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Review

Recent Trends in the Clinical Translation of Liposomal Drug Delivery Systems: Application from Conventional Therapeutics to Gene-Based Nanomedicine

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ABSTRACT

Objective: This review aims to summarize recent advances in liposome-based drug delivery systems and their applications for diverse therapeutic agents, including conventional chemotherapy, natural compounds, and gene-based therapeutics.

Method: We comprehensively analyzed recent studies focusing on various liposomal formulations and strategies designed to enhance delivery performance. This review focused on strategies involving surface ligand conjugation, ion-pairing, cell-mimetic designs, and charge modulation.

Result: Emerging liposomal strategies demonstrated enhanced drug retention, improved intracellular delivery, and increased target specificity while addressing critical challenges, including premature drug leakage, controlled drug release, non-specific tissue distribution, and multidrug resistance. Novel designs employing co-encapsulation, sustained-release platforms, and biomimetic coatings have demonstrated superior efficacy in preclinical models of cancer, infection, and inflammatory diseases. In addition, the development of liposomes for genetic therapeutics such as DNA, mRNA, and siRNA delivery has provided a promising avenue for precision therapy.

Conclusion: Recent developments in liposome-based delivery technologies offer promising solutions to the limitations of conventional drug formulations. By integrating innovative structural designs and active targeting strategies, these systems demonstrate significant potential for clinical translation across diverse therapeutic contexts. However, further work is required to ensure scalable manufacturing, reproducibility, and regulatory compliance to fully harness their clinical potential.

Keywords Liposome, Drug delivery, Conventional chemotherapy, Chemical drug, Natural compound, Gene

INTRODUCTION

Liposomes, first described by Alec D. Bangham and colleagues in the 1960s, are spherical vesicles composed primarily of phospholipid bilayers that closely resemble the structure of biological membranes.[1] Their unique amphiphilic structure enables the encapsulation of both hydrophilic and hydrophobic molecules, making them distinguished versatile carriers for a wide range of bioactive agents.[2] Liposomes have gained significant attention as one of the most established nanocarrier systems due to their unique ability to encapsulate diverse therapeutic agents, improve pharmacokinetics, and enhance treatment efficacy. Over the past several decades, liposomes have emerged as one of the most extensively studied and clinically validated nanocarrier systems in the fields of drug delivery, diagnostics, and biotechnology.[3,4]

The fundamental appeal of liposomes lies in their biocompatibility, biodegradability, and ability to protect encapsulated agents from degradation, thereby enhancing the therapeutic index of drugs.[5] Their structural similarity to cellular membranes allows for efficient fusion and uptake by target cells,

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facilitating improved intracellular delivery.[6] Furthermore, liposomes can be engineered with surface modifications such as PEGylation, ligand attachment, or charge alteration to enhance circulation time, target specific tissues or cells, and modulate immune interactions.[7] These attributes have propelled liposomes to the forefront of nanomedicine, exemplified by the clinical success of liposomal formulations for anticancer drugs (e.g., Doxil®), antifungal agents (e.g., AmBisome®), and most recently, mRNA-based vaccines for infectious diseases such as COVID-19.[8]

The versatility of liposomes extends beyond drug delivery. In the realm of basic research, liposomes serves as model systems for studying membrane dynamics, protein-lipid interactions, and cellular processes. Their tunable size, lamellarity, and surface chemistry allow researchers to mimic various biological environments and investigate fundamental biophysical phenomena. In diagnostics, liposomes are employed as carriers for biosensors and as platforms for targeted delivery of contrast agents, thereby improving the sensitivity and specificity of disease detection.[9,10]

Recent advances in nanotechnology and materials science have further expanded the potential of liposomes. The development of stimuli-responsive liposomes, capable of releasing their payload in response to specific physiological triggers (such as pH, temperature, or enzymatic activity), has opened new avenues for controlled and site-specific drug delivery.[11] Multifunctional liposomes, incorporating imaging agents, targeting ligands, and therapeutic molecules within a single platform, are being explored for theragnostic applications, enabling simultaneous diagnosis and treatment of diseases.[12] Furthermore, the integration of liposomes with other nanomaterials, such as polymers, inorganic nanoparticles, or biomolecules, has led to the creation of hybrid systems with enhanced functionality and performance.[13,14]

Given the remarkable versatility and adaptability of liposomes, it is essential to explore their diverse applications across biomedical and pharmaceutical fields. In this review, we will examine the various ways in which liposomes have been utilized from conventional drug delivery and vaccine platforms to cutting-edge roles in gene therapy, and beyond (Fig. 1). By highlighting both established and emerging uses, this review aims to provide a comprehensive understanding of the current landscape and future potential of liposome-based technologies.

