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REVIEW

Inhaled nucleic acid delivery systems for the treatment of pulmonary interstitial diseases: Challenges and opportunities



Jingwen Dong[†], Jiahui Chen[†], Junmei Mu[†], Yinuo Fan, Jinsu Wang, Qiyan Zhang, Lin Zhang, Zhanwei Zhou^{*}, Minjie Sun^{*}

NMPA Key Laboratory for Research and Evaluation of Pharmaceutical Preparations and Excipients, State Key Laboratory of Natural Medicines, Department of Pharmaceutics, China Pharmaceutical University, Nanjing 210009, China

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microenvironment

Abstract The treatment of interstitial lung diseases (ILDs) was important to reduce the inflammation or fibrosis within the interstitial space. In recent years, a variety of undruggable ILDs targets were emerged for anti-inflammation and anti-fibrosis therapy. The development of RNA delivery system provided the potential for undruggable targets to the lung *via* inhalation. However, the RNA delivery systems still faced the challenges, including the protection of the stability of RNA platform, increasing the effective delivery to the targeted cells, and selective escaping of RNA molecules into the cytoplasm. In this review, we first summarized the physiological and biological barriers of RNA inhaled platform. Subsequently, the progress of inhaled RNA delivery system and their therapeutic efficiency have been systematically addressed for the application of ILDs. Finally, in the design of an inhaled RNA delivery system, key factors needed to consider and perspectives are discussed.

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*Corresponding authors.

E-mail addresses: zwzhou@cpu.edu.cn (Zhanwei Zhou), msun@cpu.edu.cn (Minjie Sun).

[†]These authors made equal contributions to this work.

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1. Introduction

Respiratory diseases are prevalent and their incidence has been rising annually, particularly following the outbreak of the COVID-19 pandemic^{1,2}. Globally, approximately 5 million individuals succumb to lung diseases each year³. The progression of these conditions often leads to the development of interstitial lung diseases (ILDs), characterized by varying degrees of abnormal cell proliferation, inflammation, and collagen fiber⁴. Currently, the respiratory ailments that are increasingly being treated using inhalation therapy⁵, such as asthma⁶, interstitial lung disorders, chronic obstructive pulmonary disease (COPD)⁷, cystic fibrosis (CF)⁸, and acute respiratory distress syndrome (ARDS)^{9,10}. With advancements in molecular pharmacology and the identification of novel disease targets, inhaled nucleic acid-based therapies have emerged as highly promising candidates for lung diseases. These effects include (i) high nucleic acid concentration in the lung tissue; (ii) a large surface area for drug absorption; (iii) avoiding first-pass hepatic metabolism; and (iv) protecting RNA molecules from systemic nucleases (low enzymatic activities in lung lining fluid) from degrading them^{11,12}. These promote inhaled nucleic acid medicines as a non-invasive administration route to be an alternative to intravenous/intramuscular injection^{13,14}. Notably, the increasing application of inhalation therapy in recent years has opened new avenues for the delivery of nucleic acid drugs (Table 1).

However, the clinical application of nucleic acid drugs remains challenging due to their heavy reliance on efficient delivery systems and difficulties in achieving targeted accumulation within the lungs¹⁵. Currently, the targeting of drugs within the lungs is suboptimal, with less than 20% of the administered drugs being deposited in the pulmonary region, while the majority are deposited in the oral cavity, pharynx, and stomach^{16,17}. The deposition behavior of drugs is intricately linked to the size and morphology of drug particles, as well as characteristics such as mucosal binding within the airways^{18,19}. Consequently, it is essential to investigate the development of an inhaled nucleic acid drug delivery system tailored to the specific disease characteristics and inhalation challenges associated with ILDs.

In this article, we first reviewed the pathogenesis of ILDs to research the main pathologic hallmarks of lung diseases. Subsequently, we reviewed the therapeutic barriers that exist in pulmonary drug delivery, including the airway barrier, alveolar barrier, and inflammatory barrier, then discussed the optimization

of drug delivery systems against lung-related targets. Finally, we discussed the engineering of inhalable nucleic acid delivery systems to break through barriers for development of engineered inhaled nuclei acid delivery system and effective treatment of ILDs.

2. Physiology barriers for inhaled nuclei drug delivery system

The effective action of inhaled nucleic acid drugs relies on safe, efficient, and stable delivery mechanisms that guard against degradation and guarantee cellular uptake and release²⁰. Nanoparticle formulations are the most commonly used delivery systems, enabling precise targeting of nucleic acid drugs to lung cells. The efficient deposition and retention of inhaled nucleic acid formulations in the lungs are hindered by the intricate structure of the lung and lung clearance mechanisms^{21,22}. A physical barrier and pathogen-sensing ability are provided by the lung's epithelial membrane^{23,24}. Inhaled nanoparticles are often adsorbed by the airway mucus layer or cleared by mucous cilia, thereby hampering the transport of inhaled particles to lesion^{25,26}. The pulmonary surfactant, a lipid-protein system that sits on the thin aqueous layer lining the alveolar surface²⁷, will form a biomolecular surfactant corona around the nanoparticles, which in turn regulates the fate of the inhaled nanoparticles²⁸. There are abundant macrophages and dendritic cells in the lower respiratory tract, which activate the innate defense function of the respiratory tract through the biological interaction with nanomedicine. Enhanced inflammatory cytokines interact with inhaled substances to affect endocytosis and may trigger an immune response; in addition, excessive deposition of extracellular matrix (ECM) can hinder drug delivery. Therefore, it is essential to study the lung physiological barrier, optimize and engineer the inhaled nucleic acid delivery systems efficient cytoplasmic transport, and protein translation²⁹. This section primarily discusses the therapeutic barriers of inhaled nucleic acid delivery systems (Fig. 1).

2.1. Airway barrier of the lung

The process of gas exchange, which is in direct contact with the outside world and includes dangerous stimuli like bacteria, allergies, cigarette smoke, and other air pollutants, is mediated by the respiratory tract's many bronchi and alveoli. When COPD, CF,

Table 1 Inhaled delivery of drugs for ILDs.

Disease	Type	Representative drug	Acting	Manufacturer
Acute asthma relief	Short-acting β_2 agonists (SABA)	Salbutamol (Ventolin)	Short-acting	GlaxoSmithKline (GSK)
Long-term control of asthma and COPD	Long-acting β_2 agonists (LABA)	Formoterol (Foradil)	Long-acting	Novartis
Asthma	Inhaled corticosteroids	Fluticasone (Flovent)	Long-acting	GlaxoSmithKline (GSK)
	Anticholinergics	Tiotropium (Spiriva)	Long-acting	Boehringer Ingelheim
Severe allergic asthma	Monoclonal antibody therapy	Omalizumab (Xolair)	Long-acting	Genentech (Roche)
	PDE-4 inhibitors	Roflumilast, Daxas	Long-acting	AstraZeneca
	LABA + anticholinergic combination therapy	Fluticasone/Formoterol/Tiotropium (Breo Ellipta)	Long-acting	GlaxoSmithKline (GSK) /AstraZeneca
Cystic fibrosis lung infections	Inhaled antibiotics	Tobramycin (TOBI)	Long-acting	Novartis
Asthma, allergic rhinitis	Inhaled immunosuppressive drugs	Montelukast (Singulair)	Long-acting	Merck & Co.

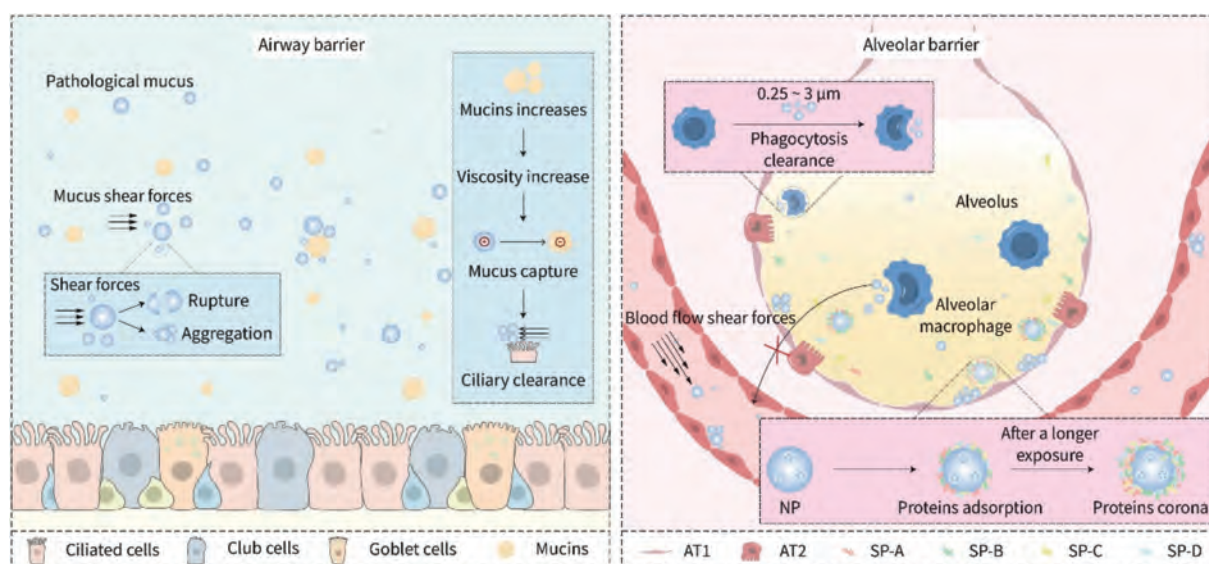


Figure 1 Physiology barriers for inhaled nucleic drug delivery system.

and IPF are present, the function of the airway barrier may alter to effectively maintain the integrity of the airway. The first line of defense against foreign substances are the airway epithelial cells (AECs), which are exposed to a variety of inhaled gases and particles at the interface between the internal and external environment. Development and structure of the airway epithelium form strong extracellular airway barriers, including mucociliary clearance, mucus secretion, epithelial polarity and transcytosis.

The active defensive system known as mucociliary clearance makes sure that inhaled particles stuck in mucus are continuously expelled. The C-terminal and N-terminal regions of mucins contain thiol-rich monomers that can form dynamic network structures through disulfide bonds, electrostatic interactions, and hydrophobic forces³⁰. Due to the mucous viscoelasticity and high negative charge properties, when nanoparticles are inhaled, the negative charges of the mucus attract the positively charged nanoparticles electrostatically, while hydrophobic interactions and hydrogen bonding in the mucus may also lead to the retention of nanoparticles^{31,32}. Research has shown that particles with a neutral surface and a negative charge have stronger mucus permeability compared to those with a positive charge³³.

Ciliated epithelial cells in the lung clear foreign substances through the mucociliary clearance mechanism^{34,35}. Therefore, drug particles captured by the mucus may be cleared from the airways by the movement of cilia or even directed to the gastrointestinal tract, limiting the effective delivery of the drug³⁶. Pathological mucus, such as that in CF, is more dehydrated and viscous, containing higher concentrations of glycosylated mucins, which carry strong negative charges and form a denser network structure, further increasing the difficulty of drug penetration^{37–39}. In addition, during aerosolization, drug particles are subjected to fluid shear forces, which may lead to aggregation or rupture. This is a challenge in the pulmonary delivery of nucleic acid drugs. Therefore, it is necessary to select appropriate physicochemical devices and optimize drug formulations to reduce drug degradation or inactivation during aerosolization and ensure the effectiveness of the drug⁴⁰.

Particle size, surface charge, and surface hydrophilicity are critical physicochemical factors influencing the mucus-penetrating

capacity of particulate delivery systems. Reducing the surface charge of carriers through material engineering, such as PEGylation, represents a promising strategy to enhance transmucosal penetration. Polyethylene glycol (PEG), a hydrophilic polymer, effectively shields nanoparticle surface charges and minimizes mucoadhesive interactions, thereby improving mucus penetration and reducing mucociliary clearance. In a study by Guo et al.⁴¹, silica nanoparticles (SNPs) modified with amine, carboxyl, or PEG groups were systematically analyzed using multiple particle tracking (MPT) technology. The results demonstrated that high-density PEGylation induced a brush-like conformation on SNP surfaces, which significantly attenuated nanoparticle–mucin interactions and enhanced mucus penetration efficiency compared to charged surfaces. These findings highlight the importance of surface PEGylation density in optimizing nano-carrier design for effective mucosal drug delivery.

Kumari et al.⁴² developed a mucus-penetrating delivery system for nucleic acid therapeutics, featuring a dual-functional coating composed of the hydrophilic biopolymer chondroitin sulfate A (CS-A) conjugated with the mucolytic agent mannitol. The hydrophilic CS-A layer reduces surface charge and minimizes interactions with negatively charged mucins, while the conjugated mannitol residues disrupt mucin–mucin crosslinking or reduce mucus viscosity by enhancing water influx into the mucosal matrix. This dual-action mechanism effectively overcomes mucus barriers, facilitating the transport of nucleic acids to pulmonary tissues.

In addition to engineering delivery systems for mucus-penetrating capabilities, researchers have developed mucus-degradable carriers to facilitate nucleic acid transport across mucosal barriers. Sharma and colleagues demonstrated that N-acetylcysteine (NAC) facilitates mucus barrier modulation by cleaving disulfide bonds (S–S) within mucin glycoproteins of the mucus gel, thereby reducing its viscoelastic properties and optimizing pore size distribution. Notably, the synergistic application of NAC with mucus-inert surface engineering strategies (*e.g.*, polyethylene glycol [PEG] coating) achieves dual suppression of electrostatic/hydrophobic interactions between nanoparticles and mucopolysaccharide-rich mucus components. This combinatorial

approach significantly enhances drug penetration efficiency across the physiological airway mucus barrier, offering a promising therapeutic strategy for overcoming drug delivery challenges in muco-obstructive respiratory disorders⁴³.

