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Review article

Inhalable materials and biologics for lung defence and drug delivery

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Abstract

Airway mucus has a crucial role in protecting against inhaled pathogens and regulating water homeostasis, but it can also diminish the efficacy of therapeutic pulmonary delivery. Recent development in inhalable materials and biologics has introduced strategies to modify mucus properties, strengthening mucosal protection, advancing drug delivery and targeting and supporting effective water regulation. In this Review, we thoroughly examine the structure and function of airway mucus, along with the challenges and opportunities it presents for inhaled treatments. We explore new methods that enhance the protective role of mucus through physical reinforcement, pathogen neutralization, muco-trapping and rehydration, as well as strategies that overcome the mucus barrier to improve drug delivery, including physical modulation, mucoadhesive design, muco-penetrating design, mucolytics and active targeting. Finally, we discuss the clinical implications of these promising strategies, emphasizing the need to balance mucosal function with optimized therapeutic delivery. We seek to explore prospective ways to improve inhalation therapies for both infectious and chronic lung diseases by reviewing recent progress in inhalable materials and biologics.

Sections

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Introduction

Inhalation has a vital role in facilitating safe gas exchange for human survival. However, during the breathing process, airborne particulates enter the respiratory tract. To mitigate potential harm, the airways are lined with a mucus barrier that serves as a primary defence mechanism. This airway mucus layer performs several essential functions: it mediates water evaporation in variable environmental conditions[1] and prevents the penetration of inhaled pathogens and particulates into the airway epithelium by entrapment and mucociliary clearance (MCC)[2–4].

However, protection of the airways often fails owing to gaps between mucin fibres, slowing of MCC caused by dehydration and other factors and the adaptive mechanisms of pathogens[3,5]. Mucosal dysfunction has motivated the development of strategies to strengthen mucus as both a physical barrier and a facilitator of particle clearance and hydration.

In addition to being a protective barrier, airway mucus represents both a formidable challenge and a potential target for inhaled drug delivery[6]. Pulmonary drug delivery for treatments targeting the respiratory tract offers many benefits over other routes of delivery, including higher drug concentration at the site of action, reduced overall dosage and, importantly, improved accessibility in low-resource settings[7–9]. Mediated by the pulmonary mucosa, these treatments can often be optimized, and even enabled, by managing and improving mucosal barrier function[10]. MCC clears pathogens and drugs in a manner sensitive to environmental conditions and the nature of the treatment, potentially impeding or modulating the efficiency of pulmonary drug delivery[10–12] (Fig. 1). As a result, there is a critical need to overcome the mucus barrier without compromising its protective and hydrating functions[13].

For many decades, advancements in inhaled therapies — typically delivered using nebulizers or dry powder inhalers (Box 1) — have focused on modulating mucus to support both lung defence and drug delivery (Fig. 2). Recent advances, particularly in response to the COVID-19 pandemic, have accelerated their translation potential. At the same time, growing environmental challenges, such as rising air pollution and exposure to dry air, underscore the urgent need to strengthen airway defences. These developments make this a critical moment to review emerging materials and delivery strategies.

In this Review, we provide a comprehensive overview of methods and materials aimed at modulating airway mucus for protection and pulmonary drug delivery through the mucus barrier (Table 1). We start by introducing the composition, structure and functions of airway mucus, which help in understanding its necessity in both host defence and therapeutic delivery. The first main section discusses strategies to reinforce the mucus barrier to improve defence against inhaled pathogens, which is especially relevant to respiratory illnesses and environmental pollutants. The second main section discusses designs to break through the mucus barrier for drug delivery, focusing on enhancing drug retention, penetration and epithelial absorption; this strategy is particularly relevant for asthma, cystic fibrosis, pulmonary fibrosis and lung cancer. We highlight how these strategies relate to maintaining water homeostasis and limiting inflammation in various environments. Finally, we discuss the clinical implications for balancing mucus protection with effective drug delivery and outline key factors that could help to speed up the applications of these new technologies in clinical settings.

Airway mucus

The respiratory epithelium is coated by a multicomponent secretion produced by the airway epithelium, which behaves as a viscoelastic fluid[14] (Fig. 1b). This airway lining fluid exists in two distinct layers: a mucin-rich hydrogel layer that overlays a periciliary layer. In the periciliary layer, cilia beat with variable frequency, and tethered mucins have a critical role in defence and in regulating osmotic water efflux[1,6]. Mucins, which range in size from 200 kDa to 200 MDa, form an important fraction of the airway mucus layer and have a key role in barrier function[5]. They are heavily glycosylated[15,16] to resist microbial protease. Mucins primarily consist of O-glycans, which are initially modified by *N*-acetylgalactosamine, with further conjugation of glycan moieties such as galactose, *N*-acetylglucosamine, fucose and sialic acids[15]. Mucins can absorb more than 1,000 times their mass in water. The resultant viscoelasticity is necessary for effective MCC[17], the primary mechanism to clear both inhaled pathogens and therapeutic agents (Fig. 1c).

Respiratory mucus also contains globular proteins and other substances with inhibitory activity against inhaled pathogens. These include lysozyme, lactoferrin, proteases and protease inhibitors such as leukoprotease inhibitor and antichymotrypsin, nitric oxide and hydrogen peroxide[18]. In hydrated and healthy conditions, globular proteins (mass fraction ~0.005) exert an osmotic pressure of about 350 Pa. Together with the osmotic pressure created by cilia-tethered mucins and the apical epithelial membrane (about 180 Pa), this pressure steadily draws water from the underlying epithelial cells into the airway lining fluid, supporting the replacement of water lost through evaporation as the airways humidify inhaled air[1]. When evaporation rate in the airways is too high, as it happens when breathing in cold air (<0 °C), hot air (>35 °C) or dry air (<40% relative humidity) of any temperature, globular proteins are unable to supply sufficient water entry into the airways, leading to mucosal collapse onto cilia, until the mesh size between the tethered mucins grows sufficiently small to increase osmotic pressure to the point where water homeostasis is restored. In this condition, the dehydrated, concentrated mucus layer retards MCC and compresses airway epithelial cells, promoting an inflammatory cascade that promotes cough, bronchoconstriction and chronic respiratory disease conditions.

Enhancing the barrier function of mucus

The airway mucus barrier serves as the first line of defence against inhaled particulate matter, including pathogens, allergens and carcinogens. The porosity of the mucus hydrogel, the speed of MCC and the constantly evolving strategies used by pathogens to breach the mucus barrier all contribute to the effectiveness of the mucosal barrier in preventing epithelial cellular toxicities and infections (Box 2). These features are inevitably sensitive to moisture and particulate content of the inhaled air. Enhancing the protective capacity of the mucosal barrier while maintaining immune defence and hydration is therefore often crucial for maintaining respiratory health and preventing pathogen invasion. To enhance the protection of the mucus barrier, different strategies have been developed, including physical reinforcement, pathogen neutralization, muco-trapping, mucus rehydration and MCC enhancement (Fig. 3).

Physical reinforcement

Nasal sprays containing virus-killing agents emerged as an early approach to inactive inhaled pathogens that failed to be naturally cleared. However, their high cytotoxicity — possibly compromising epithelial integrity — greatly limited their clinical translation. As a result, attention shifted towards physically strengthening the airway mucus barrier, utilizing drug-free components to build an ‘internal mask’.

Before the COVID-19 pandemic, reinforcing the mucus barrier against pathogens had not been widely explored, although foundational research on its rheological properties was conducted[19]. During the pandemic, the urgent need for viral prevention spurred the introduction of various polymers as nasal sprays aimed at increasing mucus density. For example, bentonite, a clay mineral consisting of aluminium

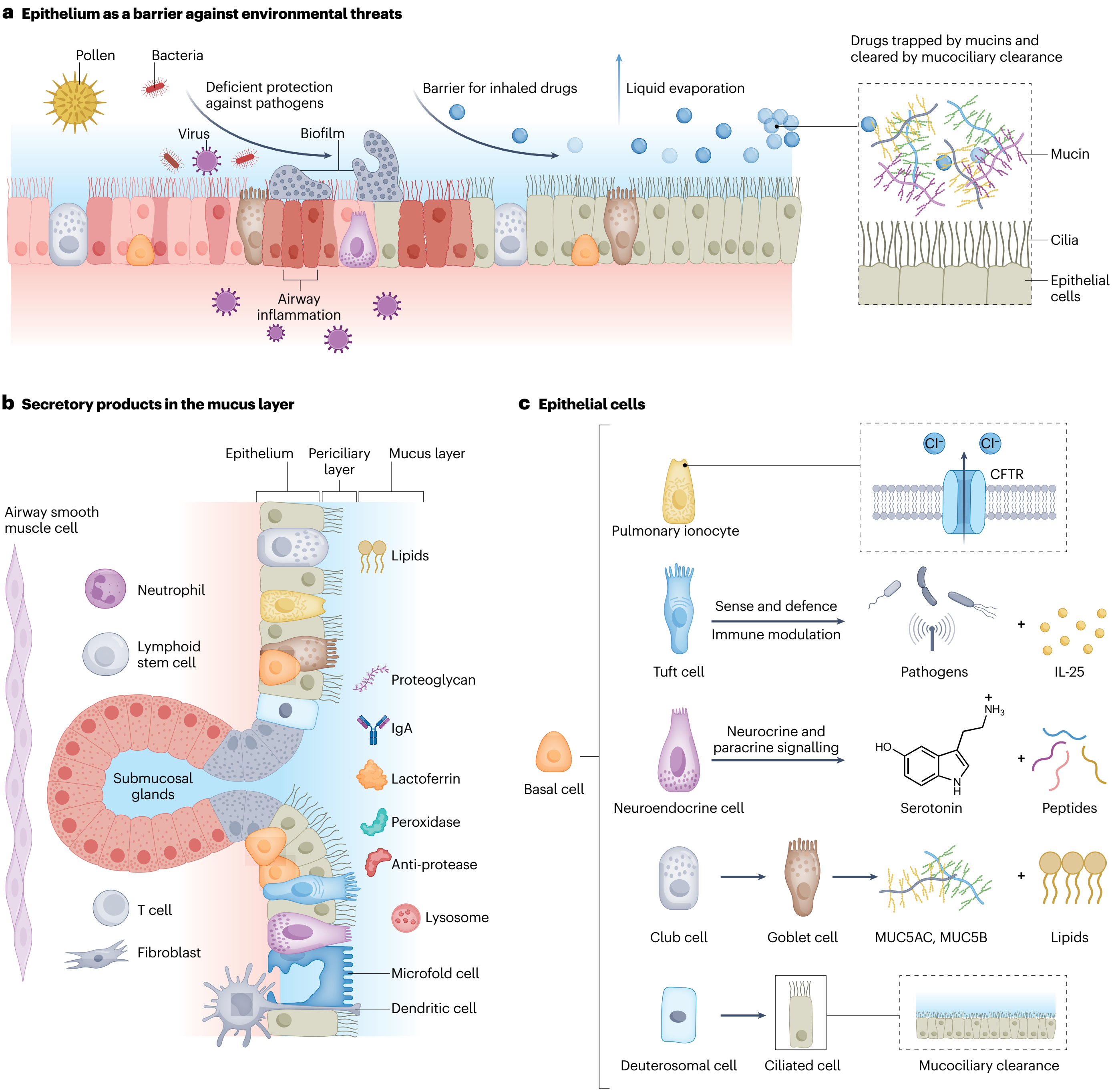


Fig. 1 | Airway mucus structure and function. a, The airway lining fluid includes a mucus-rich mucosal barrier onto which inhaled particles and pathogens deposit. Water evaporates from this layer, and inhaled drugs often traverse through it. **b**, Structure of airway epithelium and mucus. The epithelium is composed of several cell types that regenerate the mucus barrier by secreting mucosal components. **c**, Map of epithelial cell development and function. Pulmonary ionocytes, tuft cells, neuroendocrine cells, club cells and deuterosomal cells can be differentiated from basal cells. Pulmonary ionocytes allow ion exchange. Tuft cells are involved in pathogen sensing and contribute to defence and immune modulation. Neuroendocrine cells can release neurocrine and paracrine signals. Differentiated from club cells, goblet cells produce mucins and lipids to maintain mucus barrier function. In healthy hydrated circumstances, ciliated cells propel mucociliary clearance by beating at the frequency of 5–50 Hz. CFTR, cystic fibrosis transmembrane conductance regulator.

silicate sheets with a high surface area, was formulated into a nasal spray (AM-301) to protect against SARS-CoV-2 and other airborne pathogens[20]. Similarly, ethyl lauroyl arginine hydrochloride[21], as a functional excipient, and glycerol (ColdZyme)[22,23] were separately applied as nasal sprays to target multiple viruses, including rhinovirus, respiratory syncytial virus, influenza and SARS-CoV-2. Polysaccharide-based sprays were developed as jets to sterically block viral uptake into cells. For instance, different ratios of gellan and λ-carrageenan were explored based on their viscometric properties[24]. Hydroxypropyl methylcellulose was also used, either alone[25] or in combination with gellan, pectin, carboxymethyl cellulose and Carbopol[21], to act as a physical barrier to viral entry. Collectively, these spray-based strategies represent a shift towards preemptive physical defence – reinforcing mucus through biocompatible polymers without relying on cytotoxic virucidal agents.

In addition, mucoadhesive polymers were included as spray components to improve adhesion of the physical barrier to the mucosal surface. ι-Carrageenan exhibits antiviral properties, making it a candidate for nasal sprays, but its limited spray coverage and poor mucoadhesion restrict its effectiveness[26]. Low-acyl gellan was added as an excipient to enhance its performance[27]. Beyond passive polymer barriers, quaternary ammonium chitosan was found to reduce the infectivity of SARS-CoV-2 owing to its mucoadhesive properties, chemical stability and electrostatic binding to the viral spike proteins[28]. Although mucoadhesive artificial barriers enhance mucus concentration and retention, their potential interactions with MCC should be thoughtfully evaluated to ensure the preservation of the role of mucus in mediating water evaporation and assuring airway water homeostasis[1].