RESULTS

1. Liposome

Liposomes are spherical vesicles composed of phospholipid bilayers with an internal aqueous core, typically ranging in size from several tens to a few hundred nanometers.[15] The constituent phospholipids possess hydrophilic head groups and hydrophobic tails, enabling their spontaneous self-assembly into stable bilayer structures in aqueous environments.[16] In this configuration, the hydrophilic heads orient toward the surrounding aqueous phase, while the hydrophobic tails align inward, forming the bilayer. This structure allows liposomes to encapsulate both hydrophilic and hydrophobic therapeutic

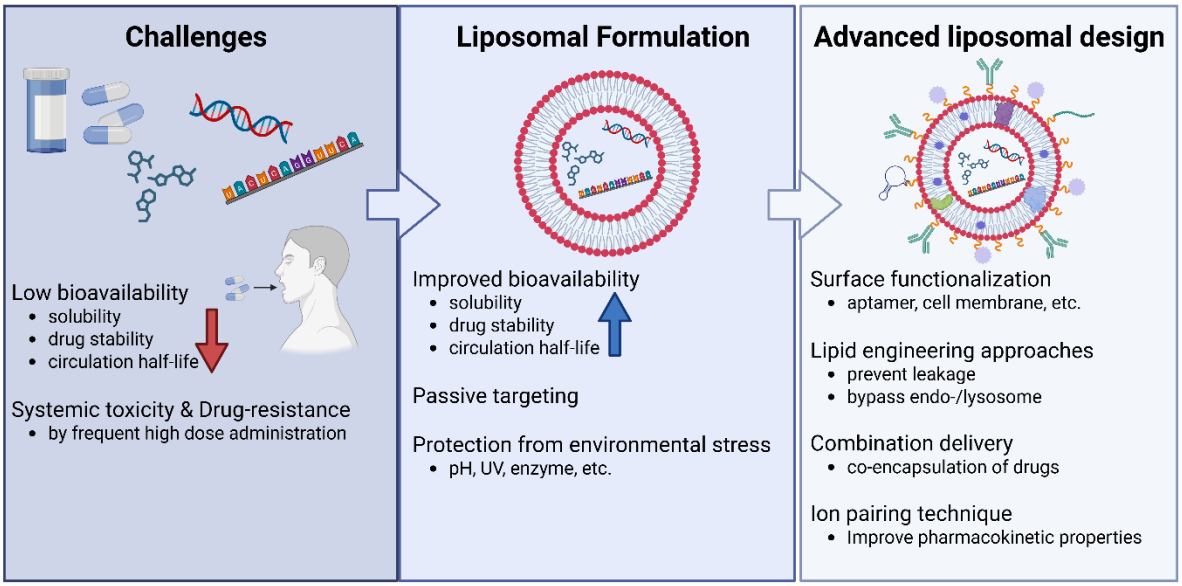

Fig. 1. Conceptual Overview of Liposomal Strategies to Overcome Drug Delivery Challenges. Conventional drugs (chemical drugs, natural compounds, gene-based therapeutics) often suffer from low bioavailability due to poor solubility, instability, and short circulation, leading to the need for frequent high-dose administration that can cause systemic toxicity and drug resistance. Encapsulation into liposomes can improve bioavailability by enhancing solubility and drug stability, prolonging systemic circulation, and passively targeting diseased tissues while protecting the payload from environmental stresses (e.g., pH, enzymes, UV). Further advances in liposomal design include surface functionalization (e.g., aptamer, cell membrane) for active targeting, lipid composition engineering to minimize premature drug leakage and evade lysosomal degradation, co-delivery of multiple drugs for synergistic effects, and ion pairing strategies to improve pharmacokinetic profiles.

agents simultaneously within a single particle.[17] Moreover, the physicochemical properties of liposomes—such as surface charge, membrane fluidity, and stability—can be finely tuned by selecting specific lipid components, which directly influence drug loading efficiency, release kinetics, and in vivo behavior.[18] Due to their biocompatibility, self-assembling nature, and capacity for functionalization, liposomes have emerged as attractive drug delivery vehicles. Encapsulation within liposomes can protect drugs from premature degradation, inactivation, and dilution in circulation, thereby enhancing bioavailability and therapeutic efficacy. These advantages have led to the clinical application of liposomal formulations in various fields, including oncology, antifungal therapy, and photodynamic therapy (Table 1). Notably, DOXIL®, the first FDA-approved liposomal anticancer drug in the United States, incorporates doxorubicin into a PEGylated liposome and was approved in 1995 for the treatment of ovarian cancer and multiple myeloma.[19] The clinical success of DOXIL® validated the potential of liposome-based platforms and spurred the development and approval of subsequent liposomal drugs, such as DepoCyt® for cancer and Epaxal® for vaccination.

Despite these advances, liposomes still face several limitations. Their interaction with serum proteins may lead to rapid clearance by the reticuloendothelial system (RES), particularly by the liver and kidneys, and insufficient stability in circulation may result in premature drug release. Additionally, liposomes administration can provoke innate immune responses, including activation of the complement system, which may cause complement activation-related pseudoallergy (CARPA), a side effect reported in numerous clinical cases.[20,21] These challenges have motivated ongoing research efforts not only to utilize liposomes to overcome the limitations of conventional drugs, but also to engineer next-generation liposomal systems that address the intrinsic shortcomings of traditional formulations. Given these challenges, the design of next-generation liposomal systems must also consider the physicochemical properties and therapeutic limitations of the encapsulated drugs.

