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Nano-delivery in Airway Diseases: Challenges and Therapeutic Applications

Indrajit Roy, MS, Ph.D.[1] and **Neeraj Vij, MS, Ph.D.**[2,3,#]

[1]Institute for Lasers, Photonics and Biophotonics, Department of Chemistry, State University of New York, Buffalo, NY

[2]Department of Pediatric Respiratory Sciences, Johns Hopkins School of Medicine, Baltimore MD

[3]Institute of NanoBioTechnology, Johns Hopkins School of Medicine, Baltimore MD

Abstract

We discuss here the challenges and therapeutic applications of nano-delivery systems for treatment of airway diseases. Therapeutic applications of nano-delivery in airway diseases involve targeted delivery of DNA, siRNA, drugs or peptides to hematopoietic progenitor cells and pulmonary epithelium to control chronic pathophysiology of obstructive and conformational disorders. The major challenges of nano-delivery involve physiological barriers like mucus and alveolar fluid for intranasal and reticuloendothelial (RES) system for systemic delivery. It is necessary that the nano-particles are biodegradable and capable of providing the sustained drug delivery to the selected cell type. Once inside the cell the nano-particle should be capable of escaping the endocytic degradation machinery. In addition, for effective gene-delivery nuclear entry and chromosomal integration are critical. We have also discussed the strategies to overcome these pathophysiological barriers as an attempt to synchronize the efforts of pulmonary biologists, chemists and clinicians to develop novel nano-delivery therapeutic(s) for airway diseases.

Keywords

Nano-delivery; Gene Therapy; Drug Delivery; Airway Diseases and Therapeutics

Introduction

Nanoparticles (NPs) are solid colloidal drug delivery systems that are submicron in size (1 to 1000 μm) and used to deliver drugs, genes or vaccines to specific tissue or cells by targeted delivery or systemic route. Besides systemic delivery, they also offer other non-invasive routes of administration, such as the nasal route. The nasal drug products that are currently in the market are in routine use for the treatment of various pulmonary conditions such as allergic rhinitis, asthma and chronic obstructive pulmonary disease (COPD) as they provide rapid and

#**Address for correspondence**: Neeraj Vij, MS, PhD, Assistant Professor, 600 N. Wolfe Street, CMSC 3-122, Baltimore, MD 21287, Ph No.: 410-955-9783 (off); 410-955-9778 (lab), Fax: 410-502-8007, E-mail: nvij1@jhmi.edu.

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specific therapeutic effects. The nanoparticulate systems provide novel systems for selective delivery of both low and high molecular weight drugs to nasal epithelia and lungs. Many low molecular weight, non-polar drugs (<250D) in solution form are able to effortlessly infiltrate the nasal or lung epithelium for sub-epithelial delivery. The proposed mechanism of absorption of these low molecular weight drugs is via aqueous channels. Molecules higher than 250D, have trouble in absorption in the airway, similar to that in gastrointestinal-tract. The absorption through lung epithelium also requires bypassing through excessive mucus layer especially in obstructive airway disease conditions. Absorption enhancers are being used to enhance absorption of drug across the nasal and lung epithelia. In addition, coating of NPs with mucolytic agents or compounds to open epithelial tight junctions and increase membrane fluidity may help in sub-epithelial delivery. Currently, there are several nasal drug delivery systems available in various phases of development for epithelial and sub-epithelial absorption [1,2]. There are many different challenges to treating airway diseases, which depend on the route of delivery, the exact disease being treated and the progression of the disease. Based on the disease state, therapeutic approach and site of disease, nanoparticulate systems can help in drug or gene delivery to both upper and lower airways.

As discussed above major challenge in delivery and therapeutic efficacy of delivery systems in chronic obstructive airway conditions is severe inflammation and mucous hypersecretion[3]. Mucous hypersecretion is a hallmark of chronic obstructive airway diseases, including asthma, COPD and cystic fibrosis (CF). These diseases have distinct etiologies and different inflammatory responses that drive mucous hypersecretion. In asthma, inflammation appears to be mediated by allergen-specific Th2 cells, leading to eosinophilia, while in CF and COPD, the inflammatory response is neutrophilic and may be induced by infection or components in cigarette smoke. Controlling inflammation is at the root of treatment through the use of corticosteroids,antibiotics or other available drugs in these chronic obstructive inflammatory conditions yet despite therapy, challenge is the sustained delivery of drugs to target cells or tissues. In spite of wide application of nano-based drug delivery systems in chronic obstructive airway diseases and variety of other pulmonary conditions like allergy, lung cancer etc, very few are tested [4–6].

