Application of nanotechnology in drug delivery systems for respiratory diseases (Review)

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Abstract. Respiratory disease is a common disease with a high incidence worldwide, which is a serious threat to human health, and is considered a societal and economic burden. The application of nanotechnology in drug delivery systems has created new treatments for respiratory diseases. Within this context, the present review systematically introduced the physicochemical properties of nanoparticles (NPs); reviewed the current research status of different nanocarriers in the treatment of respiratory diseases, including liposomes, solid lipid nanocarriers, polymeric nanocarriers, dendrimers, inorganic nanocarriers and protein nanocarriers; and discussed the main advantages and limitations of therapeutic nanomedicine in this field. The application of nanotechnology overcomes drug inherent deficiencies to a certain extent, and provides unlimited potential for the development of drugs to treat respiratory diseases. However, most of the related research work is in the preclinical experimental stage and safety assessment is still a challenging task. Future studies are needed to focus on the performance modification, molecular mechanism and potential toxicity of therapeutic nanomedicine.

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Abbreviations: ASO, antisense oligonucleotides; AuNPs, gold nanoparticles; DCs, dendritic cells; DOX, doxorubicin; DPPC, dipalmitoylphosphatidylcholine; FDA, Food and Drug Administration; LPS, lipopolysaccharide; MERS-CoV, Middle East respiratory syndrome coronavirus; MP, methylprednisolone; NPs, nanoparticles; NSCLC, non-small cell lung cancer; PAMAM, polyamidoamide; PBCA, polybutylcyanoacrylate; PEG, polyethylene glycol; PEI, polyethyleneimine; PLGA, poly-lactic-co-glycolic acid; PS, pulmonary surfactant; PTX, paclitaxel; SARS-CoV, severe acute respiratory syndrome coronavirus; SBS, salbutamol sulfate; siRNA, small interfering RNA; SLN, solid lipid nanocarriers; RSV, respiratory syncytial virus; VLPs, virus-like particles

Key words: nanoparticle, drug delivery systems, toxicity, inhaled nanoparticles, respiratory disease

Contents

1. Introduction

With the rise in air pollution levels, rapid changes in lifestyle and frequent outbreaks of microbial infections, the morbidity of respiratory diseases is increasing, particularly among children and the elderly population with weakened immune systems (1). Almost 4,000,000 people die from respiratory diseases every year worldwide (2). The main respiratory diseases include acute and chronic respiratory infections, lung cancer, asthma, chronic obstructive pulmonary disease, cystic fibrosis and tuberculosis (2). Although the current diagnostic and therapeutic techniques have improved, effective treatment of severe and chronic disease is still lacking (3,4). In addition, it is difficult for most drugs to reach the lower respiratory tract with adequate dose and minimum side effects. Therefore, there is an urgent need to efficiently and affordably enhance the quality of treatments for respiratory disease.

Nanoparticles (NPs) refer to particles ranging between 1 and 100 nm in size (5). Due to the increase in relative surface area and quantum effects, nanomaterials have special physical and chemical properties. The nanodrug delivery system is the application of nanotechnology in the pharmaceutical field, and has shown development prospects in targeted diagnosis and treatment, delaying drug release, improving drug solubility and availability, reducing drug side effects and overcoming barriers of the human body (6). The large contact surface area of airways is constructed by alveolar cells and goblet cells, whereas the main bronchiole cells consist of bronchial epithelial cells and Clara cells (mucus-producing cells). Alveolar type I epithelial cells and endothelial cells share a basement membrane. The air-blood barrier inside the lungs, with a size of 0.1-0.2 μm, is comprised of epithelial and endothelial tissue sharing the basement membrane (7). The thin barrier and high permeability of this membrane make the lungs an optimal site for systemic and local delivery of drugs. Furthermore, pulmonary delivery offers improved bioavailability, biocompatibility and distribution of drugs to lung sites (8). The development of nanotechnology brings a novel broad perspective for improving the effects of treatment and diagnosis of respiratory diseases.

However, the possible negative effects of NPs as drug carriers should also be considered. It is well known that the toxicity of inhaled NPs has a long history. For example, some NPs, similar to fine dusts and fibers in nature, may induce respiratory and cardiovascular diseases as environmental pollutants (9,10). Although these data cannot be directly transferred to inhaled therapeutic NPs, before practical application, different *in vitro* and *in vivo* methods should be used in preclinical research and clinical trials to systematically detect the interaction between nanomedicines and various components of the respiratory system. In this context, the present review summarizes the properties of NPs; discusses the research status and main points of different nanocarriers in drug delivery systems for respiratory diseases, such as lung cancer, asthma, chronic respiratory diseases, cystic fibrosis, tuberculosis and respiratory infection; and discusses the advantages and limitations of therapeutic nanomedicine in the field of respiratory diseases.

2. Application of NPs in respiratory systems

Characteristics of NPs for efficient respiratory disease treatment design. NPs can be inhaled, diffused into the respiratory tract and deposited in the alveoli, where they can approach and interact with the epithelial cells and pulmonary surfactant (PS) (11). The characteristics of NPs, including size, shape, surface charge and wettability, serve a critical role in understanding the interaction between NPs and organisms (12). Appropriate properties can not only facilitate their direct delivery to targeted tissues and cells, but also limit their adverse side effects by decreasing drug concentrations in other tissues of the body (13).

Size. Among the different characteristics of NPs, particle size is a remarkable characteristic. Inhaled NPs are deposited on the pulmonary airway mainly via diffusional displacement by the thermal motion between air molecules and the NPs (14). The nasopharyngeal and tracheobronchial deposition of NPs have been reported to be negatively correlated with their size (15). A previous animal study in pigs performed by Murgia *et al* (16) revealed that only extremely small carboxylated NPs (<100 nm) were able to penetrate into mucus. Compared with large NPs (>100 nm), NPs with smaller size (<30 nm) were more suitable at penetrating biological barriers, including the air-blood barriers.