2.2. Alveolar barrier of the lung

Nucleic acid drug particles, after bypassing the aforementioned airway barriers, reach the alveolar region⁴⁴. However, the alveolar region contains numerous residents innate immune cells. Among these, macrophages constitute over 90% of the total pulmonary immune cells and, together with neutrophils, are primarily responsible for the phagocytosis and degradation of exogenous substances⁴⁵. Once the drug enters the lower respiratory tract, it first encounters alveolar macrophages. By controlling the size of drug particles, it is possible to avoid phagocytosis by alveolar macrophages⁴⁶. Studies have reported that particles ranging from 0.25 to 3 μm are most readily phagocytosed by macrophages, while porous particles larger than 10 μm and particles smaller than 200 nm can evade phagocytosis by alveolar macrophages^{47–49}.

Additionally, macrophages, as integral components of the innate immune system, exhibit dual roles in host defense during infections and pathophysiological states. While they contribute to immune responses and combat diverse infections, their excessive production of pro-inflammatory cytokines may drive uncontrolled inflammation and exacerbate tissue damage⁵⁰. Thus, modulating macrophage-derived pro-inflammatory cytokine release represents a promising therapeutic strategy for managing acute and chronic inflammatory disorders.

The alveolar surface lining is a thin liquid layer, approximately 0.2 μm in thickness, covering the surface of the alveoli. It is synthesized by type II alveolar cells and consists of a surfactant film (pulmonary surfactant, PS) secreted by the respiratory epithelial cells^{51,52}. PS mainly keeps the alveoli from collapsing during exhalation by lowering the surface tension at the alveolar air–water contact. Additionally, it aids in the removal of foreign chemicals, which is crucial for host defense²⁷. PS consists of approximately 90% lipids and 8% surfactant proteins. The primary function of hydrophilic surfactant proteins (SP-A and SP-D) is to regulate inflammatory responses and pulmonary innate immunity, whereas hydrophobic surfactant proteins (SP-B and SP-C) are principally engaged in preserving the physiological function of the surfactant^{53–56}. Pulmonary surfactant (SP) depletion and compositional alterations are hallmark features of pneumonia and acute respiratory distress syndrome (ARDS), with dysregulated surfactant homeostasis contributing to both clinical and subclinical pulmonary pathologies. Hydrophilic surfactant proteins (SP-A and SP-D) attenuate airway inflammation by reducing activation and recruitment of CD4⁺ T lymphocytes, eosinophils, and mast cells. These collections exert anti-inflammatory effects through dual mechanisms: suppression of Toll-like receptor (TLR) signaling pathways and inhibition of pro-inflammatory cytokine production in alveolar macrophages. SP-D modulates adaptive immunity by downregulating antigen presentation in macrophages and dendritic cells, while simultaneously suppressing T-cell activation and proliferation. Furthermore, SP-D deficiency promotes oxidative stress through impaired regulation of reactive oxygen species and matrix metalloproteinases, contributing to emphysema-like structural remodeling^{57,58}. Therapeutic interventions utilizing neutral or charged polymers (*e.g.*, polyethylene glycol, chitosan, dextran, hyaluronic acid) enhance surfactant functionality through competitive displacement

mechanisms that mimic endogenous surfactant proteins. When incorporated into therapeutic surfactant formulations, these polymers counteract the inhibitory effects of serum proteins and cholesterol on surfactant activity, thereby restoring pulmonary biophysical function^{59–61}.

Inhaled nanoparticles, due to their large surface area and high surface energy, are prone to interact with the lipids and proteins in PS. This leads to the spontaneous adsorption of these components around the particles, forming a protein corona, which in turn alters the fate of the nanoparticles and impacts the function of PS²⁸. The adsorption of proteins on the surface of nanoparticles is influenced by their affinity to the nanoparticles and interactions with surface proteins^{62,63}. The composition of the protein corona changes over time, with “hard” proteins exchanging with the external environment on an hour-scale, while “soft” proteins exchange within seconds to minutes⁶⁴. The proteins in the corona undergo adsorption and desorption through the “Vroman effect”, with the total mass of adsorbed proteins typically maintaining stability^{65,66}. The physicochemical properties of nanoparticles significantly affect the formation of the protein corona. The study by Lundqvist et al.⁶⁷ analyzed six types of polystyrene nanoparticles and found that size and surface characteristics determine the composition of the protein corona, revealing that some proteins exhibit either conserved or unique properties. The shape of the drug particles also determines the composition of the protein corona, with spherical nanoparticles being more easily covered by the protein corona compared to rod-shaped or star-shaped nanoparticles⁶⁸. Additionally, the research by Lindman et al.⁶⁹ has demonstrated that higher hydrophobicity can promote the formation of protein corona. Furthermore, since proteins typically carry a negative charge, cationic nanoparticles often exhibit a higher protein corona mass.

2.3. Inflammation barrier of ILDs

The most common cause of interstitial lung diseases is inflammation or fibrosis. To be more specific, the lung experienced granulomatous inflammation caused by chronic epithelial or vascular injuries, which resulted in clusters of fibroblasts and myofibroblasts and excessive deposition of disorganized collagen and extracellular matrix⁷⁰. This caused the lung architecture to be distorted and caused spatiotemporal heterogeneity fibrosis⁷¹. Despite immune cells like lymphocytes and macrophages being drawn to the site of lung damage and producing pro-fibrotic mediators, which accelerate the development of pulmonary fibrosis⁷². Hence, these factors ultimately lead to unregulated repair in response to complex interactions between both host and environmental factors, affecting the spatiotemporal rate of inhaled nucleic acid drugs *in vivo*. This section focuses on inflammatory cells, oxidative stress and fibrosis, which form therapeutic barriers in the lung.

2.3.1. Inflammatory cells and microenvironment

Normal wound healing depends heavily on the immune system, and several studies have shown that IPF patients exhibit a shift toward a profibrotic cellular inflammatory response. Excessive Th2-driven and M2-associated responses are often a significant component of fibrosis (Fig. 2)⁷³. Macrophages emit a range of cytokines that promote the development of scar tissue by regulating fibroblast activation, angiogenesis, endothelial cell (EC) proliferation, and extracellular matrix deposition. Among these are matrix metalloproteinases (MMPs), interleukin-1 (IL-1), IL-6, tumor necrosis factor- α (TNF- α), and transforming growth

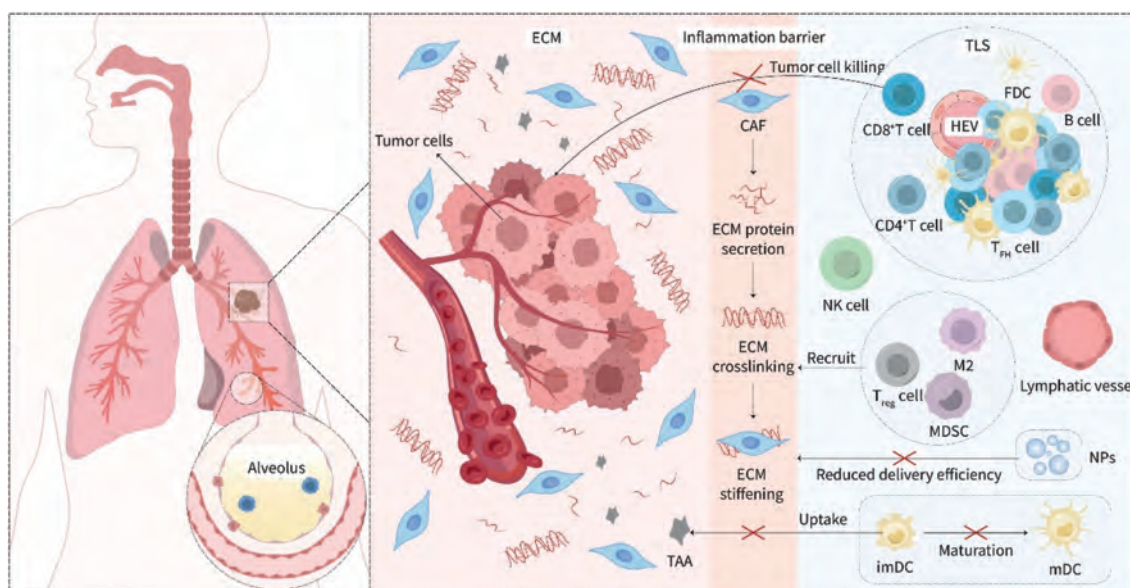


Figure 2 Immuno-microenvironment of interstitial lung diseases.

factor- β (TGF- β)^{74,75}. Indeed, pirfenidone, one of the two medications presently authorized by the US Food and Drug Administration (FDA) to treat human IPF, partially produces its anti-fibrotic effect by inhibiting TGF- β production that is important for fibroblast activation and macrophage M2 polarization. The process of fibrosis driven by the repair macrophage population also acts as an immune barrier for drug delivery, reflected in the fact that exogenous substances deposited in the respiratory area are mainly cleared by macrophages after being recognized by relevant molecular patterns, which will impair the therapeutic effect of interstitial lung disease. Additionally, fibroblasts, endothelial cells, epithelial cells, resident and migratory inflammatory cells, and other cell types undergo behavioral and morphological changes brought on by cell-signaling and repair pathways. These changes ultimately result in excessive collagen deposition, progressive lung parenchymal scarring, and irreversible loss of function^{76,77}. These immune cascades form a strong barrier in the lungs, which in turn can lead to further damage due to defective immune defenses against inhaled agents in respiratory disease, which can result in exacerbations of infections or inflammation.

2.3.2. Oxidative stress and fibrosis

Reactive oxygen species (ROS): endogenous ROS include superoxide radicals, hydrogen peroxide and hydroxyl radicals. When the lung is exposed to exogenous stimulation, the imbalance of oxidants and antioxidants inside and outside the cell leads to excessive ROS production. Then, by changing the expression of mediators (TGF- β) linked to the pathophysiology of ILDs, oxidative stress may directly harm the alveolar epithelium, encouraging fibrotic interstitial lung responses and the development of pulmonary fibrosis^{78,79}. Increased oxidative stress has been demonstrated to cause apoptosis and early cell aging^{80–82}. Whereas fibroblasts develop resistance to apoptosis and continue to be metabolically active, generating more ROS⁸³. Alveolar inflammatory cells, such as neutrophils, macrophages, and lymphocytes, in addition to fibroblasts, also produce reactive ROS in response to growth factors and cytokines. These cells are crucial for myofibroblast differentiation and collagen deposition, which

leads to pro-fibrotic events and further diminished antioxidant capacity. This fibrotic event is manifested by the deposition of ECM, which is a highly dynamic complex composed of collagen type I, elastins, laminins, and collagen IV in the lung interstitial space. Myofibroblasts may control collagen remodeling, causing collagen fibrils to reorganize spatially, raising mechanical stress and creating a stiffer ECM, resulting in inefficient delivery of nucleic acid drugs. In addition, excessive levels of ROS can non-specifically react with proteins, lipids, nucleic acids, and carbohydrates, leading to the inactivation of nucleic acid drugs and the generation of potentially toxic byproducts. These effects can further impair cellular structure and function, posing a significant challenge for inhaled nucleic acid therapies⁸⁴.

Nitric oxide: nitric oxide synthase (NOS) is the major enzyme producer of NO in the lung tissues. There are three isoforms of the NOS family: endothelium (eNOS), neuronal (nNOS), and inducible (iNOS). Unusual nitrosative stress results from fibroblasts' overexpression of iNOS and nitrotyrosine synthesis, as well as that of epithelial cells and macrophages⁷⁸. This nitrosative stress increases the expression of collagen type I and heat-shock protein (HSP)⁸⁵ facilitating fibrogenesis and progression of the disease.

Importantly, both ROS and reactive nitrogen species (RNS) may cause an imbalance between proteases and antiproteases due to their ability to activate MMPs and inactivate protease inhibitors⁸⁶. As members of the M10A subfamily of metallopeptidases, MMPs are zinc-dependent matrixins that have long been thought to be the main agents responsible for the breakdown of ECM and core matrisome proteins. However, they also play a role in controlling the activity of other proteins, such as growth factors and inflammatory mediators⁸⁷. Research has found that matrilysin (matrix metalloproteinase 7 [MMP-7]) is significantly associated with pulmonary fibrosis in animal models, and is also overexpressed in the lungs with IPF, contributing to progression and poor outcome of IPF. This highlights the importance of proteases in IPF pathogenesis⁸⁸. Thus, proteases, ROS and RNS, and a range of cytokines, chemokines and growth factors form multiple inflammatory barriers that also provide biological targets for the gene therapy of interstitial lung diseases.

Yao's group pioneered the use of triple-helix framework nucleic acids (tFNA) as an inhalable nanocarrier to develop the 3D t-Sponge system, which integrates high drug-loading capacity, immunomodulatory functions, and oxidative stress modulation. In acute lung injury (ALI) models, this system demonstrated targeted delivery of miR-155 inhibitors, significantly alleviating tissue damage while optimizing the local immune microenvironment and redox homeostasis. Mechanistically, the t-Sponge suppressed multiple immune activation pathways, including nuclear factor kappa-B (NF- κ B) signaling, thereby attenuating pro-inflammatory cytokine production and leukocyte infiltration. This biomimetic nucleic acid architecture leverages the intrinsic stability of tFNA and its spatial adaptability for coordinated therapeutic actions against pulmonary pathophysiology⁸⁹. Huang et al.⁹⁰ performed structural optimization of nimodipine to develop phosphodiesterase-1 (PDE1) inhibitors with improved activity and selectivity. The study revealed that pharmacological PDE1 inhibition effectively suppressed transforming growth factor- β 1 (TGF- β)-induced differentiation of human pulmonary fibroblasts into myofibroblasts *in vitro*. Furthermore, these optimized inhibitors demonstrated significant anti-fibrotic efficacy in a bleomycin (BLM)-induced rat pulmonary fibrosis model, reducing collagen deposition and pathological remodeling through modulation of fibrosis-related signaling pathways.

