Chapter

Applications of Chitosan in Pulmonary Drug Delivery

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Abstract

Pulmonary administration is an effective method for treating lung and other diseases. Drugs can be transported directly to the lung by the pulmonary drug delivery system (PDDS). PDDS has the advantages of maintained local drug concentration, reduced side effects, controllable drug release, promoted drug absorption, prolonged drug action time, and improved patient compliance. Polymers have been extensively utilized to prepare novel PDDS. Among these polymers, chitosan (CS) is a natural cationic polysaccharide which is rich in its source. It has many unique physicochemical properties, good biocompatibility, and satisfactory biodegradability. CS has been a popular biomaterial in pharmaceutics for decades and is widely used in drug delivery. CS contains many amino groups. The contained positive charges can interact strongly with the negatively charged mucosa membranes, thereby facilitating CS adsorption on the mucosal surface, avoiding clearance by the cilia, and improving the adhesion and penetration rate on the cell membrane. Moreover, studies have shown that CS could open cell tight junctions, which would promote drug transportation across the epithelial tissue. Thus, CS is an especially suitable material for PDDS. In this chapter, we will focus on the research progress and the applications of CS in PDDS. Many representative and advanced studies on CS-based PDDS are reviewed in detail.

Keywords: chitosan, pulmonary drug delivery, microsphere, nanoparticle, liposome, targeted delivery

1. Introduction

In recent decades, with the continuous development of medical technology, people have achieved an in-depth understanding of lung functions and characteristics. Pulmonary administration has been recognized as a simple and effective method for treating lung and other diseases [1–3]. The drug delivery system (DDS), which transports drugs directly to the lung to produce local or systemic therapeutic effects, is known as the pulmonary drug delivery system (PDDS). Oral drugs need to be absorbed by the gastrointestinal tract and further transported through the blood circulation to reach the lesion site. In this series of complex processes, serious drug loss happens after confronting acid, alkali, enzyme degradation, and liver elimination. Ultimately, only a small amount of drugs can reach the lesion site, which seriously reduces the drug bioavailability and impairs the therapeutic outcomes [4]. Parenteral administration can cause damage to the tissue and reduce patient compliance. By contrast, PDDS can deliver the drugs directly to the lung

through the trachea for the local treatment of lung diseases. Compared with systemic administration, pulmonary administration is an ideal way for treating lung disease. It can significantly reduce the dosage, and decrease the drug distribution in nontarget tissues, thereby reducing the toxicity and side effects [5]. After administration through the lung, the drugs can be transported into the systemic circulation through the thin alveolar epithelial cell layer. Thus, PDDS can also be used for systemic drug delivery. Due to the large absorption area of the lung and the lack of degrading enzymes, pulmonary administration can be used as a convenient and effective noninjection administration method for biomacromolecular drugs, such as proteins and nucleic acids [6–8]. The PDDS can maintain local drug concentration, reduce systemic side effects, control drug release, promote drug absorption, prolong the drug action time, and improve patient compliance. Therefore, PDDS has become a hotspot to prevent and treat diseases in current research [9, 10].

Chitosan (CS) is a natural and widely available polycationic polysaccharide, and it has many unique physicochemical properties, good biocompatibility, and satisfactory biodegradability [11]. CS has been a popular biomaterial in pharmaceutical research for decades and is widely used in drug delivery [12]. As a drug carrier, CS can control drug release and improve the dissolution of poorly soluble drugs. A large number of studies have reported the developments and applications of CS-based materials as drug delivery carriers with versatile functions, such as CS-based beads, films, sponges, hydrogels, microspheres, and nanoparticles (NPs) [13]. Moreover, CS contains a large number of positively charged amino groups. These positive charges can interact strongly with the negatively charged mucosa membranes and adsorb on the mucosal surface, thereby avoiding the drugs being removed by the cilia, improving the adhesion rate, reducing drug clearance, and providing conditions for the drugs to penetrate through the cell membrane. At the same time, studies have shown that CS can open the tight junctions between the cells, which will promote drug transportation through the epithelial tissues, and increase the drug absorption rate and bioavailability [14]. Thus, CS is a suitable material especially for PDDS.

In this chapter, we focus on the applications of CS in pulmonary drug delivery. The research progress of PDDS and the applications of CS in PDDS are reviewed, and many representative and advanced studies on CS-based PDDS are enumerated in detail.

2. The research progress of PDDS

2.1 The physiological basis of pulmonary administration

Lung is the respiratory organ of the human body. It cooperates with the trachea to constitute the main place for gas exchange. The bronchi and lung can provide a large absorption area for drugs, and the total number of human alveoli is as high as 5.6×10^8, and the total surface absorption area can reach up to 140 m^2 [15]. Moreover, the distance from the alveoli surface to the capillaries is only about 1 μm. By contrast, the distance is about 40 μm from the microvilli of the small intestine mucosa to the capillaries. Therefore, the transport distance required for the pulmonary absorption process is much shorter than that of intestinal absorption, and the drugs can be rapidly absorbed through pulmonary administration. At the same time, the lung contains the most abundant capillaries compared with other organs in human, and about 90% of the alveolar area is covered with capillaries. The area of pulmonary capillaries in adults can be as wide as 80 m^2. Moreover, the volume of blood flowing through the lung is very high, as almost all of the blood discharged

from the right ventricle will pass through the lung, which is up to 5 L per minute. This blood flow volume is the equivalent of the total blood flow in all other organs and tissues of the body [16]. Besides, the chemical and enzymatic degradation activity in the lung is relatively low, which can reduce the hydrolysis of the bioactive macromolecules, such as proteins, peptides, and nucleic acids. Thus, these drugs can maintain a good biological activity after pulmonary administration, which will be beneficial to improve the drug bioavailability.