After intranasal immunization of polystyrene particles (20-1,000 nm), Blank *et al* (17) compared the size-dependent cellular absorption of these particles on antigen-presenting cells at respiratory sites in BALB/c mice. In the trachea and lung parenchyma, most of the smaller particles (20 and 50 nm) were absorbed by dendritic cells (DCs) compared with larger ones (1,000 nm), and the smaller ones were also observed in lung-associated lymph nodes. However, the uptake of cells by alveolar macrophages did not depend on the size of particles and larger particles could easily be phagocytized by lung macrophages. In addition, Ghaffar *et al* (18) demonstrated that the cellular uptake of smaller polystyrene particles (50 nm) by DCs was better than that of larger ones (500 nm) through intratracheal administration in mice, which resulted in more active lymphatic transport, improved maturation of DCs and production of cytokines. Furthermore, NPs with smaller particle size had a higher surface/volume ratio and were more likely to aggregate than larger ones. The aggregation of NPs not only affects their deposition in the lung and association with PS, but also changes the clearance mechanisms of NPs.

Shape. Shape is another important property that affects the interaction of NPs and cells, and the fate of NPs in the human body. Previous studies reported that spherical particles were more conducive to cellular internalization than shaped particles (19,20). However, Gratton *et al* (21) reported that rod-shaped, cationic, cross-linked NPs modified with polyethylene glycol (PEG) were internalized at a higher rate than particles of other shapes (spheres, cylinders and cubes). In contrast, it was reported that gold nanospheres had better blood circulation and higher overall tumor accumulation rate than other shapes (nanodiscs, nanorods and nanocages) (22). Moreover, shape may also be involved in regulating the transport of NPs on the PS monolayer. A previous study revealed that NPs smaller than the thickness of the PS layer tended to be submerged and hardly transported through the PS layer, whereas NPs larger than the thickness of the lung surfactant layer tended to be encapsulated by the PS layer (23). The results of coarse-grained molecular dynamics simulations suggested that rod-like NPs exhibited stronger penetration and less adverse effects on the dipalmitoylphosphatidylcholine (DPPC) monolayer compared with other shapes (24,25).

Surface charge. The surface charge of NPs determines the interaction between NPs and anionic cell membranes. Since positively charged NPs have the potential to induce damage to cell membranes and organelles, nanocarriers with stronger positive charges may not be an ideal choice for drug delivery systems (26). For example, Mousseau and Berret (27) observed a stronger interaction between positively charged NPs and PS compared with negatively charged NPs *in vitro*, which resulted in the aggregation of NPs and reduced their transfer efficiency.

However, in some specific fields, positively charged NPs have shown obvious advantages. A previous study in mice revealed that cationic NPs were mostly associated with DCs, whereas anionic particles were mainly internalized by alveolar macrophages (28). It is possible that the different cellular uptake mechanisms of cationic and anionic NPs might lead to different immune effects following pulmonary administration. Through animal experiments in mice, Tada *et al* (29) demonstrated that cationic liposomes induced higher antigen-specific antibody levels compared with anionic and neutral liposomes. Similarly, Fromen *et al* (30) reported that cationic NPs (~37 mV) conjugated with model antigen ovalbumin induced a higher level of antigen-specific IgG and local mucosal IgA in the plasma and bronchoalveolar lavage fluid (BALF) of mice after pulmonary immunization. In addition, cationic NPs could produce a large number of $CD4^+$ T cells and a high level of chemokines or cytokines, whereas negatively charged counterparts (~-38 mV) could not induce the same level of immunity response. Notably, positively charged cationic nanocarriers are widely used in drug delivery systems for gene therapy (31).

Wettability. The differences in wettability of NPs are often associated with different treatment outcomes. Hydrophobic

NPs are deemed to interact more closely with the negatively charged cell membrane when compared with hydrophilic NPs. However, the hydrophobicity of NPs can mimic a danger signal to stimulate the immune system (32). Nanogels comprised of hydrophilic polymers [poly (sulfobetaine), PEG or poly (carboxybetaine)] were found to be effective in inhibiting immune responses after pulmonary administration, via a reduction in the degree of infiltration of inflammatory cells in the BALF and the expression of cytokines (TNF-α and IL-6) in a lipopolysaccharide (LPS)-induced inflammatory mouse model (33). Guzmán *et al* (34) reported that NPs incorporated into Langmuir monolayers of DPPC could alter the interfacial organization of the molecules. When compared with hydrophobic carbon black, hydrophilic silica had stronger influence on DPPC phase behavior.

Classification and advantages of NPs as drug delivery systems for treating respiratory diseases. NPs have great potential to be applied as pulmonary delivery systems for the diagnosis and treatment of local respiratory diseases and may even exert systemic actions, such as blood coagulation (35) and cardiovascular effects (36). Delivery of therapeutic drugs to target sites may be important for efficient treatment of tuberculosis, lung cancer, cystic fibrosis, and other acute and chronic respiratory infections. As early as 1654, an inhalation device was first designed by Bennet to produce opium vapor for cough treatment (37). The Food and Drug Administration (FDA) has already approved several materials as drug delivery systems, including liposomal, polymeric, dendrimers, inorganic and protein materials. More complex materials comprised of micelles, proteins, and a variety of inorganic or metallic materials are currently in development for assessment in clinical trials (38).

