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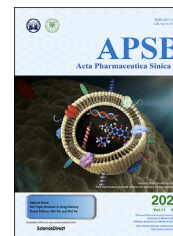
REVIEW

Pharmaceutical strategies to extend pulmonary exposure of inhaled medicines

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Abstract   Pulmonary administration route has been extensively exploited for the treatment of local lung diseases such as asthma, chronic obstructive pulmonary diseases and respiratory infections, and systemic diseases such as diabetes. Most inhaled medicines could be cleared rapidly from the lungs and their therapeutic effects are transit. The inhaled medicines with extended pulmonary exposure may not only improve the patient compliance by reducing the frequency of drug administration, but also enhance the clinical benefits to the patients with improved therapeutic outcomes. This article systematically reviews the physical and chemical strategies to extend the pulmonary exposure of the inhaled medicines. It starts with an introduction of various physiological and pathophysiological barriers for designing inhaled medicines with extended lung exposure, which is followed by recent advances in various strategies to overcome these barriers. Finally, the applications of the inhaled medicines with extended lung exposure for the treatment of various diseases and the safety concerns associated to various strategies to extend the pulmonary exposure of the inhaled medicines are summarized.

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1. Introduction

The lung with its unique physiological and anatomical features including large absorption area, highly permeable alveolar epithelial membrane, and high vascularization with limited first-pass effects has become an important route for drug administration[1]. This route has attracted increasing attention as it offers several advantages over other administration routes including rapid onset of the action, targeted delivery, reduced side effects, and improved bioavailability[2,3]. Pulmonary drug delivery (PDD) systems have been exploited not only for the treatment of some local diseases such as asthma, chronic obstructive pulmonary diseases (COPD) and respiratory tract infections, but also for the achievement of enhanced bioavailability to better treat systemic diseases like diabetes[4].
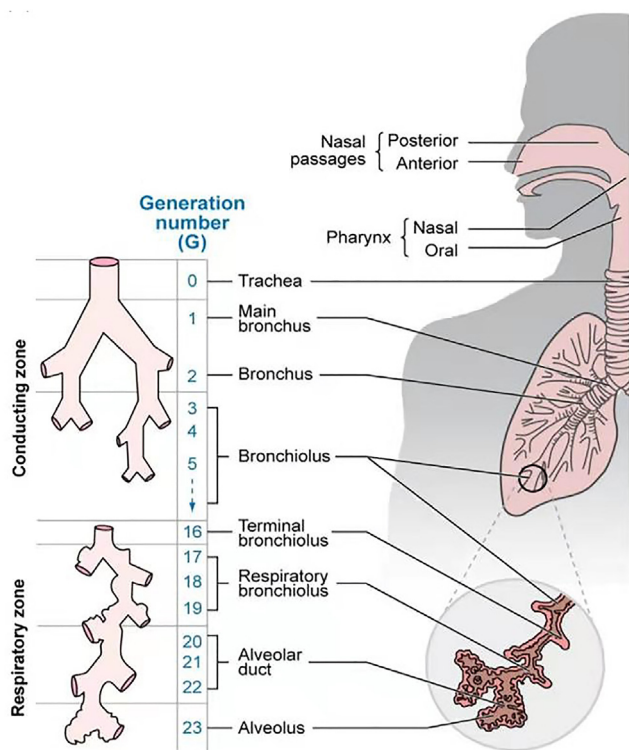
Figure 1 The physiological structure of the lungs with conducting and respiratory zones. The conducting zone (G0–G16) comprises of the trachea, bronchi, bronchioles and terminal bronchioles, which is responsible for conducting air to the respiratory regions of the lungs. The respiratory bronchioles, alveolar ducts and alveolar sacs constitute the respiratory zone (G17–G23), which facilitates the gas exchange between the airspaces and blood capillaries. Reprinted with the permission from Ref. 13. Copyright © 2019 Elsevier.

Generally, an inhaler device is indispensable to facilitate the deposition of inhaled medicine at the targeted sites of the lungs[5]. The inhalation devices current in use are nebulizers, metered dose inhalers (MDIs) and dry powder inhalers (DPIs)[6]. Different from nebulizers and MDIs, which mainly delivery the drugs in the form of solution or suspension, DPIs are used for the delivery of dry powder formulations which is typically composed of micronized drug powder and various coarse carrier particles (*e.g.,* lactose)[7]. Among them, the DPIs are gaining popularity as they are easier to handle and more favorable to the stability of drugs[8,9]. The proper selection of appropriate drug formulations and devices with specific design is extremely crucial for drugs delivery to the lungs efficiently and reproducibly. Once the drug particles are deposited, they could possibly be cleared from the lung *via* various clearance pathways including mucociliary clearance, phagocytosis by macrophages, dissolution and translocation from the airways to other sites[10]. Consequently, the local drug concentration in the lungs could decline rapidly to fail to exert optimal therapeutic effects. In order to maintain the effective drug concentration at the action sites, patients have to take the drugs frequently, which may result in poor patient compliance and adherence[11,12]. Extending the retention of inhaled particles in the lungs is an effective strategy to achieve prolonged pharmacological effect.

Herein, we start with an introduction of various physiological and pathophysiological barriers for designing inhaled medicines with extended lung exposure, which is followed by recent advances in various physical and chemical strategies to overcome these barriers. Finally, the applications of the inhaled medicines with extended lung exposure for the treatment of various diseases and the safety concerns associated to various strategies to extend the pulmonary exposure of the inhaled medicines are summarized.

2. Factors influencing the pulmonary exposure of the inhaled medicines

2.1. Lung physiology and drug delivery

As shown in Fig. 1[13], the lung is composed of conducing zone and respiratory zone and with the structure resembling an inverted tree. The trachea subdivides into two main bronchi, which successively branch into more and more narrow and short bronchioles and end at sac-like alveoli for the gas-exchange[14]. From the trachea to the alveolar sacs, there are about 23 bifurcations in total. The proximal conducting airways are constituted with pseudostratified columnar epithelium composed of ciliated cells, goblet or mucus secreting cells and basal or progenitor cells. The lower to the more distal airways are progressively replaced with a simple cuboidal cell layer and there is a very thin epithelial lining in the alveoli[5]. The fate of the inhaled medicines is highly dependent on the site where they are deposited in the lungs. For example, the large surface area, highly permeable bio-membrane, and good blood supply of the alveolar region are favorable for rapid

absorption, while the epithelial cells in the conducting airways constitute a strong barrier for systemic absorption[15].

There are three main mechanisms controlling the deposition of inhaled drug particles including inertial impaction, gravitational sedimentation and Brownian diffusion. The aerodynamic diameter (D_a) of the particles is the main factor deciding the site of deposition and which mechanism is followed[16]. Generally, the large drug particles ($D_a > 5$ μm) are mainly deposited in the upper airways (*i.e.*, mouth, trachea and main bronchi, where the air velocity is relatively high) by inertial impaction mechanism as these particles could not able to follow the change of the airstream flow direction. The drug particles with D_a within the range of 1−5 μm could be deposited *via* the gravitational settling mechanism in the central and distal tract, where the air velocity is quite low. The drug particles with $D_a < 1$ μm could remain suspended in the air and are mostly exhaled. The ultrafine particles (<100 nm) could largely be deposited in the respiratory tract by random Brownian motion. The particles with diameter <100 nm could reach the alveolar region, while the particles with size <10 nm could readily be deposited in the tracheo-bronchial region of the lungs due to their high diffusion coefficients[17].