In summary, the huge absorption area, abundant capillaries, and minimal transport distance together contribute to the rapid absorption of pulmonary administration. After being quickly absorbed in the lung, the drugs will directly enter the systemic blood circulation, avoiding the first-pass effect of the liver, which is beneficial to improve the bioavailability of the drugs. And due to the low enzyme activity in the lung, the drug's adverse reactions during local administration can be reduced, which is especially suitable for the patients who need long-term administration of the medicine [17–19].

2.2 The characteristic and development of pulmonary administration

Pulmonary administration has been initially used to relax the tracheal smooth muscles to treat the acute exacerbation of asthma, and the disease is treated by inhaling the drugs through the trachea and the lung in the forms of drug aerosol particles or dry powder particles [20]. In general, there are two therapeutic purposes for pulmonary administration. One is the treatment of diseases in the lung, such as bronchial asthma and chronic obstructive pulmonary disease. The other purpose is to achieve systemic treatment through the pulmonary absorption of the drugs. Pulmonary inhalation administration can deliver the therapeutic agents directly to the lesion site, which can reduce the drug distribution in nontarget tissues. Therefore, for the treatment of pulmonary diseases, inhalation administration has a higher therapeutic index and fewer side effects than oral administration. And pulmonary inhalation is the preferred administration method for bronchodilators, β2-receptor agonists and corticosteroids [21].

With the deep understanding of lung functions and lung diseases in medicine field, pulmonary inhalation is recognized as a simple and effective administration route for the respiratory tract and other disease treatments. The types of the pulmonary inhalation-treated diseases have been gradually increasing, such as the insulin aerosol for treating diabetes, and the salmon calcitonin powder for the treatment of osteoporosis and osteoarthritis [22]. In recent years, the number of studies on pulmonary inhalation of macromolecular drugs, such as proteins and peptides, has been increasing. These macromolecular drugs (such as insulin, growth hormone, vaccines, and cytokines) can be formulated into pulmonary administration preparations for local or systemic treatment. However, most of the pulmonary inhalation drugs currently used in clinical treatment is short-acting preparations, which require frequent administrations (about 3–4 times a day). Therefore, the long-acting preparations should be developed, because they can maintain stable blood concentrations and also increase the compliance of the patients [23]. At the same time, the sustained or controlled drug release preparations for pulmonary administration can effectively regulate drug release behavior and promote drug absorption rate, thereby contributing to the achievement of ideal therapeutic effects [10].

The particle size of the pulmonary inhalation preparations directly affects the deposition form and deposition site in the lung [24–26]. The particles of sizes >5.0 μm produce inertial impact and are deposited in the pharynx, larynx, and upper respiratory tract. Particles of sizes 1.0–5.0 μm mainly reach the deep part of the respiratory tract, trachea, bronchi, and alveolar surface by gravity deposition.

The particles of sizes 0.5–1.0 μm are deposited on the respiratory bronchioles and alveolar walls. The particles of sizes <0.5 μm will be discharged out with airflow due to the Brownian motion, and typically 80% of them will be expelled out of the respiratory tract. Therefore, the particles with a size range of 1.0–5.0 μm have the highest deposition rate in the bronchioles and alveoli, and they are generally selected as the main components of the pulmonary inhalation preparations. Studies have shown that the pulmonary absorption of the drugs is a passive process, a small molecular weight contributes to fast drug absorption, and the absorption of the macromolecular drug is relatively slow [16]. The drugs with molecular weight below 1000 Da present short absorption half-life and good bioavailability. As the alveolar wall is very thin, macromolecular drugs can also be absorbed through the large gap between the cells or be swallowed into the lymphatic system by the macrophages in alveoli, before finally entering the blood circulation [27].

In recent years, with the development of biomaterial science, biotechnology, and medical technology, the research of new PDDS for drug-loading has focused on polymer-based microspheres, liposomes, and NPs, which can be inhaled into the lung and deposited in the lung mucosa through atomization and in the form of dry powder or other forms [28]. Compared with the atomized injections currently used in clinic, the pulmonary administration preparations based on new PDDS have the advantages of convenience, sustained or controlled drug release, prolonged drug action time, enhanced bioavailability, and improved therapeutic efficiency. PDDS with better efficacy will be designed with the development of new materials and the advancement of pharmaceutical preparation technologies. Pulmonary administration will have broad prospects for disease treatment in the medical field.