Liposomes. The application of liposomes as a drug delivery system has a significant impact on pharmacology. Liposomes are a class of lipid vesicles composed mainly of phospholipids and cholesterol. This colloidal form is comprised of a self-assembled lipid bilayer with amphiphilic domains, including an inner aqueous core and an outer shell of the lipid bilayer (39). According to the physical properties of the drug, liposomes can encapsulate drugs with different solubility in the water core or bilayer interface of the phospholipid bilayer, and enhance the solubility of the loaded drug through the co-solubility effect (37). The lipid bilayer of liposomes is similar to the composition of cell membranes in the body, which can not only reduce its toxicity, but can also enable liposomes to cross numerous biological barriers (40), thereby increasing absorption and ultimately enhancing the therapeutic effect of loaded drugs. In addition, liposomes can be used as carriers for other functional groups, such as targeted ligands, to create new properties for the delivery of therapeutic drugs (41). Furthermore, a previous study by Garbuzenko *et al* (42) tested a variety of nanomaterials to select the best inhalation carrier for anticancer drugs, and revealed that compared with non-lipid-based carriers, lipid-based nanocarriers had advantages in terms of accumulation and retention time in the lungs. Based on these advantages, liposomes became the earliest nanocarriers approved by the FDA in 1995, including liposome formulations of doxorubicin (DOX; Doxil®) (43) and amphotericin B (44). In the past few decades, nanomedicine based on the liposome delivery system has generated the interest of scientists and clinicians in different fields of respiratory diseases (Table I). For example, in an orthotopic mouse model of human lung A549 non-small cell lung cancer (NSCLC) cells, Garbuzenko *et al* (45) compared the effects of intravenous and intratracheal administration of liposome-encapsulated DOX, antisense oligonucleotides and small interfering RNA (siRNA) on lung cancer, and demonstrated that compared with systemic administration by intravenous injection, intratracheal administration resulted in much higher peak concentrations and longer retention time of three drugs in the lungs, which indicated that local intratracheal administration was better than systemic administration of the same drug. Similarly, Koshkina *et al* (46) proved that the pulmonary delivery of paclitaxel (PTX) in liposome aerosol formulations was more efficient than intravenous injection in mice. In a carbamate-induced lung tumor mouse model, Fritz *et al* (47) showed that clodronate encapsulated with liposomes reduced the number of macrophages by 50% after 4-6 weeks of treatment and significantly weakened the proliferative ability of tumor cells. Besides, a phase I clinical trial carried out by Wittgen *et al* (48) explored the application of cisplatin liposomal formulation in lung cancer. Their results indicated that this drug delivery system could enhance the drug accumulation and reduce the systemic side effects.

Several types of antimicrobials for the treatment of airway infections can also be delivered by liposomes. A double-blind, randomized, phase II clinical trial conducted by Olivier *et al* (49) applied inhaled liposomal amikacin in the treatment of nontuberculous mycobacterial lung disease; the results revealed that the drug promoted the negative conversion of sputum and induced lower toxicity compared with parenteral amikacin. Similarly, Zhang *et al* (50) explored the efficacy of liposomal amikacin in nontuberculous mycobacteria both *in vivo* (rat model) and *in vitro*, and their results showed that this nanodrug could effectively enter bacterial biofilms, improve cellular uptake of amikacin in macrophages and inhibit the distribution of amikacin to other tissues. Additionally, through a randomized controlled clinical trial, Okusanya *et al* (51) reported that liposomal amikacin improved lung function and reduced bacterial density in the lung of patients with chronic *Pseudomonas* infection.

Furthermore, liposomal drug delivery systems have been applied to inflammatory respiratory diseases. For example, Konduri *et al* (52) investigated the effect of liposomal budesonide on the treatment of asthma using a mouse model; the results revealed that this drug delivery system significantly improved lung inflammation and reduced the toxicity of inhaled steroid asthma drugs. Chen *et al* (53) designed liposomes to encapsulate salbutamol sulfate (SBS) in aerosol form and demonstrated that the complexes exhibited longer anti-asthmatic effects than free SBS. Furthermore, Ng *et al* (54) demonstrated that liposome-encapsulated curcumin exerted an inhibitory effect on LPS-induced airway inflammation via cell experiments *in vitro*. A recent study performed by Komalla *et al* (55) through cell and animal experiments found that empty liposomes (UTS-001) could be used to treat chronic respiratory diseases by inhibiting epithelial pro-inflammatory cytokines and reducing the number of eosinophils.

Table I. Brief application of liposomes in drug delivery systems for the treatment of respiratory diseases.

Author, year	Colloidal system	Application	Object of the study	Drug	Characteristics	Method of administration	Key findings	(Refs.)
Fritz *et al*, 2014	Liposomes	Lung cancer	Mice	Clodronate	NA	Intravenous injection	Reduced the number of macrophages and attenuated the proliferation ability of tumor cells	(47)
Garbuzenko *et al*, 2009	Liposomes	Lung cancer	Orthotopic mice model of human lung A549 NSCLC cells	DOX/ASO/ siRNA	DOX: 130±10 nm; -10±2 mV; ASO: 130±10 nm; -10±2 mV; siRNA: >500 nm; 4±2 mV	Intratracheal/ intravenous administration	Extended the retention time of the drug in the lung and enhanced the efficacy of the drug	(45)
Koshkina *et al*, 2001	Liposomes	Lung cancer	Mice	PTX	230±170 nm	Aerosol/ intravenous administration	Reduced number of visible tumor foci on the lung surfaces, prolonged survival and enhanced the efficacy of the drug	(46)
Wittgen *et al*, 2007	Liposomes	Lung cancer	Patients with lung carcinoma	Cisplatin	NA	Inhalation	Enhanced drug accumulation and reduced the systemic side effects	(48)
Olivier *et al*, 2017	Liposomes	NTM lung disease	Patients with persistently positive NTM culture	Amikacin	NA	Inhalation	Promoted the negative conversion of sputum and induced lower toxicity	(49)
Zhang *et al*, 2018	Liposomes disease	NTM lung	THP-1 human peripheral blood monocytes/rat	Amikacin	221±98 nm	Nose-only inhalation	Effectively entered bacterial biofilms, improved cellular uptake of amikacin in macrophages and inhibited the distribution of amikacin to other tissues	(50)
Chen *et al*, 2012	Liposomes	Asthma	Rat	SBS	33-58 nm	Intratracheal administration	Increased the concentration and retention time of SBS in the lungs and displayed a longer anti-asthmatic effect than free SBS	(53)

Table I. Continued.