By targeting the nose, nasal spray reinforcement technologies have relatively rapid clearance, limiting potential disruption of water movement between the airway epithelium and the air lumen in the process of humidifying inhaled air. However, by failing to bolster the barrier function of tracheal, bronchi, bronchiole and more distal airway surfaces, these nasal technologies do not provide comprehensive coverage of the airway epithelium. They also lose effectiveness with chronic mouth breathing, recently estimated to affect 40% of school children and to increase with age[29]. Inhaled viruses travel on inhalation beyond the nose and mouth and can penetrate deep into the pulmonary airways, beyond the reach of nasal spray aerosols. To address this issue, a hydrogel-based bioadhesive dry powder has been developed for pulmonary inhalation, with particles smaller than 5 µm in diameter, allowing for deeper lung deposition[30,31]. Preclinical studies in monkeys further demonstrated the feasibility of this approach and its promise for clinical translation.

Careful tuning of physical reinforcement is critical to avoid unintended impacts such as altered hydration dynamics or airway obstruction in susceptible individuals[32,33]. Preventive mucus physical reinforcement may, indeed, not be suitable for all individuals – thickened mucus can lead to life-threatening chronic infections for patients with cystic fibrosis, for example.

Pathogen neutralization

An active approach to reinforcing mucus defences against inhaled pathogens involves incorporating agents that directly neutralize these pathogens. Traditional antiviral methods, such as those altering lung pH[34] or introducing positively charged compounds[35], have been applied to reduce pathogen load[36]. However, these approaches are nonspecific to airborne viruses and often disrupt the physiological balance of the lung. Consequently, targeted neutralization focusing on pathogen-specific entrees has gained momentum as they reinforce mucus defences without compromising lung health.

At the onset of the COVID-19 pandemic, efforts to harness antibody-based neutralization for both prevention and treatment were intensified. Antibodies derived from patients or clinical source were identified, formulated and administered via inhalation to target the receptor-binding domain (RBD) of the virus, thereby blocking its interaction with angiotensin-converting enzyme 2 (ACE2) receptors, which are widely spread on human epithelium[37–44]. Similar strategies were also used for other respiratory viruses[45,46].

However, the emergence of viral variants, particularly Omicron strains with more than 30 mutations in the spike protein, has diminished the efficacy of several marketed antibodies, driving the development of therapeutics with broader neutralizing capabilities[47]. Beyond antibodies that target conserved viral sites, ACE2 molecules offer unmatched efficacy against diverse spike mutations, leading to the marketing of inhalable ACE2 decoys for COVID-19 treatment[48]. To clarify, these ACE2 decoys are bioactive agents, not falling in the category of biomaterials. Here, we focus on biomaterials-based ACE2 reinforcement of airway mucus.

Extracellular vesicles (EVs) are heterogeneous membrane-bound structures secreted by nearly all human cells. They can be engineered to carry exogenous proteins or nucleic acids, offering stability, safety, and a biomimetic nature[49]. Studies have shown that ACE2-expressing EVs derived from patients with COVID-19 can neutralize SARS-CoV-2 by competing with ACE2-bearing cells[50]. Notably, EVs extracted from ACE2-expressing cells have been nebulized as ACE2 decoys[51–54]. Lung stem-cell-derived EV-based decoys, retaining maternal cellular features, demonstrate improved therapeutic efficiency over those derived from human embryonic kidney (HEK) cells[55]. EVs are becoming one of the most desirable candidates as drug delivery vehicles, particularly for targeted drug delivery, owing to their intrinsic advantages.

Lipid nanoparticles (LNPs), instrumental as mRNA delivery vehicles in COVID-19 vaccines, also have a role in blocking the interaction between SARS-CoV-2 and ACE2 (ref. 56). For example, a liposomal nanotrap platform functionalized with recombinant ACE2 protein or SARS-CoV-2 neutralizing antibodies was developed for inhalation to prevent viral entry[57].

Cell-membrane-based systems have also showed promise because of their biomimetic characteristics[58]. Various cell membrane sources, including red blood cells, platelets, cancer cells, immune cells and bacterial cells, have been explored for pulmonary drug delivery[59]. When delivered with positively charged thermosensitive hydrogel material in aerosols, nanoparticles expressing ACE2 extruded from HEK-293T-ACE2 cells can trap and neutralize inhaled viruses in the airway[59,60]. Additionally, cell-membrane-coated

Box 1 | Administration methods for inhaled therapies

- Nebulization: converts liquid drug formulations into aerosol droplets using compressed air, ultrasonic vibrations or mesh technology, allowing the medication to be inhaled deep into the lungs
- Dry powder inhalation: delivers micronized or porous drug particles in a dry, solid form through a breath-actuated inhaler, relying on the patient's inspiratory effort to disperse and transport the powder into the airways

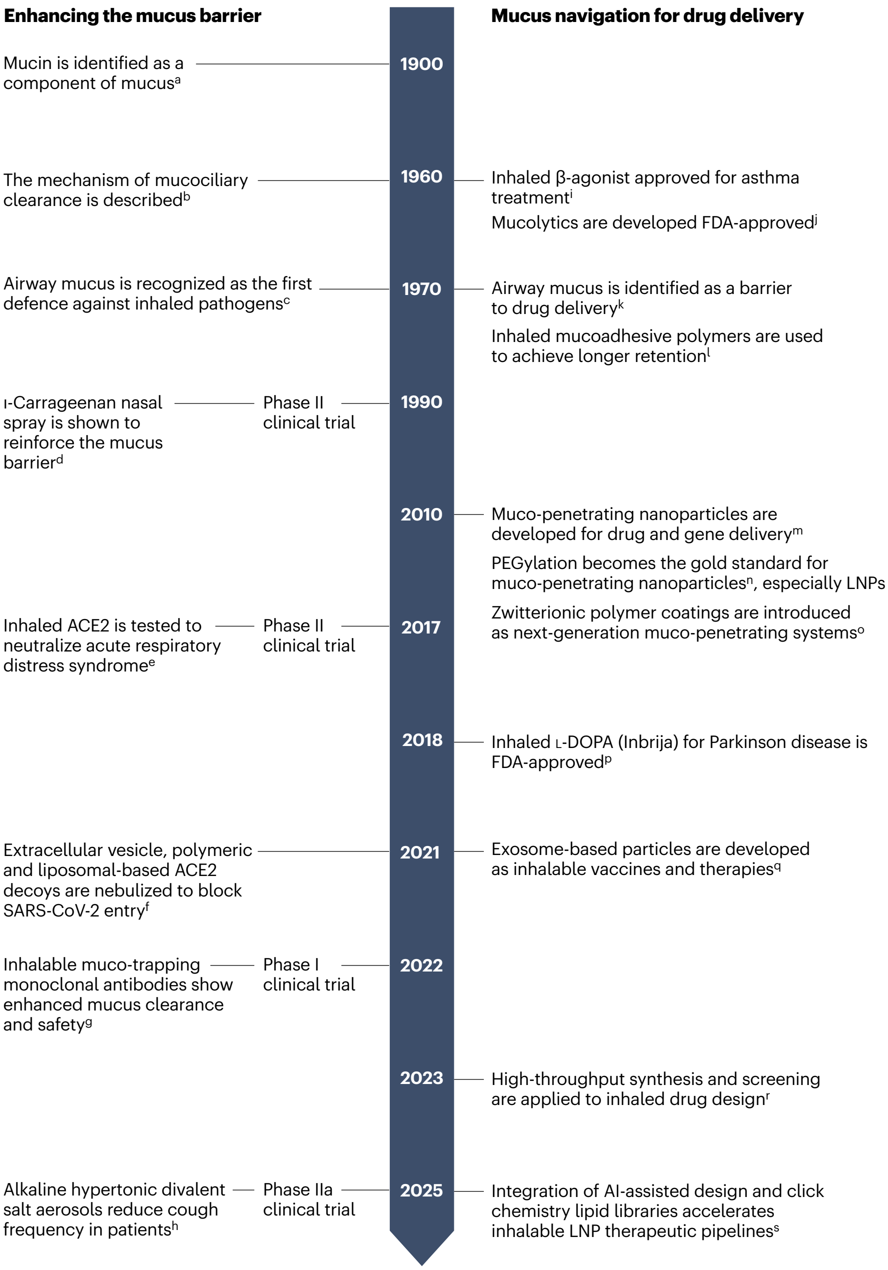


Fig. 2 | Timeline of inhalable materials and biologics development for mucus barrier enhancement and pulmonary drug delivery. Summary of key milestones in inhalable biomaterials, detailing the understanding, active ingredients and clinical status. ACE2, angiotensin-converting enzyme 2; AI, artificial intelligence; LNP, lipid nanoparticle. [a]Ref. 298, [b]ref. 299, [c]ref. 300, [d]ref. 301, [e]ref. 302, [f]refs. 55,213,303, [g]ref. 80, [h]ref. 247, [i]ref. 304, [j]ref. 305, [k]ref. 306, [l]ref. 307, [m]ref. 308, [n]ref. 309, [o]ref. 310, [p]ref. 107, [q]ref. 216, [r]ref. 273, [s]ref. 254.

poly(lactic-co-glycolic acid) nanoparticles from ACE2-expressing human lung epithelial type II cells or macrophages were developed as nanodecoys for aerosol delivery[61]. Nanocatchers containing human ACE2 and hyaluronic acid, a mucoadhesive excipient, were also formed as dry powders[62]. To mitigate COVID-19-induced cytokine storms, THP-1 cell-derived nanovesicles and ACE2-engineered 293T cells were fused to create potent decoys[63]. Further studies have explored a microfluidic microsphere-based inhalable aerosol, in which uniform methacrylate hyaluronic acid hydrogel microspheres were precisely fabricated to encapsulate hybrid nanovesicles with ACE2 and macrophage membranes[64]. As a result of the application of microfluidic technology, the microspheres can be carefully optimized for aerodynamic size, enabling even distribution across the entire respiratory tract. Their porous structure and negative surface charge contribute to prolonged residence time by promoting cilia-mediated retention and slowing enzymatic degradation.

Polymer-based nanoparticles also offer an option for COVID-19 prevention and treatment. Heparin blocks SARS-CoV-2 infection by preventing S protein binding to host cell receptors[65] and can be modified onto cell membranes[66]. As demonstrated in formulations of heparin and low-molecular-weight heparin, pulmonary delivery showed comparable bioavailability to subcutaneous administration[67]. Consequently, inhalable heparin decoys were also developed to inhibit viral interaction with heparan sulfate on host cells[68,69]. Chitosan and its derivatives

Table 1 | Inhalable materials and biologics summary for mucus barrier enhancement and drug delivery

Goal	Strategy	Mechanism	Materials	Disease applications	Advantages	Limitations
Enhancing the barrier function of mucus	Physical reinforcement	Increase mucus density/viscosity to block pathogen penetration	Polymers: gellan, λ-carrageenan, HPMC, bentonite, bioadhesive dry powders (e.g. hydrogels)	COVID-19 prevention, respiratory infections	Simple formulation design, established manufacturing processes, no need for targeting ligands, improved efficacy for systemic delivery	Risk of airway obstruction, impaired MCC, unsuitable in CF; efficacy varies by mucus turnover rate
	Pathogen neutralization	Bind or block pathogens using decoys/antibodies	ACE2 decoys (EVs, liposomes, nanoparticles), antibody aerosols, chitosan conjugates, heparin	COVID-19, influenza, RSV	Direct pathogen neutralization, synergy with other barriers, leverages validated biologics	Loss of efficacy with viral mutations, manufacturing complexity, potential immune responses
	Muco-trapping	Antibody–mucin multivalent binding traps pathogens in mucus	IgG/IgA, Fc-glycan-engineered antibodies, polyphosphates	Influenza, RSV, SARS-CoV-2, Ebola (VLPs)	Immobilizes pathogens for easier clearance, prevents pathogen–cell interaction, promotes rapid removal via MCC	Short mucin–antibody bond lifetime, rapid mucus turnover limits duration
	Mucosal hydration	Rehydrate mucus to improve clearance, reduce infection	Hypertonic saline, hypertonic divalent salts (Ca^{2+}, Mg^{2+})	CF, chronic cough, COPD	Improves MCC, reduces mucus plugging, generally well tolerated, rapid onset of action	Short duration of action, limited to upper airway, potential irritation
	MCC regeneration	Restore cilia function, promote mucin production	α-Helical peptides, hyaluronan	Primary ciliary dyskinesia, CF	Enhances MCC, reduces infection risks, restores epithelial function in chronic conditions	Technical complexity, cost, slow cilia regrowth, not scalable for prevention
Navigating the mucus barrier for drug delivery	Physical modulation (particle engineering)	Optimize size, density, shape for deeper deposition	Large porous particles, pollen-like designs	Asthma, IPF, lung cancer	Simple formulation design, established manufacturing processes, no need for targeting ligands, proven efficacy for systemic delivery	Patient variability, macrophage clearance
	Mucoadhesion	Prolong retention by binding to mucins	Chitosan, hyaluronic acid, polyacrylic acid, alginate	Vaccines, local delivery for asthma, COPD	Prolongs residence time on mucosal surfaces, enhances localized drug concentration	Rapid clearance with normal MCC, superficial binding
	Mucus penetration	Avoid adhesion, diffuse through mucus	PEGylated LNPs, zwitterionic coatings (PDA, PMPC), virus-mimic nanoparticles	CF, asthma, lung cancer gene therapy	Efficient penetration through mucus, avoids MCC and reaches epithelial targets more effectively	PEG: immune responses, aggregation; zwitterion: manufacturing challenges, reduced cellular uptake
	Mucolytic adjuvants	Locally degrade mucus to allow penetration	DNase (Pulmozyme), NAC, papain, trypsin	CF, COPD, mucus-plugged asthma	Reduces mucus viscosity and crosslinking, enhances particle diffusion through mucus	Risk of inflammation, barrier loss, slow regeneration of protective mucus
	Active targeting	Bind specific cell receptorstoenhance uptake	ICAM1, mannose, EpCAM ligands; exosomes; nanobodies	Lung cancer, IPF, asthma, infections	Enables cell-specific targeting, enhances therapeutic efficiency, minimizes systemic side effects	Expensive production, low conjugation stability, rapid MCC clearance of small formats

ACE2, angiotensin-converting enzyme 2; CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; EpCAM, epithelial cell adhesion molecule; EV, extracellular vesicle; HPMC, hydroxypropyl methylcellulose; ICAM1, intercellular adhesion molecule 1; IPF, idiopathic pulmonary fibrosis; LNP, lipid nanoparticle; MCC, mucociliary clearance; NAC, *N*-acetylcysteine; PDA, polydopamine; PEG, polyethylene glycol; PMPC, poly(2-methacryloyloxyethyl phosphorylcholine); RSV, respiratory syncytial virus; VLP, virus-like particle.

have emerged as promising polymer-based antiviral agents. Sulfated chitosan and sulfated oligochitosan also exhibit broad-spectrum antiviral effects by binding to viral surface glycoproteins or capsid proteins, thereby inhibiting virus–host cell fusion[70–72]. Other chitosan conjugates achieve viral neutralization through receptor mimicry. Chitosan-sialyllactose conjugates, for example, bind with high affinity to the haemagglutinin of influenza virus, blocking its ability to attach to host cells[73].