2.2. The barriers—pulmonary clearance pathways

It is challenging to develop inhaled medicines with extended pulmonary exposure to exert prolonged pharmacological effect. It is because there are various elimination pathways, including coughing, mucociliary transport, phagocytosis by macrophages and translocation into the cells, blood and lymph (Fig. 2[18]), through which the inhaled drugs can quickly be removed from the lungs. Some physiological factors, the pathophysiological conditions of the patients and physicochemical properties of the inhaled drugs all have significant influence on the retention of inhaled medicine in lungs by interfering these pathways. In this section, various clearance pathways that influence the duration of pulmonary exposure of the inhaled drugs are reviewed.

2.2.1. Mucociliary escalator
For the inhaled drugs deposited in the upper and middle airways, the mucociliary escalator (MCE) may be the dominant clearance mechanism[19]. This MCE is a self-clearing mechanism of the airways, which is functioning through the cilia in the mucociliary layers to keep the airways clear of mucus and dirt, and to propel sperm. The mucociliary layer is composed of ciliated columnar cells and goblet cells covered by a lung lining fluid. The lung lining fluid, consisted of a periciliary sol layer and an overlying mucus gel layer, plays an important role in the clearance of the inhaled drugs. The mucus layer is composed of mucin, proteins, lipids, salts, and water. The mucus and cilia work together for the removal of foreign objects from the lungs. When the cilia rhythmically swings, it can expel the particles trapped in the mucus layer out of the lungs[20]. The MCE works efficiently for eliminating larger particles, while smaller particles can penetrate through mucus and enter the bronchial epithelium, escaping from mucociliary clearance[21]. The lung lining fluid gets thinner as the diameter of the airways become smaller. Finally, in the alveoli, the thickness of the lung lining fluid can be less than 0.1 μm, where the mucus layer disappears and it is replaced by surfactant layer composed of lipids, cholesterol and proteins[22,23].

2.2.2. Phagocytosis by macrophages
The phagocytosis by the pulmonary macrophages is another important mechanism to eliminate the particles from the lungs besides MCE, which is believed to be the dominant clearance mechanism in the deep lungs[24]. The air side surfaces of each of the 500 million alveoli in the human lungs are usually "patrolled" by 12−14 alveolar macrophages, which engulf and digest any insoluble particles deposited in the alveoli[3]. The size of particles and molecules are important factors that control the macrophage uptake. In general, the macromolecules with molecular weight (MW) ≤25 kDa are rapidly cleared, while MW ≥ 40 kDa are slowly cleared by the macrophages[14]. The optimal D_a for lung deposition is 1−5 μm, whereas particles within the geometric diameter range 0.5−3 μm are readily endocytosed by
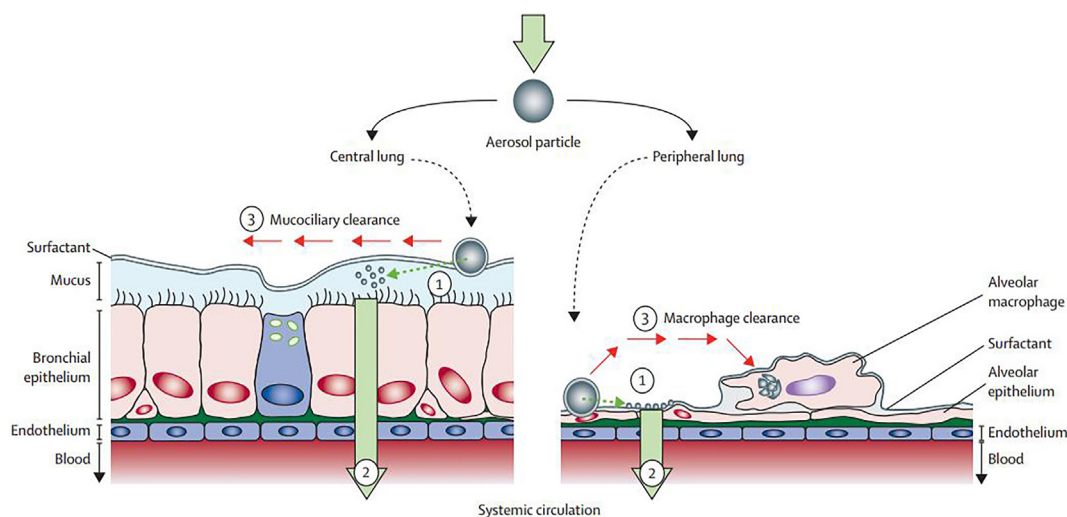
Figure 2 The fate of aerosol drugs after deposition in the lungs. (1) Active pharmaceutical ingredient (API) is released from the deposited aerosol particles when these come in contact with the lung lining fluid. Both physicochemical properties of the drug molecular and the formulation forms could control this process. (2) The particle absorption process *via* translocation into the cells, blood and lymph. (3) The particle clearance by the mucociliary escalator and phagocytosis of macrophages. Reprinted with the permission from Ref. 18. Copyright © 2013 Elsevier.

macrophages[25]. For macromolecule drugs, *e.g.*, peptides, proteins and nucleic acids are readily aggregated with the lung surfactants and eventually cleared by macrophages, which is an important cause of drug loss[14].

In addition, macrophages could also manipulate the fate of inhaled particles by stimulating the immune response against the inhaled drugs[26]. Naturally, antigens from the invading pathogens processed by alveolar macrophages are transferred back to regional lymph nodes and come in contact with T lymphocytes and cofactors, leading to the activation or suppression of cellular and humoral immunity[27]. Such immune responses can largely avoid the harm caused by the inhalation of pathogens that enter into the deep lungs. However, the administration of drugs with immunogenicity such as polysaccharides, proteins or peptides to the lungs may stimulate an immune response in the lungs, and subsequently produce anti-drug antibodies, which can reduce the efficacy of these therapeutic molecules significantly[28]. Therefore, macrophages play an important role in controlling the residence time and the pharmacological efficiency of the inhaled drugs in deep lung.

2.2.3. Translocation into cells, blood and lymph

The translocation process of the drug molecules includes transcytosis into the epithelial cells and/or across the respiratory epithelia into the interstitium and then to blood and lymph[29]. Generally, drug molecules are absorbed into the systemic circulation more quickly *via* the lung than any other non-invasive drug administration routes[30,31], which is one of the reasons for the short pulmonary exposure of inhaled medicines. When small, lipophilic drug molecules are deposited from the air into the lung lining fluid, they may firstly contact with the lung surfactants, which could facilitate the dissolution of drugs and thereby enhance their absorption[32]. For macromolecular drugs, it will take longer time (within an hour) to be absorbed into the systemic circulation[33], but relatively high bioavailability could be expected for inhalation compared with other nonparenteral routes.