2. Conventional Chemotherapy

Conventional chemotherapy refers to the use of chemically synthesized drugs for the treatment of various diseases (anti-tumor, anti-inflammatory, etc.).[22] Since its widespread adoption in the mid-20th century, it has remained a cornerstone of modern clinical practice.[23] Compared to biologics such as monoclonal antibodies, antibody-drug conjugates, and peptides, chemical drugs offer several advantages, including ease of synthesis and production, lower manufacturing costs, improved storage stability, flexible routes of administration, and rapid onset of action.[24,25] However, these agents often exhibit poor bioavailability due to limited stability in systemic circulation and unfavorable pharmacokinetic properties, necessitating repeated dosing.[26] Such repeated administration can increase the risk of nonspecific toxicity and, in some cases particularly with anticancer or antibiotic agents contribute to the development of drug resistance.[27]

With advances in liposomal technologies, numerous liposome-based formulations incorporating chemical drugs as active pharmaceutical ingredients (APIs) have entered clinical use.[28] The limitations of conventional small-molecule drugs have highlighted the need for advanced delivery systems capable of improving biodistribution, enhancing target specificity, and minimizing off-target effects. In this context, liposomes have emerged as a leading platform. Their application has extended beyond anticancer agents to encompass a broad range of chemical drugs, including anti-inflammatory and antimicrobial therapies, underscoring the versatility and growing clinical relevance of liposomal drug delivery systems.

2.1. Anti-Tumor

Bangham et al. first demonstrated that phospholipids spontaneously form bilayered spherical vesicles in aqueous environments, laying the foundation for the concept of liposomes through electron microscopy observations.[29] Initially, liposomes were utilized as biomimetic membrane models.[30] However, owing to their ability to encapsulate drugs within their aqueous core and regulate release through the lipid bilayer, along with their high biocompatibility, they later emerged as promising platforms for drug delivery.

While conventional chemotherapeutics remain the standard of care, their clinical application is limited by factors such as poor aqueous solubility, short half-life, drug resistance, and severe systemic toxicity.[31] To address these issues, liposomal drug delivery systems have been developed to enhance drug stability, prolong therapeutic effects, and improve tissue specificity, some of which have already entered clinical use.[32] Nevertheless, most liposomal formulations still rely on passive targeting, which may lead to variable therapeutic efficacy and nonspecific tissue accumulation.[7] To overcome this limitation, active targeting strategies utilizing antibodies, proteins, or aptamers are under investigation. Aptamers, in particular, are short DNA sequences (~30 kDa) that minimally affect the physical properties of nanoparticles.[33] For example, liposomes loaded with doxorubicin and modified with AS1411, an aptamer targeting nucleolin on tumor cells, significantly enhanced tumor-specific accumulation (~8-fold) and improved antitumor efficacy while reducing systemic toxicity. This formulation also induced immunogenic cell death, promoting $CD8^+$ T cell infiltration into tumors.[34]

Hydrophilic chemotherapeutics such as doxorubicin or gemcitabine present additional challenges when encapsulated in liposomes, including low loading efficiency and premature drug leakage from the aqueous core.[35,36] Such rapid release may result in off-target toxicity or reduced therapeutic efficacy.[37] Therefore, stable encapsulation and controlled release are essential for effective delivery.[38-40] In one study, "aromatized liposomes" were developed using synthetic phospholipids (Ph-DPPC, CM-DPPC) with aromatic substitutions at the terminal carbon chains to enhance π–π stacking interactions, thereby reducing membrane fluidity and permeability.[41] Compared to conventional liposomes, these formulations achieved a 19–60%

Table 1. Table of Liposome-Based Drugs Approved by the FDA (Food and Drug Administration, US) or EMA (European Medicines Agency, EU)

Drug type	Regulatory approval number (FDA/EMA)	Clinical products (approval year, area)	API (mechanism)	Lipid composition (molar ratio)	Indication	Company	ref
Anti-cancer	NDA050718	Doxil® (1995, US)	Doxorubicin (intercalates into DNA and inhibits topoisomerase II)	HSPC,cholesterol, PEG2000-DSPE (56:39:5)	Ovarian cancer, Kaposi's sarcoma, multiple myeloma	Sequus Pharmaceuticals	(19)
	NDA050704	DaunoXome® (1996, US)	Daunorubicin (intercalates DNA and inhibits topoisomerase II)	DSPC,cholesterol, daunorubicin (10:5:1)	Kaposi' s sarcoma	NeXstar Pharmaceuticals	(137)
	NDA207793	Onivyde® (2015, US)	Inrinotecan (inhibits topoisomerase I)	DSPC, PEG2000-DSPE (3:2)	Pancreatic adenocarcinoma	Merrimack Pharmaceuticals Inc	(138)
	NDA202497	Marqibo® (2012, US)	Vincristine (binds to tubulin and inhibits microtubule)	Sphingomyelin, Cholesterol (60:40)	Leukemia	Talon Therapeutics, Inc.	(139)
	NDA021041	DepoCyt® (1999, US)	Cytarabine (inhibits DNA polymerase)	Cholesterol,triolein,DOPC,DPPG (11:1:7:1)	Lymphomatous meningitis	SkyPharma Inc.	(140)
	NDA209401	Vyxeos® (2017, US)	Danuorubicin + Cytarabine (combines topoisomerase II inhibition with DNA synthesis inhibition)	DSPC,DSPG, cholesterol (7:2:1)	Leukemia	Jazz Pharma	(141)

