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.

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 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 Margia 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 amphoterocin 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        | NTM lung disease     | 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 3774 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-SLN, 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 biodeshesive 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 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.
| 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)    |
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 polymeric 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 polyamidoamine (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
| 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/citrate 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.
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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