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Advances in Nebulized Lipid Nanoparticles (LNPs) for Nucleic Acid Delivery

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Abstract. Lipid nanoparticles (LNPs) exhibit significant potential as highly efficient carriers for nucleic acid therapeutics in the treatment of pulmonary diseases. Nebulized inhalation delivery, which directly targets the lungs through respiratory tract, represents an ideal pulmonary administration route. However, the clinical translation of nebulized LNP-based delivery still faces several critical challenges. Intense shear forces during nebulization impair the structural integrity and stability of LNPs, leading to nucleic acid leakage. The mucin network within the pulmonary mucus layer forms a physical barrier that restricts LNP diffusion, while non-specific phagocytosis by alveolar macrophages further decreases delivery efficiency. In recent years, various design strategies have been developed to address these limitations. Modifying LNP component ratios, optimizing buffer formulations, and functionalizing LNP components have collectively improved stability, enhanced mucus penetration, reduced macrophage uptake, and increased cellular uptake by epithelial cells. This systematic review analyzes the key challenges of nebulized LNP delivery, summarizes recent breakthrough, and outlines future research directions, thereby providing theoretical insights for developing efficient pulmonary nucleic acid delivery systems.

Keywords: lipid nanoparticles (LNP), nebulized inhalation, nucleic acid delivery, lung-targeted delivery

1. Introduction

Nucleic acid therapeutics are therapeutic agents that take DNA or RNA as their core molecular scaffold. Owing to their unique potential to selectively target virtually any gene, they have emerged as pivotal representatives of the latest generation of precision therapeutics, holding broad clinical application prospects for an array of intractable diseases [1]. In contrast to conventional therapeutics (e.g., small-molecule chemical drugs and antibody-based biologics), nucleic acid therapeutics are featured with shorter development cycles and higher success rates in clinical translation, thus providing distinct advantages in cost-effectiveness and target specificity [2]. However, nucleic acid therapeutics are inherently confronted with stability challenges. Naked or unmodified nucleic acid molecules are highly prone to enzymatic and chemical degradation, which gives rise to extremely short circulatory half-lives and thus severely compromises their therapeutic efficacy [3]. Consequently, developing efficient and safe delivery systems to improve the in vivo stability of

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nucleic acid therapeutics has become a critical technological bottleneck as well as a major research priority for advancing their clinical translation [4].

LNPs currently represent the most widely used carrier system for nucleic acid drug delivery. At physiological pH (7.35–7.45), LNPs display a near-neutral surface charge, which significantly reduces toxicity and enhances their safety profile [5,6]. Although LNPs are typically administered intravenously, they show predominant accumulation in the liver, limiting targeted delivery to other organs. To attain pulmonary targeting, LNPs can be delivered via nebulized inhalation. This method transforms LNP suspensions into inhalable aerosol particles, permitting non-invasive and direct action on lung tissue. It bypasses rapid hepatic clearance and minimizes off-target delivery to other organs. Moreover, this approach circumvents the pulmonary endothelial barrier, offering an efficient and precise route for pulmonary targeted delivery of nucleic acid therapeutics [7].

Nevertheless, nebulized inhalation delivery systems encounter multiple challenges in the delivery of LNP-mediated nucleic acid drugs. First, the intense shear forces induced during nebulization pose a significant threat to the structural integrity and stability of LNPs, which may result in leakage of the encapsulated nucleic acid drugs. Second, the complex composition and structure of the pulmonary mucus barrier impede the diffusion of LNPs, limiting their effective migration to the surface of pulmonary epithelial cells. Additionally, macrophage-mediated phagocytosis in the pulmonary innate immune system remarkably compromises delivery efficacy by reducing the intracellular uptake of nucleic acid therapeutics.

Therefore, developing LNP systems with simultaneous nebulization stability, robust targeting capability, and high transfection efficiency is paramount for nucleic acid-based therapies against pulmonary diseases (e.g., cystic fibrosis, acute lung injury, and lung cancer). This review focuses on the core obstacles in nebulized inhalation delivery of LNP-based nucleic acid therapeutics. It systematically summarizes recent innovative solutions, design strategies, and relevant mechanistic research advances targeting these challenges in three key technical directions: optimizing LNP component ratios, designing nebulization-compatible buffer systems, and introducing functionalized modifications. This review is intended to provide theoretical insights for the development of highly efficient, lung-targeted LNP delivery systems.