3. The applications of CS in PDDS

3.1 The basic properties of CS

CS is a nontoxic natural polymer that is the only basic polysaccharide found in nature. It has good physical and chemical properties. CS has many advantages, such as widely available, nontoxic, biocompatible, biodegradable, and structure modifiable, and its derivatives also have some unique properties. CS has been widely used in environmental engineering, textile industry, papermaking, food industry, cosmetics, medicine, biotechnology, pharmacy, and also many other fields [29, 30]. In recent years, with the rapid development of the advanced DDS, CS and its derivatives have received extensive attention as drug carrier materials, especially for sustained and controlled drug release, and they have become popular research topics in this field [31, 32].

As a drug carrier, CS has the following advantages compared with other materials. (1) CS contains a large amount of amino groups, which easily become positively charged via combination with the H^+ in solution. Thus, CS has a strong adsorption effect on carrying drugs with different characteristics [33, 34]. (2) The polysaccharide chain of the CS and the lipopolysaccharide (LPS) structure can be recognized by the macrophages, thereby activating and triggering local immune responses and enhancing the targeting effect to specific tissues or cells [35]. (3) CS and its derivatives have good antibacterial activity and can inhibit the growth and reproduction of some fungi, bacteria, and viruses [36]. (4) CS is a polycation, which can easily interact with the negatively charged groups on the cell membrane surface, thereby changing the fluidity and permeability of the cell membrane [37]. (5) CS is especially suitable for local administration because of its good adhesion property and histocompatibility, which can particularly meet the requirements for drug

retention after mucosal administration [38]. Therefore, CS has a wide range of applications in drug delivery. It can increase the stability of the drug, prolong the drug action time, change the administration route, increase the targeting ability of the drug, control the drug release, improve the dissolution of drugs with poor solubility, and adjust the cell membrane permeability of the hydrophobic drugs. At the same time, the positively charged CS can be easily adsorbed on the mucosal surface and also hard to be removed by the cilium, thereby providing conditions for the drug to penetrate the cell membrane. Moreover, CS can open the tight junctions between the cells, which will promote drug transportation in the epithelial tissues and increase the drug absorption rate and bioavailability. Thus, CS is especially applicable for PDDS [39, 40]. CS also has inherent immunogenicity, which is absent in other polymers, and this enables its use as an adjuvant for vaccine delivery into the lung [5]. Therefore, research on the applications of the CS-based PDDS has attracted great attention all over the world.

3.2 Novel PDDS based on CS and its derivatives

Traditional pulmonary administration preparations have drawbacks such as relatively short drug onset time, high frequency of administration, and poor patient compliance. In order to overcome these problems, research has been focused on the development of new PDDS with sustained or controlled drug release properties, also with active targeting abilities, for increasing the drug retention time in the lung, improving the drug concentration in treated areas, reducing the damage to normal tissues or cells, and enhancing the bioavailability of the drugs. The new formulations for PDDS in recent studies mainly include microspheres, polymeric NPs, liposomes, and active targeted systems [41]. In the following content, we will introduce the above-mentioned formulations one by one.

3.2.1 CS-based microspheres

Microspheres are microparticulate disperse systems formed by drugs dispersed or adsorbed in the polymer matrix. Microspheres have some unique advantages as a DDS for pulmonary administration [42]. They can be deposited in the lung, delay the drug release, and protect biomacromolecules, such as proteins and peptides from hydrolysis by enzymes. By optimization of the preparation process, a microsphere with an aerodynamic diameter of 1–5 μm and with suitable shape and porosity can be obtained, for meeting the requirements of pulmonary administration. In addition, microspheres usually have good stability with high moisture resistance ability. These characteristics have determined the wide applications of microspheres in pulmonary administration formulations [43].

There are many kinds of carrier materials for preparing microspheres. At present, the use of biodegradable microsphere as controlled release carrier is popular in DDS research [44]. Poly(lactic-co-glycolic acid) copolymer (PLGA) and CS are the commonly used biodegradable materials for microsphere preparation [45]. The conventional methods for preparing CS microspheres include emulsion crosslinking, solvent evaporation, ion induction, and spray drying [46–49]. Among these, crosslinking is the most commonly used method in the preparation of drug-loaded CS microspheres with controlled-release property. The reaction can be carried out under mild conditions, and it also can be industrially prepared easily [50]. Moreover, as CS is positively charged, it can combine with the negatively charged drugs by electrostatic binding interaction to form a complex, which can help to improve the drug loading capacity of the microspheres.

Weifen Zhang's group had developed a series of CS-based microspheres for pulmonary administration. They reported the preparation of CS and β-cyclodextrin microspheres as PDDS [51–55]. The microspheres were prepared by the spray drying method, and theophylline was loaded into the microspheres as a model drug. These microspheres possessed spherical shape with smooth or wrinkled surfaces, and had suitable aerodynamic diameters, which were suitable for inhalation. The microspheres had high drug entrapment and encapsulation efficiency. They can remain stable under storage conditions. The microspheres could also easily penetrate the membrane with a high permeation rate. The results showed that these microspheres had good potential as a sustained drug release carrier for pulmonary administration.

