Emerging trends in pulmonary delivery of biopharmaceuticals

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Over the years, a tendency toward biopharmaceutical products as therapeutics has been witnessed compared with small molecular drugs. Biopharmaceuticals possess greater specificity, selectivity and potency with fewer side effects. The pulmonary route is a potential noninvasive route studied for the delivery of various molecules, including biopharmaceuticals. It directly delivers drugs to the lungs in higher concentrations and provides greater bioavailability than other noninvasive routes. This review focuses on the pulmonary route for the delivery of biopharmaceuticals. We have covered various biopharmaceuticals, including peptides, recombinant proteins, enzymes, monoclonal antibodies and nucleic acids, administered via a pulmonary route and discussed their rewards and drawbacks.

Keywords: Pulmonary delivery; Biopharmaceuticals; Peptides; Proteins; siRNA; Gene delivery

Introduction

Biopharmaceuticals are complex biotechnology-based products consisting of proteins, nucleic acids, sugars and tissues derived from transgenic plants, animals or microorganisms to produce highly potent therapeutic compounds.[1] They are mainly involved in producing vaccines, therapeutics, diagnostics and regenerative medicines to treat various life-threatening diseases such as cancer, HIV/AIDS, diabetes and autoimmune diseases. In 1982, Humulin®N was the first biopharmaceutical product approved for therapeutic use and, to date, nearly 300 products are available in the market.[2] Afrezza® (MannKind Corp.) and Pulmozyme® (Roche) containing human insulin and Dornase alfa, respectively, are two commercial products currently available in the niche market of inhalational biopharmaceuticals. In the 21st century, the development of these therapeutics offers numerous advantages over small-molecule drugs, such as limited interference with biological processes, high selectivity and potent therapeutic efficacy with fewer side effects and low immunogenicity.[3]

In the past few years, the biopharmaceutical market has been growing faster and the global biopharmaceutical market is forecasted to grow at a compound annual growth rate (CAGR) of 13.8% between 2018 and 2025.[4] The increased elderly population and high occurrence of genetic, metabolic or lifestyle disorders are major drivers for the growth of this market. Yet, biopharmaceuticals have shortcomings, including low thermodynamic stability, structural complexity and a difficult manufacturing process.

Although the parenteral route is primarily reported for the administration of biopharmaceutical products, other routes such as pulmonary, nasal, ocular, oral and vaginal are also studied. The pulmonary route is mainly used to deliver small molecules to the lungs because of rapid absorption, high bioavailability and specific targeting. The pulmonary route has been studied for the delivery of several biopharmaceutical products and this review focuses on recent developments, challenges and future perspectives of pulmonary delivery of biopharmaceuticals. The latest patents and clinical trials available for pulmonary delivery of biopharmaceuticals are listed in Table 1.

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TABLE 1

List of latest patents and clinical trials for pulmonary administration of biopharmaceuticals (2011–2021).

Patents

Sr. No.	*Patent title*	*Patent number*	*Applicant/assignee*	*Publication date*
1.	Nebulization of immunoglobulin	WO2015150510A1	Csl Behring Ag, Pari Pharma Gmbh, Medizinische Hochschule Hannover	08-10-2015
2.	Pulmonary delivery of mRNA to non-lung target cells	EP2858679A1	Ethris GmbH Translate Bio Inc.	15-04-2015
3.	An inhalable dry powder formulation comprising glp-1 for use in the treatment of hyperglycemia and diabetes by pulmonary administration	EP2211842B1	Mannkind Corp	12-08-2015
4.	Pulmonary administration of immunoglobulin single variable domains and constructs thereof	US20160280799A1	Ablynx NV	08-08-2017
5.	Anti-interleukin-4 receptor antibodies	US8679487B2	Immunex Corp.	25-03-2014
6.	Lung-targeting nanobodies against pulmonary surfactant protein A and their preparation	US9228010B2	Shanghai Pulmonary Hospital Tongji University School of Medicine	05-01-2016
7.	Insulin derivative formulations for pulmonary delivery	US8900555B2	Nektar Therapeutics	02-12-2014
8.	Processes and compositions for liposomal and efficient delivery of gene silencing therapeutics	EP2349210B1	Marina Biotech Inc.	18-03-2015