Drug type	Regulatory approval number (FDA/EMA)	Clinical products (approval year, area)	API (mechanism)	Lipid composition (molar ratio)	Indication	Company	ref
Anti-bacterial	NDA50740	Ambisome® (1997, US)	Amphotericin B (binds to ergosterol in fungal membranes, increasing permeability)	HSPC:Cholesterol: DSPG (2:1:0.8)	Amphotericin B	Astellas Pharma	(142)
Vaccine	SE/H/0111/001 (EMA)	Epaxal® (1994, EU/Asia)	Hepatitis A antigen (induce immune activation by presenting viral antigens)	DOPE:DOPC (25:75)	Hepatitis A	Crucell Berna Biotech	(143)
	BLA 125742	Comirnaty® (2021, US/EU)	BNT162b2 mRNA (mRNA encoding SARS-CoV-2 spike protein)	ALC-0315, ALC-0159, DSPC, Cholesterol	COVID-19	Pfizer/BioNTech	(144)
	BLA 215742	Spikevax® (2022, US/EU)	mRNA-1273 (mRNA encoding SARS-CoV-2 spike protein)	SM-102, DMG-PEG, DSPC, Cholesterol	COVID-19	Modema	(145)

increase in encapsulation efficiency and nearly a two-fold reduction in initial drug leakage. However, due to their large average diameter (~1 μm), these liposomes may face limitations in tumor penetration, indicating the need for further size optimization or the incorporation of tumor-targeting strategies.

Another major limitation of chemotherapeutics is multidrug resistance (MDR) resulting from repeated high-dose administration. When drugs are administered at the maximum tolerated dose (MTD), cancer cells may develop resistance through mechanisms such as enhanced drug efflux, DNA repair, or evasion of apoptosis, thereby reducing therapeutic efficacy and increasing the risk of relapse.[42] To address this issue, co-delivery systems that simultaneously encapsulate two agents are emerging as promising strategies.[43,44] A recent example is a multifunctional "nanocracker" liposome co-loaded with pantoprazole (PZ) and paclitaxel (PTX), designed to overcome both P-glycoprotein (P-gp)-mediated resistance and tumor microenvironment (TME) barriers. PZ and PTX were encapsulated in the aqueous core and lipid bilayer, respectively, enabling sequential release—PZ within 12 hours and PTX over 48 hours. This approach enhanced drug uptake in P-gp-overexpressing cells and improved intracellular PTX accumulation and sensitivity via local pH modulation. As a result, the IC50 was reduced by 10.5-fold compared to single-agent liposomal formulations in vitro, and 80% complete tumor regression was achieved in a P-gp-overexpressing fibrosarcoma mouse model, demonstrating the potential of this strategy for MDR cancer therapy.

2.2. Anti-Inflammatory

Non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen and piroxicam, widely used for their anti-inflammatory properties, reduce prostaglandin synthesis and alleviate inflammation, pain, and fever offering the advantage of rapid symptom relief.[45] However, NSAIDs are associated with gastrointestinal toxicity, such as gastritis and ulcers, due to reduced mucosal protection, and long-term use may increase the risk of adverse effects, including osteoporosis, myocardial infarction, and stroke.[46] To address these limitations, liposomes have been investigated as drug carriers to improve the safety and efficacy of NSAID therapy. Encapsulation of NSAIDs in liposomes can reduce systemic toxicity by concentrating on the drug at the target site, enhance local anti-inflammatory effects, and mitigate adverse systemic reactions.[47]

In particular, intra-articular (IA) administration of NSAIDs is frequently employed for arthritis treatment to achieve localized drug effects and minimize systemic side effects.[48] However, repeated IA injections can lead to cartilage damage, and high-dose administration may result in rapid systemic absorption, causing off-target toxicity.[49] To overcome these challenges, sustained-release liposomal formulations have been developed to improve the pharmacokinetic and pharmacodynamic profiles of NSAIDs.[50] For example, a drug-fortified liposome incorporating 6-methoxy-2-naphthylacetic acid (6-MNA) in an ion-pair form with the basic lipid 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE) was designed to enhance local delivery and prolonged release.[51] The ion-paired form of 6-MNA has reduced aqueous solubility, enabling extended release even in aqueous environments, thereby reducing the frequency of IA injections. This formulation demonstrated prolonged retention within the joint space following IA administration and provided a sustained drug release profile, supporting its potential as a liposomal platform for localized and safer treatment of arthritis.

While exploiting low aqueous solubility offers a strategy for sustained drug release, it may also limit broader drug applicability.[47] Therefore, research efforts have also focused on enhancing the targeting efficiency of liposomes to inflamed tissues. One such approach involves the development of biomimetic macrophage membrane-coated liposomes (PM/TN-CCLP) for targeted therapy of aortic dissection (AD).[52] These liposomes, co-loaded with anti-inflammatory agents curcumin and celecoxib, exhibited enhanced homing ability to AD lesions characterized by macrophage infiltration. This targeted accumulation reduced off-target distribution and effectively attenuated the progression of both acute and chronic AD, highlighting the therapeutic potential of biomimetic liposomal platforms for inflammatory diseases.

3. Natural Compounds

Natural compounds are chemical compounds derived from living organisms found in nature, such as plants, animals, and microorganisms, and many of them have exhibited pharmacological or biological activity, making them valuable sources of therapeutic agents since ancient times.[53] These compounds possess a wide range of bioactivities including anticancer, anti-inflammatory, antioxidant, and antimicrobial effects and several clinically approved drugs, such as paclitaxel and artemisinin, have been directly derived from natural sources.[54] Despite their therapeutic potential, the clinical application of many natural compounds has been limited by poor aqueous solubility, instability, and consequently low bioavailability.[55] For instance, compounds such as curcumin, resveratrol, and artemisinin have shown promising bioactivity but have faced challenges in clinical translation due to pharmacokinetic limitations.[56,57] In the case of curcumin, although its safety was confirmed in a Phase I clinical trial with doses up to 12 g/day, achieving therapeutic plasma concentrations remained difficult.[58]