In addition to drug delivery, the nanoparticulate systems have a novel application in therapeutic gene delivery. Although the efficiency of gene delivery and expression using viral vectors are superior over that of NPs, the latter are devoid of the health risks associated with the former. There has been some progress in improving efficacy of viral and nanoparticulate gene delivery systems in diseases such as CF and alpha-1-trypsin deficiency (ATD). However, further preclinical evaluation and improvements in design of delivery systems are warranted for translation to human subjects for clinical evaluation [7]. Delivery of CFTR (CF transmembrane conductance regulator) or other therapeutic genes to the lungs is an attractive strategy to correct CF as well as other pulmonary dysfunctions like ATD, pulmonary hypertension, asthma and lung cancer 8–10. Different delivery routes such as intratracheal instillation, aerosol and intravenous injection have been tested with varying degree of efficiency in mice and humans 11. Both viral and nanoparticulate vectors or delivery systems, with their respective strengths and weaknesses, have achieved significant levels of transgene expression in the lungs 12. However, the application of gene and drug delivery for the treatment of pulmonary and other systemic diseases has been handicapped by various physiological barriers to the carrier NPs such as serum proteins and liver macrophages during intravenous delivery, and surfactant proteins, mucus and alveolar macrophages in airway lumen during intranasal application [3]. Both intranasal and intravenous nano-particulate delivery systems are currently tested for pulmonary conditions depending on the bioavailability requirement and target tissue in the airway. Immune and cytokine response against the delivery vehicle is another major problem, encountered in pulmonary or systemic gene or drug delivery. Despite these short-comings some progress has been made to enhance the efficiency, as well as lower the toxicity of

nanoparticulate vehicles for pulmonary gene therapy [4]. Moreover, nano-delivery systems have important application in obstructive airway disease with severe inflammation and impaired mucociliary clearance, where nebulizers fail to provide sustained delivery of aerosolized drug. The use of NPs for pulmonary drug delivery is a novel therapeutic strategy (Table 1) but demands disease and drug specific fine-tuning of delivery system (Table 2). Progress in design and therapeutic potential as well as challenges of nano-based gene and drug delivery systems are discussed.

Challenges Faced by Nanoparticulate Pulmonary Carriers and Their Remedies

Although till date a number of nanoparticulate drug or gene carriers have been described, they can be broadly placed into three categories, liposomes, polymers and inorganic nanomaterials [13–16]. The interaction of genetic materials or drug with the nanoparticles involves either electrostatic compaction or encapsulation. The entire process of drug delivery or expression of a foreign gene within appropriate cell types can be broken down into a series of steps, each of which offer considerable resistance for successful delivery. An ideal nanoparticulate carrier should be equipped with appropriate ‘strategies’ to overcome most, if not all, of the obstacles. Listed below are the main strategies for delivering drugs and genes to the target cells or tissue and how the nanoparticulate formulations can be readjusted for successfully bypassing the homeostatic and disease specific pathophysiological barriers.

1. Overcoming Resistance by Respiratory Mucus and Alveolar fluids for Intranasal Deilvery of Nanoparticles

Following inhalation or instillation of gene or drug loaded NPs, they must overcome the obstructive barriers presented by respiratory secretions, such as respiratory mucus and alveolar fluids (e.g. pulmonary surfactants), in order to gain access to the target cells or tissue. The viscoelastic mucus is particularly tenacious during lung diseases, and it has been reported that the thickness of the mucus layer increases to more than 260 μM from the normal 2–30 μM in case of CF and other obstructive airway diseases [17]. Unless specifically designed to evade and bypass capture by the mucus, the NPs are sequestered by the mucus and eliminated via coughing 18, 19. The capture of NPs can be triggered by steric, electrostatic, or hydrophobic interactions 3.