Clinical trials

Sr. No.	*Therapeutic agent*	*Identifier number*	*Phase*	*Initial year*	*Status*
1.	Technosphere Insulin	NCT04974528	Phase III	2021	Not yet recruiting
2.	Tigerase®	NCT04459325	Phase III	2020	Completed
3.	Dornase alfa	NCT04359654	Phase II	2020	Recruiting
4.	Dornase alfa	NCT04402970	Phase III	2020	Completed
5.	Kamada AAT	NCT04204252	Phase III	2019	Recruiting
6.	Pneumostem®	NCT04003857	Phase II	2019	Recruiting
7.	Pneumostem®	NCT03392467	Phase II	2018	Recruiting
8.	Pneumostem®	NCT01632475	Phase I	2012	Active, not recruiting

Pulmonary route

Lungs are the prime organs of the respiratory system, and alveoli are the functional units of the lung composed of two types of cells: Type I and Type II. Type I cells, also known as small or Type A, are nonphagocytic, membranous pneumocytes ~5 μm in size and are responsible for exchanging gases. Type II cells, known as Type B or larger alveolar cells, are rounded, granular, epithelial pneumocytes of ~10–15 μm thickness that produce surfactant material to line the lung and repair the alveoli after damage from viruses or any chemical agents.[5,6]

The absorption of biopharmaceuticals through the respiratory tract is a complex process and depends on the hydrophobicity and the size of the macromolecules, there are three main mechanisms: paracellular diffusion; vesicular endocytosis or pinocytosis; and receptor-dependent transcytosis (Fig. 1).[7,8] The major barrier in the absorption of biopharmaceuticals is the alveolar and airway epithelium. The pulmonary absorption of biopharmaceuticals can be enhanced by coupling with specific peptide sequences that alter the biological activity and promote receptor-mediated transport by translocating through the epithelium.[9]

The pulmonary route is a noninvasive delivery route for biopharmaceuticals that enables easy self-administration, unlike the conventional parenteral route. It delivers a high dose of biopharmaceutical directly into the lung to give rapid onset of action. It avoids hepatic first-pass metabolism and the lungs show low metabolic activity locally resulting in high bioavailability compared with other noninvasive routes. However, it also shows local toxicity and immunogenicity, poor dosing reproducibility and considerable variability. Sometimes, poor coordination between actuation and inhalation activities using certain inhalation devices leads to patient discomfort.[5,10] Considering these advantages and drawbacks of the pulmonary route, biopharmaceutical delivery should be specifically designed.

Factors affecting pulmonary delivery

The efficiency of pulmonary delivery is principally related to the actual deposition of therapeutic agents in a targeted lung area. Particles are deposited in the respiratory tract by various mechanisms such as impaction, sedimentation, Brownian diffusion, interception and electrostatic precipitation after inhalation, as