2. Barriers to LNP delivery via nebulized inhalation

The nebulized inhalation route presents multiple physical and biological barriers that remarkably compromise the delivery efficacy of LNPs. In particular, insufficient LNP stability, blockage by the pulmonary mucus barrier, and macrophage-mediated phagocytosis stand out as three major challenges in the delivery process.

2.1. Stability

Drug nebulization is generally accomplished either by directly generating droplets from liquid formulations or preparing inhalable powders for aerosolization into the respiratory tract [8]. This physical process induces high-intensity shear forces and thermal stresses intrinsic to nebulization, which remarkably compromise the stability of LNPs as delivery carriers for nucleic acid therapeutics [9]. When exposed to nebulization, LNPs tend to aggregate or disintegrate, resulting in premature release of encapsulated nucleic acid cargo. Leaked nucleic acid drugs, deprived of the protective outer layer of LNPs, are highly susceptible to degradation and exhibit limited capacity to penetrate the mucus layer or enter target cells, thus losing their therapeutic efficacy. Consequently,

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the overall delivery performance is impaired, lowering the effective drug concentration at target sites and thereby reducing pulmonary transfection efficiency [10,11].

2.2. Mucus barrier

The airway mucus gel layer serves as a critical delivery barrier that impairs the efficacy of nebulized LNPs, and is a primary factor contributing to low pulmonary delivery efficiency. Within pulmonary tissue, the ciliated airway epithelium and the mucus layer constitute the mucociliary clearance system, with the mucus layer serving as a selective filter. It primarily traps drugs through size-based filtration and chemical interactions, including enzymatic degradation. Drugs are trapped in the mucus layer by steric hindrance and adhesive forces (e.g., electrostatic interactions, hydrogen bonds, hydrophobic interactions), preventing penetration into deeper pulmonary regions. Subsequently, the trapped inhaled drugs are cleared through the coordinated ciliary beating of epithelial cells [12,13]. These mechanisms synergistically reduce LNP delivery efficiency. The pulmonary mucus layer comprises a viscoelastic gel layer and a low-viscosity sol-like layer, which are responsible for particle adsorption and trapping, and for transport and clearance within the gel, respectively [14]. Mucus is a negatively charged hydrogel-like substance, with mucin as its main functional component. It consists of water, glycoproteins, inorganic salts, lipids, proteins, and DNA, in which mucin-nanoparticle interactions play a key role in regulating particulate drug delivery systems [15]. LNPs are easily trapped by airway mucus and subsequently cleared from the lung through the mucociliary clearance system [16]. In pulmonary diseases, immune responses induce excessive mucus production from the airway epithelium and submucosal glands, further exacerbating the impediments to drug delivery [17].

2.3. Macrophage phagocytosis

The human lung contains multiple cell types, including epithelial cells, goblet cells, immune cells, neuroendocrine cells, fibroblasts, endothelial cells, and smooth muscle cells [18]. Among these cell types, alveolar macrophages account for more than 90% of airway immune cells [19]. The lungs contain approximately 500 million alveoli, each containing 12–14 alveolar macrophages responsible for phagocytosing and degrading insoluble particles deposited there [20]. Studies have shown that particles with a geometric diameter of approximately1–5μm are preferentially phagocytosed by alveolar macrophage [21,22]. Alveolar macrophage-mediated immune clearance results in the recognition and elimination of LNPs, thereby reducing their pulmonary accumulation [23,24]. Furthermore, alveolar epithelial cells serve as the primary target for most pulmonary diseases. Strategies to enhance drug uptake by pulmonary epithelial cells, reduce macrophage phagocytosis, and improve epithelial cell-targeted localized accumulation are particularly critical for effective pulmonary delivery [25,26].

3. Optimization strategies for nebulized LNP delivery systems

To address the three key barriers of insufficient LNP stability, the pulmonary mucus barrier, and macrophage-mediated clearance, this review summarizes strategies focusing on three main approaches: optimizing LNP formulation ratios, optimizing buffer formulations, and modifying LNP components.