Muco-trapping

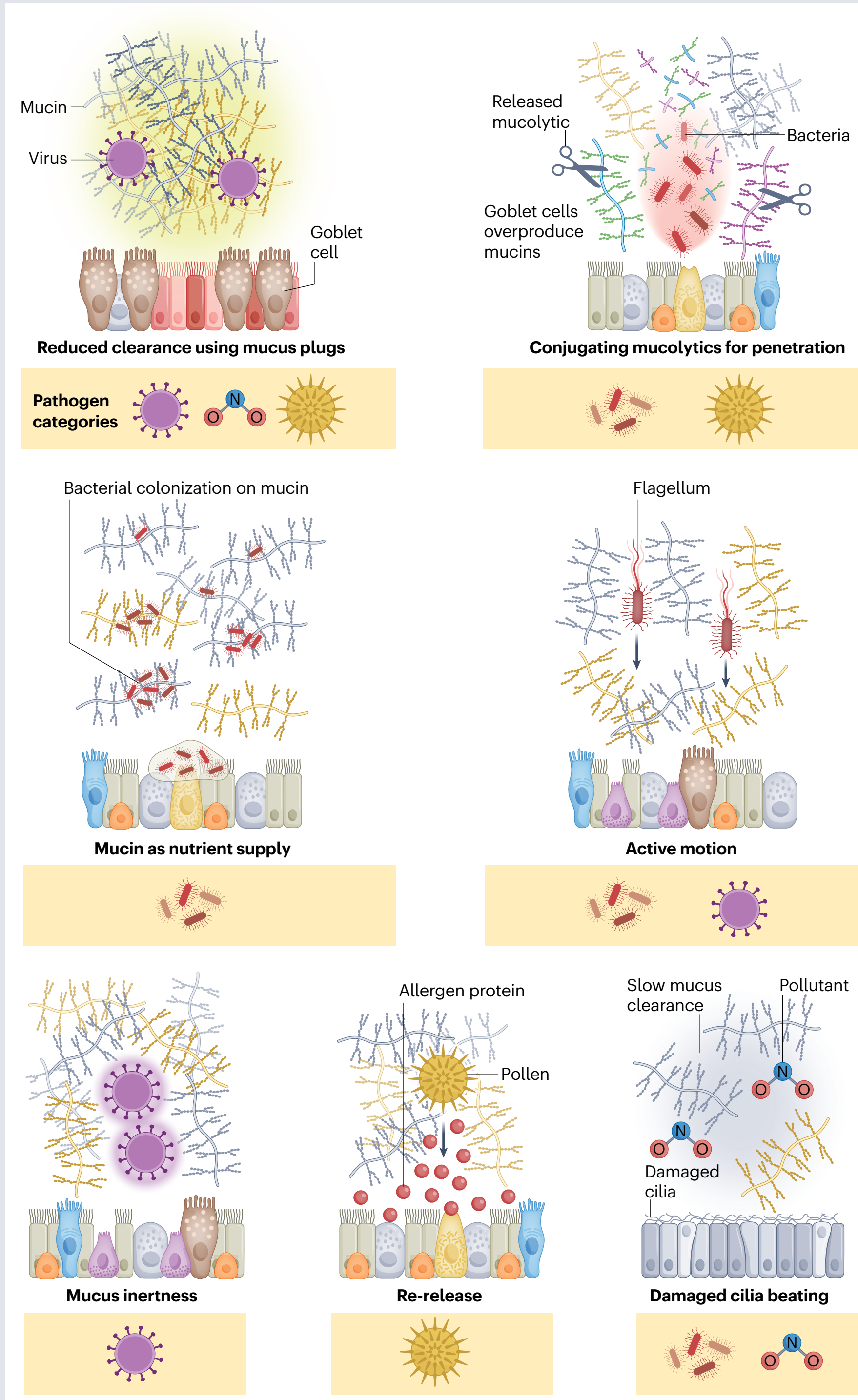
IgG antibodies possess a unique yet underutilized function in airway mucus: pathogen trapping. IgG can build multiple low-affinity bonds between pathogen-bound antibodies and the mucin network and, as a result, immobilize pathogens that are passively diffusing or actively swimming through the mucus. This universal conjunction, mediated by the IgG Fc region, can trap both viruses and bacteria in all major mucosal secretions[74].

Box 2 | Mucus as a barrier

Lung defence hinges on the tight coordination among alveolar macrophages, airway mucus and epithelial cells. In homeostasis, epithelial cells keep macrophages quiescent through direct contact and paracrine signalling[311]. Epithelial cell-derived IL-10 and transforming growth factor-β further dampen macrophage activation. Particles smaller than 200 nm often evade mucosal capture and detection by macrophages and can therefore be quickly taken up by the epithelial cells or translocated to the systemic circulation through protein-receptor-mediated mechanisms or by endocytosis through alveolar caveolae[312]. However, upon epithelial injury or detection of pathogens, macrophages shift from a quiescent to an activated, pro-inflammatory state[311], diminishing effective pathogen clearance.

Mucociliary clearance (MCC) is a critical defence mechanism, removing inhaled particles and pathogens from the airways[313,314]. Characterizing the periciliary layer, cilia are tiny, hair-like structures emanating from ciliated epithelial cells that beat in a coordinated, wave-like manner to propel the mucus and trapped substances upward towards the throat, where they can be swallowed or expelled[120,315]. It relies on the coordinated activity of hydration facilitation, mucin production and the movement of cilia[315].

Although the airway mucus barrier provides defence against inhaled pathogens, it is often insufficient to fully prevent their toxic impact, owing to mesh-like gaps among mucin fibres[316], environmental disruptions of mucus and the adaptive mechanisms of pathogens[3,5]. Various pathogens, allergens and toxins have evolved sophisticated strategies to evade and penetrate the mucosal barrier, compromising its protective function (see the figure). Disruptions in MCC can lead to chronic respiratory disease conditions, including chronic obstructive pulmonary disease, cystic fibrosis, chronic bronchitis, asthma and chronic cough, increasing risks of inflammation and infection[317].



Bacterial mucus invasion

Bacterial pathogens have evolved mechanisms to navigate and penetrate the mucus barrier. To allow active motility in mucus, bacteria adapt flagellar beating to move through the viscoelastic mucus. Some bacteria break down mucins by producing degradative enzymes, such as glycosulfatase and sialidases, decreasing mucin density as a result[318]. *Entamoeba histolytica*, for instance, can be

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released to cleave MUC2, resulting in enlarged mucin pore size and higher susceptibility for bacterial passage[318].

Conversely, some bacteria have evolved to bind tightly to mucins to facilitate their colonization. Although these bacteria are ultimately cleared through MCC, their initial binding still aids in favourable niche establishment. At the same time, mucin oligosaccharides even serve as a nutrient source for bacterial metabolism[318,319]. For example, *Pseudomonas aeruginosa* expresses adhesins such as pili and flagella to bind to sialyl-Lewis moieties on mucins[5,320,321].

Viral mucus invasion

Many non-enveloped viruses — viruses that lack a surrounding lipid membrane and are instead encased in a rigid protein shell — have special surface chemistry to allow minimal biochemical mucosal interactions[319]. In addition, their mixed positive and negative surface charges result in a net neutral charge — minimizing electronic static adhesion to mucins — whereas their hydrophilicity further reduces interactions with hydrophobic mucins[319]. Interestingly, and somewhat problematically, influenza virus can even penetrate the mucus barrier actively. For example, influenza A utilizes haemagglutinin to bind to sialic acid residues, facilitating initial host cell attachment[5]. Neuraminidase, another viral surface protein, cleaves sialic acids to prevent entrapment and release progeny virus simultaneously. Additionally, respiratory syncytial virus increases tumour necrosis factor levels, stimulating the production of MUC5AC and MUC1 to form mucus plugs[322]. As mucus plugs are hard for MCC to clear, viral load increases in return. Similarly, SARS-CoV-2 can disrupt mucus ion homeostasis, leading to mucus thickening and impaired MCC, thereby enhancing viral persistence and worsening respiratory health[319,323–325].

Pollutants mucus invasion

Air pollutants such as small particles and gases also adapt distinct mechanisms for mucus penetration[326–328]. Nitrogen oxides can disrupt ciliary beat frequency, resulting in impaired MCC and pollutant accumulation in the airways[192]. Nitrogen oxides also increase mucus viscosity, irritating bronchial nerve fibres and triggering inflammation, thereby further compromising the protective functions of the airway[329]. Additionally, some pollutants can activate the EGFR–PI3K–AKT signalling pathway, leading to mucus hypersecretion and reduced clearance[192,330].

Allergen mucus invasion

Mucosal invasion by allergens, such as pollen, represents a critical step in the pathogenesis of allergic airway diseases, including allergic rhinitis and asthma. Upon hydration in the mucus after being inhaled, pollen grains release allergenic proteins and enzymes[331,332]. These enzymes, often proteolytic in nature, degrade mucin glycoproteins to weaken the structural integrity of the mucus and facilitate deeper invasion of allergens[332,333]. Additionally, many pollen grains burst in the airway, releasing more submicron allergenic particles that diffuse through the mucus more readily than intact grains, further exacerbating mucus invasion[334].

Once pollen allergens traverse the mucus barrier, they encounter the airway epithelial barrier underneath, where they can cause direct damage[331]. Proteolytic activity from pollen allergens disrupts tight junctions between epithelial cells, increasing permeability and enabling allergens to reach underlying immune cells[335]. Furthermore, allergen invasion also alters mucus production and composition, impairing MCC and creating a favourable environment for prolonged allergen exposure[336,337].

This concept was first illustrated with the herpes simplex virus, in which herpes simplex virus-specific IgG successfully trapped herpes simplex virus in human cervicovaginal mucus, thereby inhibiting vaginal herpes simplex virus transmission in mice[75]. This approach has since been applied to airway mucus protection, where the reduced mobility of the influenza virus was correlated with endogenous influenza-binding antibodies in mucus. Reduced mobility was also found for influenza virus-like particles that cannot bind to sialic acids on mucins, demonstrating that mucus, rather than mucin, hinders virus mobility and transmission[76].

Several other studies have highlighted the effectiveness of this strategy to enhance viral clearance. Intranasally administered IgG monoclonal antibodies were reported to clear non-infectious, Ebola virus-like particles within just 30 min (ref. 46). Upon inhalation, a muco-trapping variant of motavizumab – an antibody targeting respiratory syncytial virus with improved binding – decreased the fraction of fast-moving respiratory syncytial virus in mice, rat and lamb models, in an Fc-glycan-dependent manner, by ~20–30-fold[77]. Regdanvimab, a potent neutralizing monoclonal antibody approved for COVID-19 treatment, has shown efficiency in trapping SARS-CoV-2 virus-like particles in fresh human airway mucus[78]. This antibody was later reformulated for nebulization (IN-006) and showed no serious adverse events in phase I trials[79,80]. Computational modelling has also been used to optimize antibody design for muco-trapping. Key design features include optimized Fc-glycan composition, low mucin-binding affinity to prevent free antibody immobilization and sufficient structural flexibility to facilitate dual binding[81].

Polyphosphate (PolyP) is another molecule showing potential in reinforcing mucus defence. It not only prevents viral contact to target cells but also enhances epithelial integrity and mucus barrier strength. PolyP acts as a scaffold within the airway mucus layer and also stimulates mucin production by human alveolar basal epithelial cells[82].

The application of antibody-based mucus protection was historically overlooked because it was believed to be insufficient for anchoring pathogens within mucus. Indeed, the diffusion coefficient of IgG and IgA antibodies trapped in healthy, hydrated human mucus only decreases 10% compared with that of free form in water[83,84]. This may be antibody–mucin bonds that are transient, only lasting seconds or fractions thereof, and easily disrupted by thermal motion[83]. However, other studies have implied that multiple IgG molecules can bind to the same pathogen and that the antibody array on the surface of pathogen allows for multivalent interactions with the mucin network, creating an avidity effect sufficient to effectively trap pathogens[85].

Mucus rehydration

The key to airway mucosal barrier function is its healthy hydration. Many chronic respiratory disease conditions are characterized by dehydrated, highly concentrated mucus[86]. However, healthy human airways, from the upper airways into the bronchioles, can also present dehydrated mucus owing to the mouth breathing of dry air, which is typical of both indoor air that is heated in the winter or cooled in the summer and increasingly of outdoor air as the atmosphere warms. This dehydration, beyond triggering cough, bronchospasm[87] and a cascade of inflammatory consequences implicated in the pathogenesis of chronic respiratory disease, slows down MCC by an order of magnitude or more, permitting pathogens, allergens and other particulate matter to reach the airway epithelium. A common strategy in the treatment of cystic fibrosis, in which dehydrated mucus results from a genetic defect in the cystic fibrosis transmembrane conductance regulator gene, is to inhale hypertonic salt aerosols[88], which draw water by osmosis from the airway epithelium, lifting mucus off compressed cilia and enabling it to move freely and function effectively. Although moderately effective in the treatment of cystic fibrosis, hypertonic saline aerosols can hydrate healthy airways for short periods of time, typically around 30 min.

Longer-acting hydration by hypertonic salt aerosols is possible by using divalent salts, as divalent cations are cleared by a paracellular route more slowly than sodium, which transports across the apical epithelial membrane. Inhaled hypertonic divalent salt aerosols, comprising calcium and/or magnesium chloride with median droplet size of 8–15 µm, were introduced at the start of the COVID-19 pandemic[29]. After inhalation, these aerosols deposit in the nose, larynx and trachea, where they reduce the number of exhaled respiratory droplets[89] for up to 6 h (compared to less than 1 h with hypertonic saline of similar hypertonicity) owing to prolonged hydration of the upper airways. More recently, hypertonic divalent salt aerosols with high alkalinity have been observed to provide therapeutic relief in the treatment of refractory chronic cough[86]. This effect has been attributed to their ability to increase airway surface liquid osmolarity and to exert mild antiseptic and anti-inflammatory effects through elevated pH and calcium and/or magnesium ion interactions with the airway epithelium and mucus structure.