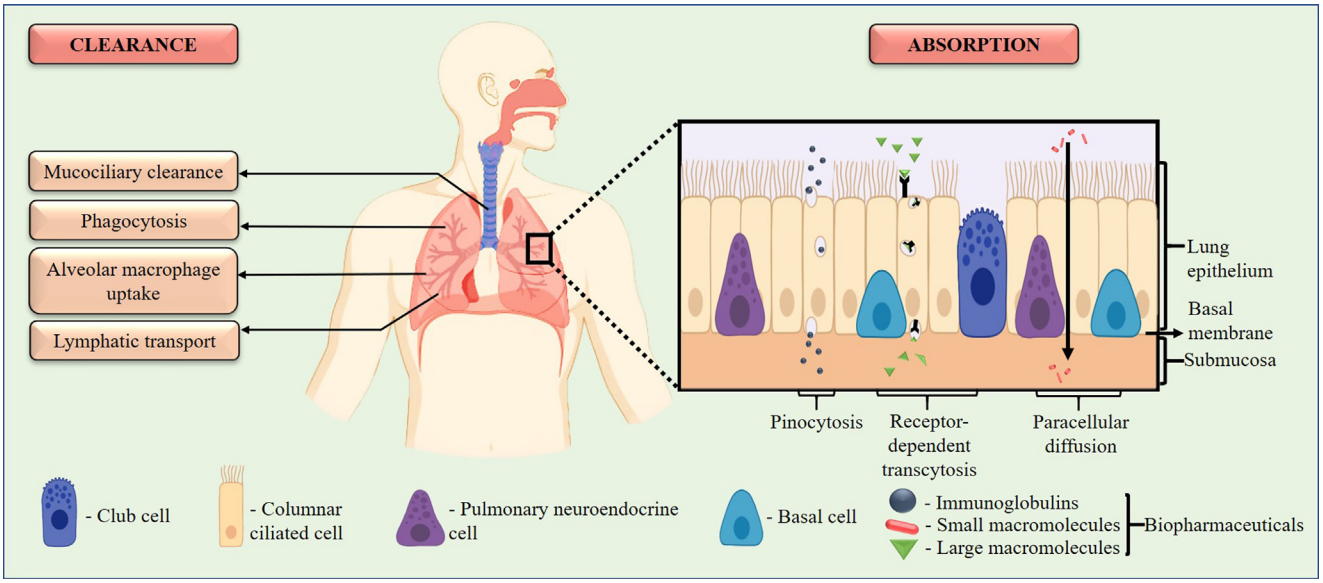

FIGURE 1
Mechanisms of absorption and clearance of biopharmaceuticals through the respiratory system.

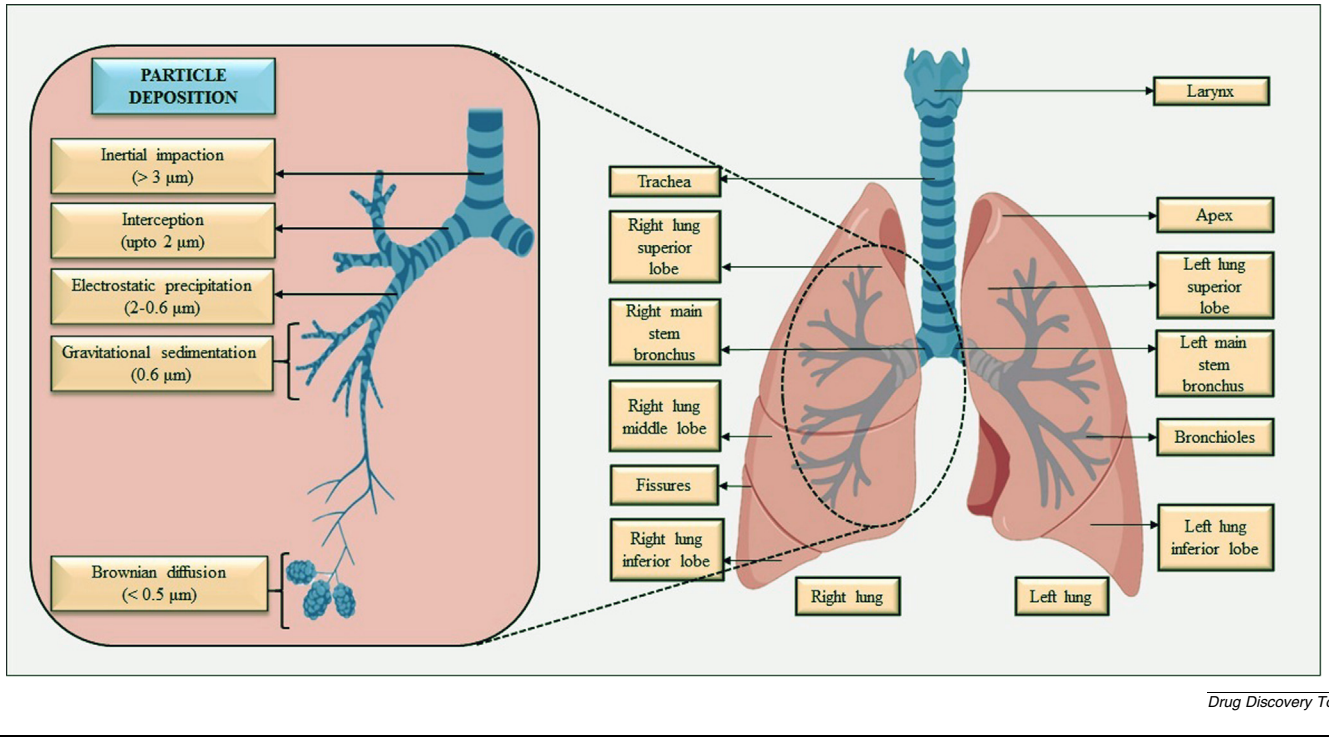

FIGURE 2
Schematic representation of human lung anatomy and mechanisms of particle deposition at various sites of the respiratory tree in lungs.

shown in Fig. 2.[5] This deposition can be varied with particle properties like size, shape, density, charge and physiological parameters such as airway geometry, breathing pattern, flow rate, lung morphology, lung surfactant and mucociliary clearance.[10,11]

Particle aerodynamic diameter (Dae) is one of the most crucial design variables for determining aerosol performance. Aerosols should have an aerodynamic diameter between 0.5 μm and 5 μm to reach the lower respiratory tract.[12] It has been observed that aerodynamic diameter is low for small as well as porous par-

ticles. The aerodynamic diameter increases with the aspect ratio for elongated particles and can be controlled by a short axis rather than a long axis. Different particle engineering techniques such as milling, spray drying, spray freeze drying and microcrystallization are available to achieve superior pulmonary delivery with an effective aerodynamic diameter.