Delivering nucleic acid drugs to the lungs successfully faces several physiological barriers that must be carefully addressed to optimize therapeutic outcomes. These barriers include the airway and alveolar barriers, which are protected by complex systems such as mucociliary clearance, macrophage-mediated phagocytosis, and the alveolar surfactant crown. Furthermore, in diseases such as ILDs, the inflammatory microenvironment presents additional challenges, as the accumulation of immune cells and extracellular matrix components can hinder effective drug delivery. Factors such as oxidative stress, dysregulation of proteases,

ROS, and RNS contribute to the complexity of these barriers, ultimately affecting the efficiency and safety of nucleic acid-based therapies. Therefore, overcoming these barriers requires a deeper understanding of pulmonary physiology and the adoption of innovative strategies in nanoparticle design to improve the efficacy and safety of inhaled nucleic acid therapies.

3. Inhaled nucleic acid delivery system

RNA delivery systems are essential for advancing precision medicine and personalized therapies. However, the intrinsic instability of RNA and challenges associated with inefficient delivery have hindered its clinical application^{91,92}. In the treatment of pulmonary diseases, traditional systemic delivery methods may lead to off-target effects, whereas inhalation delivery offers enhanced RNA stability and transfection efficiency in the lungs³⁰. Nonetheless, this approach still faces significant challenges, including RNA degradation, low cellular uptake, limited endosomal escape, and insufficient pulmonary deposition⁵. Consequently, optimizing RNA modifications, delivery systems, and inhalation strategies has become a critical area of research to improve therapeutic outcomes (Fig. 3).

3.1. Optimization of RNA stability

3.1.1. Process engineering for enhanced stability

The naked form of nucleic acids is inherently unstable during delivery due to their large size, high negative charge, rapid degradation by ubiquitous enzymes (*e.g.*, endonucleases), low cellular uptake and transfection efficiency, shear sensitivity, and high viscosity caused by secondary structure formation in solution⁹³. Additionally, during storage, transportation, and use RNA delivery systems may encounter degradation or physical-chemical instability^{94,95}. To address these challenges, the rational design

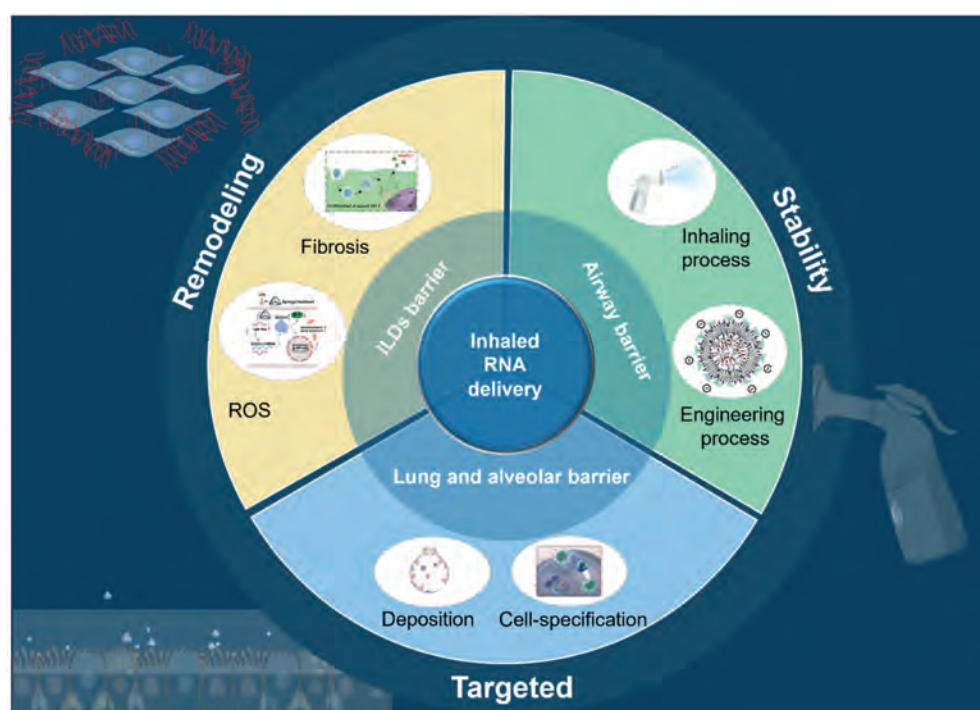


Figure 3 Illustration of the barriers of inhalation nucleic acid delivery system.

and development of inhalable nucleic acid nanodelivery systems is imperative. Such systems must not only protect nucleic acid therapeutics from enzymatic degradation and enhance cellular uptake, but also withstand shear forces generated during nebulization, penetrate the mucus barrier, and minimize alveolar macrophage clearance. Furthermore, they should facilitate target cell uptake and enable efficient lysosomal escape to ensure intracellular therapeutic efficacy⁹⁶. Critical progress involves three synergistic strategies: (1) lipid nanoparticle (LNP) engineering with charge-assisted stabilization⁹⁷, (2) low-shear nebulization device optimization⁹⁸, and (3) formulation buffering with shear-protective excipients⁹⁹. Optimization of the RNA sequence itself is also critical to enhance its stability and immunogenic potency¹⁰⁰.

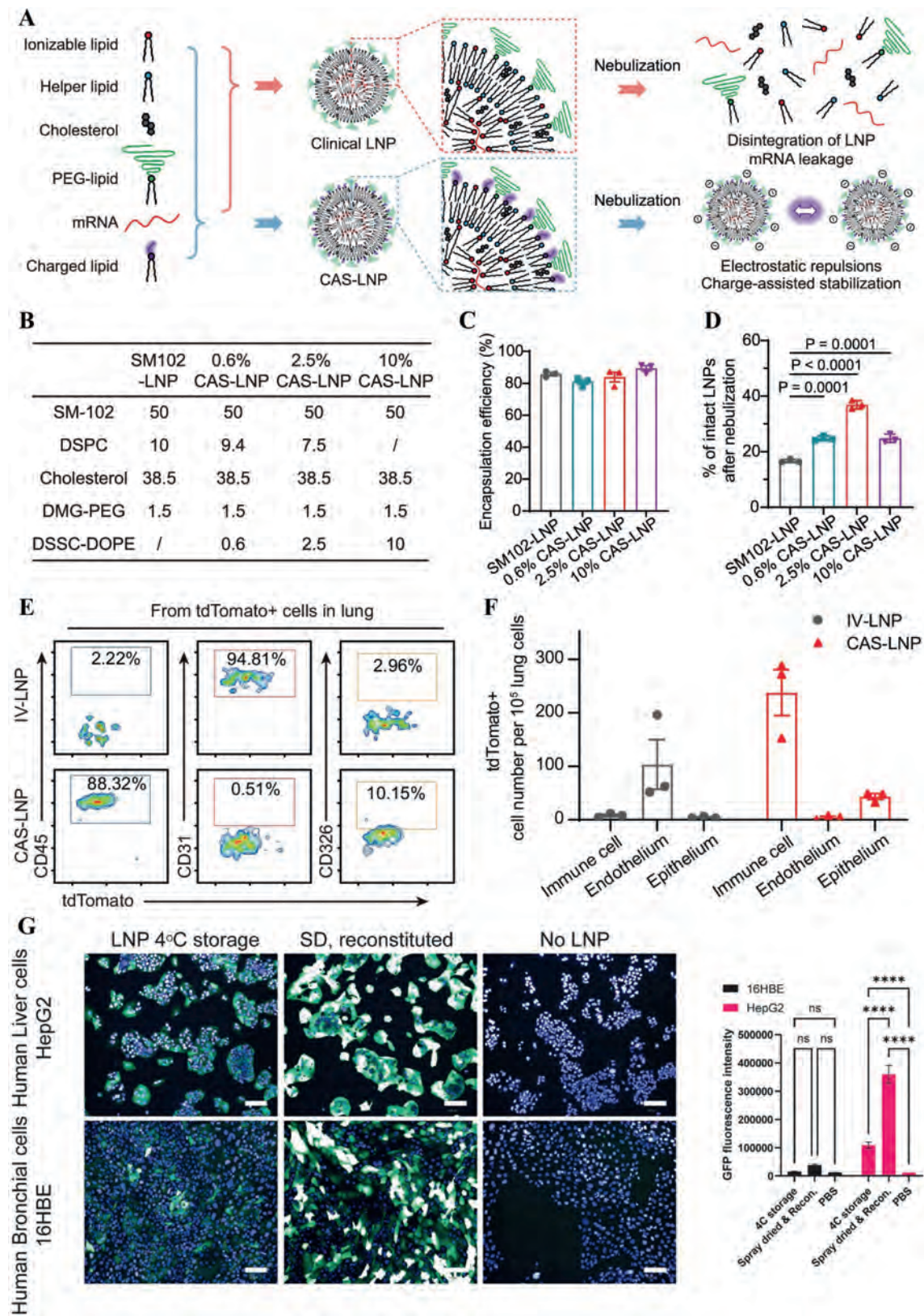
Chemical modification: to enhance the *in vivo* stability and translational efficiency of RNA, researchers have developed a variety of chemical modification strategies. Among them, pseudouridine (Ψ) and N¹-methylpseudouridine (m 1Ψ) are widely used in messenger RNA (mRNA) vaccines, as they can effectively reduce immune recognition and improve molecular stability¹⁰¹. Additionally, modifications such as 5-methylcytidine (m⁵C) and N⁶-methyladenosine (m⁶A) can prolong RNA half-life by modulating RNA secondary structure and its interactions with RNA-binding proteins^{102–104}. On the other hand, 2'-O-methyl modifications, which act on the ribose ring, enhance resistance to nucleases and are commonly employed as stabilizing strategies in small interfering RNA (siRNA) and antiviral RNA therapeutics¹⁰⁵. In addition to chemical modifications, rational formulation and excipient optimization play a crucial role in enhancing RNA stability. When encapsulating RNA, the structural design of nanocarriers must provide effective protection. For instance, lipid nanoparticles (LNPs) can encapsulate RNA within an ionically crosslinkable lipid core, thereby preventing degradation by nucleases and significantly improving *in vivo* stability¹⁰⁶. To maintain structural integrity during the drying process, polyhydroxy compounds such as sucrose and mannitol can be incorporated into the formulation¹⁰⁷. These compounds form stable hydrogen-bond networks during lyophilization, protecting RNA from degradation. Moreover, by controlling particle size (typically in the range of 50–150 nm), surface charge, and incorporating PEGylation, it is possible to extend the retention time of RNA in the lungs while minimizing nonspecific interactions with the immune system¹⁰⁸.

Nebulized inhalation: nebulized inhalation is considered one of the most promising methods for nucleic acid delivery, with numerous studies demonstrating the feasibility of inhaled delivery using LNPs^{109–111}. However, despite the success of LNPs in systemic delivery and intramuscular injection, they are prone to structural instability during nebulization and often fail to adapt to the pulmonary microenvironment, which limits their application for lung delivery¹¹². Jang et al.¹¹³ identified an ionizable lipid–mRNA lipid complex (iLPX) that exhibits greater stability during nebulization and can better adapt to the low-serum and pulmonary surfactant environment of the lungs. Another study employed a charge-assisted strategy to optimize the surface charge of LNPs and utilized peptide–lipid conjugates to enhance electrostatic repulsion between LNPs, thereby improving their colloidal stability. The optimized LNPs demonstrated excellent stability during nebulization and efficiently delivered mRNA to the lungs of mice, dogs, and pigs. Inhalation of Charge-assisted stabilization of lipid nanoparticles (CAS-LNPs) primarily transfected dendritic cells and triggered strong mucosal and systemic immune responses⁹⁷ (Fig. 4A–F). The delivery of

nucleic acid drugs to the cytoplasm has been suggested using poly (D, L-lactide-co-glycolide) (PLGA) nanoparticles (NPs). Cells may ingest these NPs by endocytosis, and after they have partially escaped from endosomes, they can release the medication¹¹⁴. Cortez-Jugo' group¹¹⁵ developed a surface acoustic wave (SAW)-based atomization platform for the pulmonary delivery of siRNA, which simplifies the drug formulation process. The aerosol droplets formed after atomizing liquid siRNA are of a size suitable for pulmonary delivery, and siRNA undergoes minimal degradation during the atomization process.

Dry powder inhalers: dry Powder Inhalers (DPI) have gained significant attention as a novel pulmonary drug delivery platform in the field of RNA delivery¹¹⁶. Compared to traditional aerosol systems, DPIs offer advantages such as high stability, convenient storage, and transport, making them particularly suitable for patients with interstitial lung diseases¹¹⁷. Jensen et al.¹¹⁸ developed a dry powder formulation of cationic lipid-modified PLGA nanoparticles that enhance gene silencing efficiency while maintaining both the biological activity of siRNA and the stability of the nanoparticles during the spray-drying process. In addition to optimizing delivery carriers, improving manufacturing processes can also enhance the stability of RNA delivery systems. Proper control of nanoparticle size, optimization of storage methods, and refinement of processing techniques are essential for improving stability. Spray drying, which allows precise control of processing parameters, is commonly used for the preparation of inhalable dry powder formulations¹¹⁹. Friis et al.'s⁹² research demonstrated the proof-of-concept for using spray drying to prepare LNP mRNA formulations, showing that this process can effectively improve the stability of mRNA drugs while retaining their functionality. Compared to liquid formulations stored at 4 °C for two weeks, spray-dried powder formulations demonstrated superior performance. Following intratracheal administration of spray-dried LNPs encapsulating *eGFP* mRNA in mice, robust expression of eGFP protein was observed in multiple cell types, including bronchial epithelial cells, macrophages, and type II alveolar cells. These findings highlight the potential of this approach for protein replacement therapy, gene editing, and vaccination⁹² (Fig. 4G).