In Weifen Zhang's another work, paclitaxel (PTX) and quercetin (QUE) were respectively loaded in the NPs, which were synthesized with the oleic acid-conjugated CS (OA-CTS). And these drug-loaded NPs were further used in the preparation of polymeric microspheres (PMs) by the spray-drying method (**Figure 1**) [56]. The microspheres could help prolong the retention time of PTX in the presence of QUE, for bypassing the P-glycoprotein drug efflux pumps. The diameters of the PMs ranged from 1 to 5 μm, and they had a uniform size distribution. The PMs displayed slow-release characteristics at pH levels of 4.5 and 7.4. In vivo pharmacokinetic and biodistribution studies suggested that the PMs exhibited a prolonged circulation time and a markedly high accumulation in the lung. The PMs could serve as a promising PDDS for combined therapy using hydrophobic drugs.

Recently, Ludmylla Cunha et al. developed inhalable CS microparticles for simultaneous delivery of isoniazid and rifabutin in lung tuberculosis treatment [57]. Spray-dried CS microparticles were obtained with adequate flow properties for deep lung delivery (aerodynamic diameter of 4 μm) and high drug association efficiencies (93% for isoniazid and 99% for rifabutin). No cytotoxicity effect was found in human alveolar epithelial (A549) cells. The CS microparticles could activate macrophage-like cells, inducing cytokine secretion well above basal levels. Moreover, the uptake level of macrophages to internalize microparticles was over 90%. The microparticles also inhibited bacterial growth by 96%, demonstrating that the microencapsulation preserved drug antibacterial activity in vitro. The dual drug-loaded CS microparticles demonstrated to be potential candidates for inhalable therapy of pulmonary tuberculosis.

3.2.2 CS-based NPs

As a potential DDS, NPs have been widely used in medicine and other fields, and it has already become a research hotspot for decades [58–60]. NPs can improve the solubility and stability of the drugs, prolong the half-life, and enhance the drug absorption rate. NPs can also help to realize sustained or controlled drug release, prolong drug acting time, reduce administration frequency, and improve patient compliance. Moreover, NPs can target the drugs to specific organs and cells by the passive or active targeting ability of the multiple functionalized NPs. The NPs can be modified to avoid the phagocytosis of the macrophages or the removal of mucosal cilia, thereby improving the bioavailability of the drugs.

Drug delivery by NPs is an effective approach for the pulmonary administration of insoluble drugs [61, 62]. The surface of the NPs can be modified to prolong the drug residence time in the lung and to achieve appropriate release property for improving the therapeutic effect. The pulmonary administration route for NPs is mostly by inhalation in the form of aerosolized colloidal solution. However, when the NPs are administered directly into the lung, some of them may be discharged out with the breath due to their small particle size, thereby resulting in low