Formulation considerations that should be contemplated in the design of pulmonary delivery of biopharmaceuticals mainly include the composition of inhalation products. It is directly related to the performance of inhaled drugs and affects their stability, deposition and absorption. Depending upon the liquid or powder formulations, excipients are selected for pulmonary products. Biopharmaceuticals are labile drugs, so there is a chance of physical and chemical instability during production and storage. Thus, antioxidants, metal chelators and enzyme inhibitors that reduce proteolytic enzyme activity are added to improve the stability of biopharmaceuticals. Also, salts and sugars can increase their thermal stability and nonionic surfactants, polymers are used to reduce formulation aggregation. The compounds metabolized quickly or cleared rapidly should be preferred for pulmonary drug delivery because the lung has a low buffer capacity.[8]

The pulmonary formulations are administered via various delivery devices depending on the clinical circumstances. Several patient-friendly inhalation devices were developed by technological advancements considering patient comfort. Nebulizers, smart nebulizers, soft mist inhalers, pressurized metered-dose inhalers (pMDIs) and dry powder inhalers (DPIs) are the currently available inhalation devices. Nebulizers convert liquid formulations into suspended droplets in a gaseous environment and are mainly used to provide ventilatory support to critically ill patients. Despite existing limitations, pMDIs and DPIs are commonly used owing to their portability, rapid delivery and compactness. pMDIs involve the use of propellents such as a drug dispersion medium and offer consistent dosing. In recent years, ozone-depleting propellants, chlorofluorocarbons (CFCs), have been replaced with safe hydrofluoralkane (HFA) in pMDIs. However, pMDIs require good coordination between actuation and inhalation for effective delivery to the lungs. DPIs are easy to manufacture, more patient-friendly than MDIs and were improved for efficient delivery at varied inspiratory flow rates, especially for patients with chronic obstructive pulmonary disease (COPD), having a low inhalation flow.[13,14]

Pulmonary delivery of biopharmaceuticals

In the following sections, we discuss the case studies of various biopharmaceuticals administered via the pulmonary route focusing on the treatment of respiratory diseases (pulmonary fibrosis, asthma, lung infection, lung cancer, etc.) as well as systemic diseases (diabetes, pseudocholinesterase deficiency, etc.).

Peptides and recombinant therapeutic proteins

Peptides have gained attention to treat chronic diseases with their target specificity and lower toxicity. Their large size and unstable nature lead to poor gastrointestinal tract (GIT) absorption and, thus, the pulmonary route has been explored for delivery of peptides having a short half-life and rapid clearance.[15]

The functionalized quercetin-loaded liposomes with T7-peptide were prepared to target the transferrin receptors in lung cancer therapy. Surface-functionalized formulation displayed higher *in vitro* cytotoxic effects on lung cancer cells as compared with bare liposomes. Further, tumor growth was inhibited in tumor-implanted mice receiving T7-quercetin liposomes via the pulmonary route more than the nontargeted formulation and free quercetin.[16] In another study, polymeric nanoparticles were loaded with esculetin-1a peptide to treat an antimicrobial lung infection. A single intratracheal administration of peptide-loaded PLGA nanoparticles in a mouse model of *Pseudomonas aeruginosa* resulted in a 3-log reduction of a pulmonary bacterial burden compared with nonencapsulated peptides for up to 36 h.[17]

Sharma et al. developed IDR-1018 peptide and *N*-acetyl cysteine (NAC) co-loaded microspheres as an adjunctive tuberculosis therapy via synergistic activity. The co-loaded inhalation formulation exhibited enhanced mucus penetration and bacterial biofilm disruption promoting efficient delivery of NAC to lungs.[18] Caveolin scaffolding domain peptide delivered as micronized inhalation powder showed remarkable *in vivo* efficacy suggesting a potential treatment option for idiopathic pulmonary fibrosis.[19] In another study, mucus-penetrating peptides were used as nanoparticle surface modifiers to potentially surpass the mucociliary clearance and achieve improved pulmonary delivery. These peptides promoted uniform nanoparticle distribution and lung retention extending its reach to lung epithelia for efficient therapeutic activity.[20] The peptide drug delivery via the pulmonary route would be beneficial to target localized diseases such as lung cancer, lung fibrosis, lung infection, among others.