Author, year	Colloidal system	Application	Object of the study	Drug	Characteristics	Method of administration	Key findings	(Refs.)
Konduri *et al*, 2005	Liposomes	Asthma	Mice	Budesonide	NA	Inhalation	Improved lung inflammation and reduced the toxicity of inhaled steroid asthma drugs	(52)
Ng *et al*, 2018	Liposomes	Asthma	BCI-NS1.1 cell line	Curcumin	271.3±3.06 nm; PdI=0.512±0.003; -61.0±0.68 mV	NA	Anti-inflammatory effects on lipopolysaccharide-induced airway inflammation	(54)
Komalla *et al*, 2020	Liposomes	Chronic respiratory diseases	Human epithelial virus-transformed cell line BEAS-2B/mice	NA	173.23±1.62 nm; PdI=0.13±0.01; -0.82±0.24 mV	Injection	Suppressed pro-inflammatory cytokines, decreased eosinophil number and reduced airway hyperresponsiveness	(55)
Okusanya *et al*, 2009	Liposomes	Cystic fibrosis	Patients with cystic fibrosis with chronic pseudomonal infection	Amikacin	NA	Inhalation	Improved lung function and reduced bacterial density in the lung	(51)
Nahar *et al*, 2014	Starch-coated magnetic liposomes	Pulmonary arterial hypertension	PASMCs/rat	Fasudil	130.4±3.98 nm; PdI=0.05±0.04; -9.58±1.74 mV	Intratracheal administration	Enhanced the absorption of PASMCs to liposomes; reduced the proliferation of PASMCs, and the optimized liposomes appeared to be safe; extended the half-life of magnetic liposomes	(56)
Wijagkanalan *et al*, 2008	Mannosylated liposomes	Alveolar macrophage-related respiratory diseases	Alveolar macrophages and alveolar epithelial type II cells/rat	NA	90-125 nm; PdI=0.14-0.35; -9-15 mV	Intratracheal administration	Enhanced the uptake of alveolar macrophages compared with bare-liposomes	(57)
Cryan *et al*, 2006	Octaarginine-coated liposomes	Respiratory diseases in lung	Calu-3 cells	Dextrans	213.4±44.3 nm	NA	Increased intracellular targeting, improved cellular uptake and reduced drug toxicity	(58)

ASO, antisense oligonucleotides; DOX, doxorubicin; NA, not available; NSCLC, non-small cell lung cancer; NTM, nontuberculous mycobacterial; PASMCs, pulmonary arterial smooth muscle cells; PdI, polydispersity index; PTX, paclitaxel; siRNA, small interfering RNA; SBS, salbutamol sulfate; SLN, solid lipid nanocarrier.

In addition to simple liposomal nanocarriers, numerous groups have successfully modified liposomes to improve their properties, including cellular uptake, stability and targeting. For example, Nahar *et al* (56) demonstrated that starch-coated magnetic liposomes could be used as an inhalable carrier to deliver fasudil to treat pulmonary hypertension through *in vitro* cell experiments and rat animal models. In addition, a previous study reported that the cellular uptake of mannosylated liposomes by alveolar macrophages was higher compared with that of non-modified ones after intratracheal administration both *in vivo* and *in vitro* (57). Through *in vitro* experiments, Cryan *et al* (58) showed that octaarginine-coated liposomes could increase intracellular targeting, improve cellular uptake and reduce drug toxicity in airway cells.

Solid lipid nanocarriers (SLNs). SLNs are another type of lipid-based material, which are slightly different from liposomes in structure. SLNs may represent an alternative to traditional carrier systems due to their numerous advantages, including targeted drug delivery, controlled-release, high drug stability, high drug loading, encapsulation of hydrophilic and lipophilic drugs, low carrier toxicity, avoidance of organic solvents in production (such as high-pressure homogenization) and large-scale industrial production (59,60). Nassimi *et al* (61) evaluated the toxicity of SLNs as potential nanocarriers in *in vitro* and *ex vivo* lung models, and their results showed that SLN20 (20% phospholipids included in particle lipid matrix) could be used as a safe pulmonary drug delivery system.

In the past few years, as a colloidal drug delivery system, SLNs have promoted the development of the treatment of respiratory diseases (Table II). For example, Videira *et al* (62) investigated the antitumor effect of PTX-loaded SLNs on lung cancer, and revealed that the pulmonary delivered nanodrug efficiently reduced cellular toxicity and suppressed the progression of lung metastases *in vitro* and *in vivo*. In addition, Castellani *et al* (63) designed SLN-encapsulated grape seed-derived proanthocyanidins to treat chronic respiratory diseases, and confirmed that the complex could inhibit oxidative stress and inflammation in airway epithelial cells through cell experiments and mouse models.

Moreover, SLNs can be modified to improve their targeting ability, thereby increasing the accumulation of drugs in targeted sites and reducing systemic toxicity. For the treatment of tuberculosis (64), Maretti *et al* (65) used SLN modified with mannose derivatives as nanocarriers of rifampicin, and tested the anti-tuberculosis ability of the novel drug in J774 murine macrophage cells. Their results showed that SLNs modified with the surfactants (mannose derivatives) could improve the absorption capacity of macrophages for their encapsulated drugs. A similar study was carried out by Nimje *et al* (66), which revealed that mannose-conjugated SLNs could deliver rifampicin more effectively than bare-SLNs, which increased the therapeutic effect and reduced the side effects of the drug.

Polymeric nanocarriers. A polymer is a type of large molecule chemical compound, which is composed of numerous smaller homogeneous molecules. Polymers can be natural (albumin, gelatin, alginate, collagen, cyclodextrin and chitosan) or synthetic [poly-lactic-co-glycolic acid (PLGA), polyacrylates, polyethyleneimine (PEI), PEG, polyanhydrides and poly-l-lysine] (67). Polymers with particular biological and physicochemical advantages are used for the formulation of nanocarriers to deliver therapeutic and diagnostic drugs. Polymer-based nanocarriers can deliver different agents, which are inserted into the surface of the polymer or dispersed in the polymeric matrix (68).