Therefore, it is evident that modification of NP size and surface-properties plays a crucial role in rendering them a safe passage across these barriers. With regard to the size, small sized NPs are less sterically impeded and therefore better suited to cross the mucosal barrier when compared to larger ones. Rytting et al. have studied the transport of negatively charged polystyrene nanospheres through the sputa of CF patients 4. They have observed that the smallest NPs (diameter 120 nm) moved very efficiently through the sputum layer, while the mobility of the larger ones (diameters 270 and 560 nm) were significantly impeded. With respect to surface functionalization, the most popular strategy, that not only modifies the surface charge of NPs to anionic or neutral but also renders their surface hydrophilic, is by coating with inert and biocompatible polymers such as polyethyleneglycol (PEG) 4. PEGylation not only enhances the transport of NPs across mucosal barrier, but also safeguard them against clearance by alveolar macrophages present in the deep lung 3. Moreover, PEGylation of NPs can provide other interesting properties to these nano-delivery vehicles, as shown by Lai et al., larger PEGylated polystyrene NPs (diameter 500 nm) can traverse more efficiently as compared to smaller ones (diameter 100 nm) across the fresh undiluted human mucus 20.

Other, lesser-known suggested strategies to evade mucosal barrier include fabricating NPs with doped magnetic properties, so that they can be guided across the mucosal barrier by using external magnetic force, as well as the use of mucolytic agents such as hydrolyzing enzymes for the disruption of mucosal network [21–23].

While bypassing the mucus is a major challenge in obstructive airway conditions such as CF and COPD, on the other hand, there is a need to develop mucoadhesive drug delivery systems for treating non-obstructive pulmonary conditions such as allergy, lung cancer etc. One prominent example is the fabrication of chitosan-DNA polyplexes, which are commonly used for oral and nasal gene therapy [24]. Such mucoadhesive particles promise several advantages by providing the localization at a given target site, prolonged residence time at the site of drug absorption, and an intensified contact with the mucosa increasing the drug or vaccine concentration gradient, uptake and bioavailability [25].

2. RES Evasion of Nanoparticles

Nanoparticulate formulations targeting the lungs, whether delivered systemically in the blood or topically, are vulnerable to recognition and subsequent destruction by cells of the defense system of the body (reticuloendothelial system, RES), such as hepatic and splenic macrophages in systemic delivery and alveolar macrophages in topical delivery 26. The bigger size of NP, as well as the presence of net positive charge and hydrophobic groups on the NP surface, mainly triggers this RES recognition 6,27. Similar to the mucosal evasion approach, it has been indicated that a few inert polymers such as PEG and polyvinylpyrrolidone (PVP), when combined with the nanoparticulate carrier, form a protective-brush, shielding any positive surface charge as well as hydrophobicity, thus preventing their RES capture 3,6,27. Such 'stealth' NPs like poly(lactic-co-glycolic acid)-PEG (PLGA-PEG) and PEGylated lipid NPs exhibited substantially improved RES evasion profile in comparison to their non-PEGylated counterparts 28, 29.

While RES evasion is a major challenge for drug or gene delivery for most of the target diseases, the capture of nanoparticulate therapeutics by macrophages of the lung may have some beneficial effects in certain pulmonary conditions such as lung cancer [30]. Indeed, a number of studies, mostly involving systemic delivery of radiolabelled NPs, have shown varied levels of NP accumulation in the lungs, though with far lower efficiency than that observed in the liver and spleen [26].

3. Cell Specific Targeting of Nanoparticles

A freely circulating nanoparticulate carrier diffuses to various tissues and organs, particularly to areas undergoing inflammation and neoplastic development, via the enhanced permeability and retentivity (EPR) effect [31]. While therapy of cancer and inflammatory diseases may benefit from this 'passive targeting', this method is inefficient for targeting most other organs like liver, lungs, brain etc. Organ-specific drug or gene targeting is enhanced by several orders of magnitude with the help of targeting 'ligands' attached to the nanoparticulate carriers which specifically recognizes the corresponding 'receptors' overexpressed at the target cells or tissues. So far, a large number of targeting ligands have been successfully tested for delivery, including monoclonal antibodies, folate, growth factors, lung-surfactant proteins, integrin-binding motifs, to name a few 32–36.