The thickness of the lining fluid in the airways is about 5−10 μm[33] and its main components are water (96%), salt, phospholipids and proteins[34], which may improve the dissolution rate of the hydrophilic drugs. The thickness of the lining fluid gradually decreases distally and it is about 0.01−0.08 μm at the alveoli[33]. The lining fluid at the alveoli is composed of phospholipids and proteins (alveolar surfactant), which would increase the wetting, the solubility, and the dissolution rate of poorly water-soluble drugs[35]. The absorption rates of the drugs with different solubility could be enhanced in the lungs due to the presence of these physiological substances.

In addition, the huge surface area of the alveoli (more than 100 m[2]) and the extremely short distance from the gas side to the blood side in alveoli (0.1−0.2 μm) also make big contribution to the rapid drug absorption in the lung[14]. The thinness of the alveolar region offers great potential for the rapid systemic absorption of drug molecules within seconds or minutes[11]. Moreover, there are abundant capillaries around the alveoli[14]. The drugs deposited in the alveoli can quickly pass through the gas-blood barrier and be absorbed into the blood[3]. In contrast, the epithelial cells in the airways constitute a strong barrier to the absorption of the drug molecules into the bloodstream. The fast absorption of the drugs in the lungs is also related to the presence of high levels of drug transporters expressed in the lungs, such as carrier-mediated and vesicle-mediated transports, which play vital roles in lung absorption[36]. The vesicle-mediated transport is mainly involved in macromolecular transport across the alveolar epithelium[37]. These transporters facilitate the transport of their substrates through the epithelial cells, thereby shortening the residence time of drugs in the lungs.

For the purpose of local treatment, rapid clearance will lead to the decline of the local drug concentration in the lungs. Once the drug concentration is too low to maintain the effective therapeutic concentration, the desired therapeutic outcome would not be expected. For example, in the case of bacterial infections to the lungs, the epithelial lining fluid (ELF) is a susceptible site for pathogens[38]. Maintaining certain concentrations of the inhaled antibacterial agents in the ELF is a key factor to achieve their optimal therapeutic effects and suppress the resistance[39,40]. If the antibacterial agents were easily adsorbed into the systemic circulation or eliminated from the lungs by various clearance mechanisms, there would not be sufficient antibacterial drug concentration in the ELF and eventually the antibacterial agents would not exert their killing and inhibition effects. Even in a worse case, this may result in the development of serious antibiotic resistance. In short, more and more attention should be paid to the research of inhaled medicines with extended pulmonary exposure.

3. Strategies to extend the pulmonary exposure of the inhaled medicines

One of the strategies to extend the pulmonary drug exposure may be exploiting sustained release formulations for pulmonary administration[41]. These sustained release formulations could confer an extended time of the drug action, reduce the frequency of dosing and consequently improve the patient compliance and even reduce their side effects[11,43]. Besides the inhaled sustained release formulations, there are also some other physical and chemical approaches including particle engineering and chemical synthesis and modification that have been exploited to extend the pulmonary exposure of the inhaled medicines to bring clinical benefits to the patients recently. These strategies have been largely based on our increasing understanding of the physiological and pathophysiological features of the lungs and the pulmonary clearance mechanisms, including mucociliary clearance and phagocytosis by macrophages, systemic absorption and metabolic degradation[21]. With the suitable structural modification of the drug molecules and delivery carriers, the pulmonary exposure of the inhaled medicines could be extended by minimizing the elimination rate of the drugs through the powerful airway clearance mechanisms[1]. In the following subsections, several strategies adopted to overcome the clearance of inhaled drugs from the lungs in order to provide extended pulmonary drug exposure are systematically reviewed. A summary of these strategies are presented in Fig. 3.

3.1. Molecular modification

3.1.1. Decreasing the solubility and dissolution rate
Inhaled drugs must be dissolved in the lung lining fluid for subsequent diffusion, cellular uptake, absorption, and interaction with the receptors[42]. The difference in the solubility and the dissolution rate of the drug compounds may result in different clearance pathways. After inhalation, water-soluble drugs may quickly dissolve in the lung lining fluid and diffuse through the airway epithelium, and disappear from the lungs by absorption[43]. However, in the case of poorly water-soluble drug, sufficient dissolving of the inhaled drug particles might not be reached due to the
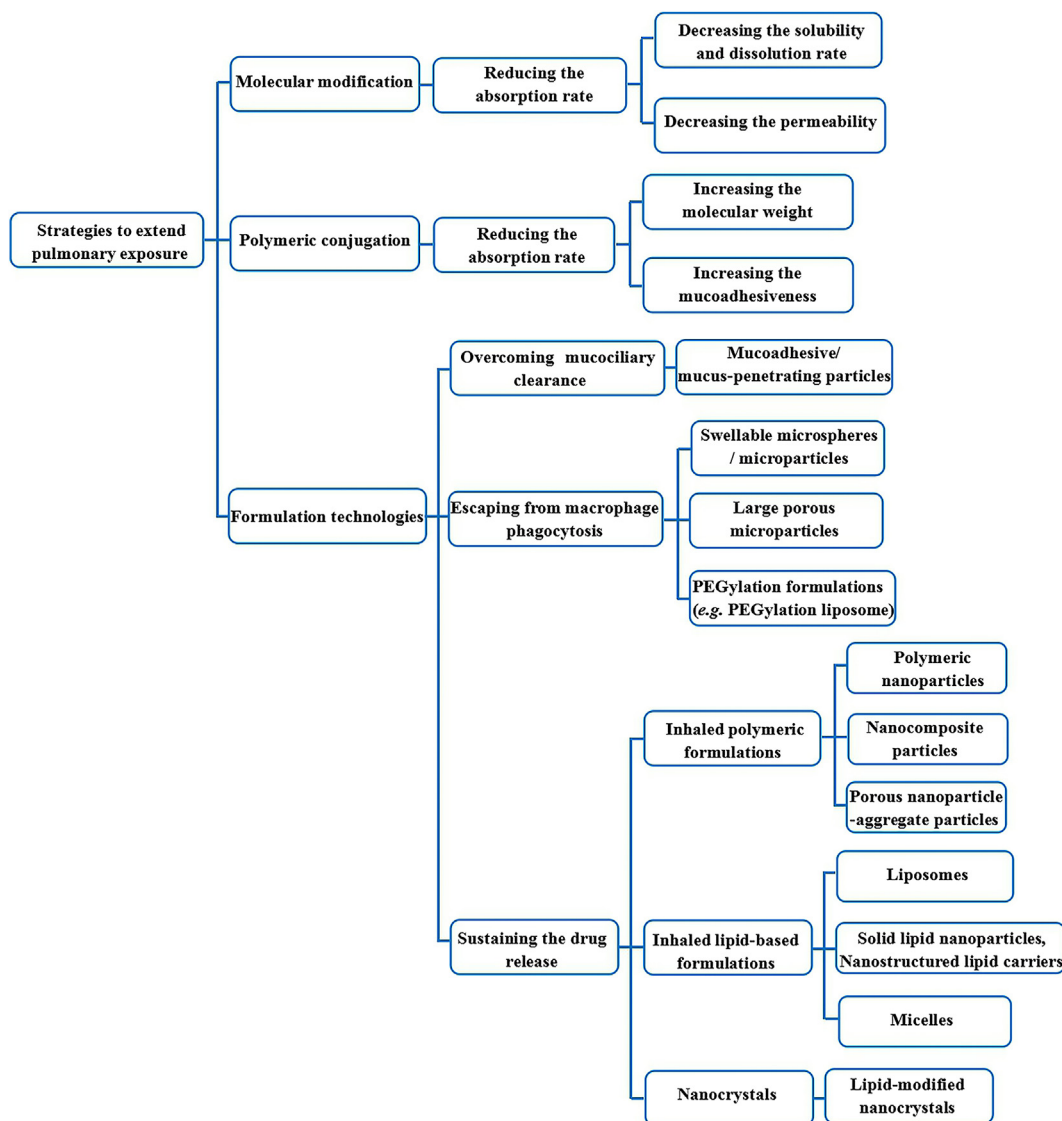
Figure 3   A summary of various strategies to extend the pulmonary exposure of the inhaled drugs.