Aliphatic polyesters are the most commonly used polymer nanocarriers due to their excellent biocompatibility, controlled-release properties and sufficient biodegradability under physiological conditions (69). Various forms of polymeric nanocarriers have been used in preclinical experiments for the treatment of respiratory diseases (Table III). Among them, PLGA has been approved by the FDA for use as a drug delivery system. Türeli *et al* (70) prepared PLGA NPs loaded with ciprofloxacin and tested their therapeutic effects on bacterial infection-induced cystic fibrosis in Calu-3 and CFBE41o$^-$ cells. The results showed that the nanomedicine had high drug loading and permeability, which could not only achieve high and persistent local drug concentration, but also decrease the drug dose to reduce side effects. Through *in vitro* and *in vivo* experiments, Kim *et al* (71) revealed that the sustained-release inhalation system assembled by DOX and PLGA had high encapsulation efficiency and good nebulization ability, could effectively inhibit the growth of tumor cells and was suitable for the treatment of metastatic lung cancer. A previous study in guinea pigs performed by Pandey *et al* (72) showed that the encapsulation of PLGA prolonged the elimination half-life and average residence time of three anti-tuberculosis drugs, thereby increasing the bioavailability and reducing the frequency of administration. Besides, Tomoda *et al* (73) demonstrated that PLGA NPs loaded with TAS-103 enhanced drug toxicity to A549 lung cancer cells and increased the drug concentration in the lungs of rats. For gene transfer applications in the treatment of respiratory disease, PLGA is also considered a good choice. An *in vitro* study by Zou *et al* (74) reported that negatively charged bioadhesive PLGA NPs could be used as an efficient non-viral vector for gene therapy in the treatment of lung cancer.

Although PLGA has numerous advantages, it also has several limitations as a pulmonary delivery system. For example, the slow degradation rate of PLGA may result in excessive accumulation of PLGA in the respiratory tract (75). The degradation rate of the drug depends on the composition and molecular weight of polymeric nanocarriers, and the release period varies from several weeks to several months. Moreover, continuous hydrolysis of PLGA may generate an acidic core within the drug delivery device, which lowers the pH of the microenvironment and damages pH-sensitive encapsulated proteins, such as peptides and proteins (76). Additionally, due to the extreme hydrophobicity of PLGA, the encapsulation efficiency of low-molecular-weight hydrophilic drugs may be undesirably low and the hydrophobic surface may cause rapid protein adsorption, leading to the clearance of PLGA by alveolar phagocytes (77).

Solutions have been applied to optimize the design and overcome the problems of PLGA delivery devices. To overcome the accumulation of PLGA carriers, several polymers with faster degradation rates have been synthesized for drug delivery. Polybutylcyanoacrylate (PBCA) is a noncytotoxic and biodegradable NP that can be used for pulmonary

Table II. Brief application of SLNs in drug delivery systems for the treatment of respiratory diseases.

Author, year	Colloidal system	Application	Object of the study	Drug	Characteristics	Method of administration	Key findings	(Refs.)
Videira *et al*, 2012	SLN	Lung cancer	MXT-B2 tumor cells/rat	PTX	92.6±6.0 nm; PdI=0.106±0.030; -15.7±0.6 mV	Inhalation	Suppressed the progression of lung metastases	(62)
Castellani *et al*, 2018	SLN	Chronic respiratory diseases	Airway epithelial cell line H441/mice	Grape seed-derived proanthocyanidins	243±24 nm; PdI=0.41-0.51; -14.5±1.0 mV	Spray instillation	Dampened oxidative stress and inflammation of airway epithelial cells	(63)
Maretti *et al*, 2019	SLN modified with mannose derivatives	Tuberculosis	J774 murine macrophage cell line	Rifampicin	400±20 nm; PdI=0.43±0.09; -35.33±0.29 mV	NA	Improved the absorption capacity of macrophages for the encapsulated drugs	(65)
Nimje *et al*, 2009	Mannosylated SLN	Tuberculosis	J774 murine macrophage cell line/rat	Rifampicin	389±2.3 nm; PdI=0.357; -11.7±0.8 mV	Intravenous injection	Targeted delivery of rifampicin, increased the therapeutic effect and reduced the side effects of the drug	(66)

NA, not available; PdI, polydispersity index; PTX, paclitaxel; SLN, solid lipid nanocarrier.

Table III. Brief application of polymeric nanocarriers in drug delivery systems for the treatment of respiratory diseases.

Author, year	Colloidal system	Application	Object of the study	Drug	Characteristics	Method of administration	Key findings	(Refs.)
Türeli *et al*, 2017	PLGA	Cystic fibrosis	Calu-3 cells and CF bronchial epithelial cells (CFBE41o$^-$)	Ciprofloxacin	190.4±28.6 nm; PdI=0.089	NA	High drug loading and permeability, achieved high and sustained local drug concentration, reduced drug dosage and side effects	(70)
Kim *et al*, 2012	PLGA	Metastatic lung cancer	B16F10 melanoma cells/mice	DOX	14.1±2.1 μm	Inhalation	High encapsulation efficiency and good nebulization ability, inhibited the growth of tumor cells	(71)
Pandey *et al*, 2003	PLGA	Tuberculosis	Guinea pigs	Rifampicin, isoniazid and pyrazinamide	1.88±0.11 μm	Inhalation	Prolonged the elimination half-life and average residence time, improved the bioavailability and reduced the frequency of administration	(72)
Tomoda *et al*, 2009	PLGA	Lung cancer	A549 NSCLC cells/rat	TAS-103	241.2 nm	Inhalation	Enhanced the toxicity to cancer cells and increased the drug concentration in the lungs	(73)
Zou *et al*, 2009	Bioadhesive PLGA	Lung cancer	A549 NSCLC cells	DNA	126±5 nm; PdI=0.105±0.004; -27.83±3.27 mV	NA	Efficient non-viral vector for gene therapy	(74)
Melguizo *et al*, 2015	PBCA	Lung cancer	A549 and LL/2 lung cancer cell lines/mice	DOX	~75 nm; PdI=0.064; -25 mV	Injection	Enhanced the cellular uptake, improved the drug antitumor activity, and increased the survival rate of mice	(79)
Howard *et al*, 2006	Chitosan	Systemic and mucosal disease	NIH 3T3 cells, H1299 human lung carcinoma cells and murine peritoneal macrophages/mice	siRNA	176.4-319.4 nm; PdI=0.20-0.51; 18.8-31.1 mV	Nasal administration	High transfection efficiency	(31)

Table III. Continued.