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3.1. Optimization of LNP component ratios

LNPs typically consist of ionizable lipids, cholesterol, helper lipids (e.g., phospholipids) and PEG lipids. These components synergistically protect, deliver, and release nucleic acid therapeutics, and the ratios of these components exert a decisive effect on delivery efficacy [27]. Optimizing the component ratios of LNPs can enhance their stability, improve mucosal penetration, and increase targeting specificity to pulmonary epithelial cells, thereby improving delivery and transfection efficiency.

PEG lipids play a critical role in modulating LNP particle size. In 2021, Melissa P. Lokugamage et al. characterized six LNP clusters with distinct compositions, molar ratios, and zeta potentials to systematically evaluate the impact of LNP composition on delivery efficacy. Using the oligomer-lipid conjugate 7C1 as the ionizable lipid, a total of 56 distinct LNPs were prepared. Dynamic light scattering (DLS) analysis revealed that LNPs lacking PEG-lipids or containing low PEG levels displayed hydrodynamic diameters exceeding 200 nm, resulting in poor stability and failure to meet experimental criteria. Moreover, LNPs formulated with low molar ratios of PEG-lipids tended to form larger particle sizes than those with high molar ratios, indicating that particle size is correlated with both the presence and concentration of PEG. Adjustment of the LNP formulation alone resulted in a 28-fold difference in delivery efficacy between the top-performing and poorest-performing LNPs, highlighting the critical role of formulation optimization. The top five performing LNPs contained high PEG molar ratios (25%, 30%, 35%, 45%, 55%, and 60%) and demonstrated significantly higher delivery efficiency, as indicated by the fold change in total flux (FC-TF), whereas, LNPs with low PEG molar ratios (2.5%, 6.5%, and 15%) showed relatively poor performance. Furthermore, experimental data demonstrated that the combination of cationic helper lipids with high PEG content improves mRNA delivery efficiency after nebulization. Based on these findings, researchers selected an LNP designated NLD1-formulated with high PEG-lipid content and cationic helper lipids for further analysis, with a component ratio of 7C1: cholesterol:C14PEG2000:DOTAP(35:5:55:5). Biodistribution studies showed that NLD1 efficiently delivered mRNA throughout the lung tissue and was internalized by four major epithelial cell subtypes. It was also capable of delivering mRNA encoding broadly neutralizing antibodies, thereby protecting mice against lethal H1N1 influenza infection [28]. These studies confirm that the rationally increasing in the PEG-lipid molar ratio of LNPs can effectively improve both stability and delivery efficiency.

In 2024, Xin Bai et al. developed a four-step workflow for the 'LOOP' platform, enabling the generation of LNPs with high shear resistance and robust luciferase expression without extensive screening. Seven distinct formulations with varying molar ratios of ionizable lipid components (AA3-Dlin), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and 1,2-dimethylethanolamine-rac-glycero-3-methoxy polyethylene glycol-2000 (DMG-PEG2000) were designed for LNP screening. By evaluating encapsulation efficiency (EE), particle size and polydispersity index (PDI) before and after nebulization across the seven formulations, the LNP with a molar ratio of AA3-Dlin:DSPC:cholesterol:DMG-PEG2000 (60:20:19:1) was identified as having optimal shear resistance [29].

In 2024, Belal Tafech et al. employed Brownian kinetic simulations to investigate the multifactorial influences on LNP diffusion rates, including mucin concentration, salivary acidity, pH, and PEG concentration in both cystic fibrosis (CF) patients and healthy individuals. The researchers varied the molar concentration of DMG-PEG 2000 between 1% and 5%. The results indicated that higher PEG concentration reduced LNP particle size. At low PEG levels, LNPs aggregated into larger clusters, whereas a denser PEG layer on the LNP surface suppressed such

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fusion events, preventing particle aggregation during storage and circulation and preserving particle size [30,31]. These findings suggest that optimizing PEG-lipid concentration may be critical for substantially enhancing LNP diffusion rates in the mucus of CF patients.