The endothelium represents an important therapeutic target for controlling oxidative stress, thrombosis and inflammation involved in pulmonary diseases [26]. It has been shown that NPs conjugated with antibodies against constitutive endothelial cell adhesion molecules (CAMs) can be preferentially targeted to endothelial cells [37]. On the other hand, NPs containing sugar residues can target lectins that are expressed on the surface of airway epithelial cells 38.

Macrophages or neutrophils can be similarly targeted by using NPs conjugated to antibodies against macrophage or neutrophil markers.

4. Cell Entry and Endosomal Escape of Nanoparticles

A number of mechanisms govern the entry of a NP inside the cell, which includes clathrin-mediated endocytosis, caveolae-mediated endocytosis, macropinocytosis, phagocytosis, etc [39]. Clathrin-coated endocytosis is the primary mechanism of entry, which is mostly, but not exclusively, 'receptor-mediated'. This mechanism involves the interaction of bioconjugated NPs with specific cell-surface receptors as discussed above, or using the electrostatic interaction between cationic NPs with the ubiquitous anionic receptors like heparin sulfate proteoglycans (HSP) present on the surface of most cell types [40]. Once endocytosed, the NP is transferred from the clathrin-coated endocytic vesicles to the endosomes and lysosomes, where the local acidic pH and presence of enzymes such as nucleases and proteases are often responsible for the degradation of the NP and its loaded therapeutic cargo.

Therefore, in order to prevent such endo-lysosomal degradation of drug or gene loaded NPs internalized through clathrin-mediated endocytosis, it is essential that such endocytic vesicles are disrupted for intracellular release of the NP. Certain cationic polymers like PEI and polyamidoamine dendrimers have non-protonated amino groups, which can buffer the acidic pH by attracting free protons 13› 41. A direct consequence of this 'proton-sponge' effect is the influx of excess chloride ions and water within the vesicles, leading to endosomal or lysosomal burst 42. The 'helper' phospholipid components of many liposomes, such as 1,2-dioleoyl-3-phosphatidylethanolamine (DOPE), are also responsible for fusion with the endosomal membranes, leading to the membrane destabilization and consequent release of the entrapped DNA 43›44. PLGA NPs are also shown to possess endo-osomolytic properties owing to selective reversal of the surface charge of NPs (from anionic to cationic) in the acidic endo-lysosomal compartment 45. In other cases like polylysine, where the nanoparticulate vector does not have any intrinsic endosome-rupturing capacity, additional 'endosomolytic' agents such as viral peptides have been used to aid the endosome disruption and release of the NPs 46.

Apart from clathrin-mediated endocytosis, caveolae-mediated endocytosis has also been shown to be a major mechanism of receptor-mediated NP-uptake, particularly in endothelial cells [39]. Here, following binding to the cell surface, NPs are coated with dimeric proteins, called caveolins, and are ferried intracellularly by caveolar vesicles. Since these vesicles do not contain nucleases and proteases, this is a preferred route for the delivery of drug-loaded NPs that are prone to endo-lysosomal degradation. Also, for effective cellular delivery, this mechanism does not rely on the inclusion of potentially toxic lysosomolytic factors with the NP formulation. From the perspective of pulmonary delivery, this is a major uptake mechanism since caveolin is also abundantly expressed on lung capillaries and Type I alveolar cells [6].

Macropinocytosis is another mode of endocytotic uptake of NPs by cells, which occurs non-specifically [47]. Finally, phagocytosis is the non-endocytotic uptake process that involves the coating of NPs by opsonin proteins, which are subsequently captured by specialized cells, called phagocytes, and are ferried to phagocyte-rich organs such as liver and spleen. These cellular uptake processes and their implications in drug and gene delivery are reviewed in detail elsewhere [39].