Recombinant therapeutic proteins are highly potent for treating diabetes, cancer and inflammatory disease. The therapeutic efficacy of proteins can be enhanced by encapsulation within microparticles, chemical modification with hydrophilic polymers and recombinant protein engineering.[21]

Biomimetic zwitterionic phosphatidylcholine-chitosan-based nanoparticles were formulated as a nanocarrier to deliver msFGFR2c protein as a treatment approach in lung fibrosis. The *in vitro* study against MRC-5 cells indicated an effective decrease in α-SMA expression compared with msFGFR2c solution. Also, an effective antifibrotic and anti-inflammatory effect was observed with increased bioavailability and survival rate in bleomycin-induced lung fibrosis rats upon orotracheal administration of a protein-loaded formulation compared with free protein.[22] Similarly, insulin was microencapsulated into chitosan nanoparticles to deliver to deep lung tissues aiding its absorption into the systemic circulation. A prolonged hypoglycemic effect was seen in the Sprague–Dawley rat group induced with nanoparticles compared with the control group. Dry powder of insulin-loaded chitosan nanoparticles exhibited greater stability over liquid insulin formulations.[23] Thus, peptides, proteins and their formulations become a promising therapeutic approach for pulmonary delivery owing to their low toxicity, selective targeting and better bioavailability.

Enzymes

Enzymes such as butyrylcholinesterase, phospholipase A2 (PLA2) and carbonic anhydrase (CA) are biological catalysts with high

molecular weights that are present in the body fluids and tissues and help treat lung cancer and thus are considered as promising biomarkers.[24] Oral delivery is generally prone to enzymatic degradation by peptidases and proteases present in the GIT reducing therapeutic activity. Liposomes were loaded with a synergistic combination of an antibiotic and proteolytic enzyme, namely, levofloxacin and serratiopeptidase, respectively. The pulmonary delivery of combinative formulation showed 80–90% inhibition by levofloxacin against *Staphylococcus aureus* augmented by disruption of the bacterial biofilm membrane by serratiopeptidase. Thus, direct targeting was achieved at the site of action with a reduced dose of antibiotic via inhalation route.[25]

To treat chronic inflammatory lung disease, multistage trypsin-responsive and neutrophil elastase-responsive polymeric nanoparticles in microgel systems were evaluated via pulmonary delivery. Lower macrophage phagocytosis was observed *in vitro* with the use of trypsin- and elastase-responsive microgel. The nano-in-microgels showed minimal uptake by alveolar macrophages in rats, thereby avoiding rapid macrophage clearance at early time points.[26] Pulmonary delivery of recombinant human deoxyribonuclease I upon PEGylation revealed greater activity in lungs than unconjugated enzyme owing to increased lung residence time, presence in the airspaces and decreased systemic absorption providing a remarkable mucolytic activity in cystic fibrosis.[27] Butyrylcholinesterase particles were formulated using particle replications in non-wetting templates (PRINT) with suitable aerodynamic properties for ideal airway deposition. Further, distribution of butyrylcholinesterase particles after dry powder insufflation indicated better residence times of 48 h and 72 h in bronchoalveolar lavage fluid and lungs, respectively, compared with the control groups. PRINT was identified as a promising technique for pulmonary delivery of active enzymes.[28] The pulmonary route promotes enzyme-responsive lung delivery at higher concentrations, as well as improved biological activity with minimal systemic side effects.