To address these limitations, recent efforts have focused on leveraging liposomal delivery systems to improve the pharmacokinetic profiles of natural compounds.[59] Liposomes are capable of encapsulating both hydrophilic and hydrophobic molecules, enhancing drug stability, enabling targeted delivery, and allowing for sustained release. These properties make liposomes particularly well-suited for overcoming the physicochemical drawbacks of natural compounds. Since the 1980s, liposomes have been actively explored as a promising strategy for improving the bioavailability and therapeutic efficacy of natural compounds, thereby broadening their

potential for clinical use.[60,61]

3.1. Polyphenol

In 1981, R.R. New et al. first applied liposomal drug delivery strategies to natural compounds by encapsulating amphotericin B and other antifungal agents into lecithin-based liposomes, thereby enhancing their anti-leishmanial activity.[62] Notably, liposomal amphotericin B enabled selective delivery to infected macrophages while reducing systemic toxicity. Compared to its non-liposomal counterpart, liposomal amphotericin B exhibited at least a six-fold increase in antileishmanial activity, with a parasite clearance rate of approximately 70%. This pioneering work is considered one of the earliest demonstrations of the practical potential of liposomes for natural compound delivery. Since then, this strategy has been extended to various natural compounds, particularly structurally unstable bioactive agents such as polyphenols.

Although polyphenols can be isolated through conventional precipitation techniques, they often suffer from structural instability during storage, leading to degradation via oxidation or hydrolysis and a consequent loss in bioactivity.[63,64] Liposomal encapsulation offers a protective barrier against environmental stressors (e.g., pH, light, digestive enzymes), thereby enhancing the stability and therapeutic potential of polyphenols.[65] For example, oleuropein, a polyphenol extracted from olive leaves (Olea europaea), was encapsulated into DOPC/Chol/GLT1 liposomes to improve its chemical stability and bioavailability.[66] This formulation demonstrated enhanced antibacterial activity against both wild-type and methicillin-resistant Staphylococcus aureus (MRSA) compared to free extract and remained stable for up to 90 days under ambient conditions.

Natural compounds and liposomes exhibit a complementary relationship. When polyphenols are encapsulated into liposomes, they can integrate into the lipid bilayer, improving encapsulation efficiency while their intrinsic antioxidant properties suppress lipid peroxidation, enhancing both physical stability and functional performance.[67,68] In particular, polyphenols derived from green tea have been shown to mitigate oxidative degradation of the lipid membrane and improve liposomal stability, despite their inherent poor solubility and instability.[69] Additionally, the incorporation of polyphenols from Centella asiatica into β-sitosterol-containing liposomes improved structural stability by modulating membrane fluidity through β-sitosterol, a cholesterol analogue.[70] This system also exhibited enhanced surface charge, suppressed lipid oxidation, and prolonged stability up to 28 days, preserving over 50% of the active ingredients and improving resistance to oxidative and thermal degradation. These findings suggest that polyphenols can function beyond antioxidants, serving also as stabilizers for oxidation-prone lipid-based formulations.

The complementary nature of natural compounds and liposomes has recently evolved into co-delivery strategies, where two or more natural compounds are encapsulated within a single liposome. This approach enables the synergistic or additive therapeutic effects of agents with distinct mechanisms of action. Numerous studies have reported that such systems improve bioavailability and enhance the functional performance of individual compounds.[68,71] For instance, the polyphenols naringenin (NRG) and trans-resveratrol (t-RES) exhibit broad-spectrum biological activities including anti-inflammatory, anticancer, antimicrobial, and anti-aging effects but their clinical translation is hindered by instability. While t-RES is susceptible to photodegradation, NRG is prone to oxidation.[72,73] To overcome these limitations, a co-delivery strategy encapsulating both compounds into a single liposome was proposed.[74] This formulation enhanced their stability and antioxidant capacity compared to their single-loaded counterparts, as the liposomal structure suppressed t-RES photodegradation and NRG oxidation. Consequently, antioxidant activity, as measured by DPPH radical scavenging and reducing power, increased up to two-fold, supporting the potential of liposomal co-delivery systems for improving the therapeutic efficacy of natural compounds.

3.2. Flavonoid

Fisetin (3,3′,4′,7-tetrahydroxyflavone) is a naturally occurring flavonoid derived from plants that exhibits anti-angiogenic and anticancer effects through multiple mechanisms, including inhibition of cyclin-dependent kinases, suppression of urokinase activity, activation of p53, and downregulation of the NF-κB signaling pathway.[75] However, its extremely low aqueous solubility (~0.01 mg/mL) and rapid metabolism in vivo result in poor bioavailability, which has limited its clinical translation.[76] To overcome these limitations, a PEGylated liposomal formulation encapsulating fisetin was developed, resulting in prolonged circulation time and enhanced anticancer efficacy.[77] The liposome, composed of DSPC, cholesterol, and DSPE-PEG2000, encapsulates fisetin within the hydrophobic bilayer based on its physicochemical properties. This formulation enhanced cellular uptake in various cancer cell lines, including Lewis lung carcinoma (LLC) cells, induced cell cycle arrest, and promoted apoptosis. In mouse models administered via intravenous (I.V.) or intraperitoneal (I.P.) injection, the liposomal fisetin showed a two-fold increase in plasma concentration and a ~47-fold improvement in relative bioavailability compared to free fisetin, highlighting the potential of natural product-based anticancer agents for clinical applications.