5. Nuclear Entry of Released DNA for Nanoparticle Mediated Gene Delivery

For NP-mediated gene therapy to be effective, it is necessary that the endocytosed NP-DNA complex (nanoplex), or the released DNA in the cytosol, should enter the nucleus of the target cell. However, the nuclear membrane, which is much more specialized and selective than the

cellular membrane, forms a formidable barrier for nuclear entry. It has been observed that it is easier to transfect *in vitro* cells undergoing active cell division (mitosis) as compared to relatively quiescent, post-mitotic cells, primarily owing to transient opening of the nuclear membrane during mitosis that may allow the nanoplex or the released DNA to passively 'sneak' within the nucleus 48. However, delivery in the airway diseases is more complicated as airway cells are post-mitotic and therefore non-dividing. Thus, this passive nuclear entry is highly inefficient and the only way for the entry of the nanoplex or the released DNA is by diffusion across the nuclear pore complex (NPC) 49. Here, nuclear entry is mediated by the active diffusion across the NPC with the help of nuclear proteins like nuclear localization signals (NLS) incorporated with the nanoplex or the released DNA 50,51. Nuclear targeting peptide scaffolds with lipid based nanoparticulate vectors have been reported to enhance the transfection efficiency by 63-fold in non-dividing mammalian cells in culture 52.

6. Chromosomal Integration of Released DNA for Nanoparticle Mediated Gene Delivery

Another main challenge for successful nanoparticulate gene delivery is the stable integration of the foreign DNA within the chromosome of the host cell. Without chromosomal integration, gene expression will be always transient, which is impractical from the point of view of most genetic diseases that require long-lasting gene expression such as CF and ATD. Chromosomal integration can gain by the use of certain primordial genetic components called transposons or 'jumping genes', that are short pieces of DNA that move around the chromosome by 'cut, copy and paste' mechanism [53]. Recently, a highly efficient, transposon-based nanoparticulate gene transfer system called 'sleeping beauty' has been described, consisting of a transposon vector and the associated transposase enzyme that help with the successful integration of foreign DNA into the chromosome of 5–6 % transfected cells. This process, called 'transposition', has shown substantial enhancement of long-term gene expression [54, 55]. However, subsequent research has also indicated the pitfalls of such a system as it may act as cancer causing 'insertional mutagens', evidenced by their ability to generate spontaneous tumors in mice [56].

Therapeutic Application of Nanoparticulate Gene and Drug Delivery in Pulmonary Diseases

1. Cystic Fibrosis (CF)

CF is a consequence of mutation(s) in the CFTR gene, resulting in imbalanced ion and water movement in the airway epithelium. This leads to accumulation of mucus, bacterial infection and chronic inflammation in the lungs [7]. The most common mutation, ΔF508-CFTR, is a temperature-sensitive, trafficking mutant with reduced chloride transport and exaggerated immune response 57–59. Since CF is a monogenic disease, gene therapy of CF is an extremely promising avenue that aims to reverse this disease by introducing exogeneous CFTR gene in the airway epithelial cells via the pulmonary route. Viruses that can transfect non-dividing cells, like adenovirus or adeno-associated virus, have already demonstrated partial correction of the CF in pre-clinical and clinical studies. However, the transgene expression subsided within weeks, and repeated administration triggered adverse immune response 60.

It has been speculated that correcting the CFTR expression of as low as 10 % of the target cells is enough to bring about therapeutically significant outcomes in CF gene therapy 61. Therefore nanoparticulate gene delivery vectors, albeit with their low efficiency, are considered as an attractive option owing to their non-immunogenicity and feasibility for repeated dosing. A number of CF clinical trials involving nanoparticulate gene transfer have been conducted using a variety of cationic lipids, either by aerosolized delivery or direct instillation in the nose 62. Although no toxic side effects could be observed, the trials resulted in little or no functional correction of CF. Some encouraging results were obtained upon nebulized delivery of the lipid-gene complex directly into the lungs, whereby about 25 % of restoration of chloride

conductance towards normal values were demonstrated [63]. However, current levels of CF gene transfer efficiency and correction of chloride conductance were not sufficient to achieve any clinical significance. Most of these gene therapy studies have used CFTR channel function to record the level of correction and may explain why no significant clinical pulmonary function outcome was recorded in spite of 25% correction in chloride conductance. The rescue of pulmonary function (FEV% predicted) is the best measure of pulmonary outcome and need to be used as a standard practice for evaluating the viral and nanoparticulate gene delivery as it will not only demonstrate the correction of chloride conductance but overall lung disease.