Furthermore, optimization of the spray-drying process can maximize the retention of both the biological activity and colloidal properties of nanoparticles. Key factors include the spray-drying conditions and the selection of excipients used in nanoparticle production¹¹⁹. Zimmermann et al.¹²⁰ discovered that spray-drying with a 5% lactose solution ensures that the LNP powder maintains RNA's biological activity, with a moisture content of less than 5% and an RNA loss of less than 15%. Xu et al.¹²¹ created a DPI formulation that stabilizes tumor necrosis factor- α (TNF- α) siRNA-loaded lipid-polymer hybrid nanoparticles (LPNs) by adding stabilizing excipients like trehalose (Tre) and dextran (Dex) along with shell-forming dispersion enhancers like leucine (Leu). They found that when Leu content reached 40%, the formulation achieved suitable aerosol characteristics for inhalation, while maintaining nucleic acid integrity in the solid state. This DPI formulation also enabled uniform deposition in the lungs. Spray freeze-drying (SFD) is another effective method for producing inhalable powder formulations. One study used SFD technology to prepare inhalable mRNA-LNP powders without deliquescent excipients. The interaction between mRNA and LNPs was enhanced by using acidic pH-adjusting agents, ensuring a robust encapsulation structure even after SFD processing. Although the powder form of mRNA-LNPs exhibited lower transfection efficiency compared to the liquid form, it



demonstrated higher transfection efficiency after long-term storage¹²².

3.1.2. Process inhaling for enhanced stability

Avoiding clearance by the mucociliary escalator, avoiding phagocytosis by macrophages, and possible shear force damage during atomization are all obstacles that must be addressed for an inhaled nucleic acid delivery method to be successful⁸¹. Simultaneously, it must ensure that the nucleic acid is effectively transported into the cytoplasm to exert its intended function²⁹.

To address the issue that LNP might disintegrate during aerosolization due to shear forces, Kim et al.¹²³ enhanced the stability of LNP by increasing PEG-lipid and added the cholesterol analogue β -sitosterol to improve endosomal escape. The gene transfection efficacy was increased by the improved LNP's fast mucosal diffusion, polyhedral shape, and uniform particle dispersion. Inhaled LNP could lead to local protein production in the lungs of mice without causing pulmonary or systemic toxicity. Two years later, the same group introduced a translatable microfluidic aerosol platform (MAP) capable of generating a uniform aerosol of lipid nanoparticles containing mRNA, thereby avoiding the shear forces typically encountered in traditional atomization methods. This platform, which is connected to an open-type reservoir *via* a microfluidic chip, allows for precise dose control and on-demand droplet generation, making it suitable for chronic treatments and large-scale vaccination. Compared to ultrasonic nebulizers, MAP generates droplets with minimal shear impact and is particularly well-suited for delivering macromolecular therapies such as nucleic acids, proteins, and nanoparticles⁹⁸ (Fig. 5A–D).

More recently, a study by Bai et al.¹²⁴ introduced an innovative “LOOP” platform designed for the development of inhalable lipid nanoparticles (iLNP-HP08^{LOOP}) specifically for pulmonary mRNA delivery. By optimizing the ratio of helper lipids, acidic dialysis buffer, and excipients, this platform significantly improved both the expression and stability of mRNA. In animal studies, iLNP-HP08LOOP effectively delivered mRNA encoding the single-chain fragment of Interleukin-11 (IL-11), which significantly inhibited fibrosis. This approach outperformed both intravenous and inhaled administration of IL-11 single chain fragment variable (IL-11 scFv) in preventing fibroblast activation and extracellular matrix deposition. Moreover, the HP08LOOP system is compatible with the commercially available ALC0315 LNP, providing a robust platform for developing inhaled mRNA therapeutics targeting respiratory diseases such as idiopathic pulmonary fibrosis¹²⁴ (Fig. 5E–H).

3.1.3. Mitigating shear stress during nebulization

Mucus in the pulmonary airways is extremely sticky and viscous, which makes it difficult to distribute nucleic acid medications to the airways. This is especially noticeable in individuals with CF and COPD¹²⁵. Due to the tightly arranged mucin fiber network and the small pore size of the mucus, it is challenging for nucleic acid drug carriers to effectively penetrate these layers, thus limiting their therapeutic efficacy¹²⁶.

One promising strategy to overcome this challenge is to coat the surface of nanoparticles with components of pulmonary surfactant, which can significantly improve the diffusion of drugs through the pulmonary mucus barrier. This approach not only

facilitates drug traversal across the mucus but also enhances targeting to deep-seated lung tissues and promotes uptake by epithelial cells, ultimately improving drug delivery efficiency¹²⁷. Wang et al.¹²⁸ developed an innovative LNP system incorporating phospholipid, derived from pulmonary surfactant. This modification improved the mucus permeability of LNPs, enabling more efficient pulmonary delivery of mRNA. The formulation was further optimized by incorporating γ -aminobutyric acid (GABA)-based ionizable lipids, allowing for the co-delivery of multiple mRNAs (*e.g.*, BMP4 and CYB5R3) to regulate stem cell function and enhance alveolar structure in the lungs. This LNP system effectively alleviated pulmonary fibrosis and prolonged survival in a mouse model, demonstrating its potential in treating pulmonary diseases¹²⁸ (Fig. 6).

The research by Suk et al.¹²⁹ introduced a synthetic gene vector platform capable of penetrating human CF mucus. With a high-density PEG coating, these mucus-penetrating gene vectors were able to effectively cross the mucus barrier, achieving uniform airway distribution and prolonged lung retention. Similarly, Kim et al.¹³⁰ developed DNA-loaded Mucus-Penetrating Particles (DNA-MPPs), which could efficiently penetrate dehydrated and concentrated mucus layers, significantly enhancing gene transfer efficiency. Specifically, when carrying the ENAc-silencing plasmid, these particles helped reduce mucus accumulation in the lungs. Additionally, the inhalation strategy involving bottle-brush polymer-DNA conjugates (pacDNA), as discovered by Fang et al.¹³¹, also exhibited excellent mucus permeability, enabling uniform and sustained pulmonary distribution in mice.

The aforementioned RNA stability optimization strategies are concretely demonstrated in clinical inhaled nucleic acid therapeutics. ARCT-032, developed through Arcturus' LUNAR[®] lipid-mediated nebulization platform, delivers CFTR mRNA to the lungs, targeting pulmonary disease, the leading cause of morbidity and mortality in CF patients. The LUNAR particles bind to target cell membranes and are rapidly internalized *via* endocytosis. Once internalized, the LUNAR formulation becomes entrapped in endosomes. Progressive endosomal acidification triggers pH-mediated membrane disruption, enabling release of the RNA payload into the cytoplasm following rapid biodegradation of the LUNAR components, thereby exerting therapeutic efficacy. Ethris' investigational inhaled mRNA therapeutic ETH47, currently in clinical studies for asthma, encodes interferon lambda 1 (IFN λ 1) mRNA delivered *via* nasal administration. This modality enhances IFN λ levels in the respiratory tract to induce innate immune defenses at mucosal viral entry sites while suppressing viral replication. Developed using Ethris' proprietary Stable Non-Immunogenic mRNA (SNIM[®] RNA) platform, ETH47 employs its stabilized nanoparticle (SNaP) LNP delivery system, which effectively addresses the challenge of direct mRNA delivery to pulmonary tissues.

ReCode Therapeutics has developed inhaled mRNA therapies targeting diseases such as CF and primary ciliary dyskinesia (PCD). Their proprietary selective organ targeting (SORT) LNP platform enables precise delivery of diverse genetic payloads to extrahepatic organs and cells, including the lungs, spleen, and liver, with specific tropism for epithelial cells, endothelial cells, B cells, T cells, and hepatocytes. The SORT-LNP technology innovatively incorporates a fifth lipid component (SORT molecules)

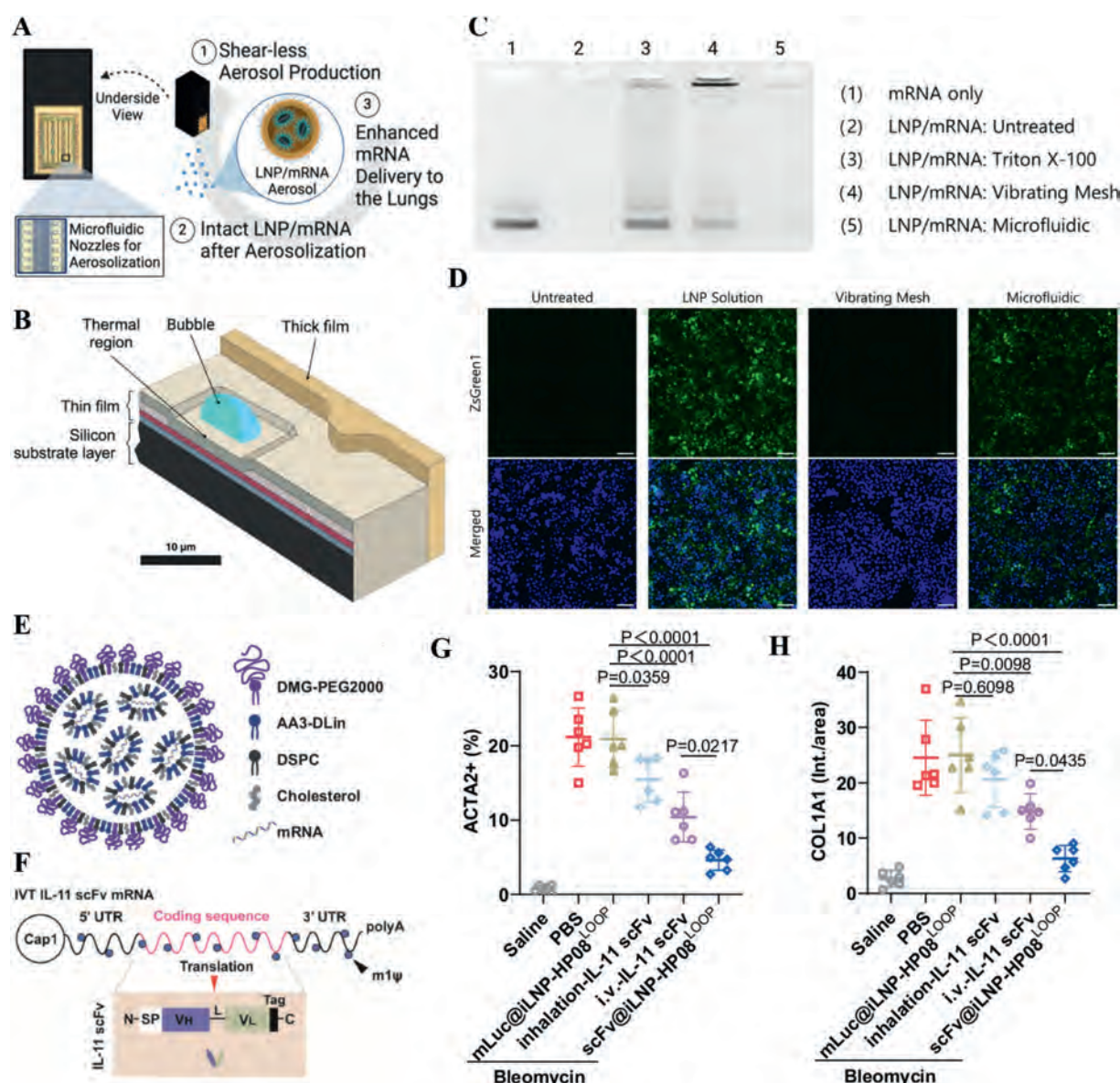


Figure 5 Demonstrates a microfluidic aerosol platform (MAP) for low-shear mRNA delivery. (A) Microfluidic aerosol platform for efficient lung delivery of gene therapy. (B) Illustration of the bubble-containing microfluidic chip's layer structure. (C) Illustration of agarose gel electrophoresis analysis under different treatment circumstances. (D) Fluorescence microscopy pictures of HeLa cells under different circumstances. Reprinted with permission from Ref. 98 Copyright © 2024 American Chemical Society. (E) Schematic representation of mRNA@iLNP-HP08LOOP. (F) Diagrammatic illustration of the mRNA structure of IL-11 scFv. (G, H) Quantitative analysis and representative immunofluorescence pictures of COL1A1 and ACTA2 in mouse lung sections treated with various techniques. Reprinted with permission from Ref. 124 Copyright © 2024 Springer Nature.

into conventional LNPs, creating a unique lipid formulation design. The base four-component “mDLNP” formulation comprises 4A3-SC8, DOPE, cholesterol, DMG-PEG, and mRNA at a lipid molar ratio of 23.8:23.8:47.6:4.8, with a total lipid/mRNA ratio of 40:1 (*wt/wt*). For SORT-LNP production, SORT molecules are added at defined quantities to this base formulation while maintaining constant relative molar ratios of the core four components to preserve optimal RNA encapsulation and endosomal escape capabilities. Tissue tropism modulation depends on the chemical functionalities (*e.g.*, permanent cationic, anionic, ionizable cationic, or zwitterionic properties) and physicochemical characteristics of the SORT molecules, as well as their

incorporation ratios. The microfluidics-based LNP manufacturing process enables mass-controlled production with high reproducibility, making it suitable for both preclinical animal studies and clinical translation^{132,133}.