Mucociliary clearance enhancement

Dysfunctions of the airway mucus barrier can arise owing to infectious diseases, chronic respiratory conditions and physical injuries such as respiratory tract burns. To alleviate these issues, reinforcing the mucus barrier by regenerating mucins or MCC has also been explored.

As a strategy, regulating mucus' rheology has shown promise. For example, low-molecular-weight alginate oligosaccharides can bind mucus, altering mucosal surface charge and porosity to modify the viscoelasticity of sputum[90]. Glutathione and sodium bicarbonate nanoparticles can also decrease mucosal viscosity by alkalizing the airway surface liquid and reducing oxidative crosslinking[91]. Among mucin subtypes, overexpression of MUC5AC alone does not cause airway obstruction or inflammation, as MCC is preserved. Because MUC5AC overexpression produces an 'expanded' rather than a concentrated mucus layer, infection rates of PR8/H1N1 influenza virus were lowered in mice models[92].

In addition to mucus overproducing, diseases such as cystic fibrosis are further exacerbated by impaired MCC. Considering the delicate balance of mucin subtypes, improving MCC becomes a beneficial option for mucus barrier reinforcement[93]. Tissue-engineered

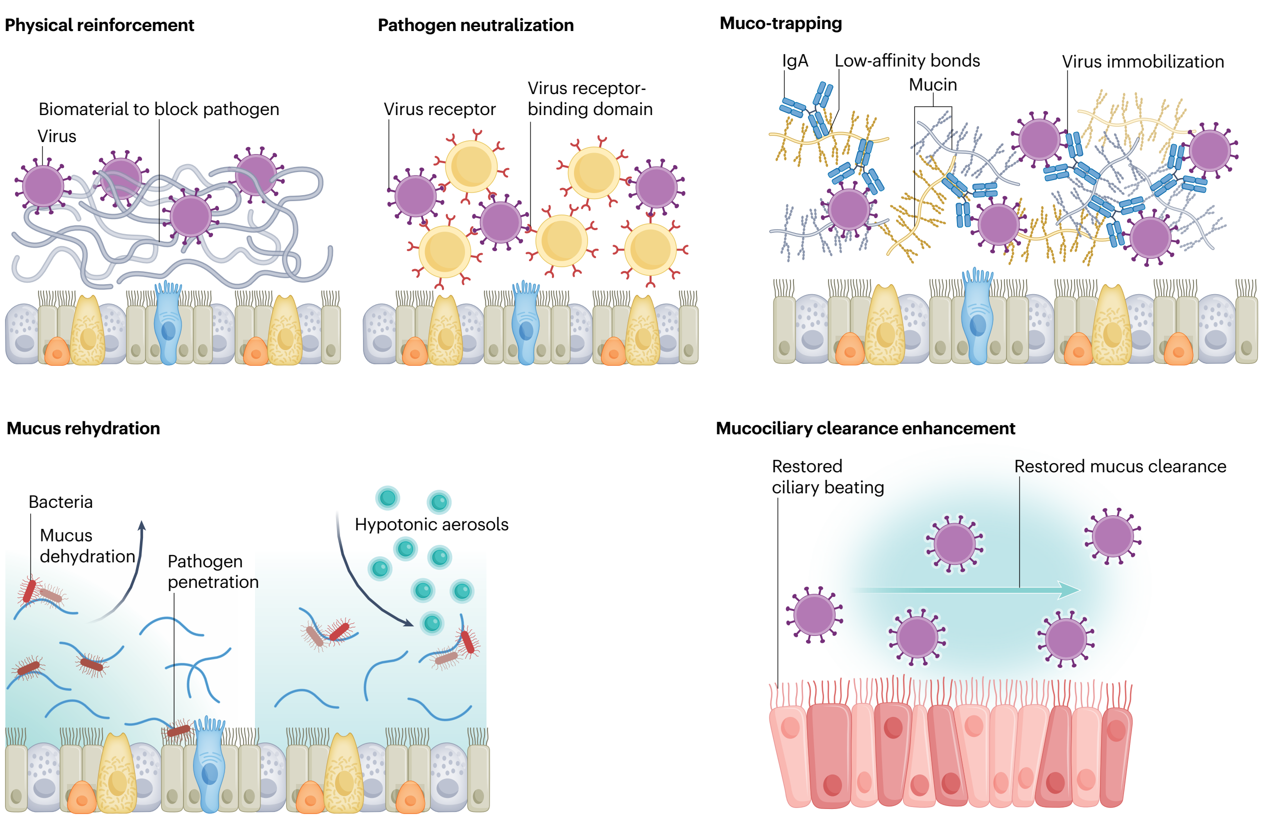

Fig. 3 | Strategies to enhance mucus barrier function. Strategies to enhance the mucus barrier using inhalable biomaterials include physical reinforcement, pathogen neutralization, muco-trapping, mucus rehydration and mucociliary clearance enhancement.

implants were explored as a potential solution. However, considering its invasiveness, complexity and high cost, implantation is clinically impossible for either preventative or therapeutic purposes. Gene therapy offers an alternative avenue. For instance, lentiviral vectors – engineered viruses used to stably deliver genes into target cells for gene therapy – have been used to target the dynein axonemal intermediate chain 1 gene in airway epithelial cells, restoring ciliary beating for primary ciliary dyskinesia treatment. However, considering this gene only accounts for 10% of airway obstruction cases[94], its clinical importance is limited. By contrast, MMP7 and MMP9 are involved in modulating mucociliary differentiation, and their inhibition has been shown to disrupt this process, limiting the ability to restore function in dysfunctional tissues[95]. For promoting ciliogenesis, using α-helical peptides on plasma-treated polymers has also shown promise by enhancing peptide adsorption, which supports epithelial attachment, tight junction formation and cilia regeneration[96]. Furthermore, hyaluronan, known for its lubricating properties, has been shown to increase intercellular adhesion molecules and to enhance MCC through both ciliary action and coughing[97]. These explorations underscore the potential to reinvigorate MCC through distinct strategies: targeting specific pathways and molecules and restoring cilia beating or encouraging epithelium ciliation.

Navigating the mucus barrier for pulmonary drug delivery

Although airway mucus serves as a critical line of defence for the respiratory tract, it also poses considerable challenges for efficient therapeutic pulmonary delivery. Effective modulation of both the delivered drug and the mucus barrier is thus crucial to enhance delivery efficiency.

Pulmonary administration both permits direct drug delivery to the lungs for respiratory applications and offers a non-invasive alternative to intravenous administration for systemic drug delivery[98–100]. Typically formulated as a dry powder aerosol or droplet mist, inhaled drugs can be administered by nasal or oral inhalation and even by pulmonary instillation[101,102]. The lungs' large surface area – comparable to a singles tennis court – provides an expansive, thin and highly vascularized interface for gas exchange. This anatomical feature also facilitates rapid absorption of inhaled therapeutics, bypassing the digestive system and avoiding first-pass liver metabolism. As a result, drugs that are otherwise poorly absorbed orally or require injection can achieve good bioavailability through pulmonary delivery. To optimize the bioavailability of systemically targeted inhaled drugs with favourable aerodynamic properties, dry powder aerosols have been engineered as large porous particles, thereby simplifying inhaler design[103–105]. This approach has led to one of the early programmes for inhaled insulin and eventually to FDA-approved inhaled L-DOPA for the treatment of Parkinson disease[106,107].

Inhalation of aerosols for the treatment of respiratory diseases ensures that the medication directly reaches the therapeutic target, maximizing therapeutic impact[99,108–110]. Direct delivery of respiratory drugs to the lungs bypasses systemic circulation, allowing higher local drug concentrations and reduced overall dosage need, thereby minimizing systemic side effects[98,99,108,109,111]. This local delivery also ensures rapid symptom relief, which is particularly valuable for respiratory diseases such as asthma, chronic obstructive pulmonary disease and cystic fibrosis[99,111,112].

Another major benefit of inhaled drug administration is its potential suitability for low-resource settings, in which simpler delivery methods and reduced need for medical infrastructure can be especially valuable. The non-invasive nature of inhalation allows for ease of self-administration without the need for specialized equipment or health-care personnel[99,113].

Despite these numerous advantages, inhaled drug delivery encounters considerable barriers that limit its overall effectiveness[13,114] (Fig. 4). One major challenge is the physical barrier imposed by airway mucus[10,115], which traps and clears all inhaled foreign particles indistinctively through MCC[116]. This MCC reduces the retention time of therapeutic agents whose function requires long presence in the airways, making it difficult for drugs to reach and remain at their target site for optimal treatment efficacy[10,13,115]. Additionally, the crosstalk of macrophage–mucus–epithelial cells presents a double-edged sword for drug delivery. Macrophages in the mucus layer can rapidly recognize and engulf inhaled therapeutics, leading to premature clearance or degradation. Although this innate immune surveillance is essential for host defence, it poses a critical hurdle for effective drug deposition and cellular uptake in the distal lung[117]. The respiratory epithelium also acts as a barrier, with tightly connected epithelial cells regulating the passage of substances into and out of the lungs[118,119]. Although epithelial-based and macrophage-based barriers operate later, MCC acts earlier and impacts all formulations, making it the primary barrier to effective inhaled drug delivery[120–122]. Therefore, we focus here on addressing challenges associated with MCC.

To enable efficient pulmonary drug delivery and tissue uptake, several strategies have been developed (Fig. 5a). Some, such as physical modulation, mucoadhesive particles and muco-penetrating particles, are relatively mature. Others, such as mucolytic agents and active targeting strategies, are multifaceted approaches that remain in active development. Collectively, these strategies are promising to improve clinical outcomes.

In addition, the inhalable delivery format is very diverse and includes directly inhaled enzymes, chemicals and nanobodies, as well as delivery assisted by carriers such as lipid-based nanoparticles or exosomes[123–125] (Fig. 5b,c).

Physical modulation

Similar to how the physical properties of particles influence their deposition patterns[126], they also influence their ability to traverse the mucus barrier. Among these properties, aerodynamic diameter is the most critical factor. Aerodynamic diameter is defined as the diameter of a spherical particle with a density equal to that of water that would have the same aerodynamic behaviour – such as deposition in the airways – as the actual particle, regardless of its true shape, size and density. The aerodynamic diameter of a particle of arbitrary physical properties dictates where the particle will land in the airways through three mechanisms: inertial impaction, gravitational sedimentation and diffusion[127]. Particles with aerodynamic diameters larger than 5 μm typically cannot adjust their trajectory within the airstream and are deposited in the upper airways through inertial impaction[128]. Particles with aerodynamic diameters between 1 μm and 5 μm are more likely to settle in the lower airways (bronchioles and alveoli) through gravitational sedimentation[129]. By contrast, particles with aerodynamic diameters smaller than 1 μm often remain suspended in the airstream and are exhaled without deposition, with diffusion being the primary mechanism for their deposition[129]. Consequently, particles with aerodynamic diameters between 1 μm and 5 μm are generally preferred for inhaled drug delivery[130]. It is, however, possible to design particles that are larger than 5 μm in diameter but still have very low mass density. Also, large porous particles are less susceptible to macrophage uptake,

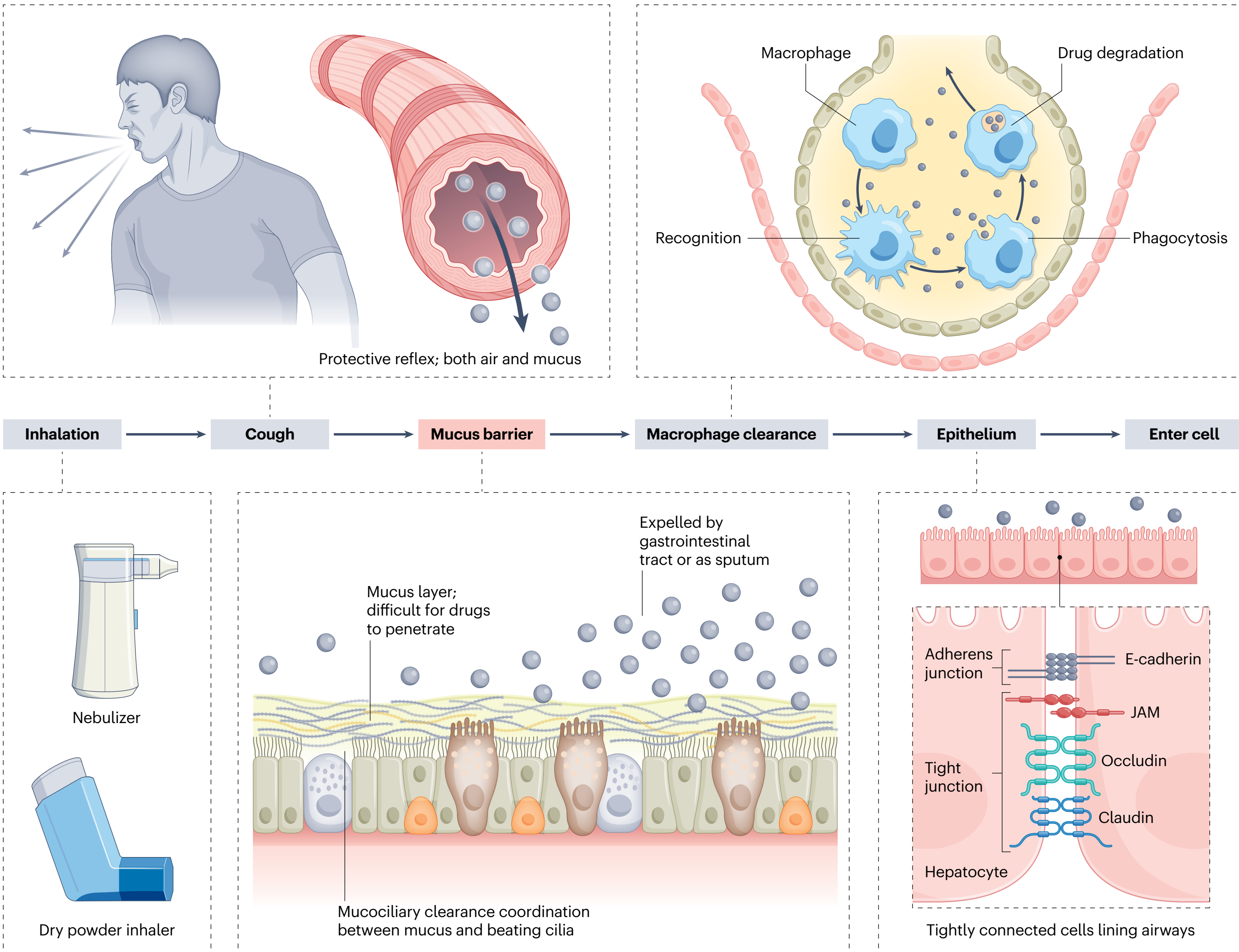

Fig. 4 | Physical and biological barriers for pulmonary drug delivery. Among the potential barriers that hinder drug transport from deposited aerosol to epithelial or systemic targets – such as mucociliary clearance, cough-induced mucus propagation, macrophage clearance and epithelial tight junctions – mucus is the first biological obstacle and, directly or indirectly, is most implicated in drug clearance. JAM, junctional adhesion molecule.

thereby enhancing pulmonary drug retention and supporting sustained release of inhaled therapeutics[103–105].