A critical factor responsible for the poor CF nanoparticulate gene transfer is the large size (several hundred nanometers) of the vector-DNA complex, which precludes their entry into the nucleus of the virtually non-dividing airway epithelial cells, as discussed previously. In order to circumvent this problem, Copernicus Therapeutics have recently developed ultra small nanoparticulate DNA complexes (size less than 25 nanometers), which can passively enter the nucleus through the NPC and transfect post-mitotic cells. Recently, a clinical trial was conducted using a single CFTR plasmid molecule compacted with PEG substituted polylysine (overall size less than 25 nanometers) by intranasal administration [64, 65]. Some evidence of functional correction of CF could be observed in 7 out of 12 patients with no signs of toxic side effects, though the results are viewed with caution owing to the absence of placebo controls.

In addition to gene therapy, other therapeutic applications of nano-delivery in CF include selective and sustained delivery of small molecules. As an example, we recently proposed the therapeutic application of extremely potent, stable, reversible, and selective proteasome inhibitor drug, bortezomib (Velcade or PS-341) for treatment of conformational and inflammatory pathophysiology in CF [66]. The recent approval of bortezomib as a drug for refractory multiple myeloma [67–69] has initiated the examination of protein catabolism for potential therapeutic intervention in various conformational protein disorders. Proteasome modulators were also recently shown to have dual therapeutic importance in pharmaco-gene therapy of CF airway 70. A major concern in considering the proteasome as a therapeutic target is the theoretical risk that multiple processes may be affected by proteasome inhibitors. The nano-based approach may provide controlled and sustained drug delivery at lower doses with selective inhibition of proteasome mediated homeostatic processes. Moreover, recent studies have identified several novel “correctors” and molecular targets for functional rescue of misfolded protein or chronic inflammatory state [71–76]. The design of novel nano-based biodegradable therapeutic vehicles will provide the controlled drug delivery and selective gene modulation to rescue the pathophysiology of chronic lung diseases without affecting the homeostatic function(s).

2. Alpha-1-antitrypsin (AAT) deficiency (ATD), COPD, Emphysema and Asthma

ATD is another monogenic lung disease amenable to treatment with the synthetic vectors and is characterized by low levels of AAT, the principal antiprotease present in the human plasma, leading to enhanced susceptibility of the lungs to COPD and emphysema [77]. Cationic lipids, with their ability to transfect the lung parenchyma, can be used for localized delivery of the AAT gene in the lung. Alternately, the liver can also be transduced to produce AAT which can reach the lung through systemic circulation.

A single clinical trial was conducted with intranasal delivery of the AAT gene in the form of a lipoplex. Although only transient enhancement of the AAT levels was observed, that too peaking to only about one third of the normal level, some anti-inflammatory effect was observed as evidenced by the decreased IL-8 levels in the nasal fluid [78]. Though such results are not sufficient for drawing much clinical attention, a better future of the synthetic carriers against this disease can be expected, with modifications similar to that required for CF gene

therapy. The similar nano-based gene and drug delivery systems are also studied for therapeutic application in other airways diseases like asthma.

Therapeutic Application of Nanosystems for siRNA Delivery in Pulmonary Diseases

An experimental and potentially powerful way to fight disease, called RNA interference (RNAi), could now be closer to reality, as a key obstacle to effectively delivering the short interfering RNA (siRNA) molecules to targeted airway cells has been addressed using nanotechnology 79. In a collaborative project between Alnylam Inc and group from MIT, researchers report a method for quickly synthesizing more than a thousand different lipid-like molecules and screening them for their ability to deliver siRNA molecules to cells. In tests on mice, the best of these delivery agents were 10 times as effective at delivering siRNA to treat a respiratory ailment, compared with existing methods that deliver the siRNA directly to the lungs without it being encapsulated 80. Another recent study demonstarted that siRNA-mediated knockdown of p38 MAP kinase mRNA in mouse lung is enhanced by the use of nano- delivery vehicles containing cholesterol and the cell penetrating peptides (CPP) TAT and penetratin 81. These high throughput and gene specific approaches hold a promise for the development and translation of the efficient and selective nano-siRNA therapeutics for treatment of airway disease as an alternate to small molecules.