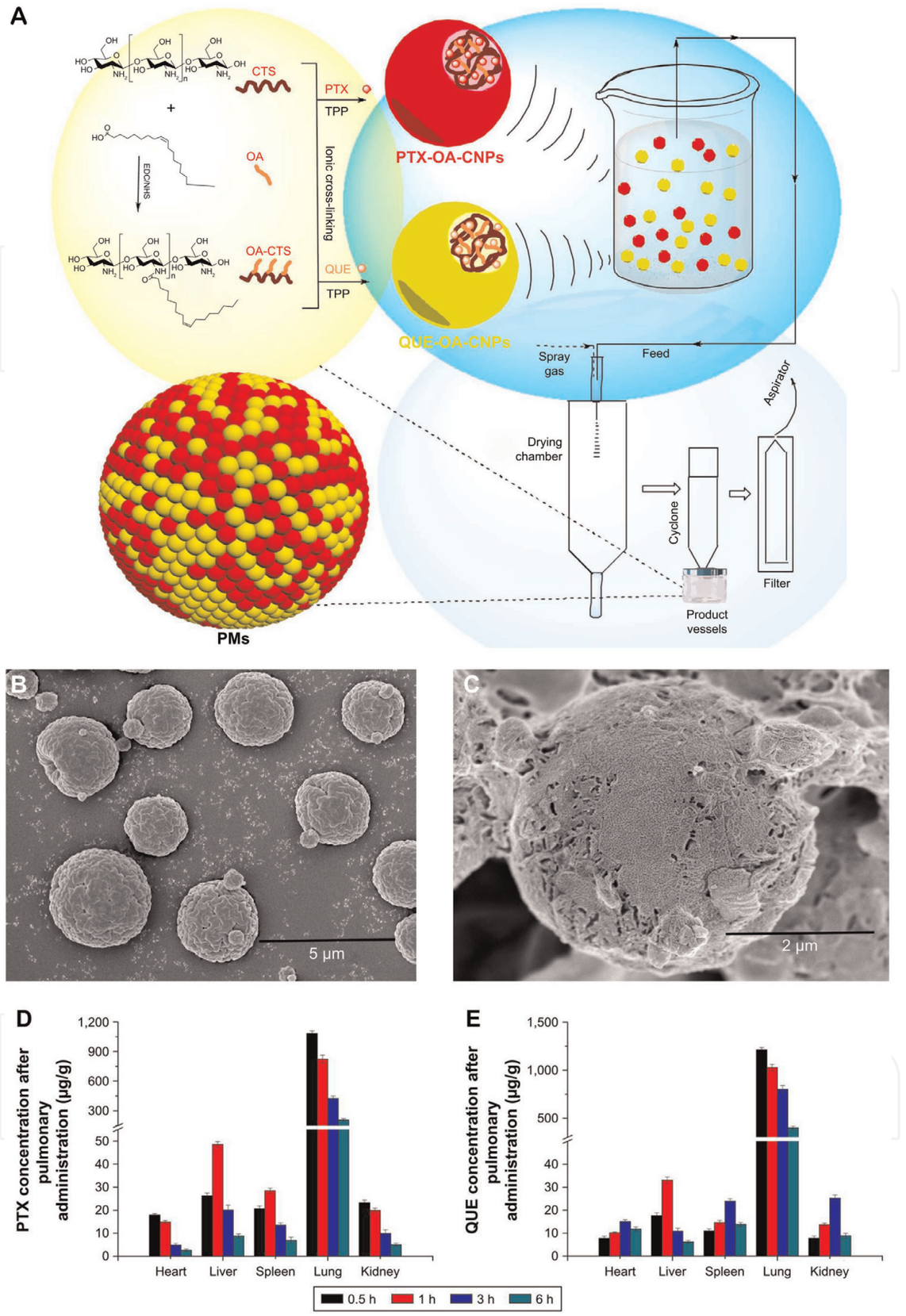

Figure 1.
(A) The synthesis and preparation route of the PMs. (B and C) SEM image of the PMs with a scale bar of 5 and 2 μm. (D and E) The concentration of PTX and QUE accumulated in different organs measured by HPLC at 0.5, 1, 3, and 6 h after pulmonary administration.

deposition in the lung and discounted effect. Studies have shown that coating the surface of the NPs with biocompatible polymers, such as CS, can prolong the residence time of NPs in the lung [5]. Sometimes the surface energy of the NPs is

relatively high, and the weak stability will lead to aggregations and interactions between the NPs. Thus, special surface modification will also be an effective way to improve the stability of the NPs.

Kenneth A. Howard et al. had developed CS-based siRNA-loaded NPs for pulmonary RNA interference therapy [63]. The negatively charged siRNA was complexed by the positively charged CS to form the polyelectrolyte complex NPs. The particle size ranged from 40 to 600 nm. These NPs showed a rapid uptake (1 h) into NIH 3T3 cells. The NPs could mediate efficient knockdown of endogenous enhanced green fluorescent protein (EGFP) in both H1299 human lung carcinoma cells and murine peritoneal macrophages in vitro. Moreover, effective in vivo RNA interference was also realized in the bronchiole epithelial cells of transgenic EGFP mice after nasal administration. The results indicated that this kind of CS-based complex NPs has great potential in RNA interference therapy for systemic and mucosal disease.

A series of CS/fucoidan (CS/F) NPs had been designed and prepared as PDDS for gentamicin (GM) in Yi-Cheng Huang's study (**Figure 2**) [64]. The NPs presented a biphasic release feature, with a zero-order release of GM for the first 10 h, followed by a sustained release of up to 72 h, and the cumulative release value reached 99%. The GM-loaded CS/F NPs exhibited multiple antimicrobial capabilities against *Klebsiella pneumoniae*. The intratracheal administration of the GM-loaded CS/F NPs displayed a higher area under the curve (AUC) and lower minimum inhibitory concentration ratio than the free GM that was administrated by intravenous injection. These results had showed that the CS/F NPs had superior antimicrobial efficacy and lower risk of systemic toxicity, and this GM pulmonary delivery system has exhibited good potential for pneumonia treatment.