Following deposition in the respiratory tract, nanoparticles first interact with the pulmonary surfactant layer before being internalized by alveolar macrophages. These immune cells sequester foreign particles in lysosome-containing vesicles for enzymatic degradation or transport them to the mucociliary escalator and lymphatic system for clearance. To prevent premature elimination of therapeutic particles before reaching target sites, delivery carriers must overcome these dual barriers, highlighting the critical

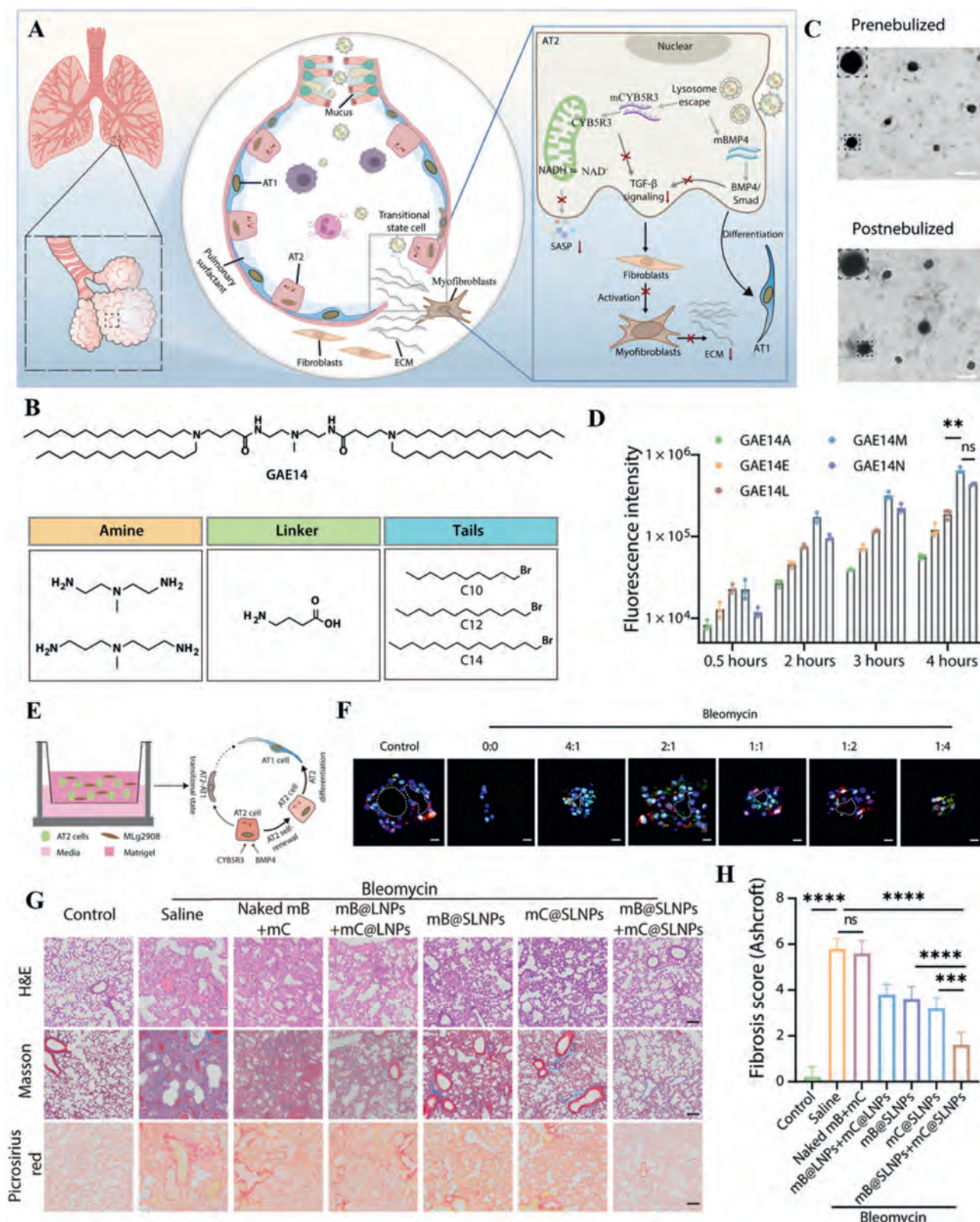


Figure 6 Validates mucus-penetrating capacity of surfactant-mimicking LNPs and their efficacy in reversing alveolar stem cell depletion *via* mRNA delivery for pulmonary fibrosis. (A) Diagram showing the inhalation of mRNA-LNPs to AT2 cells to reverse the depletion of epithelial stem cells in order to cure IPF. (B) Construction of lipid compound combinatorial library. (C) Representative TEM images of GAE14N LNPs prior to and during nebulization. (D) Optimum GAE14 LNP formulations' penetration across the artificial mucus layers ($n = 3$). (E) Schematic of mouse 3D alveolar organoid culture. (F) Representative organoid immunofluorescence pictures (AT1: AQP5, red; AT2: SFTPC, green). (G) Representative images of lung tissue section stained with H&E, Masson's trichrome, and picrosirius red. (H) Lung sections with an Ashcroft scale toward fibrosis. Pulmonary fibrosis scores ($n = 5$). Reprinted with permission from Ref. 128 Copyright © 2024 The American Association for the Advancement of Science.

role of optimized delivery systems. Clinical data robustly demonstrate that rational engineering of nanocarriers (*e.g.*, composition optimization, formulation ratio screening, and precise control of manufacturing parameters) enables effective pulmonary delivery of nucleic acid therapeutics by mitigating these clearance mechanisms.

To achieve efficient pulmonary delivery of nucleic acid drugs, key elements such as RNA chemical modifications, delivery system design, and aerosolization techniques must be integrated. Modifications like pseudouridine (Ψ) and N¹-methylpseudouridine (m1 Ψ) enhance RNA stability and translation. Delivery platforms such as LNPs and DPIs offer good biocompatibility and efficiency. Challenges like mucociliary clearance, macrophage uptake, and shear stress during nebulization can be mitigated by optimizing LNP components (*e.g.*, increasing PEG-lipid content, adding β -sitosterol) and using microfluidic aerosol platforms (MAPs). For diseases with thick mucus barriers like CF and COPD, strategies such as surfactant-modified LNPs, densely PEGylated carriers, and mucus-penetrating nanoparticles improve drug diffusion and retention, enhancing local delivery and therapeutic effect.

3.2. Precision targeting in pulmonary delivery

The complex structure and physiological characteristics of the lungs, including the airway barrier and clearance mechanisms, present significant challenges to the efficient deposition of drugs and their targeted delivery to specific lung cells¹³⁴. As a result, enhancing the lung-targeting ability of inhaled nucleic acid delivery systems has become a central focus of research in this field¹³⁵. This section discusses strategies to improve the lung-targeting efficiency of inhaled nucleic acid delivery systems, with an emphasis on two main aspects: enhancing the lung deposition of nucleic acid drugs and improving their targeting of specific cells and the lung microenvironment.

3.2.1. Optimizing deposition dynamics

The structure of the pulmonary airways influences the deposition patterns and regions where particles of different sizes are deposited. The particle size dictates how deeply they can penetrate the lungs and where they are likely to be deposited¹³⁶. Particles between 1 and 5 μm are most suitable for reaching the alveoli and accessing the deeper regions of the lungs¹³⁷. Larger particles tend to deposit in the upper respiratory tract, while smaller particles are often cleared from the respiratory tract. Therefore, controlling the particle size within the 3–5 μm range optimizes lung deposition and targeting efficiency, enhancing the overall drug delivery effect¹³⁸. Traditional shelf freeze-drying is used to prepare dry powders of siRNA-encapsulated lipid particles, but its aerosol performance still requires improvement. Additional treatments are often needed to optimize particle size¹³⁹. Thin-film freeze-drying (TFFD) is an ultra-rapid freezing technology commonly applied in the preparation of small molecules, proteins, and vaccines. In this process, an API (Active Pharmaceutical Ingredient) solution is deposited onto a low-temperature surface, rapidly frozen, and then dried in a freeze dryer. The resulting dry powder has a porous and fluffy structure, which confers excellent aerosol properties, making it ideal for pulmonary administration *via* DPIs¹⁴⁰. Wang et al.¹⁴¹ demonstrated the feasibility of using TFFD technology to prepare dry powder formulations of solid lipid nanoparticles (SLNs).

To further improve pulmonary drug deposition efficiency, selecting appropriate carbohydrate excipients and optimizing spray drying parameters are also essential. Sarode et al.¹⁴² developed a dry powder product (DPP) of mRNA-LNPs for treating pulmonary diseases. By optimizing excipients, lipid composition, and mRNA concentration, they achieved effective pulmonary delivery. Specifically, leucine, or its combination with mannitol, improved formulation efficacy, increased mRNA yield, and reduced particle size. In a mouse model, DPP successfully delivered mRNA and expressed luciferase *via* intratracheal administration, demonstrating its potential for mRNA-based therapies in pulmonary diseases. Xu et al.¹⁴³ introduced an innovative siRNA pulmonary delivery system combining cell-penetrating peptides, novel excipients, and spray-drying technology. By selecting suitable excipients, such as a lactose and trehalose mixture, they ensured nanoparticle structural integrity during spray drying and improved lung deposition. The study optimized spray-drying conditions to yield particles smaller than 5 μm , enhancing pulmonary delivery efficiency. The results showed that the SAR6EW/TNF- α siRNA complex, after spray drying, maintained its structure and gene-silencing activity, successfully alleviating ALI-induced pneumonia and inflammation in mice¹⁴³ (Fig. 7A and B).

The surface charge of the carrier significantly affects drug distribution and absorption in the lungs. Particles with cationic surface modifications are more likely to interact electrostatically with cell membranes in the lungs, enhancing the pulmonary deposition of nucleic acid drugs. A novel inhalable drug delivery method was developed by Zhao et al.¹⁴⁴ utilizing cationic liposomes (CLPs) modified with RGD-TAT dipeptide and loaded with the gap junction regulator all-trans retinoic acid (ATRA) and miR-34a. This system, delivered *via* DPIs, effectively targets lung tumors and enhances gene transfection efficiency. ATRA improves drug accumulation in the lungs by upregulating gap junctions between tumor cells, which facilitates the intercellular trafficking of miR-34a and increases gene expression in deep tumor.

3.2.2. Cell-specific and microenvironment-responsive delivery

Targeting specific cells or the pathological microenvironment can enhance RNA delivery efficiency, reduce non-specific distribution, and improve RNA therapy^{146–148}. LNPs are a commonly used platform for inhaled delivery. Owing to their lipid-like structure, LNPs can efficiently encapsulate RNA, facilitate cellular uptake, and promote endosomal escape, thereby achieving high transfection efficiency. Studies have shown that well-designed LNPs can induce robust gene expression in the lungs of mice, primarily targeting pulmonary endothelial and immune cells. Their particle size and surface characteristics can be tuned to regulate pulmonary retention time, with particles around 70 nm demonstrating superior retention and transfection efficiency¹⁴⁹. Although LNPs may elicit immune responses¹⁵⁰, immune activation can be effectively minimized through chemical modifications and formulation optimization¹⁵¹. Moreover, by refining their composition and preparation methods, LNPs can exhibit low cytotoxicity both *in vitro* and *in vivo*. Polymer-based carriers are also common delivery platforms. For example, high molecular weight Polyetherimide (PEI) offers high transfection efficiency but is associated with significant toxicity¹⁵². Novel brush-shaped polymers enhance transfection efficiency through steric hindrance and show promising results¹³¹. Polymers exhibit good adhesion properties, allowing for slow nucleic acid release, which is beneficial for sustained effects, but they may be rapidly cleared by alveolar

macrophages through phagocytosis. Additionally, non-biodegradable polymers may lead to accumulation and toxicity risks with long-term administration, while positively charged materials can potentially disrupt cell membranes. Choosing biodegradable polymers, especially those that degrade into non-toxic natural metabolites, helps improve biocompatibility and reduce toxicity¹⁵³. Table 2^{50,89,97,99,110,124,131,144,145,154-169} compare different delivery platforms for inhaled nucleic acid drugs.

Macrophages, crucial to pulmonary immune defense, can also contribute to inflammation and tissue damage, playing a role in various diseases. Therefore, regulating the excessive inflammatory response triggered by macrophages presents a promising strategy for treating inflammatory diseases¹⁷⁰. To deliver nucleic acid drugs specifically to alveolar macrophages, Zhao et al.⁵⁰ screened 112 different kinds of LNPs and conjugated the macrophage-specific antibody F4/80 to the LNP's surface to create an improved LNP formulation. This formulation effectively delivered siRNA targeting TAK1, suppressed NF- κ B activation, and reduced the release of pro-inflammatory cytokines, thereby alleviating lung injury induced by influenza infection. The study underscores the potential of LNP-mediated, macrophage-targeted therapy in mitigating inflammation and presents a promising strategy for treating pulmonary inflammatory diseases⁵⁰ (Fig. 7C–E). Another study employed mannose-modified engineered RNA nanoparticles (Man-EV-miR-511-3p) to deliver the miR-511-3p mimic *via* intratracheal inhalation, successfully targeting macrophages and reversing airway inflammation¹⁶⁶.

Tang et al.¹⁵⁸ proposed an innovative dual-targeted mRNA nanomedicine formulation. Upon inhalation, this system not only targets macrophages but also accumulates in lung tumor cells. By combining cationic lipids with hyaluronic acid, the formulation effectively expresses target proteins (such as p53, luciferase, and green fluorescent protein). This dual-targeted mRNA nanoparticle demonstrates excellent stability and efficient lung tissue transfection, enabling the *in vivo* expression of specific proteins. This design opens new possibilities for developing mRNA-based inhaled drugs or vaccines, with significant potential for treating lung diseases. Another study developed an inhalable, mucus-permeable nanoparticle system (siIL11@PPGC NPs) for delivering siRNA targeting IL-11 to treat idiopathic pulmonary fibrosis (IPF). The nanoparticles, which self-assemble from PLGA-PEG copolymers and a custom cationic lipid, G0-C14, efficiently deliver siRNA across the mucosal barrier. The results showed that siIL11@PPGC NPs inhibited the ERK and SMAD2 signaling pathways, preventing fibroblast differentiation and reducing extracellular matrix (ECM) deposition. This approach significantly improved lung function in a mouse model of bleomycin-induced pulmonary fibrosis, without causing systemic toxicity¹⁴⁵ (Fig. 7F–O).

β -Adrenergic agonists, with their cationic amphiphilic properties, can enhance the cytoplasmic delivery of siRNA in target lung cells, especially when mediated by nanocarriers like nanogels. Previous studies have shown that the long-acting β 2-adrenergic agonist salmeterol can promote siRNA delivery in lung epithelial cells *via* nanogels¹⁷¹. To explore whether other β -adrenergic agonists or different nanocarriers could play similar roles, Merckx et al.¹⁶⁹ found that common clinical β 2-adrenergic agonists—such as salbutamol, formoterol, salmeterol, and indacaterol—can all enhance siRNA delivery. Notably, salmeterol and indacaterol significantly boosted siRNA delivery in both lung epithelial cells and macrophages, with salmeterol showing a particularly strong effect in both cell types.