Recently, Dr. Mohapatra and his team have developed nasal drops containing nanoparticulate vectors capable of delivering siRNA molecules. These molecules were then encapsulated within chitosan NPs that are miniscule naturally occurring polymeric particles that stick to mucous-producing cells lining of the lungs. The RNA produced is specifically designed to suppress the specific protein NS1. Without NS1, the host antiviral defense remains intact and the virus cannot reproduce. Mice treated intranasally with the gene-silencing NPs, before and after infection with RSV, showed significantly lower levels of virus in the lung and less airway inflammation and hyper-reactivity than untreated mice [82]. We and others are evaluating similar strategies aiming at novel molecular targets using the above discussed nano-based delivery systems for rescue of chronic and obstructive lung pathophysiology.

Conclusions

The effective and safe translation of nano-based delivery systems to human subjects require improvement in the design of therapeutic vehicles based on the disease pathophysiology, followed by preclinical evaluation and standardization for airway delivery. The NP designing approaches should include modifications of the already established gene or drug carriers, cationic lipids and polymers, as well as developing new types of biodegradable materials. The field is further reinforced with the availability and development of various novel nano-sized inorganic materials (spheres, rods, tubes etc) as therapeutic delivery vehicles. The successful therapeutic translation of nano-based delivery system demands a concerted and synchronized effort from synthetic chemists, biomedical engineers, biologists, and clinicians. Moreover, translation of nano-therapeutic strategy to the clinic mandates extensive therapeutic development involving pre-clinical and clinical evaluation, and approval by regulatory agencies such as the US Food and Drug Administration (FDA) for human use. Finally, immuno-toxicological studies of these synthetic systems is strictly required for evaluating their potential short and long term toxic effects and associated health hazards.

Perspective

The development of novel nano-systems for pulmonary gene or drug delivery may provide a convenient, noninvasive method for the administration of gene or drugs to the lungs and provide

sustained site directed delivery to specific disease cell type or tissue bypassing the obstructive pathophysiological barriers (Table 1 & Table 2).

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Table 1

Therapeutic Application of Nano-Delivery in Airway Diseases

Disease	Therapeutic Application
Cystic Fibrosis (CF) [4,7,8,10,12,62–64,66,70,80]	Gene Therapy, Drug or siRNA Delivery
Alpha-1-Antitrypsin Deficiency (ATD) [4,10,77,78,80]	Gene Therapy, Drug or siRNA Delivery
COPD [4,10,80]	Small molecule, Drug or siRNA Delivery
Emphysema [4,10,80]	Gene Therapy, Drug or siRNA Delivery
Asthma [4,10,79,80]	Small molecule, Drug or siRNA Delivery
Infectious Diseases [4,10,80]	Small molecule, Drug or siRNA Delivery
Lung Cancer [4,7,10,80]	Gene Therapy, Drug or siRNA Delivery

Table 2

Challenges of Nano-Therapeutics in Airway Diseases

Nano-Therapeutics	Disease	Therapeutic Challenge
Gene	Cystic Fibrosis (CF), Alpha-1-trypsin Deficiency (AAT), Emphysema, Lung Cancer	Mucus, Alveolar Fluid, Endosome Escape, Nuclear Transport & Chromosomal Integration
si/shRNA	CF, COPD, Infectious Disease, Asthma, Lung Cancer	Mucus, Alveolar Fluid, Endosome Escape, Nuclear Transport and Stable or Transient Knock Down Efficiency
Peptides	CF, COPD, Infectious Disease, Asthma, Lung Cancer	Mucus and Alveolar Fluid, Endosome escape, Sustained Delivery
Drug	CF, AAT, COPD, Emphysema, Infectious Disease, Asthma,	Mucus and Alveolar Fluid, Endosome escape Sustained Delivery