Author, year	Colloidal system	Application	Object of the study	Drug	Characteristics	Method of administration	Key findings	(Refs.)
Germershaus *et al*, 2008	Trimethyl chitosan/ PEGylated trimethyl chitosan	Lung cancer	A549 NSCLC cells	DNA	334/142 nm; 6.47±0.27/0.01± 0.02 mV	NA	Improved cellular uptake and transfection efficiency	(84)

DOX, doxorubicin; NA, not available; NSCLC, non-small cell lung cancer; PBCA, polybutylcyanoacrylate; PdI, polydispersity index; PEG, polyethylene glycol; PLGA, poly-lactic-co-glycolic acid; siRNA, small interfering RNA.

administration; in addition, it is pH-sensitive and can be catalyzed by enzymes (78). Compared with free DOX, *in vitro* and *in vivo* studies by Melguizo *et al* (79) revealed that PBCA-encapsulated DOX significantly enhanced the drug uptake of lung cancer cells, improved the antitumor activity of drugs and increased the survival rate of mice. Another approach is to use hydrophilic polymers to reduce accumulation of polymerics in the body. For stabilization of proteins within PLGA, pH-sensitive drugs could be pre-mixed with zinc or antacid excipients could be added to buffer the vehicle microclimate (80).

Polymers are important delivery carriers for nano-gene drugs in gene therapy of respiratory diseases; as well as the aforementioned modified PLGA, PEI is also a promising polymer for delivering recombinant genes to mammalian cells due to its high transfection efficiency, biocompatibility and biodegradability (81,82). This polymer with positively charged groups is able to closely interact with negatively charged genes. Similarly, chitosan is another nanocarrier commonly used in gene therapy for drug delivery. It is positively charged under neutral and acidic pH conditions and is a biodegradable polymer synthesized through the deacetylation of chitin (83). In 2006, Howard *et al* (31) synthesized a NP system composed of siRNA and chitosan, and revealed that the complex had high transfection efficiency *in vitro* and *in vivo*, and it was considered a potential genetic medicine for mucosal disease. Beyond that, in order to improve the transfection efficiency of gene drugs, a variety of improved polymer carriers based on chitosan have been proposed. Germershaus *et al* (84) compared the performance of chitosan, trimethyl chitosan and PEGylated trimethyl chitosan as DNA carriers, and the results showed that compared with unmodified chitosan, both modified forms of chitosan exhibited improved cellular uptake and transfection efficiency. In addition, the quaternization of chitosan could effectively inhibit the pH dependence and aggregation of DNA complexes, and PEGylation could further improve the stability of colloids.

Dendrimers. A dendrimer is a type of polymer nanostructure that is different from traditional polymers. It has a highly branched monodisperse three-dimensional structure. The multiple functional groups distributed on the surface of a dendrimer increase its versatility and biocompatibility as a nanocarrier (85). In addition, their external functional groups can be modified by other charged compounds through electrostatic interaction, and dendrimers with both hydrophobic and hydrophilic group structures can deliver a large number of drug molecules with different solubility (86). During the delivery process, the loaded drug can be combined with the functional groups on the surface of the dendrimer, or it can be wrapped in the molecular cavity of the dendrimer (87).

Based on these advantages, dendrimers have attracted the attention of researchers in the field of drug delivery due to their unique structural and physicochemical properties (Table IV). For example, a previous *in vitro* study by Bellini *et al* (88) reported that fourth-generation polyamidoamide (G4-PAMAM) dendrimers containing the anti-tuberculosis drug rifampicin had high stability under physiological pH conditions, and the PAMAM dendrimers could be used as a pH switch to rapidly release drugs in the acidic area of

Table IV. Brief application of dendrimers in drug delivery systems for the treatment of respiratory diseases.

Author	Colloidal system	Application	Object of the study	Drug	Characteristics	Method of administration	Key findings	(Refs.)
Bellini *et al*, 2015	G4-PAMAM	Tuberculosis	NA	Rifampicin	2.03±0.02 nm	NA	High stability and rapid pH-dependent release	(88)
Rajabnezhad *et al*, 2016	G3-PAMAM	Tuberculosis	Rat	Rifampicin	6.21±0.03 nm	Intratracheal administration	Sustained release, and improved drug absorption and bioavailability	(89)
Conti *et al*, 2014	G4-PAMAM	Lung cancer	A549 NSCLC cells	siRNA	254±52 nm; PdI=0.5±0.1; 33±3 mV	NA	Targeted lung alveolar epithelial A549 cells and silenced target genes	(90)
Zhong *et al*, 2016	G4-PAMAM	Lung cancer	Mice	DOX	4.7-9.7 nm; 13.8±7.0 mV	Intratracheal administration	Prolonged the accumulation and retention time, reduced the systemic toxicity and enhanced the efficacy of the drug	(91)
Inapagolla *et al*, 2010	G4-PAMAM	Asthma	Mice	Methylprednisolone	NA	Inhalation	Prolonged the drug residence time, enhanced the ability of the drug to inhibit inflammation	(92)

DOX, doxorubicin; NA, not available; NSCLC, non-small cell lung cancer; PAMAM, polyamidoamide; PdI, polydispersity index; siRNA, small interfering RNA.

macrophages. Similarly, Rajabnezhad *et al* (89) synthesized different generations of PAMAM dendrimers encapsulating rifampicin to produce inhalable nanodrugs for the treatment of tuberculosis. Their results showed that compared with intravenous administration, third generation PAMAM dendrimers achieved sustained drug release, and significantly improved drug absorption and bioavailability. For the application of dendrimers in lung cancer, Conti *et al* (90) used an amine-terminated G4-PAMAM dendrimer (G4NH2) loaded with siRNA to decrease the expression of enhanced green fluorescent protein in a model of A549 cells, indicating that G4NH2-siRNA could not only target alveolar epithelial cells, but could also effectively silence the target gene. Furthermore, Zhong *et al* (91) explored the effect of a complex composed of DOX and carboxyl-terminated G4-PAMAM dendrimers in lung metastasis, and confirmed that compared with intravenous administration, the complex prolonged the accumulation and retention time of the drug in the lung and reduced systemic toxicity, thereby enhancing the efficacy of DOX on melanoma lung metastasis and increasing the survival rate of mice. In addition, dendrimers have been used to treat inflammatory respiratory diseases related to asthma. Through a mouse lung inflammation model, Inapagolla *et al* (92) revealed that G4-PAMAM conjugated with methylprednisolone (MP) might enhance the ability of MP to inhibit inflammation by prolonging the residence time of the drug within the lung.