Monoclonal antibodies

Monoclonal antibodies (mAbs) are identical immune system proteins produced by a single clone of cells derived from hybridoma. They are directed against a specific epitope on an antigen and used as diagnostics and therapeutics agents for various diseases.[29]

Lin et al. developed triptolide-loaded liposomes surface modified with anti carbonic anhydrase IX (CA IX) antibody (CA IX-TPL-Lips) for targeted therapy in lung cancer. The cellular uptake study of CA IX-TPL-Lips performed in CA-IX-positive and -negative A549 cells showed effective cellular uptake in the former over the nontargeted TPL-Lips, whereas no significant difference was seen in the latter, suggesting its efficient targeting to CA-IX-overexpressing cells. The CA IX-TPL-Lips treated group suppressed tumor growth more efficiently to extend the lifespan of mice compared with the nontargeted TPL-Lips group.[30] In another study, conjugation of murine anti-IL-17A Fab′ fragment with a two-armed 40 kDa PEG via site-selective thiol PEGylation was investigated to determine residence time after pulmonary delivery for respiratory diseases. The *in vitro* activity of anti-IL-17A Fab′ indicated that its PEGylation improved the biological activity by 8.5-fold. Also, the pharmacokinetic study suggested that PEGylation increased the residence time of anti-IL-17A Fab′ in the lungs along with a reduced clearance rate.[31]

Respaud et al. developed an anti-ricin neutralizing mAb (IgG 43RCA-G1) and a device for alveolar delivery to treat pulmonary ricin intoxication. A 60-fold increase in lung distribution was observed after inhalational delivery compared with the systemically delivered formulation.[32] Likewise, anti-hemagglutinin anti-influenza mAbs abministered via the pulmonary route showed 10–50-times greater efficacy in treating viral infections than systemic delivery.[33] Amino-acid-stabilized adalimumab-incorporated microparticles were developed for the treatment of asthma by pulmonary delivery. Effective neutralization of tumor necrosis factor (TNF)-α activity was observed against L-929 cells with cell viability > 92% and reduced inflammation of bronchioles. Spray freeze dried adalimumab microparticles stabilized by leucine and phenylalanine imparted 3 months stability at accelerated conditions.[34] mAbs, mainly administered via the parenteral route, are exposed to issues such as pH sensitivity, degradation and instability. Conversely, pulmonary delivery of enzymes establishes prolonged residence time and greater biological activity in the treatment of various respiratory diseases.

Nucleic acids

Nucleic acids have been identified as a new potential therapeutic category in the treatment of various pulmonary diseases.[35] Upon intravenous administration, nucleic acids have to traverse physiological barriers limiting their efficient delivery to the target site. Gene therapy is the most promising nucleic-acid-based therapy, mainly used to transfer genetic information into host cells to obtain potential therapeutic effects. It has become successful in developing vectors essential for delivering genes to cells.[36] Cholesterol-conjugated polyamidoamine (PAMAM) micelles were co-loaded with phytochemicals such as curcumin, resveratrol and heme oxygenase-1 gene for combination therapy in inflammatory lung injury. Their synergistic effect reduced cytokine levels, thereby promoting an anti-inflammatory effect.[37,38] Another study reported higher attenuation of growth in lung cancer xenografts via a combination of aerosol and systemic delivery of the AT2R gene complexed with peptide and plasmid DNA.[39]

Small interfering RNA (siRNA) interferes with the expression of specific genes with complementary nucleotide sequences by degrading mRNA after transcription or cleaving mRNA before translation, thus preventing the translation process.[40] Cationic nanolipoplexes loaded with siRNA, specific for the myeloid cell leukemia sequence 1 (Mcl1) gene, demonstrated effective cellular delivery and *in vivo* gene silencing ability after being administered by pulmonary inhalation.[41] Hybrid nanoparticles loaded with siRNA were developed as an inhalation system to determine airway internalization using the triple cell co-culture model. In addition to successful internalization, prolonged inhibition of sodium transepithelial channel protein was observed.[42] Inhalation delivery of siRNA loaded into Curosurf®-coated nanogel was demonstrated by Merckz et al. for treatment of respiratory diseases. Coating of pulmonary surfactant enhanced the particle stability, long-term storage and its intracellular delivery.[43]

PAMAM dendrimers were investigated for delivery of siRNA to silence the eGFP gene overexpressed on lung epithelial cells. The

TABLE 2

Novel biopharmaceutical formulations for delivery via pulmonary route.