limited volume of lung lining fluid. If the undissolved fraction of inhaled particles successfully escaped from the MCE or phagocytosis of microphages clearance, the slow dissolution rate may result in prolonged retention time in the lungs. For example, fluticasone propionate, an water-insoluble steroid anti-inflammatory agents used for managing asthma, shows an extended absorption time in pharmacokinetic profiles due to its poor dissolution rate, which leads to a prolonged lung exposure[44]. Several poorly water-soluble drugs such as sex steroids and antifungal drugs typically exhibit slow absorption and elimination rates from the lungs[44,45]. These drugs often dissolve slowly in the lung lining fluid, resulting in slow absorption to the blood circulation and extended retention time in the lungs[45]. The same phenomenon has also been observed in the case of inhaled biologics such as insulin. It is well known that the monomeric or dimeric form of insulin could convert to a hexamer in the presence of zinc ions, affording an insoluble complex. The hexamer of insulin could act as a reservoir and gradually be depolymerized into a monomer, the only absorbable form of insulin. Consequently, the retention time of inhaled insulin and absorption time in the lungs was prolonged significantly.

Vanbever et al.[46] loaded insoluble insulin complexes into large porous particles and delivered them into the lungs of rats, which resulted in 12 h of plasma insulin concentration. Therefore, lowering the solubility or dissolution rate of inhaled drugs *via* physical modification could potentially extend the pulmonary exposure and pharmacological effects.

3.1.2.   Decreasing the permeability
The permeability of a drug compound is another important physicochemical property that influence the residence time of the drug compound in the lungs. Low permeability of inhaled drugs is generally preferred to extend the residence time of the drug in the lungs by diminishing the diffusion of the drugs through the lung epithelium into the interstitium of the lungs or systemic circulation[47]. Lipophilic drugs are mainly absorbed *via* passive diffusion through the cells, whereas the hydrophilic drugs are absorbed by the diffusion through the tight junctions[48]. The former causes a faster absorption through the respiratory epithelium into the bloodstream than the latter. The drugs with poor membrane permeability are not easily passed through the lung epithelial

cells, and thus, a relatively high drug concentration may be achieved in the lungs. Several studies have shown that the drugs with low permeability, such as colistin, aztreonam could retain a high drug concentration in the lungs after inhalation[49]. This can be critically meaningful for the inhaled antibiotics to maintain sufficient high concentration in the lungs to combat respiratory infection.

For those inhaled drugs with high permeability, scientists have attempted some molecular engineering techniques to alter the polarity of molecules. Ciprofloxacin is a potent antibacterial agent, and widely employed to eradicate the bacterial infections in the respiratory tract. However, the pulmonary delivery of ciprofloxacin causes a rapid drug diffusion through the lung epithelium, offering marginal or no advantage over the systemic administration[50]. It was reported that the systemic and pulmonary exposures of ciprofloxacin were almost equivalent after intravenous and pulmonary administration of ciprofloxacin solution to rats[51]. In order to reduce the permeability of ciprofloxacin, Brillault et al.[50] formulated a complex of the drug with metal cations, which can limit the diffusion through the epithelium, reduce its absorption, and maintain a high concentration in the lungs. Furthermore, Lamy and colleagues[52] showed that the complexes formed between copper ions and ciprofloxacin could slow the absorption rate of ciprofloxacin from the ELF into plasma, resulting in high and sustained drug concentrations in ELF of the lungs. The attachment of the metal ions with the drug molecules could often ionize the molecules structure[50], which can increase the polarity of the molecule, can diminish the permeation of the molecules through respiratory epithelium.

Besides the strategy of modifying the polarity of drug molecules, increasing the affinity between drug molecules with the lipophilic group of receptors localized at the surface of lung cells also could extend pulmonary exposure. Salmeterol is one of the examples. It is a long-acting β2-adrenoceptor agonist (LABA) and belongs therapeutically to β2-sympathomimetics. It binds to G-protein coupled β2-receptors in the lungs and inhibits the contraction of the smooth muscle[53,54]. Compared to another β2-adrenoceptor agonists salbutamol, which duration of action is 4−6 h, the effect of salmeterol lasts 12 h. Although both molecules have the same lead structure, salmeterol has longer nonpolar group, i.e., hydrophobic N-substituents with saligenin ethanolamines, than salbutamol. When the active sites of salmeterol bind to the β2-adrenoceptor, the nonpolar group of salmeterol can bind to nearby nonpolar region of cell membrane or hydrophobic area of the receptor protein[55]. Thus with this molecular modification, salmeterol can maintain its binding site, extend the lung exposure and activate the β2-adrenoceptor repeatedly to exert prolonged pharmacological responses[56].

3.2. Polymeric conjugation

3.2.1. Increasing the molecular weight

The synthesis of polymer−drug conjugates is another approach to diminish the elimination of inhaled drugs from the lungs and extend their lung exposure. By synthesizing polymer−drug conjugates, the molecular weight and hydrophilicity of the molecules can be increased and consequently their lung absorption could be retarded and the residence time of the drugs in the lungs can be prolonged[57]. In this context, polymers such as polyethylene glycol (PEG) and polyethyleneimine have been utilized[58,59]. PEG is a water-soluble polymer containing ethylene glycol repeating units and its molecular weight is related to the degree of

polymerization. To achieve optimal residence time of the drug conjugates in the lungs, the selection of the PEG with appropriate molecular weight is important. One study showed that if the molecular weight of PEG is less than 2 kDa, it can be cleared from the rat lungs within 2 days, but it can reside into the lungs up to 7 days when its molecular weight is greater than 5 kDa[60]. As compared to the pure prednisolone, a steroid medication used to treat allergies, inflammation, autoimmune disorders and cancer, its PEG-prednisolone conjugates showed a 7.7-fold of the reduction of the absorption rate of the drugs from the lungs, which could prolong the retention of inhaled drug in the lungs, improve its pharmacokinetics profiles and also reduce its systemic adverse effects[61]. Apart from a decrease in the permeability by enlarging molecule weights of inhaled drugs via PEGylation, it was also reported that PEGylated insulin may be able to evade phagocytosis of macrophage and resulting in sustained insulin delivery after pulmonary administration[62].