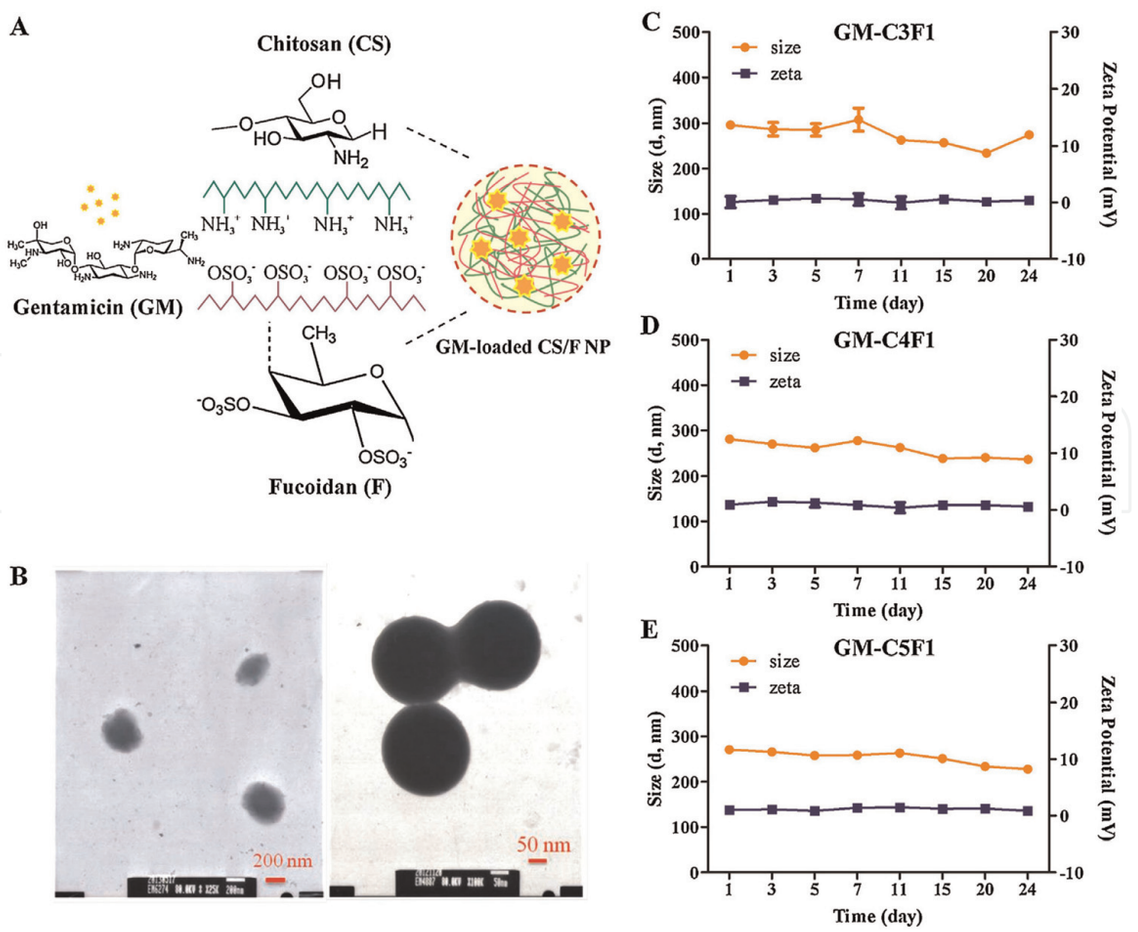

Figure 2.
(A) The preparation of the GM-loaded CS/F NPs. (B) TEM of the GM-C5F1 NPs. (C–E) Long-term stability of the series of CS/F NPs.

Abdallah Makhlof et al. synthesized a thiomer derivative of glycol CS (GCS), which was coupled with thioglycolic acid (TGA) [65]. The NPs were prepared with GCS and GCS-TGA by ionic gelation for the pulmonary delivery of peptides. The NPs were positively charged and had sizes in the range of 230–330 nm. They also showed high calcitonin entrapment. After intratracheal administration, the mucoadhesion capacity of the GCS-TGA NPs was much better than that of nonthiolated NPs in rats. The toxic effect of the NPs with lung tissue was confirmed with negligible epithelial damage and toxicity. The NPs could enhance the pulmonary absorption of the delivered peptides, and the calcitonin-loaded GCS and GCS-TGA NPs showed efficient hypocalcemic effect. The GCS and its thiomer derivative could be used as potential PDDS for delivering peptides.

In another work, Adriana Trapani et al. had prepared CS and GCS NPs containing Lipoid S100 for the systemic delivery of low molecular weight heparin (LMWH) by pulmonary administration [66]. The NPs were prepared by ionotropic gelation method. The NPs were positively charged and with nanoscale size. Efficient drug entrapment and good mucoadhesive property could be achieved by using these NPs. The LMWH was effectively delivered into the lung by the aerosolization of the drug-loaded NPs. These results revealed the promising characteristics of the CS-based NPs, which were highly applicable for PDDS.

Tejal Rawal et al. designed a NP-based dry powder formulation of rifampicin (RFM) for achieving local and sustained targeting of anti-tubercular drugs to reduce dose and frequency [67]. The drug-loaded NPs were formulated by the ionic gelation probe sonication method. The optimized formulation had a small particle size of 124.1 nm with an entrapment efficiency of 72%. No toxicity was found by in vitro and in vivo experiments. The pharmacokinetic assay verified that the NP formulation would serve a better alternative because it could minimize the frequent dosing schedule than conventional dry powder inhalation and market formulation. The RFM-loaded NPs open new avenues for developing therapeutic interventions for lung tuberculosis.

3.2.3 CS-modified liposomes

Liposomes are primarily used as PDDS for the treatment of respiratory distress syndrome and other lung diseases [68]. The drugs carried by liposomes mainly have stable physicochemical properties and strong lipophilicity. Liposome-based drug carrier has been one of the research hotspots in pharmaceutics [69, 70]. At present, drug-loaded liposome preparations, such as for amphotericin B, daunorubicin, doxorubicin, cytarabine, and morphine, have been successively developed [71]. The liposome preparations of these drugs have many unique advantages compared with their common preparations. Liposome-based pulmonary administration has the advantages of rapid absorption, little irritation, good tolerance, high safety, controllable drug release, and improved bioavailability [72]. The encapsulation of the drug in liposomes affects the pharmacokinetic property of the drug and prolongs drug half-life. Moreover, 85% of the components of the alveolar surface are phospholipids, which can facilitate the liposomes accumulate and enter the lung tissue. Liposome-loaded macromolecules with low lipophilicity can significantly improve bioavailability, and liposomes can also reduce the side effects of the toxic drugs to normal tissues by pulmonary administration.