Sr. No.	Therapeutic moiety	Delivery system	Indication	*In vitro* cell line	*In vivo* model	Refs
Peptides and recombinant therapeutic proteins						
1.	T7 peptide, quercetin	Liposomes	Lung cancer	A549 cells	A549-Luc orthotopic lung tumor-bearing BALB/c nude mice	16
2.	Esculentin-1a (Esc) peptide	PLGA NPs	Lung infection	*Pseudomonas aeruginosa* ATCC 27853	C57BL/6J female mice	17
3.	IDR-1018 peptide, *N*-acetyl cysteine	Microspheres	Tuberculosis	RAW 264.7 macrophages	Tuberculosis-induced Balb/c mice.	18
4.	Caveolin scaffolding domain peptide	Dry powder inhalation	Idiopathic pulmonary fibrosis	–	Female C57BL/6J mice	19
5.	Mucus-penetrating peptides	PEGylated nanoparticles	Cystic fibrosis	CuFi-1 bronchial epithelial cells	Female BALB/c mice	20
6.	msFGFR2c	PCCs-NPs	Idiopathic pulmonary fibrosis	MRC-5 cells	Bleomycin-induced lung fibrosis Wistar rat	22
7.	Insulin	Chitosan NPs	Diabetes	–	Sprague–Dawley rat	23
Enzymes						
8.	Levofloxacin and serratiopeptidase	Liposomes	Respiratory tract infection	–	Wistar rat *Staphylococcus aureus* infection model	25
9.	Trypsin and elastase	Nano-in-microgel	Chronic inflammatory lung diseases	RAW 264.7 macrophage cells	BALB/c mice	26
10.	PEGylated recombinant human deoxyribonuclease I	Dry powders	Cystic fibrosis	–	Female Swiss mice	27
11.	Butyrylcholinesterase	Microparticles	Pseudocholinesterase deficiency	–	Nude C57BL/6 BALB/c mice	28
Monoclonal antibodies						
12.	Triptolide	Liposomes	Non-small-cell lung cancer	A549 cells	A549-Luc orthotopic lung cancer BALB/c nude mice	30
13.	Anti-IL-17A Fab'	PEGylated antibody fragments	Respiratory diseases	NIH/3T3 cells	Murine model of allergic asthma	31
14.	IgG 43RCA-G1	-	Pulmonary ricin intoxication	–	Murine lung challenge model and Cynomolgus macaques model	32
15.	Anti-influenza monoclonal antibodies	-	Influenza viral infections	–	Influenza virus-infected female BALB/c mice	33
16.	Adalimumab	Microparticles	Asthma	L-929 cells	–	34
Nucleic acids						
17.	Curcumin and heme oxygenase-1 gene	PAMAM micelles	Acute lung injury (ALI)	L2 cells	LPS-induced ALI BALB/c mice model	37
18.	Resveratrol and heme oxygenase-1 gene	Cholesterol-conjugated PAMAM micelles	ALI	L2 cells	ALI model	38
19.	AT2R Gene	TAT peptide-plasmid DNA-Ca^{2+} nanoparticles	Lung cancer	A549 cells, HeLa cells, HEK-293 cells, Lewis lung carcinoma cells	LLC tumor-bearing mice, H358 graft-bearing mice	39
20.	siRNA	Cationic nanolipoplexes	Lung cancer	B16F10 and murine Lewis lung carcinoma cells	Metastatic lung cancer mouse model	41
21.	siRNA	Lipid/polymer nanoparticles	Lung diseases	Triple cell co-culture model	–	42
22.	siRNA	Nanogel	Respiratory diseases	H1299-eGFP cells	–	43
23.	siRNA	PAMAM dendrimers	Lung diseases	A549 cells	–	44
24.	siRNA	Triphenylphosphonium-modified PAMAM dendrimers	Lung diseases	A549 cells	–	45
25.	siRNA	PAMAM dendrimers	Acute lung inflammation	RAW 264.7 macrophage cells	LPS-induced acute lung inflammation model	46
26.	mRNA	PEG12KL4 peptide	–	A549 cells and BEAS-2B cells	Female BALB/c mice	47
27.	DNA complexes	LAH or LADap peptides	–	A549 cells	–	48
28.	Plasmid DNA	PEGylated nanoparticles	Cystic fibrosis	CFBE41o cells	Sprague Dawley rat	49

aerosolized formulation of siRNA dendriplexes showed efficient lung cell targeting properties and maintained gene silencing biological activity.[44] Another study conducted with a similar approach indicated that the pMDI and DPI aerosolized formulation of siRNA-loaded triphenylphosphonium PAMAM dendrimers showed desired FPF values of 50% and 39% required for deep lung deposition.[45] Similarly, PAMAM dendrimers were synthesized for delivery of anti-TNF-α directed siRNA in the treatment of lung inflammation. The formulated dendriplexes exhibited high cellular uptake and could mediate specific TNF-α silencing. An effective *in vivo* inhibition of TNF-α by siRNA via the pulmonary route suggested PAMAM dendrimers as promising carriers for local delivery of siRNA in treating acute inflammatory lung diseases.[46]