3.2.2. Increasing the mucoadhesiveness

Researches also designed mucoadhesive polymer-drug conjugates to extend the residence time of the inhaled drugs in the lungs[63−65]. This modification could create an intimate contact of the drug molecules with endogenous substances such as mucin at the surface of respiratory tract and thus sustain their residence time in the lungs. Generally, the binding bond between drug and polymer is designed to be broken at certain time point to release free drug, which might slowly diffuse into the sites of action to exert their effects. Chitosan (CS) is one of the mostly studied polymers used for this purpose. The NH_2 groups present in the structure of CS can be conjugated with various functional groups to achieve further conjugation of the drug molecules and other ligands[60]. Due to the presence of COOH and NH_2 groups in its backbone, which can generate hydrogen bonds with the lung mucosa, CS exhibits mucoadhesive property at physiological pH and the capacity to prolong the residence time of the drugs in the lungs[65]. Thiolized CS has been developed to further improve the mucoadhesion property of the polymer-drug conjugates in the lungs[66]. The thiol groups of the thiolated glycol CS could form disulfide bonds with the cysteine-rich domains of the mucus[67]. Compared to pristine CS, the mucoadhesion potential of the thiolated glycol CS was increased by 2 times[66]. The mucoadhesive polymer-drug conjugates could not only overcome MCE by anchoring to the mucin but also diminish the diffusion of the chemical entities through the epithelial membrane due to the enlarged sizes and the increased hydrophilicity.

3.3. Formulation technologies

Besides the aforementioned modifications of drug molecules, various formulation technologies have been exploited to extend the pulmonary exposure of inhaled drugs, providing improved therapeutic benefits to the patients. In the following subsections, various formulation strategies to overcome the MCE and phagocytosis of microphages are described. Moreover, the recent advances in inhaled sustained-release formulations are also critically reviewed.

3.3.1. Overcoming mucociliary clearance

Inhaled drugs need to firstly pass through the mucus barriers to reach epithelial membrane at the upper airways. To reduce the elimination of inhaled drugs by MCE, researchers proposed the development of mucoadhesive formulations, which could anchor
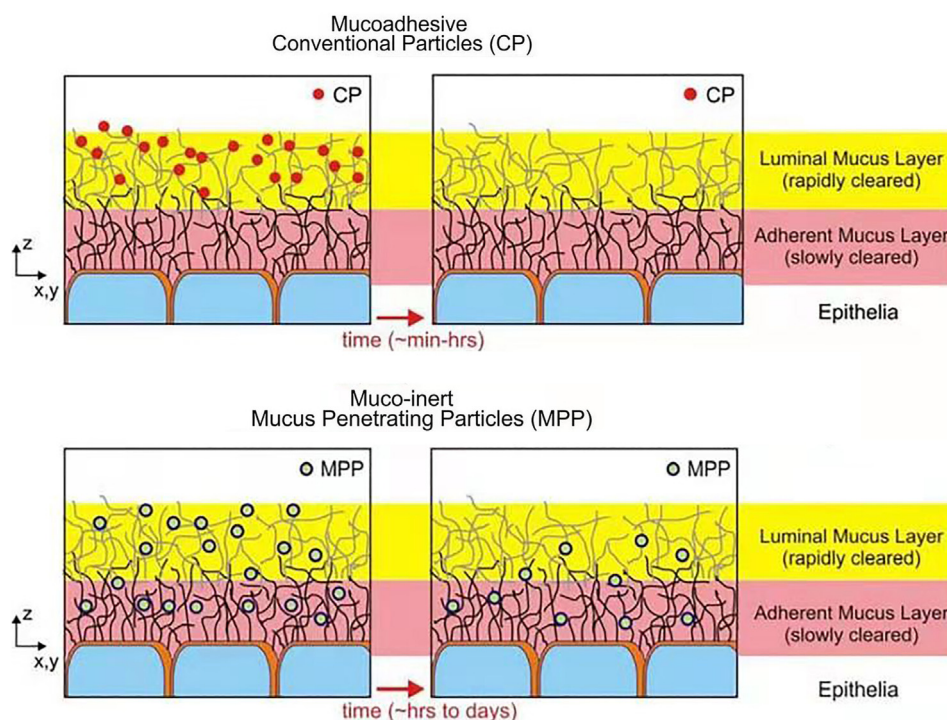
Figure 4 A comparison between the fate of the conventional mucoadhesive particles (CP) and mucus-penetrating particles (MPP) administered to a mucosal surface. The CP are largely immobilized in the luminal mucus layer and removed along with the luminal mucus layer quickly. In contrast, the MPP could enter into the adherent mucus layer and retained, leading to a prolonged residence time for the MPP at the mucosal surfaces. Reprinted with the permission from Ref. 72. Copyright © 2009 Elsevier.

with the mucin molecules *via* multivalent interactions to effectively decrease their mucociliary clearance[68,69]. The mucoadhesive polymers (*e.g.,* CS, hyaluronic acid) are commonly used in these formulations. The particle size of the formulations also has a considerable influence. For instance, the MCE could remove the particles with size larger than 6 μm from the human airways within 24 h, and the particles with diameter less than 6 μm could retain in the lungs for more than 24 h[69]. In a study, salbutamol sulphate was co−spray dried with hyaluronic acid (HA) to pro-duce biomucoadhesive microparticles with negative surface charges and D_a around 5 μm. It was found that the resultant mi-croparticles exhibited excellent mucoadhesion property in an *in vitro* model. The lung retention time of salbutamol was significantly prolonged from 2 to 8 h in a rat model after being incorporated in HA microparticles. In addition, the systemic exposure of salbutamol sulphate in the biomucoadhesive micro-particles was significantly reduced, which potentially reduced the side effect of inhaled drugs[70]. In another study, the similar strategy was used to formulate budesonide, a poorly water-soluble drug, into biomucoadhesive microparticles composed of HA with negative surface charges and D_a around 5.3 μm to extend the pulmonary retention[71]. The results showed that HA did not alter *in vitro* release of budesonide from the biomucoadhesive microparticles, whereas, the budesonide in the microparticles exhibited a significantly prolonged time of maximum concentra-tion (T_{max}) and increased bioavailability as compared to the for-mulations without HA after intratracheal administration to rats. That study demonstrates HA could provide budesonide with sus-tained pharmacological effects. This can be attributed to the prolonged retention time of the drugs by overcoming the muco-ciliary clearance with the polymers.