The shape of inhaled particles is another crucial factor influencing deposition patterns. For example, elongated particles with larger contact surfaces tend to be less effective at lung targeting, as their shape promotes stronger van der Waals forces and aggregation[131]. Among nanoparticles of identical volume but of varying shapes and densities, pollen-like particles have shown superior performance for inhalation, owing in part to their inherent porosity, which improves aerosolization, flowability and deposition, all suited for inhaled drug delivery[132].

Mucoadhesion

Following deposition, inhaled drugs and drug delivery systems traverse through mucus by diffusion[133], possibly adhering to mucin fibres. This adhesion depends on the physicochemical properties of the inhaled therapeutics, which influence hydrogen bonding, hydrophobic interactions and electrostatic interactions[134]. Mucoadhesive polymers leverage the same properties to optimize particle–mucin interactions and prolong the residence time of particles in mucosal tissues.

Various approaches have been developed to improve the adhesion of particles to mucus. One common strategy involves enhancing electrostatic interactions: because mucin fibres are negatively charged, mucoadhesive particles are often engineered with a positive surface charge[13]. Chitosan, a commonly used mucoadhesive polymer, exemplifies this approach and is typically attached to particle surfaces either through chemical conjugation or physical adsorption[135–137]. However, such electrostatic interactions are moderate in strength and can be weakened under high ionic strength or fluctuating pH conditions. Another strategy involves covalent bonding, such as the formation of disulfide bonds between thiol groups on the particle surface and mucin strands[138,139]. This approach generally offers stronger and more stable adhesion than electrostatic interactions, providing prolonged

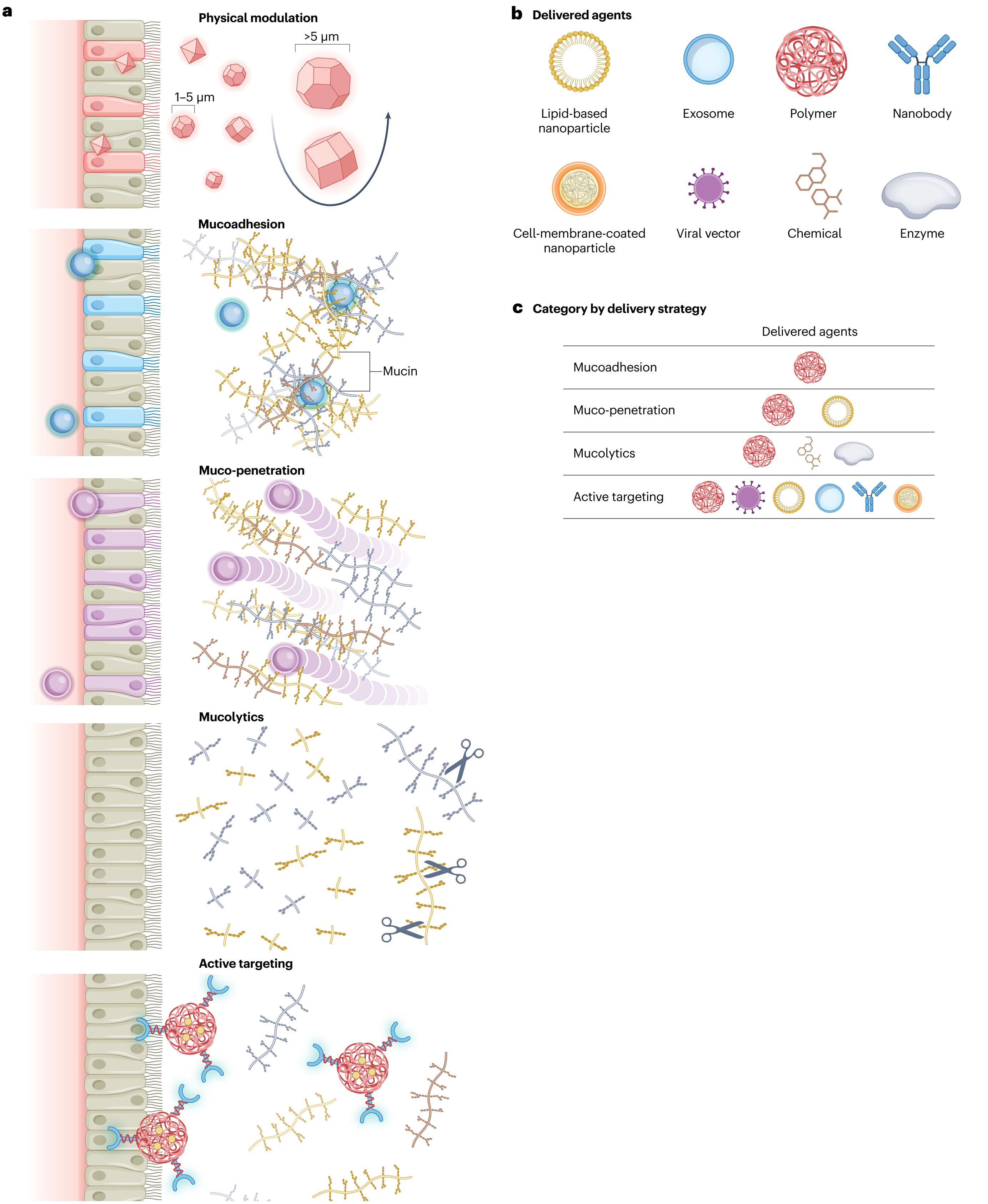
a
Physical modulation
>5 μm
1–5 μm
Mucoadhesion
Mucin
Muco-penetration
Mucolytics
Active targeting
b Delivered agents
Lipid-based nanoparticle
Exosome
Polymer
Nanobody
Cell-membrane-coated nanoparticle
Viral vector
Chemical
Enzyme
c Category by delivery strategy
Delivered agents
Mucoadhesion
Muco-penetration
Mucolytics
Active targeting

Fig. 5 | Strategies for material and biologic pulmonary delivery, categorized by delivered agent type for each strategy. a, Five strategies to navigate the mucus barrier for pulmonary drug delivery. They include modulating the inhaled particles' aerodynamic size, mucoadhesion, muco-penetration, mucolytics and active targeting. **b**, Categories of agents for pulmonary delivery: lipid-based nanoparticles, exosomes, polymers, cell-membrane-coated nanoparticles, viral vectors, chemicals, enzymes and nanobodies. **c**, Categories of delivered agents by delivery strategy. Polymeric materials can be designed to obtain mucoadhesive properties; polymeric and lipid-based nanoparticles can be designed to obtain muco-penetrating properties; polymeric particles, chemical and enzymes can be designed to obtain mucolytic properties or have mucolytics as adjuvants; polymeric particles, viral vector, lipid-based nanoparticles, exosomes, nanobodies and cell-membrane-coated nanoparticles can be delivered using active targeting.

residence time, although it requires more complex chemistry and may be susceptible to cleavage in reducing environments. Hydrophobic interactions between nanoparticles and mucus have also been utilized[140–142]. They offer a balance between simplicity and strength, but carry risks related to nonspecific binding and potential immune activation. Finally, some polymers, such as polyacrylic acid, alginates and hyaluronic acid, adhere by physically entangling with mucin fibres and are commonly used for mucoadhesive particles coatings[62,143]. Each strategy has distinct advantages and limitations, and combining multiple modes of interaction is often used to enhance overall mucoadhesive performance.

Despite these strategies, mucoadhesive particles predominantly adhere to the superficial mucus layer and, at least in the upper airways where mucus turnover is rapid with proper hydration (~10–20 min)[144,145], they are quickly cleared. This quick clearance limits the delivery of therapeutic payload over the long period of time for which the mucoadhesion is designed, reducing the bioavailability of inhaled therapeutics[145].

Muco-penetration

In contrast to mucoadhesive particles, muco-penetrating particles are designed to traverse the mucus layer without being adhered. This strategy enables them to avoid mucus turnover and clearance, reaching the underlying epithelium more effectively[133,146]. Muco-penetrating particles are also typically smaller than the pore size of mucus to prevent size exclusion[133,147]. Two commonly used surface modification approaches, PEGylation and zwitterionic design, have been explored to support this strategy.

PEGylation. A common approach for muco-penetrating particles or muco-inert particles involves coating them with brush-like polyethylene glycol (PEG), whose electric neutrality, high flexibility and remarkable hydrophilicity reduce chain entanglement and shield the hydrophobic core[148–160]. Not surprisingly, PEGylation has emerged as the gold standard for engineering muco-penetrating LNPs for stability[161,162]. Nearly all types of lipid-based nanoparticles, including solid LNPs[163], liposomes[164,165] and other LNP formulations[161,166], exhibit enhanced mucus penetration using this approach. Beyond PEG, pluronic[164], poly(vinyl alcohol)[167], poly(2-alkyl-2-oxazolines)[168] and Tween[169] have also been explored as coatings, expanding the range of useful materials.

To further improve mucus penetration and therapeutic efficacy, new coatings have been created that combine multiple functional components. For instance, magneto-sensitive iron oxide has been incorporated into PEG-solid LNPs[170], enabling external magnetic guidance to physically pull particles through highly viscous airway mucus and enhance their contact with the epithelium. In PEG-LNPs modified with chitosan, the muco-penetrating outer PEG layer reduces adhesive interactions with mucins, whereas the chitosan layer promotes mucoadhesion through electrostatic interactions[170,171]. Additionally, β-sitosterol inserted into the lipid bilayer of PEG-LNPs induces the formation of polyhedral particle shapes, which increase membrane contact area and promote membrane destabilization, thus enhancing endosomal escape after cellular uptake[172]. Peptides have also been conjugated to LNP surfaces to actively target receptors on lung epithelial cells, such as intercellular adhesion molecule 1, thereby improving cellular uptake and directing therapeutic delivery after particles traverse the mucus barrier[173].

PEGylation has an important role in extending the circulation time of nanoparticles. However, its impact on endosomal escape requires careful optimization, as PEG can hinder LNP fusion with endosomal membranes, a phenomenon often referred to as the 'PEG dilemma'[174–176]. In inhaled delivery applications, PEG lipids may undergo size changes and aggregation upon nebulization[177], and their effects on lipid hydration and membrane structure warrant consideration[178]. Additionally, PEGs have been associated with immune responses, including complement activation and the formation of anti-PEG antibodies in certain contexts[179,180]. These insights have motivated the exploration of alternative strategies that preserve LNP stability and delivery efficiency while minimizing immunogenicity. For example, mRNA could be hyperbranched with poly (β-amino esters) as inhalable polyplex vectors. These systems show outstanding nebulizing stability, efficient mRNA translation and low toxicity without the need for PEGs[181].

Zwitterionic materials. Zwitterionic materials are unique polymers with both positive and negative charges within the same molecule, resulting in a neutral overall charge. This special design makes them very hydrophilic and helps to avoid unwanted interaction with mucus or proteins[182,183].

Polydopamine coatings have become a flexible tool for breaking through mucus and epithelial barriers. These zwitterionic coatings allow rapid mucus penetration and improve uptake by epithelial cells. Silica nanoparticles coated with polydopamine exhibit pH-sensitive mucus penetration, with diffusion rates approximately three times higher at pH 5.6 – close to their isoelectric point – than at more acidic (pH 3) or neutral (pH 7) conditions[184,185].

Beyond polydopamine-based systems, zwitterionic micelles formed from block copolymers containing 2-methacryloyloxyethyl phosphorylcholine have shown considerable promise for muco-penetrating drug delivery. 2-Methacryloyloxyethyl phosphorylcholine polymer brushes efficiently prevent mucin adsorption at various pH levels. However, minor chemical modulations can affect this interaction; for example, incorporating boronic acid groups allows for specific mucin binding in acidic conditions[186]. Broader studies have shown that zwitterions such as poly(carboxybetaine), poly(sulfobetaine) and phosphorylcholine can be effective nanoscale drug delivery systems. They achieve longer circulation times and extraordinary mucus penetration and do not provoke the immune responses that PEGylated carriers typically do[187–189].

Some designs draw inspiration from viruses, which can break through mucus because of their densely charged but neutral or slightly negative zeta potentials at physiological pH levels. For instance, to mimic viral surface characteristics, nanoparticles can be synthesized using a combination of chitosan and chondroitin sulfate to create equal densities of positive and negative charges[190]. LNPs can typically be designed with a specific surface charge to either increase or reduce electrostatic interactions with mucins, such as through the incorporation of chitosan or cationic amphiphilic drugs[124,191].

Replacing the PEG-lipid component with zwitterionic polymers has improved LNP stability during nebulization, minimizing size increases and ensuring superior mucus penetration[192,193]. Mucus penetration can be further boosted by adopting a two-pronged approach, combining existing muco-penetrating strategies and zwitterionic polymers. For example, zwitterionic-based nanoparticles encapsulated by the mucolytic agent *N*-acetylcysteine have demonstrated enhanced mucus penetration, whereas the gradual release of *N*-acetylcysteine cleaves disulfide bonds within the mucus network, locally reducing its viscosity and further facilitating nanoparticle diffusion[194]. For dry powder inhalation, mucolytics such as mannitol and sorbitol are recommended to improve particle penetration through mucus by osmotically drawing water into the airway surface, thereby hydrating and loosening the mucus gel layer[195].