Plant-derived natural compounds have long been consumed in capsule or tablet form, typically administered orally.[78] While oral mucosal absorption offers the advantage of bypassing the gastrointestinal tract and enabling rapid systemic uptake, the oral bioavailability of flavonoids such as quercetin remains extremely low due to poor solubility, instability in the gastrointestinal environment, and extensive first-pass hepatic metabolism.[79] To address these limitations, numerous studies have incorporated liposomal systems to enhance oral delivery. For instance, total flavonoids extracted from Dracocephalum moldavica L. were encapsulated into complex phospholipid-based liposomes, which protected the compounds from

degradation in simulated gastric and intestinal fluids.[80] This formulation demonstrated up to a two-fold increase in stability compared to free flavonoids and sustained antioxidant and anti-inflammatory activity over an extended period.

Despite these advantages, liposomes and other nanocarriers primarily rely on passive targeting, wherein tumor accumulation is dependent on the enhanced permeability and retention (EPR) effect, leading to unpredictable delivery efficiency.[81] Tumor types with abundant extracellular matrix (ECM), such as pancreatic and hepatocellular carcinomas, often show poor drug accumulation and treatment resistance.[82] To address this challenge, a hyaluronidase-modified quercetin-loaded liposome (HQL) was developed to degrade hyaluronic acid in the ECM, thereby enhancing intratumoral penetration and delivery efficiency to tumor cells.[83] This strategy improved apoptotic activity in 3D pancreatic cancer spheroid and organoid models via enhanced tissue penetration, and consistent therapeutic outcomes were observed in vivo, demonstrating the potential of ECM-targeted liposomal delivery of natural products.

4. Gene

Gene therapy represents a groundbreaking strategy capable of treating the molecular basis of various diseases by replacing, silencing, or supplementing defective genes. However, the clinical application of nucleic acids such as plasmid DNA (pDNA), small interfering RNA (siRNA), and messenger RNA (mRNA) is hindered by their inherently poor membrane permeability, rapid degradation, and short circulation half-life, necessitating the development of efficient delivery systems.[84,85] While viral vectors currently dominate clinical gene therapy trials due to their high transfection efficiency and ability to modulate long-term gene expression, they suffer from major limitations, including restricted payload capacity and serious safety concerns, such as insertional mutagenesis and strong immunogenicity.[86] Notably, clinical use of recombinant retroviral and adenoviral vectors has been associated with fatal outcomes in two out of four reported cases.[31,87] As a result, there has been increasing interest in non-viral delivery platforms, among which liposomes have emerged as promising candidates due to their biocompatibility, tunable design, and ability to form stable complexes with various nucleic acids. In particular, cationic liposomes composed of positively charged lipids such as DOTAP, DOTMA, and DDAB are widely employed for nucleic acid delivery.[88] Despite potential cytotoxicity at high concentrations, cationic liposomes facilitate efficient cellular uptake through electrostatic interactions with negatively charged proteoglycans and promote endosomal escape via membrane fusion, thereby achieving high transfection efficiency.[89]

4.1. DNA

The concept of liposome-mediated nucleic acid delivery was first introduced by Felgner et al. in 1987, through the development of a novel synthetic cationic lipid, DOTMA (N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride), which laid the foundation for the lipofection technique.[90] DOTMA-based liposomes were capable of forming stable lipid-DNA complexes via electrostatic interactions with negatively charged DNA, promoting membrane fusion and facilitating efficient intracellular gene transfer. Compared to conventional methods such as calcium phosphate or DEAE-dextran, lipofection demonstrated up to 100-fold higher transfection efficiency, highlighting the potential of simple formulation strategies for enhanced non-viral gene delivery.

Cationic liposomes are widely considered safe and effective non-viral vectors for both in vitro and in vivo DNA delivery.[91] However, their clinical translation remains challenged by several biological barriers, including cytotoxicity from high surface charge, nonspecific interactions with plasma proteins, and poor tissue specificity.[92] To address these limitations, one study explored surface modification of cationic liposome–DNA complexes (CL-DNA NPs) using various homing peptides (e.g., linear RGD, iRGD, cyclic RGD, and linear RPARPAR), revealing that differences in peptide identity and charge density significantly influenced cellular binding, tumor accumulation, and delivery efficiency.[93] These findings emphasize the importance of optimizing surface charge and ligand presentation for effective gene delivery. Additionally, shielding surface charge using polymers or ligands has been employed to improve biocompatibility and targeting specificity.[94,95] Beyond delivery, CL-DNA complexes have shown potential to induce immunogenic cell death (ICD) in tumor cells, enhancing antigen presentation, activating dendritic cells, and promoting anti-tumor immune responses involving $CD8^+$ T cells and M1 macrophages.

Still, intracellular degradation of exogenous genes in endosomes and lysosomes poses a major hurdle.[96] To circumvent this, endoplasmic reticulum (ER)-targeted liposomes (Lipo-Par), incorporating the cationic peptide pardaxin (Par), were developed to exploit non-lysosomal caveolin-mediated endocytosis and enhance nuclear delivery.[97,98] Lipo-Par demonstrated up to 420-fold higher transfection efficiency than conventional liposomes and significantly improved the in vivo anti-tumor efficacy of therapeutic genes such as p53 and PTEN.