Optimizing the PEG ratio enables regulation of LNP particle size and enhances mucus barrier penetration, thereby improving diffusion efficiency. Studies have demonstrated that nanoparticles with smaller sizes and a dense, protective PEG coating traverse the mucus barrier more effectively [32]. PEG leverages its near-neutral charge and hydrophilicity to form a hydration layer [31], which helps the carrier repel adsorption and binding to mucins. This minimizes adhesion between mucus and nanoparticles while facilitating penetration. The molecular weight, chain length, and surface density of PEG all influence mucus penetration capacity [33]. Increasing the molar percentage of PEG-lipids in LNP formulations from 1.5% to 4.5% has been demonstrated to moderately decrease LNP aggregation and enhance diffusivity in mucus [10]. Additionally, optimizing PEG ratios can modulate macrophage-mediated phagocytosis by reducing LNP immunogenicity through inhibition of complement activation, thereby prolonging systemic circulation. Complement activation occurs when complement components bind to the surface of LNPs, triggering recognition and clearance by macrophages. PEG-lipids form a hydrophilic water layer on the particle surface, generating steric hindrance that inhibits complement activation, helps maintain therapeutic concentrations in circulation, and improves overall LNP delivery efficiency [30].

Although PEG plays a critical role in enhancing LNP stability and facilitating mucus barrier penetration, studies have shown that higher PEG content (≥3%) reduces ApoE adsorption to LNPs, thereby decreasing cellular uptake [34]. Excessive PEG can also compromise LNP stability and decrease transfection efficiency. Belal Tafech et al. assessed the transfection efficacy of LNPs with elevated PEG ratios in primary human bronchial epithelial cells isolated from CF patients. Their results indicated that higher PEG concentrations reduced functional green fluorescent protein (GFP) expression, impairing both mucosal delivery and transfection efficiency [31].

In summary, a PEG molar ratio of approximately 1–3% appears to achieve an optimal balance between diffusion efficiency and transfection efficacy. Further experimental studies are required to define precise PEG ratios for different therapeutic applications.

3.2. Optimization of nebulization and dialysis buffers in LNP delivery systems

Optimizing the nebulization and dialysis buffers, along with the pH of LNP delivery systems, significantly enhances nebulization delivery efficiency.

In 2023, Allen Y. Jiang et al. compared two nebulization buffers: 0.9% saline (pH 7.0), 100 mM sodium acetate (NaAc, pH 5.2), and phosphate-buffered saline (PBS) to optimize LNP delivery. The results demonstrated that the slightly acidic NaAc buffer protonates the ionizable lipids in LNPs, promoting electrostatic repulsion and reducing aggregation, which significantly enhances nebulization delivery efficiency. Additionally, the addition of the excipient branched PEG (bPEG20K) to either buffer mitigated the increase in particle size following nebulization. Specifically, the combination of 2% bPEG20K with the NaAc buffer resulted in the smallest LNP particles after nebulization, with morphology largely preserved [35].

In 2024, Xin Bai et al. examined the effects of dialysis buffers and excipients on the nebulization stability of LNPs. Dialysis was conducted using two buffers: phosphate-buffered saline (PBS, pH 7.4) and 4-(2-Hydroxyethyl)-1-piperazine ethane sulfonic acid (HEPEs, pH 6.0). Changes in LNP encapsulation efficiency (EE), particle size, and polydispersity index (PDI) were quantified before and after nebulization. Data indicated that using HEPEs (pH 6.0) as the dialysis buffer resulted in minimal changes in these parameters post-nebulization, identifying it as the optimal buffer for

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subsequent experiments. Ethanol, propylene glycol, and Poloxamer 188 (a non-ionic surfactant) were subsequently added to the nebulization buffer to enhance shear-induced stability. The results showed that higher excipient concentrations reduced particle size and PDI variation, increased pulmonary luciferase expression, and improved LNP stability. Both the 12% ethanol nebulization system and the 8 mg/mL poloxamer 188 nebulization system exhibited elevated pulmonary luciferase expression. In contrast, the latter showed superior safety compared to the 12% ethanol system, which generated toxic LNPs after nebulization. By measuring surface tension and viscosity across different nebulization systems, the team determined that enhanced luciferase expression is likely associated with reduced surface tension and decreased solution viscosity. Encapsulating IL-11 single-chain variable fragment (scFv) mRNA into the aforementioned LNPs to form iLNP-HP08LOOP enabled efficient delivery and secretion of IL-11 scFv into the lungs of male mice, thereby effectively inhibiting pulmonary fibrosis [29].