Zwitterionic systems still face many difficulties. Polydopamine and similar pH-sensitive zwitterions might lose their ability to prevent fouling at certain pH levels, restricting their application in different mucosal environments. Manufacturing zwitterion-coated carriers with uniform surface density is challenging and can hinder large-scale production. Achieving a balance between mucus penetration and cell uptake is still challenging because the neutral, hydrophilic surfaces that benefit mucus penetration can limit interactions with epithelial cells unless they incorporate features such as positive charges or targeting molecules. This limitation makes polydopamine coatings especially appealing because they effectively penetrate mucus layers and improve uptake by epithelial cells — an advantage not inherent to all zwitterionic systems.

Mucolytics

For pulmonary diseases in which mucus exhibits elevated viscoelasticity, mucolytics can enhance drug retention and penetration[11]. For example, Pulmozyme, a recombinant human DNase, is the most commonly used mucolytic for patients with cystic fibrosis to mitigate airway mucus obstruction. It hydrolyses DNA, which forms dense entanglements with mucin glycoproteins, reducing mucosal viscoelasticity by up to 50%[196]. However, particle diffusion is not substantially enhanced, likely because the DNA fragments generated still increase the micro-viscosity within mucus pores, ultimately limiting particle mobility[197].

As an alternative approach for mucus degradation, proteolytic enzymes such as trypsin, papain and bromelain can break down peptide bonds and cleave non-glycosylated mucin domains[198]. A bulk rheological analysis demonstrated that papain considerably reduced mucus viscosity regardless of pH[199]. As these enzymes have a key role in the natural turnover of airway mucus, high biocompatibility is promised.

In addition to enzymatic degradation, chemical methods have been used both to modify the structure of mucus for drug delivery and to treat mucus overproduction. Mucinex (*N*-acetylcysteine) is a commonly used mucolytic for the mucus barrier of patients with cystic fibrosis. It cleaves disulfide bonds in mucus, thereby limiting crosslinking and successfully decreasing mucosal viscosity[199,200]. However, it does not correct the underlying cystic fibrosis transmembrane conductance regulator defect, as shown by the lack of improvement in nasal transepithelial potential difference in cystic fibrosis transmembrane conductance regulator-deficient mice, indicating that epithelial ion transport remains impaired. Other promising mucolytic agents, including Nacystelyn[201], Gelsolin[202], thymosin β4 (ref. 203) and *S*-carboxymethylcysteine (carbocisteine)[204], also reduce mucus viscosity and are promising candidates as adjuvants for pulmonary drug delivery. Notably, certain luminal food components, such as carboxymethylcellulose and polysorbate-80, have been found to decrease mucus thickness[205], although their application to airway mucus remains unexplored.

Rather than weakening the entire airway mucus layer, inhaled mucolytic-like particles can facilitate penetration by selectively cleaving specific structural components of the mucus[205,206], preserving the protective function of the mucus layer. Disulfide-breaking agents such as *N*-acetylcysteine, *N*-dodecyl-4-mercapto-butanimidamide and 2-mercapto-*N*-octylacetamide have been incorporated into particle formulations to enhance mucus penetration[207]. Similarly, enzymes such as bromelain, papain, pronase and trypsin immobilized on particle surfaces can efficiently cleave amide bonds within mucin glycoproteins to boost particle mobility[199,208].

However, long-term mucolytics exposure is associated with inflammation, metabolic syndrome and increased microbial proximity[205]. Therefore, mucus regeneration after mucolytic treatment is crucial, albeit poorly understood. When agents such as DNase or *N*-acetylcysteine disrupt the mucus layer, goblet cells and submucosal glands begin secreting mucins to rebuild the barrier. However, this process is slow, often taking hours to days. Recovery is incomplete in diseased lungs, such as those affected by cystic fibrosis, where mucin production and structure are already abnormal[200]. Therefore, mucolytics are suitable only for temporary use in drug delivery or for symptomatic relief in conditions involving mucus overproduction. To preserve or restore the barrier, possible strategies include pairing mucolytics with hydrating agents (such as hypertonic saline or surfactants) and developing synthetic mucus mimetics or hydrogels. Compared with administering mucolytics separately as adjuvants, incorporating them directly into therapeutic particles becomes a promising option.

Active targeting

Active targeting strategies are widely applied to enhance pulmonary drug delivery by directing therapeutic agents to specific receptors, leveraging molecular recognition mechanisms to enhance cellular uptake, thereby minimizing off-target distribution and boosting therapeutic efficacy. Emerging platforms, including exosomes, ligand engineering, cell-membrane-coated nanoparticles, nanobodies and viral vectors, can enable effective targeted pulmonary drug delivery.

Extracellular vesicles. EVs are lipid-bilayer-delimited membrane structures released by cells that have a crucial role in intercellular communication and disease development. EVs have been harnessed as delivery systems for proteins and genetic materials[209]. Among the different types of EVs, exosomes represent a well-characterized subclass. Exosomes, with diameters usually between 30 nm and 150 nm, are identified by their contents. They are often studied for their role in cell communication, immune response regulation and as cargo carriers for potential treatments. It is important to note that although all exosomes are EVs, not all EVs are exosomes — the defining feature of exosomes is their endosomal origin, which sets them apart from other EVs[210,211].

One notable advantage of tissue-specific cell-derived EVs is their natural homing ability to specific sites[209]. For example, lung spheroid cells – secretome (Sec) and exosomes (Exo) – have been isolated and delivered via inhalation[212]. In studies of idiopathic pulmonary fibrosis treatment, both lung spheroid cell Sec and lung spheroid cell Exo demonstrated superior therapeutic efficiency compared with their mesenchymal stem-cell-derived counterparts, owing to longer retention in airway mucus[212]. On top of this, a COVID-19 vaccine was developed by conjugating the SARS-CoV-2 RBD to lung spheroid cell Exo[213]. Compared with liposomes, the RBD-conjugated exosomes exhibited substantially enhanced retention in the mucus-lined respiratory airway and lung parenchyma[213]. Additionally, lung spheroid cell Exo, which naturally expresses higher ACE2 levels than other cell types, was inhaled as decoys to bind and neutralize SARS-CoV-2. These exosomes remained in the lungs for over 72 h post-delivery and provided stronger lung protection than HEK cell-derived exosomes[55].

Beyond surface modification, exosome-encapsulated therapeutic agents also leverage inherent biocompatibility of exosomes to cross biological barriers. For instance, *IL12* mRNA-loaded exosomes were developed to treat lung cancer owing to potent tumour-suppressing properties of IL-12. Compared with *IL12* mRNA-loaded liposomes, aerosolized exosomes demonstrated better mucus penetration[214,215], resulting in notable immunogenicity. Similarly, for vaccine applications, SARS-CoV-2 spike protein-encoding mRNA-loaded lung spheroid cell Exo was developed for dry powder inhalation[216]. This formulation elicited stronger IgG and secretory IgA responses than mRNA-loaded liposomes and retained functional stability at room temperature for up to 1 month. Other than mRNA, lung spheroid cell Exo loaded with protein cargo showed superior biodistribution and retention in the bronchioles and parenchyma following nebulized administration compared with both HEK cell-derived exosomes and liposomes[217].

Despite the exosomes' inherent advantages of tissue homing, insufficient attention has been paid to optimizing their mucus penetrability. Unlike synthetic nanoparticles, exosomes do not inherently avoid mucus clearance. Their natural surface charge and protein composition may interact with mucus. However, emerging evidence suggests that exosomes possess tissue tropism in the context of mucus-rich environments, which may help to mediate tissue targeting despite mucus barriers[214].

Notably, surface modifications substantially enhance mucus transport rates. For example, surface-modified milk-derived exosomes exhibit improved mobility in intestinal mucus[218,219], underscoring the potential for pulmonary therapeutic delivery. However, given that strengths of exosomes lie in their natural composition, modifications must be carefully designed to avoid compromising their stability and biocompatibility.

Greater attention should be paid to the challenges of exosome heterogeneity and manufacturing scalability. Different cell batches, culture conditions or isolation methods produce exosomes with variable properties[220]. In addition, natural production yields are low, and massive cell cultures are needed to generate clinically relevant exosome doses[220]. Addressing these challenges will require regulatory frameworks that support standardization and a more consistent approach to exosome engineering.

Ligand engineering. Ligands are functional moieties – often small molecules, peptides, carbohydrates or antibodies – that bind specifically to receptors expressed on target cell surfaces. Ligand binding triggers receptor-mediated endocytosis or other forms of cellular internalization, which not only enhances drug uptake but can also modulate downstream signalling pathways that influence therapeutic outcomes.

For targeting alveolar macrophages, mannose is widely utilized owing to its interaction with the macrophage mannose receptor 1 (also known as CD206) on macrophage surfaces, making it a popular choice in pulmonary drug delivery applications[221,222], including LNPs[223], solid LNPs[223,224] and other lipid nanoformulations[225].

To target airway epithelial cells, cell adhesion molecules – a class of widely expressed transmembrane glycoproteins – are of particular interest. Their abundance under inflammatory conditions enhances their appeal as therapeutic targets, especially through interactions with intercellular adhesion molecule 1. For instance, through pulmonary administration, anti-intercellular adhesion molecule 1 conjugation enhanced DNA-loaded nanocomplexes' transfection to airway epithelial cells[226]. This conjugation was also used in small interfering RNA delivery by LNP as asthma treatment[227]. Another popular choice is epithelial cell adhesion molecule; it was shown that lipid or polymer hybrid nanoparticles conjugated with anti-epithelial cell adhesion molecule antibodies can efficiently deliver TLR7 agonists to the airway epithelium[228].

Despite their specificity and efficacy, ligands' production and conjugation are expensive, especially for the complex design of delivered therapeutics[229]. Given the difficulty of lowering ligands' cost, developing smarter and more stable coupling to drugs could help to maximize their cost-effectiveness.

Cell membrane engineering. Recent advances in pulmonary drug delivery have increasingly turned to cell-membrane-coated nanocarriers as a strategy to enhance therapeutic efficacy in the lung microenvironment. These systems harness the biological functionality of natural cell membranes to overcome barriers.

By combining *Chlamydomonas reinhardtii* microalgae with neutrophil membrane-coated poly(lactic-co-glycolic acid), microrobotic nanoparticles were developed and loaded with antibiotics[230]. The neutrophil membrane provided a biomimetic interface that shielded the payload from immune detection and enabled specific interactions with pathogens, whereas the active motility of the microrobots promoted uniform distribution and deep penetration into lung tissues.

Building on this approach, a red blood cell membrane coating was applied to doxorubicin-loaded poly(lactic-co-glycolic acid) nanoparticles onto motile algae to target lung metastases[231]. The red blood cell membrane cloaking served to extend nanoparticle circulation time and reduce immune recognition, whereas active propulsion enhanced dispersion and accumulation in the lungs.

In a further extension of membrane-coated delivery systems, inhalable microrobots were engineered for non-invasive administration. Platelet membrane-coated, vancomycin-loaded nanoparticles were attached to *Micromonas pusilla* algae and nebulized to form aerosol particles capable of reaching deep lung regions[232]. The platelet membrane coating conferred immune evasion and pathogen-targeting properties, whereas the algae's motility ensured homogeneous lung distribution and prolonged retention.

Beyond microrobots, membrane-coated nanoparticle vaccines have been explored for mucosal immunity. For example, poly(lactic-co-glycolic acid) nanoparticles coated with native *Giardia lamblia* membranes and loaded with a CpG adjuvant[233] elicited strong mucosal and systemic immunity following intranasal administration, protecting mice against *G. lamblia* infection. Bacterial membrane vesicle-coated nanoparticles and outer membrane vesicle-functionalized nanovaccines offer additional nature-inspired strategies. These platforms leverage the inherent immunostimulatory properties and pathogen-mimicking surfaces of bacterial membranes to induce robust local immune responses[234,235]. Similarly, parasite membrane-coated nanoparticles designed for mucosal delivery have demonstrated protective immunity against *G. lamblia*, highlighting the versatility of membrane-cloaked nanocarriers[233].

Nanobodies. Nanobodies, which are the smallest fragments from camelid heavy-chain antibodies, have become potential tools for targeted pulmonary drug delivery. Unlike traditional antibodies, nanobodies can be easily turned into aerosols or prepared for use in the lungs without losing their effectiveness owing to their exceptional thermal and chemical stability, resistance to proteolytic degradation and ability to remain functional under the shear stress of nebulization.

Some nanobodies and small molecules can be designed to target disease-related pathways. For example, GSK3008348 was reported to bind αvβ6 to inhibit the activation of transforming growth factor-β for pulmonary fibrosis treatment[236]. Another bivalent nanobody comprising two HuNb103 units was inhaled to target IL-4 receptor subunit-α in asthma treatment[236,237].

Nanobody-based inhaled biotherapeutics have also been explored for respiratory pathogens. ALX-0171 – a trivalent nanobody targeting the fusion protein of respiratory syncytial virus – achieves efficient deposition in the lower respiratory tract, potently neutralizing respiratory syncytial virus at early stages of infection and reducing viral replication in preclinical models[238,239].

However, because of their extremely small size, nanobodies can be easily cleared by MCC, greatly limiting their residence time. Overcoming this challenge necessitates optimization of both formulation and dosing frequency.

Viral vectors. Only a limited fraction of adeno-associated virus vectors exhibit sufficient mobility to penetrate and distribute within the airway mucus layer[240]. This physical limitation makes it more difficult to effectively target cells and requires higher viral doses, which can raise safety issues and complicate manufacturing. Improving the vectors' mucosal penetration ability is important to reduce the dosage needed while maintaining effectiveness. Adeno-associated virus serotype 6 is known to spread more efficiently in mucosal tissues, owing to surface features that reduce its adhesion to mucins. This property makes it a promising candidate for genetic pulmonary delivery[241].

Even in the absence of active motility mechanisms, some viruses have evolved surface features that enable them to navigate the mucus meshwork effectively. Influenza A virus is a notable example, in which the coordinated action of the receptor-binding protein haemagglutinin and the receptor-cleaving enzyme neuraminidase facilitates penetration of mucus while ensuring firm binding to epithelial cells once the barrier is crossed[242]. However, this system is not without limitations. Excessive neuraminidase activity can risk premature detachment from target cells, whereas insufficient activity results in entrapment within mucus. Translating these viral strategies to synthetic or gene therapy vectors will require careful tuning of surface functionality[243].