Improving the targeted delivery efficiency of inhaled nucleic acid drugs to the lungs requires optimizing both the deposition behavior of the drug in the lungs and its specific targeting ability to cells and microenvironments. In terms of deposition dynamics, precisely controlling the particle size (especially within the range of 1–5 μ m), applying TFFD techniques, and carefully selecting excipients and optimizing spray drying parameters can improve the distribution and delivery stability of the drug in the lungs. For cell-specific and microenvironment-responsive delivery, strategies such as adjusting the size and surface properties of LNPs, incorporating specific ligands (*e.g.*, RGD-TAT or F4/80 antibodies), and using biodegradable polymers or functionalized cationic materials can enable precise targeting of alveolar epithelial cells, immune cells, and fibroblasts, while reducing systemic toxicity. Additionally, enhancing the carrier's mucus penetration ability, modulating macrophage-mediated inflammatory responses, and intervening in specific cell signaling pathways provide more targeted and durable therapeutic strategies for lung diseases. The integration of these strategies is expected to significantly enhance the clinical translation potential of inhaled nucleic acid drugs in the treatment of lung diseases.

3.3. Immuno-microenvironment remodulation for enhanced efficacy

Lung diseases are often associated with complex changes in the microenvironment, including oxidative stress¹⁷², inflammatory responses¹⁷³, and abnormal metabolic activities in tumor cells¹⁵⁵. These factors can significantly impact the therapeutic efficacy of RNA-based drugs. To enhance the effectiveness of RNA delivery systems, much of the current research focuses on improving their intracellular transfection efficiency and modifying the disease microenvironment to make it more conducive to treatment.

3.3.1. Overcoming biological barriers for transfection

Viral vectors are known for their high transfection efficiency, but they face several challenges, including limited gene packaging capacity, safety concerns, and production difficulties. In contrast, non-viral vectors offer better safety profiles but require improvements in their transfection efficiency, particularly in areas such as cell uptake, targeted delivery, immune escape, and lysosomal escape¹⁵⁶. Therefore, improving the intracellular transfection efficiency of inhaled nucleic acid delivery systems is crucial for enhancing the therapeutic efficacy of RNA drugs. Strategies such as optimizing the delivery vector, targeted modification, enhancing RNA stability, designing responsive systems, and improving intracellular transport mechanisms can significantly boost RNA transfection efficiency in lung cells.

Endosomal escape is a critical bottleneck in non-viral gene delivery, as it determines whether the genetic material can be released into the cytoplasm. Common mechanisms of endosomal escape include membrane fusion or transient pore formation by cationic lipids, the formation of a hexagonal phase by ionizable lipids, and the proton sponge effect by polymers, which leads to endosomal swelling and rupture^{174–176}. The proton sponge effect is typically a key mechanism in lysosomal escape for cationic polymer materials such as PEI and poly-amidoamine (PAMAM). First-generation poly-lysine (PLL) has a relatively low transfection efficiency and typically requires auxiliary methods¹⁷⁷. In contrast, “proton sponge” polymers such as PEI perform better in promoting endosomal escape but still pose some toxicity issues¹⁷⁸. To reduce cell toxicity and improve transfection efficiency,

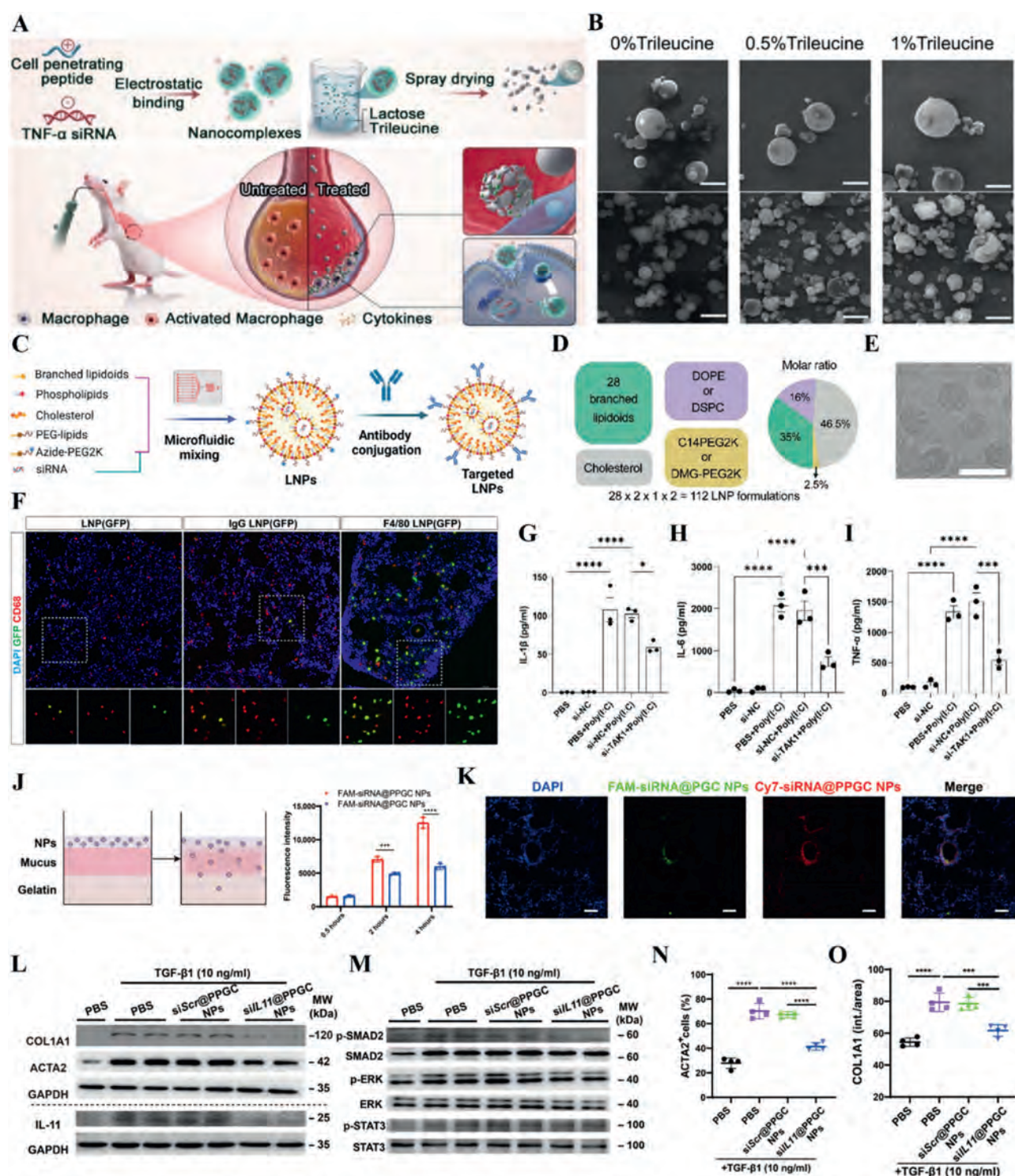


Figure 7 siRNA dry powder formulations improve lung deposition and gene silencing (A, B); antibody-modified branched LNPs target macrophages and suppress inflammation (C–I); PEG-coated nanoparticles enhance mucus penetration and anti-fibrotic effects (J–O). (A) Diagrammatic representation of the dry powder formulation of siRNA nanoparticles with high transfection efficiency and pulmonary deposition for the treatment of acute lung injury. (B) EM images of powders produced with 0% triarginine, 0.5% triarginine, and 1% triarginine. Reprinted with permission from Ref. 143 Copyright © 2024 American Chemical Society. (C) Schematic diagram of antibody modification strategy. (D) Branched lipid nanoparticles were prepared by pipetting ethanol phase containing branched lipids, phospholipids, cholesterol and PEG lipids and water phase containing siRNA. (E) A typical cryogenic transmission electron microscopy (cryo-TEM) picture of branching LNP created using microfluidic mixing method. Reprinted with permission from Ref. 50 Copyright © 2024 National Academy of Sciences. (F) Representative images of macrophages (CD68⁺) expressing GFP from each group. (G, H, I) The proinflammatory cytokines IL-1 β (G), IL-6 (H), and TNF- α (I) were measured in the supernatant using ELISA. (J) Penetration of NPs in an artificial mucus model ($n = 3$) with (FAM-siRNA@PPGC NPs)

researchers have chemically modified these polymers by introducing hydrophobic or ionizable structures, enhancing their dissociation ability and membrane disruption in acidic environments, thus further improving transfection efficiency and safety¹⁷⁹.

Lipid-based materials are commonly used delivery vectors that facilitate endosomal–lysosomal escape through fusion with the endosomal membrane. Ionizable lipids play a central role in this process: under the acidic conditions of the endosome, they become protonated and form ion pairs with anionic lipids in the membrane, triggering membrane phase transitions (*e.g.*, inverted micelle structures) that promote membrane disruption^{180,181}. The functionality of ionizable lipids can be further enhanced by incorporating unsaturated fatty acid tails, biodegradable groups, or glutathione (GSH)-responsive linkages^{182,183}. Additionally, helper lipids such as dioleoylphosphatidylethanolamine (DOPE) contribute to the formation of hexagonal phase structures that support efficient endosomal escape¹⁸⁴.

Various peptides can facilitate transmembrane transport of nucleic acids and enhance endosomal escape¹⁸⁵. Amphipathic peptides can adopt α -helical structures that disrupt endosomal membranes *via* membrane insertion or pore formation¹⁸². Although melittin possesses strong membrane-disruptive activity, its high toxicity limits its application. However, chemical modifications or pH-sensitive strategies can improve its safety profile¹⁸⁶.

During proton sponge effect–driven endosomal escape, the acidic environment poses a significant challenge to RNA stability. On one hand, the low pH in endosomes and lysosomes accelerates spontaneous RNA hydrolysis, particularly in unmodified mRNA or siRNA, where the 2'-OH group can attack the phosphodiester backbone, leading to strand cleavage¹⁸⁷. On the other hand, these organelles are rich in acidic RNases (such as RNase A and RNase T2), which can readily degrade RNA if it remains trapped for extended periods or is insufficiently encapsulated^{188,189}. Additionally, acidic conditions may induce conformational changes in RNA structures, potentially impairing downstream functional expression^{190,191}. Meanwhile, the design of proton sponge-based carriers can be optimized to enhance their acid sensitivity and endosomal escape efficiency, enabling rapid RNA release and minimizing exposure to the harsh endosomal environment. This approach improves transfection efficiency while simultaneously preserving RNA integrity and ensuring biosafety¹⁹². Especially in patients with cystic fibrosis, the altered properties of mucus make the treatment even more difficult¹⁹³. Traditional cationic gene vectors tend to be trapped and aggregated by mucus³¹. To overcome this, Osman et al.¹⁹⁴ developed a non-viral gene transfer system that integrates cell-penetrating peptide (CPP)-enhanced transfection with glycosaminoglycan-binding enhanced transduction (GET) technology. By modifying the GET peptide with PEG, they optimized gene transfer efficiency in the lungs. This PEGylated complex demonstrated excellent gene transfer effects both *in vitro* and in a mouse lung model, while also showing superior safety and gene expression compared to traditional viral vectors.

Backer et al.¹⁹⁵ optimized siRNA-loaded dextran nanogels combined with pulmonary surfactant Curosurf® to create

nanoparticles with a core–shell structure. They evaluated the ability of this hybrid nanoparticle to transfect mouse alveolar macrophages (rAM) *in vivo*. Inhalation of these nanoparticles by BALB/c mice showed that both surfactant-coated and uncoated nanogels promoted siRNA uptake by rAM cells. However, only the surfactant-coated nanogels significantly reduced the target gene's protein level, achieving a 70% downregulation of target mRNA. Notably, this system induced only a mild pro-inflammatory response and neutrophil infiltration, which could be reduced by decreasing the surfactant amount.

In addition to these systems, other strategies have been developed to improve transfection efficiency in RNA inhalation therapies. For instance, Wu et al.¹⁵⁹ found that lipid-polymer hybrid nanoparticles were more effective than liposomes in prolonging the silencing effect of the EGFP gene, and PLGA helped alleviate acute pulmonary inflammation caused by cationic lipids. Chen et al.¹⁹⁶ proposed a strategy of coupling PEG polymer chains to cationic carbon nanoclusters (TPFE) to enhance gene transfer efficiency in the lungs. By PEGylating the TPFE nanoparticles, they could penetrate airway mucus and avoid macrophage uptake, improving delivery to target lung cells. The volume reduction mechanism controlled by a hypotonic carrier also facilitated nanoparticle endocytosis by lung parenchymal cells. There are also studies that have developed small tFNAs for inhaled formulations. Compared with the state-of-the-art LNP delivery system (IR-117-17), tFNAs maintain 100% of the accompanying effect during the nebulization process. Additionally, studies have developed small tFNAs for inhaled formulations, which, despite slightly lower lung residence times, exhibited excellent dispersibility and immune response advantages. They also constructed 3D t-sponges to further improve the bioavailability of microRNA inhibitors by adsorbing microRNA and ROS⁸⁹ (Fig. 8).