In 2024, Belal Tafech et al. evaluated the diffusion rate of LNPs in mucin hydrogels across a pH range of 3–7. The result indicated that the highest diffusion rate was observed at pH 5, with a diffusion coefficient of approximately 0.7, confirming that a slightly acidic pH is optimal for LNP diffusion [31].

Targeted optimization of the composition and pH of key buffers (nebulization buffer, dialysis buffer) in LNP delivery systems significantly enhances nebulization efficiency.

3.3. Modifying the composition of LNPs

Adjusting the types and concentrations of ionizable lipids, cholesterol, helper lipids, and PEG lipids in LNPs can effectively improve their stability and delivery efficiency. Furthermore, this strategy may enhance targeting of LNPs to pulmonary epithelial cells, thereby further improving transfection efficiency.

3.3.1. Ionizable lipids

In 2023, Mae M. Lewis et al. selected three potent ionizable lipids (MC3, SM-102, and ALC-0315) to prepare three LNP variants, differing only in the type of ionizable lipid, while the ratios of other components, including helper lipids, PEG-lipids, and cholesterol, were kept constant. Screening results showed that the SM-102-containing LNP (B-1) displayed the highest transfection efficiency and mRNA expression in the lungs. Subsequently, researchers assessed the types of cells transfected by B-1 in the lungs. Flow cytometry result demonstrated that 8.9% of epithelial cells, 1.9% of immune cells, and 0.6% of endothelial cells were tdTomato-positive. Compared to other cell types, epithelial cells exhibited relatively higher uptake efficiency during nebulized delivery of B-1. In contrast, the transfection rate of endothelial cells decreased significantly from 66% to 0.6%. These findings highlight the potential of B-1 for treating pulmonary diseases such as cystic fibrosis [36]. It is clear that altering the type of ionizable lipid can effectively regulate the cellular targeting specificity of LNPs.

In 2025, Ke Huang et al. synthesized a series of degradable ionizable glycerides with branched tails and five ester bonds through a three-step esterification process. Following systemic delivery and tracheal aerosolization, luciferase expression validation showed that among the 23 glycerolipids, TG4C achieved significantly higher in vivo mRNA delivery efficiency compared to commercial MC3, SM102, and ALC0315. Compared to commercial SM102-LNP and MC3-LNP, TG4C-LNP exhibited a sixfold increase in luciferase expression. Formulation screening results indicated that LNPs formulated at a molar ratio of TG4C:DOPE:cholesterol:DMG-PEG (50:10:38.5:1.5) retained

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high stability following nebulization. In an elastase-induced mouse model of pulmonary emphysema, TG4C-LNPs loaded with hepatocyte growth factor (HGF) mRNA significantly reduced the secretion of inflammatory cytokines (interleukin (IL)-1β, interleukin (IL)-6, and tumor necrosis factor (TNF)-α) and alleviated alveolar wall thinning. Additionally, partial replacement of cholesterol with the anti-inflammatory glucocorticoid Bude in TG4C-LNPs resulted in comparable protein expression while significantly enhancing therapeutic efficacy. After nebulization, LNPs containing 25 mol% Bude exhibited comparable mRNA encapsulation efficiency, percentage of EGFP-positive cells, and fluorescence intensity to cholesterol-containing LNPs in both A549 and BEAS-2B cell lines [9]. Therefore, LNPs formulated with ionizable lipids like TG4C offer significant advantages in nebulized delivery, with their cholesterol content being partially replaced by Bude while maintaining functional equivalence.