Conclusion for active targeting. Active targeting is a term that is often misunderstood as guided missile, able to direct particle movements to target cells[244]. In reality, it only serves for selective cellular interaction. In mucus, all inhaled drugs suffer from indiscriminate MCC, even with active targeting conjugation. As a result, no dramatic changes are observed when adopting active targeting alone. To enhance delivery efficiency, active delivery is best used as an adjuvant to mucus-penetrating approaches. For example, conjugating of neonatal Fc-receptor-targeted peptides to PEGylated nanoparticles substantially enhances drug delivery efficiency[245].

Clinical implications

From the first inhaled insulin clinical programme by Inhale Therapeutics (later Nektar) in partnership with Pfizer (later Sanofi-Aventis), based on an air-gun device technology, to the porous particle-based technology developed by Alkermes and Eli Lilly, impressive advances in optimizing particle aerodynamics have led to deeper lung deposition and efficient systemic absorption. These inhaled insulin programmes, although eventually discontinued following the market challenges of Exubera[246] laid important groundwork for engineering inhalable biologics and other therapeutics aimed at systemic administration with a need for accurate control over systemic exposure. An example of the latter is inhaled L-DOPA for the treatment of Parkinson disease, the FDA-approved Inbrija that emerged out of the large porous particle technology underlying the Alkermes inhaled insulin programme. MannKind's inhaled insulin programme, which progressed successfully through phase III clinical testing and gained FDA approval as Afrezza, represents another example of advanced control of particle size, density and dispersibility to enable reliable pulmonary delivery of therapeutics. Although these formulations did not specifically aim for mucus penetration or protection, they illustrate the potential of physical modulation strategies in overcoming anatomical and physiological barriers for systemic delivery. These same principles could be extended to the design of inhaled peptide and protein therapies aimed at navigating or modulating the mucus barrier, particularly by integrating aerodynamic design with mucus-penetrating or mucus-protective features.

Efforts to enhance mucus protection by pulmonary delivery have drawn on this deep technological and clinical experience with inhaled therapeutic aerosols. For healthy mucus protection (Table 2), trials involving ethyl lauroyl arginate hydrochloride (LAEH) (NCT05768113, LAEH for COVID-19) and povidone-iodine sprays have largely remained exploratory, with relatively small trial sizes. Greater progress in the clinic has been achieved with neutralization of inhaled virus by inhaling recombinant human ACE2 (NCT04396067 phase II of retinoic acid for COVID-19 and NCT05065645 phase I for ACE2 for COVID-19) and unfractionated heparin (NCT05184101, phase II and phase III of heparin for COVID-19).

In a unique endogenous approach to mucosal engineering, Sensory Cloud has developed a family of inhaled alkaline hypertonic divalent salt aerosols for the therapeutic treatment of chronic cough, a respiratory condition estimated to afflict 10% of the adult human population, and presently without safe and adequate treatment. To date, alkaline hypertonic divalent salt aerosols have been designed to be inhaled through the mouth or nose, specifically targeting the larynx and trachea. Hypertonic divalent salt aerosols contain naturally occurring ions that hydrate the larynx and trachea through osmotic action. When these aerosols are buffered to a pH of 9, they temporally disrupt the mucus structure by raising the local pH above the pKa (~8.3) of cysteine disulfide linkages between MUC5AC and MUC5B macromolecules. This combination of hydration and disruption of the mucin interactome sharply reduces inflammatory stresses on airway epithelia and helps to prolong cough relief, owing to the osmotic effects of the released globular proteins. Acting at the top of inflammatory cascade, alkaline hypertonic divalent salt appears to reduce cough and, over time, relieves the cough hypersensitivity that underlies chronic cough

Table 2 | Recent clinical trials of reinforcing mucus barrier

Protective strategy	Details	Trial registration number	Disease implications	Active ingredient	Clinical phase
Physical reinforcement	Nasal sprays (for example, bentonite-based AM-301, ColdZyme), inhalable hydrogel-based powders	NCT05768113	SARS-CoV-2 prevention	Ethyl lauroyl arginate hydrochloride	NA
		NA[296]	Asthma	PVP-I	NA
		NCT03831763	Common colds	Glycerol (ColdZyme)	NA
		Inhalable hydrogel-based bioadhesive dry powder: developed for deep lung deposition (<5 μm particles); promising in non-human primates; enhances mucus barrier without airway obstruction			Promising preclinical approach
Pathogen neutralization	Antibody-based therapies (for example, RBD-targeting antibodies), ACE2-based decoys, nanodecoys	NCT04396067	SARS-CoV-2	Recombinant ACE2	Phase I
		NCT05065645			Phase I
		NCT05003492			Phase I
		NCT04568096			Phase I
		NCT05184101	SARS-CoV-2	UFH	Phase II
		NCT04723563			Phase II
		NCT04530578			Phase II
		NCT05255848			Phase II
		NCT04842292			Phase II
		NCT01483911	RSV	Inhaled nanobody (Nb11-59)	Phase I
		Microfluidic microspheres with ACE2 nanovesicles: enhanced residence, decoy effect			Promising preclinical approach
Muco-trapping	IgG-based antibodies for trapping pathogens, polyphosphate for mucin stabilization	NCT06287450	RSV	Bivalent RSV vaccine (IN006)	Phase I
		NCT06670937	Non-cystic fibrosis bronchiectasis	Polyclonal IgG	NA
Mucus rehydration	Hypertonic or alkaline salts, osmotic agents or small molecules to increase airway surface liquid, lower mucus viscosity and enhance clearance	NA[247]	Refractory chronic cough	Alkaline hypertonic divalent salts	Phase II1
Mucociliary clearance enhancement	Low-molecular-weight alginates, gene therapy (for example, *CFTR* targeting), tissue-engineered implants	NCT05712538	CF	Full-length *CFTR* mRNA LNP	Phase I
		NCT06429176		Antisense oligonucleotide to target splicing mutations of *CFTR* gene	Phase II
		NCT05248230		AAV-*CFTR* gene	Phase I/II
		NCT05668741		Full-length *CFTR* mRNA LNP	Phase I/II
		NCT05875025	Propellant in metred-dose inhalers	HFA-152a propellant	Phase I
		NCT06506266		HFA-134a propellant	FDA-approved
		NCT01331863	Bronchial transplantation	Airway and/or pulmonary vessels transplantation	NA
		NCT03894657	CF	Forskolin	Phase II
		NCT04732910			Phase II
		NCT05095246		KB407 (vector-*CFTR*)	Phase II
		Low-molecular-weight alginate: alters mucus properties, supports clearance; highlighted for modifying sputum viscoelasticity			Promising preclinical approach

AAV, adeno-associated virus; ACE2, angiotensin-converting enzyme 2; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; HFA, hydrofluoroalkane; LNP, lipid nanoparticle; NA, not available; P-I, povidone-iodine; RBD, receptor-binding domain; RSV, respiratory syncytial virus; UFH, unfractionated heparin.

Table 3 | Recent clinical trials of pulmonary drug delivery

Delivery strategy	Description	Advantages	Disadvantages	Trial registration number	Disease implications	Active ingredients	Clinical phase
Physical modulation	Particle engineering to physically alter aerodynamic behaviour and deposition profile without relying on biochemical interactions	Simple formulation design, established manufacturing processes, no need for targeting ligands, proven efficacy for systemic delivery	Limited control over cell-specific uptake, no inherent mucus penetration or adhesion, often rapid clearance by mucociliary action	NCT00734591	Diabetes	Insulin, mannitol, glycine, sodium hydroxide	FDA-approved
				NCT04974528		Afrezza (dry powder insulin)	FDA-approved
				NCT05904743			
				NCT01189396	Asthma, COPD, bronchitis, emphysema	Albuterol dry powder: include ProAir RespiClick and ProAir Digihaler	FDA-approved
Mucoadhesion	Chitosan-coated nanoparticles, polyacrylic acid-based particles, thiol-modified particles, dry powders	Prolongs residence time on mucosal surfaces, enhances localized drug concentration	Rapid mucus turnover (~10–20 min), limits penetration to deeper mucus layers	NA[297]		mRNA-stabilized nanoparticle	Phase I
				NCT04716569	SARS-CoV-2	Mucoadhesive budesonide	Phase III
				NCT04466280		Mucoadhesive mucodentol	Phase II
				NCT03479411	Asthma	Itraconazole dry powder	Phase II
				NCT05351086	Acute migraine	Dihydroergotamine dry powder	FDA-approved
				NCT02807675	Parkinson disease	Levodopa powder	FDA-approved
				NCT02812394			
				NCT03887884			
				NCT02352363			
				NCT03541356			
				NCT03706781	Mucositis	Mucoadhesive cetylpyridinium chloride and benzydamine HCl	Phase I
Muco-penetration	PEGylated nanoparticles, zwitterionic polymers, nature-inspired particles mimicking viral surface properties	Efficient penetration through mucus, avoids MCC and reaches epithelial targets more effectively	Potential for back diffusion, reduced epithelial endocytosis, PEG-related instability (PEG dilemma)	NCT04417036	Acute respiratory distress syndrome	Pegylated adrenomedullin	Phase IIa/b
				NCT02344004	Refractory *Mycobacterium avium* complex lung disease	Amikacin-liposome	FDA-approved
				NCT02104245	Non-CF bronchiectasis	Ciprofloxacin	Phase III
				NCT01753115	Post-exposure inhalational anthrax		FDA-approved
				NCT01331863	Airway and/or pulmonary vessels transplantation	Cyclosporine A-liposome	Phase III
				Virus-mimicking nanoparticles (chitosan/chondroitin sulfate); balanced charge for mucus penetration			Promising preclinical approach
Active targeting	Ligand-conjugated nanoparticles (for example, mannose for macrophages, ICAM1 for epithelial cells), exosome-based delivery	Enables cell-specific targeting, enhances therapeutic efficiency, minimizes systemic side effects	Limited effectiveness in overcoming MCC, high cost of ligand production, requires combined strategies	NCT04262167	IPF	Lung stem cells	Phase I
				NCT05933239	Lung cancer	Mannose	Phase I
				NCT04512027	SARS-CoV-2		Phase II
				NCT03641690	H1N1 influenza		Phase I
				NCT00132522	Non-small-cell lung cancer	EpCAM-targeted catumaxomab	Phase II
				NCT02612051	IPF	αvβ6 Integrin antagonist GSK3008348	Phase I
				NCT05124561	SARS-CoV-2	Ad5-nCoV	Phase III
				NCT05204589	SARS-CoV-2		Phase III

Table 3 (continued) | Recent clinical trials of pulmonary drug delivery

Delivery strategy	Description	Advantages	Disadvantages	Trial registration number	Disease implications	Active ingredients	Clinical phase
Active targeting (continued)				NCT00186927	Sendai virus	Sendai virus vector-GM-CSF	Phase I
				Cell-membrane-coated microrobots (for example, neutrophil membrane + microalgae): self-propelling particles loaded with antibiotics; enhanced uniformity of distribution and lung retention			Promising preclinical approach
Mucolytics	NAC, papain, bromelain, mucolytic-incorporated particles	Reduces mucus viscosity and crosslinking, enhances particle diffusion through mucus	Risk of long-term side effects (inflammation, microbial proximity), limited to temporary use	NCT04402944	SARS-CoV-2	Dornase alfa	Phase II
				NCT04432987			Phase II
				NCT01155752 NCT00179998 NCT00265434 NCT04402970	CF		FDA-approved
				NCT01046136	Colds	Guaifenesin (mucinex)	Phase IV
				NCT01114581	Acute respiratory infection		Phase IV
				NCT01537081	Acute upper respiratory tract infection		Phase IV
				NCT03000348	CF	Cysteamine	Phase I
				NCT05947955	Acute respiratory distress syndrome, infections	Gelsolin	Phase I
				NCT04140214	Bronchiectasis	Carbocisteine	Phase IV
				NCT00251056	Chronic asthmatic bronchitis, emphysema, pneumonia and pulmonary complications of CF	NAC	FDA-approved
				Mucolytic-incorporated particles (for example, NAC-loaded nanoparticles): local substructure cleavage while preserving global mucus barrier; animal models show enhanced diffusion			Promising preclinical approach

COPD, chronic obstructive pulmonary disease; EpCAM, epithelial cell adhesion molecule; GM-CSF, granulocyte–macrophage colony-stimulating factor; ICAM1, intercellular adhesion molecule 1; IPF, idiopathic pulmonary fibrosis; MCC, mucociliary clearance; NA, not available; NAC, *N*-acetylcysteine; PEG, polyethylene glycol.

syndrome. In a first exploratory clinical trial in patients with refractory chronic cough, daily nasal treatment with an alkaline hypertonic divalent salt pH 9 aerosol (SC001) reduced daily cough rate by up to 35% relative to a nasal saline control[247]. Most recently, a double-blinded, randomized, placebo-controlled phase IIa study in patients with refractory chronic cough with an alkaline hypertonic divalent salt pH 9 aerosol (SC0023) confirmed the treatment efficacy results of the exploratory trial, while further reporting a reduction in daily cough rate for patients with heavy cough (≥19 coughs h^{-1}) exceeding 70% relatively to baseline up to 3 weeks post-treatment (NCT07003347).

Efforts to improve pulmonary delivery by reinforcing the mucus barrier have also progressed in the clinic (Table 3). Although mucoadhesive particles are designed to bind to the mucus layer and extend residence time, this approach has faced challenges in clinical translation. Mucoadhesive performance varies greatly because mucus characteristics, such as composition, thickness and turnover rate, differ among patients, health conditions and body areas. This variability makes it difficult to predict and manage mucoadhesive treatments in a clinical setting. Manufacturing mucoadhesive formulations can also be complicated owing to their complex surface chemistries (such as thiolation or carbomer coating), which make consistent production harder than that of simpler muco-penetrating particle coatings. Consequently, inhaled mucoadhesive formulations have been considerably less successful than muco-penetrating methods. Muco-penetrating approaches that have made it to the clinic include PEGylated adrenomedullin (NCT04417036, phase II of BAY1097761 for acute respiratory distress syndrome), amikacin liposomes (FDA-approved for mycobacterium infections therapy) and ciprofloxacin liposomes (NCT02104245, phase III of ciprofloxacin liposomes for non-cystic fibrosis bronchiectasis), all showing better penetration and retention in the airways during delivery (Table 3).

The restricted use of mucoadhesive particle systems in the clinic teaches us an important lesson for future inhaled drug development: preclinical and clinical studies should include various cell lines, tissues and disease models that represent the complexity of human lung biology (Fig. 6a). Differences in mucus properties between patients, such as moisture level and renewal rate should be carefully evaluated because they affect therapeutic efficiency. A translationally robust strategy should therefore integrate screening of inhaled formulations and devices under conditions that capture this biological diversity, ensuring that therapies are effective across the real-world heterogeneity of patient airways.