Alternative to using mucoadhesive polymers to interact with the mucus to overcome mucociliary clearance in the upper air-ways, researchers also attempted to fabricate mucus-penetrating particles (MPP), which could exhibit prolonged lung residence time. This method was developed because of the concern of the potential negative impact of mucoadhesive particles to the airway surfaces. In order to relieve the potential negative impact of mucoadhesive particles, researchers attempted to fabricate MPP to prolonged lung residence time (Fig. 4)[72]. In addition, it was also reported that mucoadhesive particles (MP) suffered from a rapid clearance mediated *via* phagocytosis by macrophages[73] and the inhaled drugs that could not diffuse through the mucus layers will be cleared quickly by rhythmically swings of cilia to expel the particles trapped in the mucus layer[74]. Hence the inhaled drug particles have to rapidly diffuse through the mucus lining fluid and enter into the periciliary fluid layer[75]. The enhanced mucus-penetrating ability of nanoparticles and PEG modified nano-particles allowed the nanoparticles to escape from mucus and continue their journey in the mucus, which could effectively prolonging the residence time of drugs in the lungs[76,77].

3.3.2. Escaping from macrophage phagocytosis

Macrophage phagocytosis may be the next clearance pathway for those inhaled drug particles that survive from MCE. In the following section, some formulation strategies to evade macro-phage phagocytosis are reviewed.

3.3.2.1. Swellable microspheres/microparticles. Most inhal-able drug particles are in a micron range of 1−5 μm, but unfor-tunately the microparticles within this size range could be readily captured by the alveolar macrophages *via* phagocytosis

process[78]. It was reported that anti-tubercular drugs formulated into this size range microspheres could be exploited to target alveolar macrophages for the treatment of the pulmonary tuberculosis[79]. However, the inhaled drugs used to treat other lung diseases should avoid the phagocytosis by macrophages. In order to achieve it, researchers[80,81] accomplished swellable microspheres. Before deposition in the lungs, the sizes of the swellable microspheres are in an inhalable range of 1−5 μm. When landed in the lung lining fluid, these microspheres could swell and their size becomes too large to be engulfed by macrophages. These microparticles are often fabricated using some biopolymers such as CS, sodium alginate[1]. El-Sherbinyet al.[82] loaded curcumin into inhalable and expandable CS-PEG microspheres and showed that about 20% of the encapsulated curcumin was rapidly released within 1 h as the microspheres are expanded rapidly. Afterwards, the release was subsequently slowed down and lasted for about 24 h. Some other researchers[83] also reported that CS based swellable microparticles could potentially be used as nanocrystal carrier to achieve sustained pharmacological effect *via* pulmonary delivery.

3.3.2.2. Large porous microparticles.

The design of large porous microparticles is another formulation approach to bypass the phagocytosis of macrophages. The large porous microparticles with a geometric diameter larger than 5 μm, but a low density could exhibit similar aerodynamic performance as the small but compact microparticles as D_a is a combined factor of geometric particle size, density and shape as shown in Eq. (1):

$$D_a = D\sqrt{\rho_p/\rho_0 x} \tag{1}$$

where, D_a is the aerodynamic particle diameter, D represents the geometric particle diameter, ρ_p and ρ_0 symbolize the densities of the particles and water(1 g/cm^3), respectively, and x designates the dynamic shape factor[48]. According to Eq.(1), it is possible to enlarge the geometric particle diameter D, but keep the aerodynamic particle diameter D_a the same by a decrease in the densities of the particles. Therefore the large porous microparticles could be designed to evade macrophage phagocytosis by the large size, but not compromise aerosol performance by decreasing the density. In addition, some researchers[84] have proved that macrophages are not able to engulf large particles. Chvatal et al.[85] compared the performances of the inhalable non-porous fine particles with the large porous particles (LPPs) containing meloxicam. It was found that a higher proportion of LPPs reached the lower respiratory tract than the non-porous fine particles. At the same time, the LPPs could avoid phagocytosis due to their large size and exhibit prolonged residence time in the lungs. Kim et al.[86] encapsulated doxorubicin in the highly porous and large PLGA microparticles, which were administered through pulmonary route for the treatment of metastatic lung cancer. Their results showed that the large porous PLGA microparticles exhibited sustained drug release characteristics in the lungs and provided excellent therapeutic efficacy.

3.3.2.3. PEGylation.

As described in section 3.2.1, the fabrication of PEG−drug conjugates could increase the molecular weight of the drugs and diminish their elimination from the lungs. In fact, apart from the conjugation with drugs, PEGs have also been attached to particulate delivery systems to extend pulmonary exposure of inhaled drugs. Kaneko et al.[87] compared the distribution of PEGylated liposomes and unmodified liposomes in ELF

and alveoli after pulmonary administration. It was found that the PEGylated liposomal systems are more stable than the unmodified liposomes. In addition, the PEGylated liposomes could escape from the opsonization by the surfactant proteins, which is the process of recognition and removal of foreign substances by the alveolar macrophages in the lungs[87]. Consequently, the PEGylated liposomes were not obviously engulfed by alveolar macrophages as compared to the unmodified systems in the lungs, which resulted in their extended pulmonary retention. Although PEGylated formulations in general possesses various advantages over unmodified systems in terms of stability, safety, and biocompability, in a few cases, wrapping PEG layer may also result in some unfavorable features to the formulation. Li et al.[88] reported that the antibacterial activity of colistin-loaded PEGylated liposomes was lower than that of non-PEGylated liposomes, which can be partially attributed to the fact that the PEG chain hindered the direct contact of liposomes with the bacterial cell walls and eventually the drug delivery efficiency was compromised.

3.3.3. Sustaining the drug release

There are large numbers of literature reporting the design of inhaled sustained release formulations to provide therapeutic benefits to the patients. In the following section, various formulation strategies to design inhaled sustained drug release formulations are reviewed.

3.3.3.1. Inhaled polymeric formulations.

As developing other formulations, the selection of suitable excipients is a key step in the development of inhaled polymeric formulations. A large number of polymers including natural polymers such as CS, gelatin, alginate, collagen, and HA, and synthetic polymers such as polylactic acid (PLA), poly(lactic-*co*-glycolic acid) PLGA, oligo (lactic acid), poly-*ε*-caprolactone (PCL) and polyvinyl alcohol (PVA) are widely used for the PDD applications[89]. The type of the polymers used would affect the particle disposition, drug release, and the efficacy of the inhaled drugs[90]. Polymer-based inhaled formulations are often micro- or nano-particles in either dry powder form or colloidal dispersion form. Depending on the types of the polymers and characteristics of micro-, nano-particles, the inhaled drugs might experience different clearance pathways in the lungs[91].