3.3.2. Cholesterol

In 2022, Jeonghwan Kim et al. incorporated the cholesterol analog β-sitosterol into LNPs, using β-sitosterol to induce a polyhedral shape that facilitates endosomal escape. The optimized LNPs showed uniform particle distribution, polyhedral morphology, and rapid mucosal diffusion, leading to enhanced gene transfection efficiency. This strategy effectively overcame the limitations of intracellular mRNA delivery, which were caused by the PEG shell inhibiting receptor-mediated endocytosis and interfering with LNP endosomal escape through the reduction of serum protein adsorption [37,38]. The researchers selected LNP-Sito/3.5 as the optimized formulation for further studies, based on its physicochemical properties, mucosal activity, and in vivo efficacy, and named it nebulizable LNPs (nLNPs). This formulation successfully delivered mRNA encoding the cystic fibrosis transmembrane conductance regulator (CFTR) to CFTR-deficient animal models, leading to pulmonary expression of this therapeutic protein for cystic fibrosis treatment [39].

3.3.3. Helper lipids

In 2024, Shuai Liu et al. utilized charge-assisted stabilization technology to incorporate an optimized amount of negatively charged peptide-lipid conjugates into conventional four-component LNPs, generating a LNP formulation termed CAS-LNP. The team conjugated a polypeptide sequence composed of aspartic acid, two serine molecules, and cysteine (DSSC) to 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) on the outer membrane of LNPs. The amino group of DOPE was modified with N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), sequentially forming PDP-DOPE and DSSC-DOPE. By selecting DOPE instead of PEG for conjugation, the team effectively eliminated PEG-induced interference, facilitating the validation of the charge-assisted stability hypothesis in LNPs. The team varied amounts of DSSC-DOPE (0.6%, 2.5%, and 10% of the total lipid molar fraction) to regulate the surface charge of CAS-LNPs. The results showed that CAS-LNPs displayed excellent stability during nebulization, accompanied by a significant improvement in mRNA delivery efficiency. Among these formulations, CAS-LNP containing 2.5% DSSC-DOPE exhibited the highest stability during nebulization, resulting in a 6.9-fold increase in mRNA expression compared to SM102-LNP. In addition to enhanced stability, inhaled CAS-LNPs efficiently penetrated the mucus layer and transfected immune cells.

Subsequent studies illustrated that CAS-LNPs mainly target pulmonary dendritic cells, rendering them suitable for mRNA vaccine delivery. Inhaled CAS-LNPs loaded with mRNA encoding the SARS-CoV-2 B.1.1.529 spike protein induced robust systemic and mucosal immune responses. Compared with SM102-LNP, CAS-LNPs elicited a more potent IgG response. The corresponding

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mice showed enhanced serum neutralization capacity and significantly elevated IgA levels, thereby strengthening viral neutralization. This notably promotes mRNA delivery and expression of respiratory antigens. Additionally, experiments in a mouse model of metastatic lung cancer proved that CAS-LNPs have multifunctional potential as both preventive and therapeutic cancer vaccines. CAS-LNPs trigger systemic anti-tumor immune responses, and their therapeutic application significantly reduces the number of pulmonary metastases while extending animal survival. It activates pro-inflammatory macrophages and effectively inhibits tumor metastasis [11].

The modification of helper lipids can boost the biocompatibility of LNPs and reduce PEG shell interference with endocytosis, thereby improving gene delivery efficiency. These optimizations not only enhance mRNA vaccine delivery but also expand the potential of LNPs in fields such as tumor immunotherapy.

3.3.4. PEG lipids

To address LNP aggregation while enhacing stability and retaining high transfection efficiency, Allen Y. Jiang et al. fully replaced PEG lipids with zwitterionic polymer (ZIP)-lipid conjugates. The results showed that just 0.5 mol% ZIP lipids in C12–200 LNP formulations effectively prevented declines in mRNA encapsulation efficiency and LNP aggregation after nebulization. Moreover, ZIP lipids reduced cholesterol content within LNPs, with ZIP-LNPs showing superior aggregation resistance versus PEG-LNP controls. These findings confirmed that nebulized ZIP-LNPs provide enhanced mRNA delivery capacity and better nebulization stability compared to PEG-LNPs. When ZIP-LNP/9.2 was administered to a mouse model of mucus-obstructive lung disease, it caused nearly a fourfold increase in total pulmonary fluorescence intensity and significantly improved pulmonary protein expression [10].