Advancements in liposomal gene delivery have also enabled combination therapies wherein drugs and plasmid DNA are co-encapsulated within a single liposome, thereby enhancing synergy between chemotherapy and gene therapy.[99] One such strategy, designed to target cancer stem-like cells (CSCs) resistant to TRAIL (tumor necrosis factor-related apoptosis-inducing ligand), involved a dual-loaded liposome (pTRAIL/Sal/L-HA) encapsulating salinomycin (Sal) and pTRAIL while incorporating hyaluronic acid (HA) for CSC targeting.[100] Sal upregulated death receptor expression in CSCs,

sensitizing them to TRAIL-induced apoptosis and resulting in improved therapeutic efficacy.

In parallel, CRISPR/Cas9 genome-editing technology has emerged as a promising tool for correcting disease-causing genetic mutations at the DNA level.[101] Delivering CRISPR/Cas9 components such as guide RNA (gRNA) and Cas9 nuclease via pDNA offers advantages in manufacturing and gene expression stability but requires efficient nuclear transport.[102] Recently, a protamine-liposome hybrid system was developed to deliver pDNA encoding CRISPR/Cas9 for the targeted knockout of MTH1 in non-small cell lung cancer (NSCLC), effectively inhibiting tumor growth and metastasis.[103]

4.2. messenger RNA (mRNA)

The remarkable success of mRNA-based vaccines (BNT162b2, mRNA-1273) during the COVID-19 pandemic has accelerated research on mRNA delivery technologies, with a primary focus on vaccine development. As demonstrated by extensive preclinical studies and ongoing clinical vaccine programs, liposomes and other lipid-based carriers have emerged as both early and promising platforms for the delivery of mRNA and protein antigens.[104-106] In this context, novel formulations such as histidylated liposomes incorporating cationic lipids with histidine-derived headgroups have been proposed to improve mRNA delivery efficiency.[107,108] Despite these advances, all six virus-related mRNA vaccines currently in clinical trials are formulated as lipid nanoparticles (LNPs) and administered intramuscularly.[109,110] The manufacturing feasibility and efficacy of liposomal or lipid particle-based mRNA vaccines across various routes of administration remain under investigation.[111] One study employing a liposome-based SARS-CoV-2 RBD-mRNA vaccine highlighted that the immunogenicity, including virus-neutralizing capacity and T cell responses (Th1 vs. Th2 type), varies significantly depending on the administration route, emphasizing the importance of route optimization for improved efficacy and safety.[112]

To overcome the liver-targeted accumulation characteristic of liposomes, surface engineering strategies have been proposed to enhance intracellular delivery to specific immune cells and thereby promote robust immune responses.[113] In particular, liposomes engineered to function as both carriers and immunostimulatory agents have been explored.[114] For example, surface modification of DOTAP-based liposomes with a cholesterol-conjugated cationic antimicrobial peptide, DP7 (DP7-C), facilitated mRNA delivery to dendritic cells (DCs), enhanced DC maturation and $CD103^+$ DC populations, and promoted pro-inflammatory cytokine secretion, thereby improving both mRNA delivery and antigen presentation.[115]

Meanwhile, cancer immunotherapy has gained increasing attention as a strategy to combat malignancies with the highest morbidity and mortality. Tumor vaccines that elicit immune responses against tumor-associated antigens (e.g., proteins, DNA, RNA) are under active investigation, with several candidates advancing to clinical trials.[116] Most mRNA-based cancer vaccines in development are administered via intradermal, intravenous, subcutaneous, or intramuscular routes.[117] However, the inherent hepatic accumulation and rapid clearance of liposomes present challenges for RNA delivery to extrahepatic tissues.[118] To address this, lipid engineering approaches have been developed to enable tissue-specific delivery particularly to the lungs following systemic administration.[118,119] Although pulmonary delivery using non-invasive nebulizers is a promising alternative, structural instability of nanoparticles during aerosolization can hinder mRNA delivery efficiency, necessitating the development of specialized formulations.[120]

Conventional intramuscular or intravenous administration of liposomal mRNA vaccines faces limitations due to rapid systemic clearance via protein interactions and suboptimal lymphatic trafficking, which may trigger excessive systemic immune responses upon repeated dosing. To circumvent these issues, mucosal delivery strategies with strong immunostimulatory potential are being actively explored.[121] Among them, intranasal delivery offers a non-invasive route with high patient compliance and the ability to simultaneously induce local IgA-mediated and systemic IgG-mediated immunity via stimulation of nasal-associated lymphoid tissue (NALT).[122] To promote effective intranasal delivery of mRNA antigens and elicit anti-tumor immunity, a cationic liposome–protamine complex (LPC) formulation was administered, which significantly enhanced IL-12 and TNF-α secretion in DCs and activated $CD4^+$ and $CD8^+$ T cells in a Lewis lung carcinoma (LLC) mouse model.[123] These findings support the potential of intranasal liposome-based mRNA vaccine platforms to induce robust anti-tumor immune responses.