Marco Zaru et al. prepared CS-coated liposomes and used them as drug carriers for pulmonary delivery by nebulization [73]. Rifampicin (RFM) was loaded in the CS-coated liposomes with different lipid compositions. By coating with CS, the encapsulation efficiency of the liposomes increased slightly, and after nebulization, the stability also remarkably increased. The mucoadhesive capacity of the

CS-coated liposomes was much better than that of the noncoated ones. The cytotoxicity of the RFM-loaded CS-coated liposomes on A549 cells was much lower than that of the free drug. The results showed that the CS coating could significantly enhance the mucoadhesive capacity of the liposomes, and these CS-coated liposomes could be potential drug carriers for pulmonary administration by nebulization.

In another study, Mitsutaka Murata et al. investigated the surface modification of liposomes by CS for pulmonary delivery [74]. The surface of the liposomes was modified with CS oligosaccharide (oligoCS) and polyvinyl alcohol with a hydrophobic anchor (PVA-R). The association study showed that the PVA-R modification decreased the interaction between liposomes and A549 cells. By contrast, the oligoCS modification could significantly promote the cellular interaction. After pulmonary administration to rats, the peptide elcatonin (eCT) loaded oligoCS or PVA-R modified liposomes exhibited significantly enhanced therapeutic efficacy. The oligoCS-modified liposomes could adhere to the lung tissues and open the tight junctions between cells, which remarkably improved the drug absorption rate. On the other hand, the PVA-R-modified liposomes could induce a sustained absorption through the long-term eCT retention in lung fluid. The results verified that the surface-modification of liposomes with oligoCS and PVA-R could be used as effective PDDS for peptide pulmonary administration.

3.2.4 CS-based active targeting DDS

The pulmonary inhalation preparations are usually directly transported into the lung. Thus, they can passively target to the lung tissues by pulmonary administration. The deposition site of the drugs in the lung can be controlled by regulating the physicochemical and functional properties of the drug carriers to meet the disease treatment requirements. In addition to passive targeting, active targeting systems have more applications in precise and efficient disease treatment. Active targeting systems utilize ligands or antibody-modified carriers to deliver the drugs to the specifically targeted tissues or cells, even intracellular organelles, thereby improving drug efficacy and reducing toxicity and side effects [75, 76]. The drug carriers could be chemically modified with active ligands, such as sugar moieties (galactose, mannose, and glucose) or specific ligands, such as folic acid (FA), transferrin (Tf), and Arg-Gly-Asp (RGD) peptide [77–79]. Antibody-mediated active targeting is also a primary strategy for delivering the drugs to the specific parts of the body [80].

Hu-Lin Jiang et al. had prepared a folate-CS-graft-polyethylenimine (FC-g-PEI) copolymer for cancer cell-targeting gene delivery [81]. FC-g-PEI could effectively load and protect the shRNA. The copolymer also showed decreased cytotoxicity compared with the PEI control. Compared with the untargeted polymer, FC-g-PEI was a more efficient Akt1 shRNA carrier, and it exhibited effective cancer cell-targeting ability. Moreover, aerosol delivery of the FC-g-PEI/Akt1 shRNA NPs effectively inhibited lung tumorigenesis in the urethane-induced lung cancer model mice via Akt signaling pathway. The results demonstrated that the FC-g-PEI could be applied for the shRNA gene therapy via aerosol delivery.

Yongfeng Luo et al. developed an inhalable β2-adrenoceptor ligand-directed guanidinylated-CS (GCS) carrier for targeted lung delivery of siRNA [82]. GCS could effectively condense the siRNA and form complex NPs. Compared with pristine CS, the siRNA-loaded GCS NPs exhibited lower cytotoxicity and higher cellular internalization, which finally resulted in promoted gene silence efficiency. Salbutamol (a β2-adrenoceptor agonist) was further chemically coupled to the GCS to enhance the targeting specificity of the siRNA-loaded NPs. The gene silence efficacy was remarkably increased both in vitro and in vivo. The carrier could

protect the siRNA against the destructive shear forces generated by mesh-based nebulizers. Aerosol treatment also improved the size distribution of the NPs, which was beneficial in promoting the transfection efficiency. This CS-based targeting DDS had potential applications for siRNA delivery in lung disease treatment by aerosol inhalation.

Suhui Ni et al. had prepared γ-aminobutyric acid type B ($GABA_B$) receptor ligand-directed NPs for survivin siRNA delivery [83]. The NPs were synthesized by baclofen (Bac)-functionalized trimethyl CS (Bac-TMC) with tripolyphosphate (TPP) as an ionic crosslinker. GABAB receptor agonist Bac was initially introduced into TMC as a novel ligand. The Bac-TMC/TPP NPs could promote the cellular uptake of siRNA by interacting with the $GABA_B$ receptor, which further induced effective gene silence and cell apoptosis. Mannitol microparticles were further utilized for the pulmonary delivery of the siRNA-loaded NPs via pressurized metered dose inhalers (pMDI). The obtained formulation had good aerodynamic properties, which benefited the deep lung deposition. The pMDI formulation containing Bac-TMC/TPP NPs could be a potential PDDS for siRNA.