The most studied polymeric formulations for inhalation may be nanoparticle based formulations, which are used to load bioactive substances through encapsulation or adsorption. These systems protect the drugs from the degradation, modulate the dissolution rate or release of the drugs, reduce the side effect, and may facilitate targeting some specific cells or tissues in the lungs[92]. One of the most investigated inhaled nanoparticles may be for the treatment of lung cancers. For this purpose, the inhaled polymeric nanoparticles have been designed to reduce the side effects of the chemotherapeutic drugs. In addition, the inhaled polymeric nanoparticles are expected to provide targeting features to the tumor sites and extend the pharmacological effects of the anti-tumor drugs. Instead of formulating polymeric nanoparticles into colloidal dispersion forms, many researchers afforded nano-in-microparticles, nanoembedded microparticles, or nanocomposite particles in order to achieve adequate aerosol performances[93−95]. The matrices of the microparticles are commonly composed of hydrophilic sugars. After dissolution of the matrix materials in the lung lining fluid, the nanoparticles are reconstituted in the lungs to exert the therapeutic effects[96,97]. Liu et al.[98] used oleic acid-CS as the carrier to load paclitaxel and quercetin in nanoparticles, and then transformed the

Table 1 Examples of polymeric particles based sustained-release formulations for inhalation.

Polymer	The property of polymer	MW of polymer	API	Aerosol form	D_a	Performance in *in vitro* release profiles/*in vivo* PK studies	Ref.
Natural polymers							
Albumin	Naturally present at the lungs, non-immunogenic, biocompatible, biodegradable	∼66 kDa	Benzothiazinones	Dry powders	2.2–2.7 μm (geometric diameters)	The drug release was triggered by proteases, causing more than 50% of drug being released within 4 h, which was followed by a slower and sustained release profile lasting for 48 h.	111
Alginate	Biodegradable, biocompatible, mucoadhesive	–	Isoniazid, rifampicin	Dry powders	1–2 μm	The formulations exhibited an initial burst release (30%–40% within the first 4 h) followed by a sustained release pattern (90% within 60 h).	112
CS	Biocompatible, biodegradable, non-immunogenic, antimicrobial activity, mucoadhesive	–	Levofloxacin	Dry powders	Less than 5 μm	A sustained *in vitro* release profile was observed (cumulative release of 80%–90% over 24 h)	113
Gelatin	Biodegradable, biocompatible	<300 kDa	Methotrexate	Dry powders	2.2–2.9 μm	Only 35% of the loaded drug was released from the methotrexate conjugated gelatin particles within 72 h in the presence of trypsin, while the unconjugated entirely released the drug within 36 h.	114
HA	Naturally present at the lungs, non-immunogenic, biodegradable, mucoadhesive	∼2000 kDa	Insulin	Dry powders	1–4 μm	As compared to spray-dried pure insulin powders, the formulations had no immediate blood concentration peak and displayed a slower *in vivo* clearance phenomenon.	115
Synthetic polymers							
PCL	Biocompatible, biodegradable	8 kDa (Mn)	Dexamethasone	Dry powders	∼5 μm	Dexamethasone-loaded PCL microparticles exhibited a sustained *in vitro* release profile (cumulative release of 45% over 24 h) as compared to the dexamethasone prepared without PCL.	116
PLGA	Biocompatible, biodegradability could be altered by varying composition	7–17 kDa	Tobramycin	Dry powders	∼3.4 μm	A sustained release profile with a cumulative release of more than 90% over one month was achieved.	117
PVA	Ability to improve the colloidal stability, biocompatible	85–124 kDa	Ciprofloxacin	Dry powders	∼5.06 μm	A sustained *in vitro* release profile with cumulative 90% of drug released over 24 h was achieved.	118

Table 1 (*continued*)

Copolymers

Polymer	The property of polymer	MW of polymer	API	Aerosol form	D_a	Performance in *in vitro* release profiles/*in vivo* PK studies	Ref.
PLGA-PVA	Biodegradable, amphiphilic	–	siRNA	Nebulized nanoparticulate suspensions	150–200 nm (hydrodynamic diameters)	A sustained siRNA release profile with cumulative release of 85% within 8 h was acquired.	119
PLGA-PEG	Biocompatible, amphiphilic, high drug loading capacity	–	Salbutamol	Dry powders	8.24 μm (geometric diameters)	A sustained drug release profile with cumulative release of 91.1% over 12 h was achieved.	120
PEG-CS	Hydrophilic, biocompatible, biodegradable, non-toxic	CS: 190–310 kDa PEG: 5 kDa (Mn)	Bovine serum albumin	Dry powders	1.02–2.63 μm	A tri-phase drug release pattern with a burst release (25%–45%) within the first 2 h, followed by a slow sustained release up to 4 days and an even slower release in late phase.	99
PEG-PLA	Biocompatible, biodegradable	~100 kDa	Placebo nanoparticles	Nebulized nanoparticulate suspensions	129–141 nm (hydrodynamic diameters)	–	121

API, active pharmaceutical ingredient; CS, chitosan; D_a, aerodynamic diameters; HA, hyaluronic acid; Mn, number-average molecular weight; MW, molecular weight; PCL, poly-e-caprolactone; PEG, polyethylene glycol; PK, pharmacokinetics; PLA, polylactic acid; PLGA, poly(lactic-co-glycolic acid); PVA, polyvinyl alcohol; T_{max}, time of maximum concentration; –, not applicable.

nanoparticles into inhalable micropartilces using the spray drying process. Their results showed that the microparticles composed of a large number of nanoparticles exhibit adequate aerosol performance. In addition, the *in vivo* study showed that the microparticles deposited in the lung exhibited sustained drug release characteristics up to 48 h. Other research group[99] applied the coating of self-assembled co-polymers onto drug nanoparticles and subsequently encapsulated into inhalable/expandable semi-interpenetrating polymeric network microspheres, which exhibited extended pulmonary exposure. Besides these systems, another form of inhaled polymeric particles that were used to extend pulmonary exposure may be LPPs composed of nanoparticles for inhalation. This formulation strategy combined the merits of porous microparticles and nanoparticles, including high lung deposition and extended pharmacological effects[100]. One of the examples is that, rifampicin, an antibiotic drug were loaded into PLGA nanoparticles and then processed into inhalable porous microparticles for the treatment of pulmonary tuberculosis[101].

CS and PLGA may be two most commonly investigated natural polymers and synthetic polymers, respectively, in the inhaled formulations to extend the pulmonary exposure and pharmacological effects because of their biodegradability and biocompatibility. CS is a type of natural-origin polysaccharides produced by the deacetylation of chitin[102]. Due to its mucoadhesive property, CS is used either to form drug-conjugates or modify the surfaces of micro-, nano-particles to overcome MCE. It is reported that the CS can be dissociated into natural non-toxic metabolites by the lysozymes present in the respiratory secretions, which is an attractive feature for its pulmonary administration[103]. Besides the controlled-release feature, inhaled particles composed of CS were also reported to swell and evade phagocytosis of macrophages, thereby prolonging the retention of the inhaled drugs in the lungs. Various therapeutic agents such as antibiotics, anti-tuberculosis molecules, anti-tumor drugs, peptides and proteins have been formulated with CS for their pulmonary delivery[103,104]. In fact, it is also reported that CS itself has some biological activities, which can be used as antioxidants, antimicrobial and antifungal agents[105]. As for PLGA, it is a co-polymer made from the polymerization of lactic acid and glycolic acid, which can regulate its own biodegradability by varying the composition (lactide/glycolide ratio), molecular weight and chemical structure[106]. It is not a natural polymer, but its degradation products are lactic acid and glycolic acid, which can be eliminated by the citric acid cycle[107], possess excellent biocompatibility and biodegradability. However, to overcome the hydrophobicity of PLGA or modulate the drug release, hydrophilic polymers such as PEG, PVA, HA can be employed to modify PLGA in the inhaled formulations[108−110]. Various polymeric excipients used in inhaled sustained-release formulations are summarized in Table 1[99,111−121].