4.3. small interfering RNA (siRNA)

Small interfering RNA (siRNA) is a nucleic acid-based therapeutic modality that silences specific gene expression at the post-transcriptional level, with several siRNA-based drugs currently under clinical development for various diseases.[124, 125] Liposomal delivery of siRNA has been extensively investigated over the past decades, with recent studies focusing on formulation optimization and combination strategies with other therapeutic modalities. While liposomes are one of the most widely studied carriers for siRNA, they present limitations in terms of delivery efficiency, safety, and target specificity.[126] In contrast, extracellular vesicles (EVs) exhibit excellent biocompatibility and inherent targeting ability to parental cells, but their clinical translation is hindered by low loading efficiency of exogenous RNA cargo.[127] To overcome these limitations, hybrid nanoparticles composed of natural EVs and synthetic liposomes have been proposed as an emerging delivery strategy. Several studies have demonstrated that EV–liposome hybrid nanoparticles exhibit distinct profiles in cellular uptake, gene silencing, and toxicity depending on the EV source and cell type, while preserving the intrinsic functional properties of EVs (e.g., regenerative potential).[128]

While siRNA-based therapies suppress specific oncogenic gene expression in tumor cells to induce apoptosis, the

immunosuppressive tumor microenvironment (TME) remains a major barrier to their therapeutic efficacy.[129] Consequently, siRNA delivery systems are increasingly being engineered not only for gene silencing but also to modulate the immune landscape or to be co-administered with immunomodulatory agents. One such strategy involved an aptamer-conjugated liposome co-encapsulating doxorubicin (DOX), an inducer of immunogenic cell death (ICD), and IDO1 siRNA (Aptm [DOX/IDO1]) for TME remodeling.[130] The liposome surface was functionalized with aptamers targeting CD44 and PD-L1 to enhance tumor specificity and enable efficient co-delivery of both agents to breast cancer cells. DOX induced ICD and enhanced antigen presentation, while siRNA-mediated knockdown of IDO1 reversed the immunosuppressive TME, ultimately yielding a potent antitumor immune response.

Liposomal penetration into tumors is often hindered by the dense extracellular matrix (ECM), limiting delivery to target cells.[131] Furthermore, intracellular siRNA degradation within endosomes and lysosomes reduces gene silencing efficacy.[132] To address these challenges, combination strategies incorporating physical stimuli such as ultrasound or light-based therapies have been explored.[133,134] For example, to overcome trametinib (Tr) resistance in non-small cell lung cancer (NSCLC) harboring BRAF-V600E mutations, a liposome co-loaded with BRAF siRNA and Tr (Tr/siRNA@Lip-FA) was developed. In BRAF-overexpressing NSCLC models, this platform effectively suppressed resistance, and when combined with ultrasound propulsion, further enhanced tumor accumulation and therapeutic efficacy.[135] In another study, a cationic liposome–microbubble hybrid complex was formulated for ultrasound-targeted microbubble cavitation (UTMC). This formulation demonstrated higher siRNA loading capacity compared to conventional microbubbles and achieved effective site-specific siRNA delivery in squamous cell carcinoma upon UTMC treatment.[136]

DISCUSSION

Liposome-based drug delivery systems have long been studied as a versatile platform to improve the therapeutic efficacy of various agents by offering biocompatibility, protection of cargo, controlled release, and targeting capabilities. In this review, we examined recent advances and representative applications of liposomal formulations across diverse therapeutic classes, with the aim of providing insight into the future potential of liposomes as drug carriers.

Conventional liposome-based delivery systems largely rely on passive targeting, which can result in variable therapeutic outcomes due to disease heterogeneity and interpatient physiological differences. Furthermore, nonspecific accumulation can lead to unwanted side effects. To address these limitations, active targeting strategies have been actively pursued, including the surface functionalization of liposomes with targeting ligands or the design of biomimetic liposomes that emulates immune cell or cancer cell membranes. In the case of chemical drugs, therapeutic efficacy can be compromised by low encapsulation efficiency and premature drug leakage. To overcome this, strategies such as promoting lipid–drug interactions or reducing solubility by modulating drug ionization have been proposed to achieve stable encapsulation and sustained release. As a potential approach to overcome the instability of liposomes, the incorporation of antioxidant components has been proposed to improve their structural and physicochemical stability. In addition, lipid engineering approaches such as using pH-responsive lipids or lipids that facilitate non-lysosomal trafficking have been developed to circumvent lysosomal degradation and maximize cytosolic delivery. Moreover, co-delivery systems that encapsulate multiple therapeutic agents to generate synergistic effects have also garnered increasing attention.

Despite extensive research and the clinical approval of several liposomal drugs, many liposomal formulations continue to face challenges in clinical translation. Future efforts should thus focus not only on improving drug release control, targeting specificity, circulation time, and stability, but also on ensuring scalable manufacturing and batch-to-batch reproducibility to facilitate the development of clinically translatable liposomal platforms. In parallel, the establishment of standardized evaluation criteria and analytical methods will be critical to accelerate regulatory approval. In particular, with the rapid progress in gene-based therapeutics, there is a growing need to re-evaluate the potential of liposomes in the delivery of nucleic acid drugs. Although liposomes have long been investigated for delivering pDNA, mRNA, and other nucleic acids, current clinical and research trends are heavily focused on lipid nanoparticles (LNPs). Unlike LNPs, which typically require four distinct lipid components, liposomes can often be formulated using only two or three lipids, thereby reducing GMP manufacturing costs and simplifying regulatory approval processes. Therefore, continued structural and functional refinement of liposomes is essential to overcome current limitations in nucleic acid delivery and to re-establish their relevance in the evolving landscape of gene therapy. Looking forward, advancing liposomal drug delivery technologies toward clinical translation will depend on addressing regulatory approval pathways, ensuring large-scale reproducibility, and optimizing cost-effective manufacturing. These considerations are critical for

enabling broader accessibility and therapeutic applications.

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The authors declare no competing interests.

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