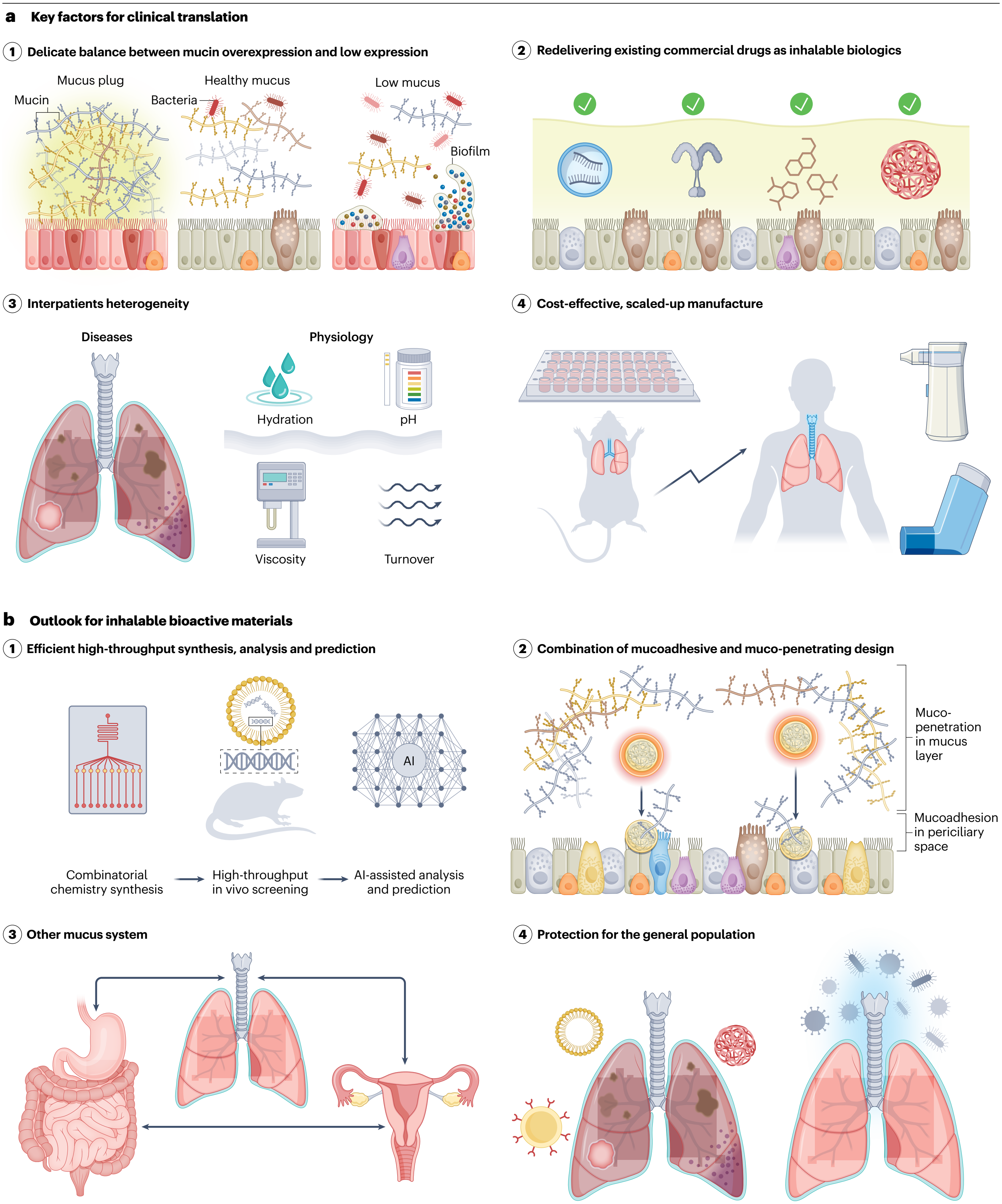
a Key factors for clinical translation
1 Delicate balance between mucin overexpression and low expression
Mucus plug
Healthy mucus
Low mucus
Mucin
Bacteria
Biofilm
2 Redelivering existing commercial drugs as inhalable biologics
3 Interpatients heterogeneity
Diseases
Physiology
Hydration
pH
Viscosity
Turnover
4 Cost-effective, scaled-up manufacture
b Outlook for inhalable bioactive materials
1 Efficient high-throughput synthesis, analysis and prediction
AI
Combinatorial chemistry synthesis
High-throughput in vivo screening
AI-assisted analysis and prediction
2 Combination of mucoadhesive and muco-penetrating design
Muco-penetration in mucus layer
Mucoadhesion in periciliary space
3 Other mucus system
4 Protection for the general population

Fig. 6 | Clinical implications and outlook of inhalable materials and biologics of next decade. a, Key factors entailing attention for clinical translation: keeping the delicate balance of mucin overexpression and low expression to prevent mucus plugs and protection loss (key factor 1); redelivering existing commercial drugs by inhalation with low cost (key factor 2); Heterogeneity between patients, including different diseases and mucus physiological characters (key factor 3); cost-effective, scaled-up manufacture (key factor 4). **b**, Strategies to enhance inhalable biomaterial design:: applying efficient high-throughput combinatorial chemistry, lipid nanoparticle DNA-barcoding for high-throughput in vivo screening and artificial intelligence (AI)-assisted prediction (strategy 1); designing delivered particles combining mucoadhesive and muco-penetrating properties for better mucus penetration and tissue uptake (strategy 2); taking inspiration from the design of delivery systems for other mucosal systems, such as the intestine or the vagina (strategy 3); and developing drug delivery systems aimed at routine airway protection in the general population, not solely for treating specific diseases (strategy 4).

Mucolytics such as dornase alfa (NCT04402944, phase II of recombinant human DNase I for COVID-19) and mannitol (NCT00251056, phase II of mannitol for cystic fibrosis) have been tested mainly to relieve mucus blockages, but they do not assist drugs breakthrough or controllability interact with the mucus effectively. Also, mucolytics can modify mucus in ways that might reduce its protective abilities, which raises concerns about a higher risk of infections or irritation, especially with long-term use. Additionally, their effects are usually temporary and greatly influenced by mucus turnover and the stage of the disease, leading to inconsistent therapeutic benefits. Cysteamine (Lynovex) offers a more advanced example, combining mucolytic, antibiofilm and antimicrobial activities to target cystic fibrosis airway infections[248]. Preclinical studies show that cysteamine disrupts *P. aeruginosa* biofilms and synergizes with antibiotics to enhance bacterial clearance[249,250]. However, although effective in biofilm clearance and mucus degradation, cysteamine does not address active restoration of the mucus barrier post-eradication. This limitation highlights the need to pair such therapies with agents that promote epithelial repair and balanced mucin production – such as cystic fibrosis transmembrane regulator modulators, growth factors or emerging mucosal regenerators – to fully restore airway defence and function[251,252].

In clinical settings, trials harnessing active targeting strategies show promise. For example, mannose-coated nanoparticles (NCT04512027, Prolectin-M for COVID-19) utilize mannose-lectin binding to enable selective uptake by immune cells. Epithelial cell adhesion molecule-targeted catumaxomab (NCT00132522, phase I of EMD 273066 for non-small-cell lung cancer) uses a bispecific antibody, which can combine epithelial cell adhesion molecules on tumour cells to enhance epithelial-specific delivery. Recombinant ACE2 (NCT04396067, phase II of soluble ACE2 protein for COVID-19) functions as a decoy receptor for SARS-CoV-2 to neutralize virus inhaled to the airway mucus. Meanwhile, inhaled nanobody treatments (NCT01483911, phase I of ALX-0171 for respiratory syncytial virus infection) apply small mucus-penetrating single-domain antibodies to reach virus-targeted airway tissues. Together, these clinical trials indicate the promise of precise cellular targeting.

Beyond novel drug formulations, redelivering existing commercial drugs for inhalation offers a cost-effective and streamlined alternative to the high costs and long timelines of developing inhalable-specific compounds. Establishing a universal adjuvant platform capable of supporting a wide range of drugs and diseases would substantially enhance the feasibility of inhalable therapeutics for broad clinical applications.

Future translation of advanced inhaled therapies requires tackling regulatory challenges. These products often face fragmented oversight, as their classification can trigger separate drug, biologics and device review requirements, increasing development time and cost. Early engagement with regulators and alignment on classification criteria will be essential to ensure efficient approval.

Outlook

Inhalable biomaterials have transformed lung protection and medication delivery. Even with important progress, research in this field is still scattered, lacking combined strategies and teamwork across different disciplines. To advance the field, better integration of innovative design, research, and clinical use is needed (Fig. 6b).

Artificial intelligence (AI)-based predictive modelling can enhance inhaled drug design by integrating formulation details, aerosol behaviour and biological factors, leading to quicker and cheaper development of therapies that penetrate mucus and target the lungs[114,253–258]. Methods such as machine-learning regression models (such as random forests[259–262] or support vector regression[263]) and deep learning techniques (such as neural networks[255,264,265] and graph neural networks[266–268] for molecular features) can be used to understand complicated, nonlinear connections between formulation factors – such as particle size, surface charge, hydrophobicity and excipient makeup – and their pharmacokinetic or pharmacodynamic profiles[269,270]. Additionally, multiobjective optimization methods such as reinforcement learning[271] and Bayesian optimization[272] can help to create formulations that optimize aerosol performance, mucus penetration and cellular uptake at the same time. These tools can integrate in silico simulations of aerosol deposition and MCC with in vivo pharmacokinetic/pharmacodynamic data to predict therapeutic outcomes and accelerate the rational design of next-generation inhalable therapies. Although AI enhances the rational design of inhalable formulations, physicochemical properties must also be carefully engineered to maximize drug retention and cellular uptake.

In parallel with AI-assisted design, high-throughput optimization platforms offer a powerful and systematic approach to rapidly test and refine inhalable LNP formulations. For example, a comprehensive workflow to evaluate formulations for pulmonary nebulization revealed that, when paired with neutral helper lipids, a low molar ratio of PEG improves LNP nebulization, whereas with cationic helper lipids, a high molar ratio benefits nebulization[177]. Using a high-throughput platform, 720 ionizable lipids based on head–linker–tail structures were synthesized and screened, and RCB-4-8 was demonstrated as most effective for pulmonary delivery[273]. Furthermore, above ionizable lipids' composition optimization, a combination library of lipid molar ratios, nebulization buffer and excipient additions was tested and optimized to achieve a formulation with 300-fold improved inhalable mRNA delivery[274]. Assisted by high-throughput technology, innovative designs of ionizable lipids such as siloxane-incorporated[275] and amidine-incorporated lipids[276] show promise, and AI could greatly pace up the analysis and prediction of larger lipid libraries[114,253–257]. Barcoding systems can also provide considerable help for more efficient and economic high-throughput in vivo testing[277–282].

Meanwhile, although muco-penetrating particles penetrate mucus more effectively than mucoadhesive particles, they may experience back diffusion owing to concentration gradients[206]. Additionally, their neutral or hydrophilic surfaces may limit epithelial endocytosis. A promising solution is the integration of mucoadhesive and muco-penetrating properties. Preliminary studies have explored particles that shift from a negative to a positive charge upon permeating the barrier, facilitated by intestinal alkaline phosphatase cleavage of phosphate residues. This approach shows potential for enhancing epithelial endocytosis[206,283], although it has not yet been investigated in the context of airway mucus. Building on these findings, further research is needed to explore similar adaptive strategies tailored for respiratory drug delivery.

Given the similarities in mucus barriers across different organs, inhalable drug delivery may explore and try to apply strategies used in the gastrointestinal and vaginal mucus to enhance diffusion and retention in the airway[284–288]. Cross-applying these insights could lead to the development of more effective formulations that balance penetration with localized drug retention.

Advancements in muco-protective strategies and drug delivery systems are paving the way for new therapeutic opportunities. However, these efforts have predominantly focused on disease-specific applications, leaving broader population-wide strategies underdeveloped. Reinforcing the airway mucus barrier for universal protection against inhaled pathogens represents a transformative opportunity, such as physical reinforcement of the mucus barrier. Especially in the past half century, global warming has driven drier environment and higher water evaporation, as a result, leading to mucus thinning, epithelial compression and subsequent inflammation[289]. Reengineering the airway water homeostasis becomes a critical consideration to enhance its protective functions, whether by increasing viscosity, modifying hydration dynamics or reinforcing its barrier properties. This approach could complement current protective strategies while expanding their applicability to diverse respiratory conditions. Together, future research should explore combinatorial strategies that integrate these strengths while preserving mucus homeostasis.

On top of good inhalable drug design, successful translation from bench to bedside requires addressing key clinical factors (Fig. 6b). Excessive mucin secretion exacerbates delivery challenges through airway obstruction and inflammation. Such pathological associations have overshadowed the protection from airway mucus in infections[290–294]. Although controlling mucin hypersecretion remains attractive for managing obstruction, prolonged mucin inhibition may not be advisable owing to their critical role in airway defence[93] (Fig. 6b).

EVs and other cell-based drug delivery strategies offer promising biocompatibility and targeting capabilities, yet their clinical application remains limited. For instance, the dendritic cell-based cancer vaccine Provenge costs approximately US$ 93,000 per patient for three doses, with only a modest survival increase of 4.1 months[295]. Given that EV recovery rates are low, their use in drug delivery may be even more expensive than Provenge. To face the challenge, synthetic pulmonary delivery systems can be designed to recapitulate key features of EVs and cell-based carriers. For example, LNP formulations can be functionalized with ligands or engineered with biomimetic surface coatings to emulate the targeting and fusion capabilities of EVs.

By fostering collaboration across disciplines and prioritizing translational research, we can unlock the full potential of inhalable therapeutics for both local and systemic treatments, driving important advancements in respiratory medicine.

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S.W.Z. searched and summarized literature for the article. All authors contributed substantially to the discussion. S.W.Z. drafted the article. All authors edited and approved the manuscript before submission.

Competing interests
D.A.E. is a co-founder of Sensory Cloud. K.C. is a co-founder of BreStem Therapeutics. A complete list of R.L.'s competing interests is provided in the Supplementary information. S.W.Z. declares no competing interests.

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