3.3.2. Therapeutic microenvironment remodeling

Lung diseases such as lung cancer, COPD, ALI and pulmonary fibrosis are often associated with hypoxia, acidic pH, and elevated ROS levels. These factors can compromise the structural integrity of delivery vehicles, accelerate mRNA degradation, and reduce expression efficiency. ROS-induced oxidative damage is considered a key mechanism in the development of various pulmonary diseases. Additionally, ROS may interfere with endosomal maturation, affecting the endosomal escape of pH-sensitive delivery systems and thereby limiting the efficacy of inhaled nucleic acid therapies^{197–200}. To address this challenge, researchers have developed various ROS-responsive or scavenging systems. For example, Fan et al.²⁰¹ developed red-emitting carbon dots (RCMNs) that can respond to ROS and possess both bioimaging and anti-inflammatory properties. In a lipopolysaccharide (LPS)-induced ALI mouse model, RCMNs reduced lung damage and inflammation, improved alveolar macrophage function, lowered inflammatory factors, and increased survival rates. Mechanistically, RCMNs alleviate ALI inflammation by enhancing mitochondrial function and regulating intracellular Ca²⁺ levels. Wali Muhammad developed ROS-responsive polymer nanoparticles

and without (FAM-siRNA@PGC NPs) PEG coating. (K) Airway mucus penetration of NPs *in vivo* ($n = 3$). (L, M) Western blotting of COL1A1, ACTA2, IL-11, phosphorylation, and total expression of SMAD2, ERK, and STAT3 in MLFs in the presence of PBS, siScr@PPGC NPs, or siIL11@PPGC NPs followed by TGF- β 1 treatment (10 ng/ml) for 24 h ($n = 2$). (N, O) ACTA2+ cells and COL1A1 immunostaining intensity/area in MLFs treated with TGF- β 1 for 24 h in the presence of siScr@PPGC NPs or siIL11@PPGC NPs ($n = 4$). Reprinted with permission from Ref. 145 Copyright © 2022 The American Association for the Advancement of Science.

Table 2 Comparison of different delivery platforms for inhaled nucleic acid drugs.

Carrier material	Delivery system		Therapeutic nucleic acid	Disease	Administration	Advantage	Ref
Hybrid	Lipid–peptide	CLPs	miR-34a	NSCLC	Intratracheal	Improve the efficiency of gene transfection	144
		Pep-LNPs	TSLP siRNA	Asthma	pulmonary	Targeting asthma AECs	154
		RGD-TAT-CLPs	miR-34a	NSCLC	Intratracheal	Tumor targeting	144
	Polymer–peptide	PEG-LA	mRNA	—	Intratracheal	Enhance the lung delivery efficiency of aerosol mRNA	155
		PEG ₁₂ KL4	mRNA	mRNA vaccines	Intratracheal	High transfection, low inflammatory response	156
	Polymer–lipid	HNPs	siRNA	NSCLC	Intratracheal	Improves mucus permeability and cell uptake	157
		HDPM	mRNA	—	Intratracheal	Targeting cancer cells and macrophages in the lung	158
		LPNs	EGFP-siRNA	—	Intratracheal	Improves lung retention time	159
		tFNA	miRNA	IPF	Intratracheal	Offers the inherent advantage of dispersibility	89
		ZIP-LNPs	CFTR mRNA	—	Intratracheal	Improve atomizer stability	99
			DNA	—	Intratracheal	Efficiently penetrate airway mucus	160
Polymer	PBAEs		pDNA	CF	Bronchoalveolar lavage	Improved atomization performance and lung deposition	161
	PEGylated PLGA-PEI		pDNA	CF	Intratracheal	Enhanced CF sputum penetration and airway gene transfer	162
	CK ₃₀ PEG _{10k}		ASO	NSCLC	Intratracheal	Efficient mucus and lung tissue penetration	131
	pacDNA		IL-11 siRNA	PF	Intratracheal	Mucus permeation characteristics	145
	PPGC NPs		mRNA	SARS-CoV-2	Intratracheal	High transfection efficiency	163
	PACE		mRNA	IPF	Intratracheal	Overcome the barrier of mucous layer penetration	110
	SORT LNPs		IL-11 scFv	IPF	Intratracheal	Enhance mRNA stability and lung expression	124
	iLNP-HP08 ^{LOOP}		mRNA	Cancer vaccines	Oropharyngeal aspiration	High mucus penetration, mRNA expression, and dendritic cell targeting	97
Lipid	CAS-LNP		mRNA	—	Intratracheal	Improve mRNA delivery efficiency in lung	164
	TG4C-LNP		miR-335	Primary lung cancer	Intratracheal	In vivo stable, highly targeted	165
	EVs		miR-511-3p	Asthma	Intratracheal	Targeting lung macrophages	166
	Man-EVs		IL-12 mRNA	Lung cancer	Intratracheal	Low toxicity, high biocompatibility	167
Functionalized nanoparticles	HEK-Exo		siTGF- β 1	PF	Intratracheal	Improve the stability of nucleic acid drugs <i>in vivo</i>	168
	M-Exos		TAK1 siRNA	Viral pneumonia	Intratracheal	Targeting lung macrophages	50
	MacLNP		siRNA	NSCLC	Intranasal	Promotes cytoplasmic drug delivery	169
	CS-siNGs						

NSCLC, non-small cell lung cancer; Pep-LNPs, a cyclic peptide that resembles part of the capsid protein of rhinovirus and binds to ICAM-1 receptors was initially conjugated with cholesterol and subsequently assembled with ionizable cationic lipids to form the LNPs; LA, lysine and histidine-rich cationic peptide LAH4-L1; PEG12KL4, PEGylated synthetic KL4 peptide; HNPs, shell–core based polymer-lipid hybrid nanoparticles; HDPM: HA-DSPE-PEG-mannose; ZIP, zwitterionic polymer; PBAEs, poly(β -amino esters); CK30PEG10k, nonviral gene carrier, composed of poly-L-lysine conjugated with a 10 kDa polyethylene glycol segment; PACE, poly(amine-co-ester); SORT, Selective Organ Targeting; HEK-Exo, human embryonic kidney cell-derived exosomes; M-Exos, milk exosomes; MacLNP, macrophage-targeted lipid nanoparticle; CS, Curosurf®; TSLP, thymic stromal lymphopoietin; EGFP, enhanced green fluorescent protein; ASO, oligonucleotide; IL-12, Interleukin-12; siTGF- β 1, transforming growth factor- β 1.

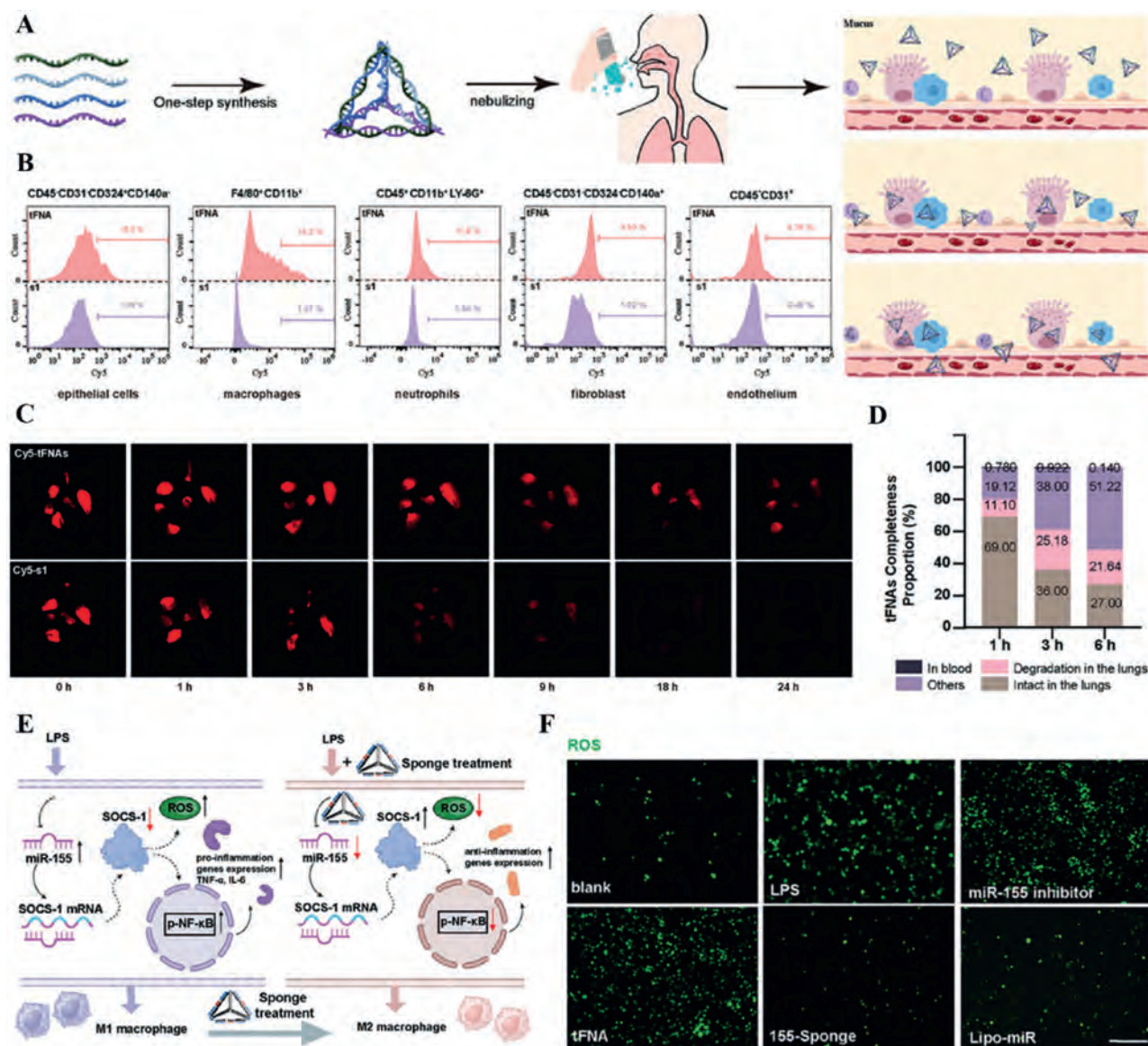


Figure 8 DNA tetrahedral microRNA sponges that penetrate the airway mucus barrier and regulate the immune microenvironment. (A) Scheme of tFNAs synthesis, nebulization, mucus, and tissue penetration. (B) Flow cytometry analysis of the uptake of Cy5-tFNA and Cy5-s1 by different lung tissue cells 24 h after nanostructure inhalation in mice. (C) Fluorescence images of mouse lungs at different times after inhalation of tFNAs and s1. (D) Distribution and integrity of tFNAs *in vivo* over time ($n = 4$). (E) Mechanistic graph of 155-Sponge regulation of inflammatory factors and ROS production in macrophages. (F) Fluorescent images of intracellular ROS content. Reprinted with permission from Ref. 89 Copyright © 2024 John Wiley and Sons.

loaded with dexamethasone (DEX) (PFTU@DEX NPs) for the treatment of ALI. Prepared using an improved emulsion method, PFTU@DEX nanoparticles can rapidly release the drug in ROS environments, effectively scavenging excess ROS, and exhibit non-hemolytic and non-cytotoxic properties. These nanoparticles are capable of converting M1 macrophages into M2 macrophages, demonstrating significant anti-inflammatory effects²⁰².

In addition, cytokines in the inflammatory microenvironment influence the effectiveness of RNA delivery systems through various mechanisms, including activation of immune responses, enhanced endocytosis, increased mucosal barriers, and transcriptional suppression. These mechanisms may lead to a reduced RNA delivery efficiency^{203–205}. To overcome these challenges, future

research will need to explore more deeply how to optimize delivery systems, reduce immune responses, and enhance their performance in inflammatory environments. Inhaled nucleic acid delivery systems offer the advantage of not only delivering therapeutic agents to the affected areas but also actively modulating the pathological microenvironment to enhance therapeutic efficacy²⁰⁶. Unmodified siRNA is typically inefficient in penetrating lung cells and mediating RNA interference through tracheal administration. However, chemically modified siRNA demonstrates significant RNA interference activity across various lung cell types while avoiding immune activation. Specifically, chemically modified siRNA is efficiently internalized by dendritic cells and alveolar epithelial cells, exerting anti-tumor or antiviral

effects by targeting and silencing specific genes. Furthermore, these modifications help resist degradation by pulmonary nucleases and lysosomes, extending the half-life of the siRNA *in vivo* and promoting its release into the cytoplasm²⁰⁷.

Tumor cells are often surrounded by an immunosuppressive microenvironment that dampens the body's anti-tumor immune response. This leads to the inhibition of immune cells such as dendritic cells, T cells, and macrophages²⁰⁸. Inhaled nucleic acid delivery systems can help overcome this by activating immune cells and reshaping the immune environment. For instance, a pH-responsive tetrahedral DNA nanomachine (CP@TDN) has been designed to deliver immunomodulatory CpG oligonucleotides and PD-L1-targeting antagonistic DNA aptamers. In an acidic tumor microenvironment, the release of the PD-L1 aptamer blocks the immune checkpoint axis, thereby activating anti-tumor immune responses²⁰⁹. In another study, Chong Qiu et al.¹⁶⁸ targeted lung cancer by delivering *IL-12* mRNA encapsulated in extracellular vesicles (EVs) *via* inhalation. This approach ensures preferential uptake by cancer cells, enhances the immune response, increases interferon- γ production, and stimulates immune effector cell expansion and antigen presentation, further strengthening the anti-tumor immune response.

IPF is associated with excessive oxidative stress, leading to cell damage, inflammation, and tissue fibrosis²¹¹. Xie et al.²¹² developed a method for treating IPF using tFNA to deliver pirfenidone

(PFD) through nebulized tracheal administration. This complex demonstrated antioxidant and immunomodulatory effects, improving lung tissue structure, reducing mortality. Additionally, an inhalable siRNA delivery system, PEI-GBZA, successfully delivers siIL-11, inhibiting fibroblast transformation, slowing the progression of pulmonary fibrosis, and demonstrating low systemic toxicity²¹³. Rui Zhang and colleagues have also developed an inhalable mRNA-based nanomedicine for IPF treatment. This formulation, composed of ribosomal protein mRNA, a keratinocyte growth factor (KGF)-modified bifunctional peptide, and a polyethylene glycol-coated protective shell, targets the ECM in the lungs, promoting alveolar epithelium regeneration. Upon inhalation, the nanomedicine is deposited in the alveoli, releasing KGF to activate matrix metalloproteinase 2 (MMP2), thus promoting collagen removal and local re-epithelialization, which leads to improved lung function²¹⁰ (Fig. 9).