Inorganic nanocarriers. There are several types of inorganic substances that have been used to synthesize NPs, including gold, silica, iron oxide, alumina and titanium dioxide. Inorganic NP carriers possess several advantages, such as high biocompatibility, high delivery efficiency, high stability, magnetic properties and resistance to microbial degradation (93). Several iron oxide NPs have been approved by the European Union. Based on the plasmonic and magnetic characteristics of inorganic materials, they can also be used for diagnosis of respiratory diseases, such as positron emission tomography, computed tomography and magnetic resonance imaging (94). The external magnetic field can not only direct these magnetic NPs to the targeted sites, but also increase the temperature of these NPs (95). High temperature can induce apoptosis of target cells, including infected cells and cancer cells (86,96).

In addition to high-temperature-based targeted drug control, gold NPs (AuNPs), as typical inorganic NPs, are often used as nanocarriers in drug delivery systems for the treatment of respiratory diseases (Table V). For example, a previous study conducted by Chen *et al* (97) demonstrated that methotrexate (MTX)-AuNPs were more cytotoxic to tumor cell lines than free MTX, and MTX could inhibit tumor growth only when under AuNP encapsulation both *in vitro* and *in vivo*. Similarly, Brown *et al* (98) confirmed that AuNPs increased the toxicity of the antitumor drug oxaliplatin to lung cancer cell lines. Apart from the application in lung cancer treatment, Codullo *et al* (99) investigated the role of AuNP-loaded imatinib in the treatment of lung fibrosis through cell experiments and mouse models, and the results showed that the complex could significantly improve the anti-fibrotic efficacy of imatinib, thereby inhibiting the proliferation of fibroblasts and macrophages.

Despite these advantages, drug delivery systems using metal NPs as carriers still have some limitations. For example, when administered by intravenous injection, positively charged AuNPs are easily combined with negatively charged serum proteins in the blood and form aggregates (96). Based on this defect, previous studies have proposed an improved solution, that is, PEG modification of the surface to prevent the aggregation of AuNPs and thus improve their stability during storage (100). Omlor *et al* (101) modified AuNPs with PEG and citrate to reduce airway inflammation in a mouse model. Furthermore, an *in vitro* study performed by Park *et al* (102) combined cell-penetrating peptides with PEG-AuNPs to enhance the cell death-inducing activity of the anticancer drug DOX. In addition, the potential concentration-dependent cytotoxicity and low excretion of inorganic nanocarriers also limit their clinical application to a certain extent. Therefore, systemic absorption and subsequent adverse events must be fully considered when designing and examining nanomedicine.

Protein nanocarriers. Protein NPs include a large number of classes, such as endogenous protein carriers conjugated with drugs, engineered proteins and combined platforms that rely on protein or peptide motifs for targeting delivery (103). Protein NPs have many advantages, including high biocompatibility and solubility, biodegradability, modifiability, controlled-release properties and targeted drug delivery (104). At present, a large number of preclinical experiments based on protein nanocarriers have been reported in the field of respiratory diseases, particularly for respiratory infection (Table VI). Among them are virus-like particles (VLPs), which are a type of protein NP assembled from viral proteins with diverse structures and functions (Table VI). The proteins of VLPs can be commercially expressed in numerous systems, such as prokaryotic systems (*Escherichia coli*) and eukaryotic systems (yeast and insect cells) (105). VLPs could be used as nanocarriers for vaccines to treat infectious respiratory diseases, such as influenza virus and respiratory syncytial virus (RSV) infection (106). For example, Coleman *et al* (107) proposed to use purified coronavirus spike protein NPs to load Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV) protein antigens for vaccination. The results showed that this strategy could produce high titers of antibodies in mouse models. Additionally, Smith *et al* (108) and Lee *et al* (109) used baculovirus vector and VLPs to combine with RSV fusion proteins to construct protein nanocarrier vaccines, respectively. The results demonstrated that in cotton rat and mouse models RSV replication was effectively inhibited in the lungs following intramuscular injection of vaccines, and nanocarriers promoted the immunogenicity of vaccines when compared with traditional formalin-inactivated RSV. In addition to the application of vaccination strategies, protein nanocarriers have been used in other respiratory diseases. A previous *in vitro* study confirmed that the encapsulation of apigenin with bovine serum albumin could inhibit lung injury induced by immune responses by enhancing antioxidant activity (110).

The advantages and limitations of therapeutic nanomedicine in respiratory diseases. Inhaled administration is a non-invasive drug delivery route. The drug is delivered through

Table V. Brief application of inorganic nanocarriers in drug delivery systems for the treatment of respiratory diseases.

Author	Colloidal system	Application	Object of the study	Drug	Characteristics	Method of administration	Key findings	(Refs.)
Chen *et al*, 2007	AuNP	Lung cancer	Cancer cell lines (LL2, ML-1, MBT-2, TSGH 8301, TCC-SUP, J82, PC-3, HeLa)/mice	MTX	14.3 nm; -7.3±2.5 mV	Intraperitoneal administration	High cytotoxicity toward numerous tumor cell lines, suppressed growth tumor	(97)
Brown *et al*, 2010	AuNP	Lung cancer	Cancer cell lines (A549, HCT116, HCT15, HT29, RKO)	Oxaliplatin	176±25 nm; 14±7.0 mV	NA	Improved the toxicity of drugs to cancer cells	(98)
Codullo *et al*, 2019	AuNP	Lung fibrosis	Lung fibroblasts and alveolar macrophages/mice	Imatinib	21.25±2.461 nm; PdI=0.255±0.023; -46.3±2.842 mV	Intratracheal instillation	Increased drug efficacy, inhibited proliferation of fibroblasts and macrophages	(99)
Omlor *et al*, 2017	PEGylated AuNP/citrated AuNP	Asthma	Mice	NA	6 nm; -43 mV/ 9 nm; -51 mV	Intranasal administration	Inhibited both inflammatory infiltrates and airway hyperreactivity	(101)
Park *et al*, 2014	P-PEG-AuNP25	Lung cancer	Cancer cell lines (A549, HeLa)	DOX	25 nm	NA	Enhanced cell death induction activity of the drug	(102)

AuNP, gold nanoparticle; DOX, doxorubicin; NA, not available; MTX, methotrexate; PdI, polydispersity index; PEG, polyethylene glycol.