PEG12KL4 peptide exhibited exceptional properties without any toxicity and inflammatory response as a delivery vector of inhalable mRNA complex. The PEGylated peptide also provided improved solubility and lung transfection efficiency by mRNA.[47] Liang et al. developed inhalational dry powders of pH-responsive peptides from the LAH and LADap series to deliver DNA to the lung tissue. Dry powders formulated by two techniques, namely, spray drying and spray freeze drying, showed good aerodynamic properties but the former showed greater *in vitro* transfection efficiency and significantly higher luciferase expression.[48] Pulmonary delivery of plasmid DNA was improved by surface modification of nanoparticles for treatment of cystic fibrosis. Improved cellular uptake and mucus-penetrating properties were attained owing to PEGylation of nanoparticles with efficient pulmonary delivery.[49] Thus, pulmonary delivery of biopharmaceuticals showed excellent *in vitro* and *in vivo* transfection efficiency, prolonged lung residence time, *in vivo* gene silencing efficiency and improved deposition characteristics.

Delivery of biopharmaceuticals via the pulmonary route is an exciting area with immense scope for expansion of lung therapeutics in the treatment of several respiratory and systemic diseases. The current studies reported for pulmonary delivery of biopharmaceuticals are summarized in Table 2.

Challenges and future perspectives

The progression of biopharmaceuticals has revolutionized the treatment of various lung diseases. Despite the beneficial features possessed by biopharmaceuticals, several challenges exist in its successful delivery. Their high molecular weight and structural complexity cause a reduction in the absorption and permeability across biological barriers leading to poor systemic effects.[50] Owing to multiple structural conformations, challenges related to denaturation, aggregation and insoluble particulate formation arise, causing a loss in activity and increase immunogenicity. Also, protein purification is a crucial step and requires strict controls on manufacturing, storage and delivery stages to avoid contamination. Proteins and peptides have shorter serum half-lives and, hence, require frequent administration to attain the therapeutic effect.[51] Similarly, nucleic acids are degraded beforehand by nucleases upon endosomal vesicular entrapment which reduces therapeutic activity.[52] Sometimes, the rapid mucociliary clearance and alveolar macrophage uptake in lungs result in short residence time, thus requiring frequent administration to attain a therapeutic effect that is not patient compliant.[53] Additionally, the limited range of FDA-approved excipients raises the cost of formulation.[54] Large-scale translational challenges are related to batch-to-batch variability and reproducibility of important formulation parameters such as particle size, porosity and charge.[55]

In the future, various novel carriers should be investigated to overcome these delivery limitations and achieve desired therapeutic performance. Techniques such as surface modifications, coating and attachment of ligands might be explored to attain better lung targeting. The biomimetic strategy can be investigated for targeted biopharmaceutical therapy in lung cancer, pneumonia and respiratory diseases such as COVID-19 and COPD via the pulmonary route. Further, the focus should be on establishing robust and sensitive in-process and finished quality testing methods to check biopharmaceuticals. Moreover, the identification of biocompatible excipients is mandatory to attain improved aerosol performance with greater absorption and stability. By contrast, the development of delivery devices specialized for biopharmaceuticals needs to address the challenges and unmet needs. Moreover, pulmonary developments must be oriented toward clinical studies to establish substantial evidence for the therapeutic use and safety profile of pulmonary delivery of biopharmaceuticals.

Concluding remarks

Biopharmaceuticals are sensitive moieties emerging as novel therapeutics over small molecular drugs. They are highly selective, noncarcinogenic agents possessing potent therapeutic efficiency with minimal side effects. The pulmonary route of delivery is a preferred alternative owing to its striking features and is thus explored as a mode of biopharmaceutical delivery. Several nanocarriers have opted for efficient pulmonary delivery of biopharmaceuticals in the treatment of lung ailments. Although the development of biopharmaceuticals has witnessed exceptional progress, certain limitations such as structural complexity, stability constraints and high cost of manufacturing restrict its commercial translation. Owing to the ongoing pandemic of COVID-19, the pulmonary delivery route is worth contemplating to treat dreadful viral diseases using biopharmaceuticals.

Conflicts of interest

The authors have no conflicts of interest to report.

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