Despite the significant advantages of nucleic acid delivery systems in protecting RNA integrity and enhancing transfection efficiency, their constituent components may pose immunogenic risks. Common materials, such as cationic lipid and polymers (*e.g.*, PEI, PAMAM), are prone to nonspecific interactions with plasma proteins or cell membranes *in vivo*, potentially triggering complement activation and subsequent inflammatory responses²¹⁴. Even relatively mild ionizable lipids may undergo protonation in the acidic endosomal environment, leading to recognition by the

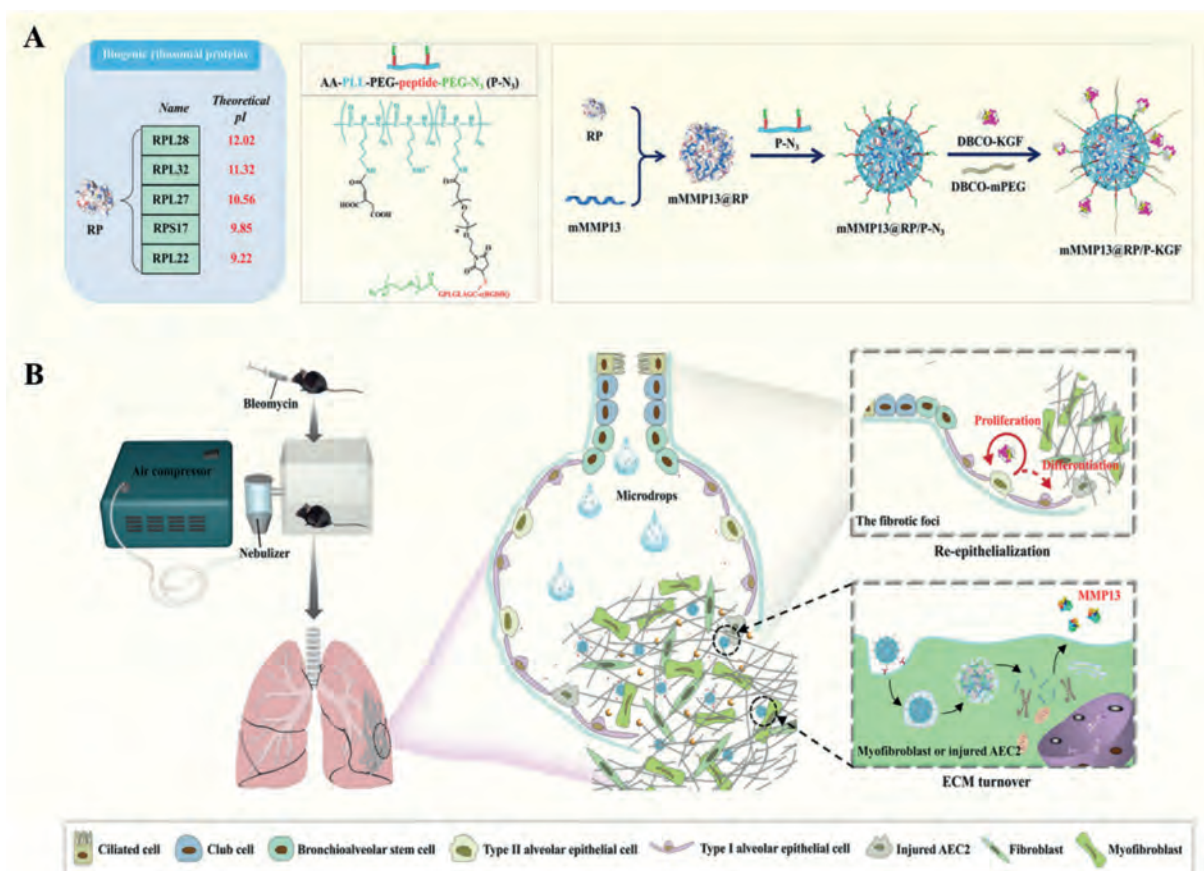


Figure 9 MMP2/pH dual-responsive mRNA nanomedicine promotes collagen degradation and alveolar re-epithelialization to reverse pulmonary fibrosis. (A) Construction of mMMP13@RP/P-KGF with MMP2 responsiveness and pH sensitivity. (B) Therapeutic mechanism of mMMP13@RP/P-KGF. Reprinted with permission from Ref. 212 Copyright © 2022 John Wiley and Sons.

innate immune system^{215,216}. Moreover, unmodified or insufficiently modified mRNA and siRNA are susceptible to detection by Toll-like receptors (TLR3, TLR7/8), resulting in the induction of type I interferons and proinflammatory cytokines²¹⁷. Moreover, fragmented nuclear DNA released into the cytoplasm due to cellular damage can be recognized by the immune system as a viral mimic, thereby triggering innate immune responses²¹⁸. These concerns are particularly pronounced with inhalation-based delivery, as the pulmonary immune barrier is highly sensitive to exogenous nucleic acids and particulate carriers²¹⁹. Alveolar macrophages and dendritic cells may initiate robust acute or chronic inflammatory responses upon exposure.

Current studies suggest that rationally designed delivery materials exhibit good pulmonary safety in inhaled nucleic acid therapies. For example, Kim et al.¹²³ improved the stability and endosomal escape capability of LNPs by incorporating PEG-lipids and β -sitosterol, and no significant toxic effects were observed following inhaled mRNA delivery. Similarly, the bottlebrush-shaped polymers developed by Fang's team showed good tolerability in mice without inducing noticeable inflammation¹³¹. Although these preliminary results are encouraging, long-term studies and clinical trials are still needed to systematically evaluate their pulmonary toxicity and safety. Alton et al.²²⁰ evaluated the efficacy and safety of repeated inhalation of a cationic lipid GL67A complexed with a CpG-free plasmid encoding human CFTR (pGM169) in mice. The treatment was administered *via* nebulization over 12 doses. The study demonstrated sustained, dose-dependent gene expression lasting over 140 days with good tolerability, supporting the potential of this approach for multi-dose, non-viral pulmonary gene therapy in patients with cystic fibrosis. In addition, off-target effects and dysregulation of gene expression are important safety concerns that need to be addressed²²¹.

Although significant progress has been made in RNA-based therapies for lung diseases, there remain key challenges in overcoming biological barriers, especially the issue of endosomal escape, and optimizing delivery systems to improve transfection efficiency. Strategies such as improving intracellular transport, altering the disease microenvironment, and developing non-viral carriers have shown potential to enhance therapeutic efficacy. However, to ensure the success of these therapies in clinical settings, safety issues such as immunogenicity, pulmonary toxicity, and off-target effects must still be addressed. Therefore, further optimization of delivery systems to improve their long-term safety and efficacy in the treatment of respiratory diseases remains a key focus for future research.

4. Overcoming bottlenecks through interdisciplinary collaboration

Inhaled RNA drug delivery systems have shown unique advantages in treating lung diseases; however, they continue to face challenges such as low delivery efficiency, limited targeting specificity, poor stability, and potential safety risks. To address these issues, recent advancements in responsive nanomaterials, biodegradable materials, and materials science are being integrated to optimize inhaled nucleic acid delivery systems for the treatment of interstitial lung diseases, which combined with cross-disciplinary innovations in computational biology, pharmacology, and clinical medicine.

Synergistic optimization of nucleic acid targeting through pharmacology and computational biology: the pathological

progression of ILDs is highly complex, and the numerous barriers faced by nucleic acid-based therapeutics during delivery underscore the need for integrated strategies that combine pharmacology with computational systems biology. By integrating single-cell omics with high-resolution imaging, researchers have uncovered the reversible dynamics between profibrotic and anti-fibrotic fibroblast subpopulations within lung tissue for identifying key regulatory factor. These findings provide a precise cellular and molecular foundation for the development of targeted nucleic acid therapies²²². Integrating single-cell omics could facilitated the rational design of biodegradable ionizable lipids, enabling LNPs to achieve efficient and specific gene editing in the lungs *via* intratracheal delivery. This approach successfully overcomes both anatomical and immunological barriers, offering a novel therapeutic avenue for diseases such as pulmonary fibrosis²²³. Together, the integration of spatial transcriptomics, artificial intelligence, and drug screening platforms holds great promise for systematically elucidating key regulatory networks involved in disease progression. This knowledge can support the design of intelligent delivery systems capable of dynamically sensing the lesion microenvironment and precisely identifying specific cellular subpopulations. Concurrently, the convergence of multi-scale modeling and *in vivo* real-time imaging technologies enables more accurate regulation of RNA-based therapies, particularly in terms of dose control, biodistribution monitoring, and immuno-compatibility optimization.

Translational applications of pharmacology and clinical medicine: recent advances in imaging technologies and cell-based therapeutic strategies have underscored the translational potential of novel drug modalities in respiratory and oncological diseases. Dynamic tracking of nucleic acid distribution in lung tissues is achieved through positron emission tomography (PET), second near-infrared window, magnetic resonance imaging (MRI) and tissue-responsive imaging agents. Additionally, organoid microarrays are employed to evaluate drug compatibility and anti-fibrotic efficacy, thereby accelerating preclinical validation²²⁴. For instance, PET tracers targeting type I collagen combined with multi-echo MRI provide a non-invasive tool for monitoring the progression of fibrosis and drug-induced interstitial lung disease (DILD). This approach enables spatial differentiation between inflammatory and fibrotic regions, enhancing clinical decision-making and longitudinal follow-up²²⁵. Additionally, NIR-II window nanoprobe, functionalized with peptides, antibodies, or nucleic acid ligands, enable deep tissue imaging and targeted therapy, while enhancing molecular specificity and resolution. This innovation facilitates the development of early diagnosis and therapeutic localization²²⁶. Moreover, radioactive-labeled CAR-T cells can be dynamically tracked *via* PET imaging, allowing for more precise evaluation of CAR-T cell delivery, homing, and retention. This method effectively correlates therapeutic outcomes with potential toxicities, such as cytokine release syndrome²²⁷. By integrating high-resolution imaging, multimodal probes, and AI analysis platforms, it will be possible to more effectively track lesion progression and early identify pathological changes, thereby providing stronger support for personalized treatment. Particularly in the fields of pulmonary fibrosis, tumor immunotherapy, and cell therapy, the above tools will optimize drug delivery, enhance therapeutic outcomes, and reduce potential side effects, taking precision medicine a crucial step closer to clinical application.

5. Conclusion

Inhaled nucleic acid delivery systems represent a transformative approach for treating pulmonary ILDs, offering targeted therapy with reduced systemic exposure. Recent advancements have addressed critical challenges in RNA stability, pulmonary deposition, and cellular uptake. Innovations in LNP engineering, such as mucus-penetrating formulations, PEGylation, and surfactant-mimicking designs, have enhanced RNA protection and alveolar delivery. Techniques like spray drying, thin-film freeze-drying, and microfluidic platforms have improved aerosol performance and stability, enabling efficient mRNA/siRNA delivery to epithelial cells, macrophages, and fibroblasts. Moreover, cell-specific targeting strategies—including antibody conjugation, microenvironment-responsive carriers, and dual-targeted nanoparticles—have minimized off-target effects while amplifying therapeutic efficacy in fibrotic and inflammatory microenvironments. Notably, inhaled *IL-11* siRNA and mRNA-based antibody therapies have demonstrated significant anti-fibrotic and immunomodulatory effects in preclinical models, underscoring the potential of nucleic acid drugs to address unmet needs in ILD treatment.

Despite these breakthroughs, challenges persist. Mucus barriers, macrophage clearance, oxidative stress, and pathological ECM remodeling remain obstacles to optimal drug delivery. Future research should prioritize the development of multifunctional carriers capable of dynamically adapting to disease-specific microenvironments, such as pH- or enzyme-responsive systems. Additionally, scalable manufacturing processes and rigorous safety evaluations are essential for clinical translation. Combining nucleic acid therapies with existing anti-fibrotic agents or immunomodulators may yield synergistic effects, while advances in CRISPR-based gene editing could further expand therapeutic possibilities. Collaborative efforts among material scientists, pharmacologists, and clinicians will be critical to harnessing the full potential of inhaled nucleic acid therapies, ultimately transforming ILD management from palliative care to disease modification.

In recent years, extensive research has focused on the development of inhaled nucleic acid drug delivery systems, addressing challenges such as poor stability, limited targeting of lung cells, and multiple physiological barriers. Current studies have demonstrated that optimizing nucleic acid structures, enhancing delivery system stability, and refining atomization processes can significantly improve the stability of nucleic acid drugs. Additionally, cellular uptake can be increased by promoting drug deposition and enhancing targeting specificity to desired cell types. Furthermore, delivery barriers in the lungs can be mitigated by eliminating reactive oxygen species and improving the fibrotic microenvironment. The integration of these strategies can effectively enhance the transfection efficiency of nucleic acid drugs and optimize inhaled lung delivery systems.

In the future, inhaled nucleic acid drugs are expected to leverage their advantages in lung-targeted therapy to treat a broader range of diseases, including lung infections, tumors, and other pulmonary conditions. For example, the development of nucleic acid drug delivery systems for diseases such as tuberculosis, influenza, and lung cancer aim to regulate the lung immune microenvironment and overcome delivery barriers posed by pulmonary fibrosis and the tumor-associated immunosuppressive microenvironment.

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Author contributions

Jingwen Dong, Jiahui Chen and Junmei Mu contributed equally to this work. Minjie Sun and Zhanwei Zhou conceived and designed the work. Jingwen Dong, Jiahui Chen and Junmei Mu co-wrote the paper. Jinsu Wang, Yinuo Fan, Lin Zhang and Qiyang Zhang commented and corrected the paper. All of the author has read and approved the final manuscript.

Declaration of AI-assisted technologies

During the preparation of this work the authors used deepseek in order to proofread grammar. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Conflicts of interest

The authors declare no conflicts of interest.

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