Table VI. Brief application of protein nanocarriers in drug delivery systems for the treatment of respiratory diseases.

Author	Colloidal system	Application	Object of the study	Drug	Characteristics	Method of administration	Key findings	(Refs.)
Coleman *et al*, 2014	Purified coronavirus spike protein	Vaccination strategies	Mice	MERS-CoV and SARS-CoV protein antigens	~25 nm	Intramuscular injection	Produced high titer antibodies	(107)
Smith *et al*, 2012	Baculovirus vector	Vaccination strategies	Cotton rat	RSV fusion proteins	40 nm	Intramuscular injection	Induced neutralizing serum antibodies and inhibited virus replication	(108)
Lee *et al*, 2017	VLP	Vaccination strategies	Mice	RSV fusion proteins	60-120 nm	Intramuscular injection	Induced distinct innate and adaptive cellular subsets	(109)
Pápay *et al*, 2017	Bovine serum albumin	Pulmonary inflammation	Franz cell apparatus	Apigenin	376±7.824 nm; PdI=0.285±0.01; -19.20±0.818 mV	NA	Effective against oxidative stress-induced lung injury	(110)

MERS-CoV, Middle East respiratory syndrome coronavirus; NA, not available; PdI, polydispersity index; RSV, respiratory syncytial virus; SARS-CoV, severe acute respiratory syndrome coronavirus; VLP, virus-like particle.

the cavity of the respiratory tract and the mucous membrane to achieve local or systemic drug delivery. The special physiological structure of the lung determines the characteristics and advantages of inhaled administration. Firstly, pulmonary inhalation can achieve effective lung-targeted medication and maintain the biological activity of the drug, which is suitable for the treatment of common respiratory diseases, including asthma, emphysema and chronic bronchitis (111). Secondly, in contrast to other routes of administration, such as oral and intramuscular injection, pulmonary inhalation takes effect rapidly (112). Thirdly, pulmonary inhalation can avoid hepatic first-pass metabolism, decrease the dosage of administration and reduce systemic side effects (113).

Based on the basic advantages of pulmonary inhalation, the application of nanotechnology in drug delivery systems has further improved the efficacy of inhalation therapy for different respiratory diseases. In general, nanocarriers enhance cellular uptake and achieve therapeutic effects in the lungs with lower drug doses (70), enhance the solubility of drugs, particularly the delivery of hydrophobic molecules (37,86), enhance the stability of drugs under physiological conditions (88), achieve controlled-release to prevent the rapid elimination of drugs (72,91,92) and result in targeted drug delivery (57,66,90).

Although nanocarriers have promoted the development of drugs related to respiratory diseases, the potential toxicity of NPs to the lung microenvironment or systemic toxicity is also the focus of current nanomedicine research. It has been reported that the size, surface charge, polarity and degradability of NPs are typical characteristics related to toxicity (114). Previous studies have demonstrated that inhalation of nanomaterials <100 nm is usually related to chronic toxicity (115,116). Therefore, in terms of particle size, the drug development process needs to fully balance the efficacy and toxicity of the nanodrug. However, a previous study evaluated the effects of particles with different sizes (50-150 nm) and different materials (PEG-ylated lipid particles, polyvinyl acetate and polystyrene) on mice, and found that acute respiratory toxicity was independent of particle size and only hydrophobic materials caused inflammation (117). In addition, Dailey *et al* (118) compared non-biodegradable polymers and biodegradable polymers of the same size, and confirmed that NPs derived from biodegradable polymers produced less toxicity and inflammatory responses. Furthermore, the application of nanocarriers in pharmaceutical preparations may change the distribution behavior of the original drugs in the body, which may cause new unpredictable adverse reactions.

Briefly, in the process of developing nanomedicine for the treatment of respiratory diseases, it is necessary to systematically explore the interaction between the nanomedicine and the respiratory system, including *in vitro* and *in vivo* detection methods to measure the genotoxicity, cytotoxicity and tissue toxicity of the drug (119), in order to thoroughly examine the safety, tolerability and therapeutic effect of NPs in treatment. Although some preclinical studies have shown promising application prospects, the current clinical trials of nanomedicine related to respiratory diseases remain limited, and the clinically known applications of nanocarriers are liposomes (48,49) in nontuberculous mycobacterial lung disease and lung cancer, and PLGA in pulmonary arterial hypertension (120).

3. Conclusions

Nanotechnology has become an important tool to overcome the defects of drugs, and to enable them to target specific cells or tissues passively or actively. The present review summarized the applications and advantages of NPs as drug delivery vehicles in respiratory diseases, such as lung cancer, asthma, chronic respiratory diseases, cystic fibrosis, tuberculosis and respiratory infection. The combination of nanotechnology has further promoted the development of drugs for respiratory diseases based on the benefits of inhaled administration. However, although preclinical studies have shown broad development prospects, most relevant studies are still in the early stage of experimentation, and their clinical effects need to be further verified. Future studies should focus on the performance modification, molecular mechanism and potential toxicity of therapeutic nanomedicine in the process of treatment.

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MXL wrote and revised the manuscript. SH contributed to the conception and design of the work. QYS contributed to the drafting of the article and literature search. MXL and QYS confirmed the authenticity of all the raw data. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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