Mincheol Jang et al. developed an ionizable liposome-mRNA lipocomplex (iLPX). This PEG-free lipid formulation forms a highly ordered bilayer structure via hydrophobic interactions, conferring resistance to external stresses and enhanced stability during nebulization. The lack of PEG enables it to effectively penetrate low-serum environments and pulmonary surfactant layers, rendering it suitable for delivery in the pulmonary microenvironment. Compared to conventional LNPs, IH-iLPX exhibited a 26.3-fold increase in pulmonary protein expression, effectively mediating lung-specific protein expression across all lobes including deep tissue regions, while preserving its physicochemical properties and transfection efficiency during nebulization. Additionally, IH-iLPX efficiently delivers mRNA to pulmonary epithelial cells, which serve as therapeutic targets for multiple diseases [40]. While PEG lipids have proven crucial for improving LNP delivery efficiency, ongoing research into alternative lipid formulations and modifications offers exciting prospects for more efficient and safer therapeutic delivery systems, especially in applications such as gene therapy, mRNA vaccines, and targeted drug delivery.

Regarding the optimization of LNP composition, precise regulation of the types and concentrations of ionizable lipids, cholesterol, helper lipids, and PEG lipids can effectively boost stability and transfection efficiency. Simultaneously, rational design of lipid components can strengthen the active targeting ability of LNPs to pulmonary epithelial cells, thereby notably improving drug internalization and transfection efficiency, and providing an optimized delivery system for targeted therapy of pulmonary diseases.

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3.4. Other approaches

In addition to modifying LNP components, optimizing delivery devices can alleviate the impact of shear forces induced by nebulization on LNP stability.

Jeonghwan Kim et al. developed a microfluidic aerosolization platform (MAP) that connects a microfluidic chip to an open reservoir holding LNP/mRNA solution, enabling on-demand droplet generation and precise dose control. This platform produces uniform aerosols that maintain the structural and physicochemical integrity of LNPs via shear-free nebulization, thereby enabling safe and effective mRNA delivery to the respiratory system. Compared to conventional vibrating mesh nebulizers, the droplet generation mechanism of MAP minimizes shear effects by volumetrically expelling droplets. This prevents LNP aggregation and mRNA leakage, boosting mRNA delivery efficiency and improving transfection efficiency across multiple cell lines. In vivo studies showed that the LNP aerosol produced by MAP successfully delivered mRNA to mouse lungs, achieving selective pulmonary transfection without inducing significant inflammation [41].

4. Conclusion

Nebulized LNP delivery serves as an ideal strategy for pulmonary nucleic acid drug delivery, with its core advantages lying in robust targeting capabilities and avoidance of systemic toxicity. However, its clinical application remains restricted by three major bottlenecks: insufficient stability during nebulization, difficulties in efficiently penetrating the pulmonary mucus barrier, and loss of delivery efficiency due to non-specific macrophage phagocytosis. In recent years, researchers have made significant progress in overcoming these obstacles through strategies such as optimizing LNP component ratios, optimizing nebulization and dialysis buffers in LNP delivery systems, and modifying LNP composition.

Despite these advances, the complex structure of the pulmonary airways, dense mucus barrier, and active immune surveillance continue to pose significant challenges. Existing research often focuses on isolated aspects, rendering it difficult to synergistically target the pulmonary physiological microenvironment. Consequently, simultaneously improving LNP delivery efficiency and in vivo stability remains unattainable. Furthermore, during LNP mass production, quality control and long-term safety assessment still lack unified standards and systematic solutions. Additionally, research into the dynamic interaction mechanisms underlying LNP nebulization performance, mucus diffusion, cellular internalization, and immune evasion remains inadequate. This gap in fundamental research results in a lack of clear theoretical guidance for LNP carrier design, hindering precise optimization.

Future strategies may entail developing pulmonary aerosolization simulation platforms using fluorescence imaging and particle size analysis to mimic airway physiological microenvironments, providing in vitro tools for carrier structural optimization. Integrating artificial intelligence algorithms to integrate multi-omics data on LNP composition, aerosolization parameters, and delivery performance could facilitate the construction of structure-activity relationship prediction models, thereby shortening optimization cycles and accelerating carrier refinement. Furthermore, establishing quality control protocols and safety assessment standards for LNP pulmonary delivery systems will be crucial. Defining critical process parameters for large-scale production and long-term toxicity evaluation metrics will provide standardized support for the clinical translation of LNP-based therapies.

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