Recently, Ting Wu et al. had developed a genipin-crosslinked carboxymethyl CS nanogel for lung-targeted delivery of isoniazid (INH) and rifampin (RMP) [84]. The dual drug-loaded nanogel particles (NGPs) had a uniform particle size from 60 to 130 nm with spherical morphology. The NGPs had sustained release behavior in simulated lung fluid. The dual drug-loaded NGPs had high antibacterial activity against multidrug-resistant *Mycobacterium tuberculosis*, and also could realize long-term antibacterial activity. Further in vivo evaluation exhibited that alveolar delivery of NGPs had satisfactory deposition of drug within the lung with lower toxicity. The results indicated that the NGPs would be a potential dosage form for treating against multidrug-resistant *Mycobacterium tuberculosis*.

4. Future perspectives

Pulmonary administration is a promising route for drug delivery to prevent and treat diseases, especially for delivering the drugs for lung diseases and some small molecule drugs with low absorption rate when in oral dosage form, also suitable for some traditional Chinese medicines and peptide or protein drugs. PDDS can effectively deliver the therapeutic drugs to the target sites, and improve the drug bioavailability and therapeutic efficacy. Inhalation is a noninvasive method for pulmonary administration, and the inhaled drugs can directly enter the blood circulation through the absorption of the alveolar epithelium. Pulmonary administration can enhance the drug absorption rate, reduce systemic side-effects, and improve the compliance of long-term medication. By transforming the drugs into dry powder inhalation formulations, drug degradation can be avoided. The therapeutic effect of the PDDS is mainly influenced by the physicochemical properties of the DDS, the dosage forms, and the administration devices, and also some other factors. The increase of the amount of drug delivered into the lung and the promotion of drug absorption rate are the key factors to improve the therapeutic efficiency of pulmonary administration. The application of sustained or controlled release preparations is an important method to prolong drug action time, enhance drug bioavailability, and improve patient compliance. In recent years, many controlled release preparations or active targeting preparations for pulmonary drug delivery have been constructed by drug-loaded microparticles, liposomes, and NPs. These multifunctional drug carriers have gained increasing popularity in pulmonary administration. It is really inspiring for us to see that some of the PDDS have already been applied to treat patients in clinic and become commercially available

commodities with promising prospects. And in the coming years, great progress will be made in PDDS research by the development of drug delivery devices and pharmaceutical preparation technology.

The PDDS constructed with biomaterials has been a hot research direction in medicine and pharmacy fields for decades. Among these biomaterials, CS, which is the only natural cationic polysaccharide, has been recognized as one of the most versatile and promising functional biomaterials. Moreover, CS is also one of the most abundant regeneration resources, second only to the cellulose. After decades of research, CS has been recognized as a nontoxic, biocompatible (especially with respiratory cells) and biodegradable polymer. Moreover, CS can accommodate both hydrophilic and hydrophobic drugs due to its amphiphilic properties [5]. The excellent performances of CS as a building component of DDS have been confirmed by many studies, and it has been identified as a novel drug delivery carrier with broad application prospects, especially for sustained and controlled drug release. Due to the unique features, CS can assist the drugs accomplishing local and systemic delivery, realizing high mucoadhesion, and efficient drug absorption in target tissues, which is especially applicative for PDDS. However, CS is insoluble in aqueous solutions at neutral and alkaline pH, and it only can be dissolved in aqueous acidic media (pH < 5). This frustrating issue has badly limited CS's application range, and there is an urgent need for developing convenient and practical modification strategies to improve its solubility. Up to now, CS has been approved as safe by US-FDA and EU for dietary use and wound dressing applications [85]. And additionally, CS has only been approved by the European Pharmacopeia as a pharmaceutical excipient for oral preparations. The safety issues of CS and its derivatives in pulmonary delivery and other administration routes have not been fully understood and still remain to be further evaluated. Therefore, the safety issue is a noteworthy research gap remained to be filled in the future work, for figuring out the potential adverse effects of CS and its derivatives in humans. At the same time, appropriate engineering of designing and modifying CS and its derivatives is highly demanded to optimize the performance of the CS-based drug carriers, for meeting the special requirements of in vivo pulmonary drug delivery. We hope that in the near future, more advanced synthesis and modification methods will be developed. And in the meanwhile, the physicochemical property, toxicity, biodistribution, biocompatibility, and biodegradability of CS and its derivatives should also be thoroughly investigated in detail.

5. Conclusions

Despite all this, CS-based PDDS has already achieved considerable success in the past decades. It is believed that in the near future, with the rapid development of material science, biotechnology, genetic engineering, medical technology, and other scientific fields, people will have a more extensive and in-depth understanding of the unique properties of CS and its derivatives. We hope that more efficient CS-based PDDS will be designed, by developing novel material preparation strategies, advanced formulation methods, and improved inhalation technology. We believe that CS-based PDDS will play more important roles in the applications of local and systemic disease treatment in the near future.

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Conflict of interest

The authors declare no conflicts of interest in this work.

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