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