3.3.3.2. Inhaled lipid-based formulations. Lipid is another commonly used group of excipients for pulmonary administration. One of the reasons is due to their biocompatibility and safety especially when unwanted immune response of inhaled formulation can be avoided using endogenous lipids[122]. Several lipid based systems such as liposomes, solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), micelles are generally formulated to render sustained-release profiles of inhaled drugs.

In liposomes, drugs are encapsulated in a lipid bilayer structured vesicles, which can be used as drug reservoir to extend the drug release profiles and lung exposure. Furthermore, the liposomes could reduce the toxicity of the encapsulated drugs and improve their tolerability, leading to better patient compliance[123].

Some clinical studies have demonstrated that inhaled liposomal formulations could be used to reduce the frequency of administration, but maintain high local concentrations in the lungs and reduce systemic side effects[98]. Liposomal formulations have been exploited to load a variety of drugs and used in the treatment of a broad range of diseases[123]. At present, the representative sustained release formulation for pulmonary delivery is amikacin liposomal inhalation suspension (ALIS; Arikayce®), which has been approved in the USA for use as part of a combination antibacterial drug regimen against *Mycobacterium avium* complex lung disease[124]. In this product, amikacin was encapsulated in liposomal vesicles composed of dipalmitoylphosphatidylcholine (DPPC, an ideal excipient used in inhaled liposomal formulations[125]) and cholesterol. As compared to pristine amikacin, the inhaled liposomal formulations exhibited higher local concentrations in the lungs and prolonged lung exposure of amikacin for several hours, resulting in a better therapeutic outcome in terms of killing respiratory bacteria[126]. Researchers[87] reported that liposomal formulations were suitable for targeting alveolar epithelial cell lining fluid to treat lung diseases in the deep lungs, where inhaled liposomes exhibited a continuous distribution in alveolar epithelial cell lining fluid. Most inhaled liposomal formulations are administered *via* nebulization as these are a liquid dosage form, *i.e.*, liposomal suspension or colloidal dispersion. The colloidal stability and also potential leakages of drugs from the liposomes during nebulization may limit their clinical applications[127]. To overcome these problems, various dehydration methods such as freeze drying, spray drying, spray freeze drying have been attempted to produce liposomal dry powders for inhalation[128−133]. As the liposomal formulations are soft as compare to polymeric particulate systems, their reconstitution from the dry powders upon deposition in the lungs is more challenging in terms of particle size distribution[128,130].

SLNs are made up of lipid shell and a solid hydrophobic core, in which the drug molecules are dispersed or dissolved in the molten solid lipid matrices[127]. The size, composition and method of delivery of SLNs determine the drug release rate and deposition in the lungs[134]. SLNs are used to control the drug release and achieve long-term drug exposure in the lungs, and they are initially used as alternatives to polymer nanoparticles[135]. It is because SLNs are in general degraded faster than polymeric nanoparticles in an *in vivo* environment, which may alleviate the burden to the lung tissues caused by slow degradation rate of polymers. Moreover, most lipids are better biocompatible than the polymers[127]. As compared to liposomes, the SLNs are much more stable under nebulization due to their rigidity[1]. Vieira et al.[136] loaded rifampicin into CS-coated SLNs, which showed high encapsulation efficiency and improved drug delivery efficacy to the alveolar macrophages, and the researchers concluded that the CS-coated SLNs were expected to be a safe and effective against pulmonary tuberculosis. The SLNs were also be used as drug carriers for pulmonary delivery of macromolecular drugs. For example, Bi et al.[137] loaded insulin into SLNs, which conferred prolonged hypoglycemic effects and relatively high insulin bioavailability after their administration into the lungs of diabetic rats through intratracheal perfusion. NLCs, which are composed of a solid−liquid mixed lipid core, are a second-generation lipid nanoparticles, have attracted increasing attentions to deliver drugs to the lungs and to extend pulmonary drug exposure. Similar to SLNs, the NLCs are more stable than the liposomes during the nebulization and storage, and are more biocompatible than the polymeric nanoparticles and other forms of nanoformulations[138,139]. Pastor et al.[140] compared the efficacy of NLCs and SLNs for the pulmonary delivery of an antibacterial drugs, *i.e.*, sodium colistimethate. Interestingly, the encapsulation efficiency of NLCs (94.8%) was significantly higher than that of SLNs (79.7%). Both NLCs and SLNs exhibited sustained release profiles, whereas, the release of SLNs were incomplete. In contrast, the drugs release from NLCs were almost complete within the sustained release time.

The micelles are another kind of lipid formulations often used for pulmonary drug administration to extend their pulmonary exposure, reduce the frequency of administrations and improve patient compliance. They are self-assembled systems with a hydrophobic inner core and a hydrophilic outer shell in a certain aqueous medium. The hydrophobic micellar core could improve the drug loading efficiency of the poorly water soluble drugs[141]. Micelles are small in the size and with the bulky hydrophilic shell, it was found they can evade the uptake of alveolar macrophages and prolong the retention of drugs in the lungs[48]. In literature, phospholipids and amphiphilic molecules including block copolymers, polymer−lipid conjugates, etc. were used to prepare micelles, and their fate in the lungs depends upon the compositions of the micelles[142,143]. Block copolymers micelles are composed of hydrophobic and hydrophilic polymer molecules. The degradation rate of the block copolymers based micelles is slow, which can resemble that of polymeric nanoparticles, may be harmful to the lungs[144]. In contrast, the micelles composed of lipid-polymer conjugates exhibit better biocompatibility in the lungs and illustrate a prolonged residence time in the lungs and improved physical stability[145]. Various hydrophilic polymers such as PEG and PVA are commonly used to afford the polymer-lipid conjugates. Among others, the PEGylated lipid may be the most studied scaffold to accomplish micelles for PDD[127]. The PEGylation is recognized as an efficient means to avoid the recognition of phagocytes, leading to a prolonged residence time of drug delivery systems in the lungs. Craparo et al.[146] loaded beclomethasone dipropionate into PEGylated phospholipid-polyaspartamide micelles where the hydrophilic shell was based on both polyaspartamide and PEG, and the hydrophobic stearoyl tails attached to phospholipids. The micelles increased *ca.* 240 times of the solubility of beclomethasone dipropionate, and exhibited excellent biocompatibility, cellular internalization and promising for the treatment of lung diseases.

3.3.3.3.   Nanocrystals. Nanocrystals are submicron colloidal dispersions of pure drug particles stabilized by excipients[147]. They are regarded as a promising formulation strategy for poorly soluble drugs due to their ability to increase the dissolution rate and saturated solubility of the drugs. However, if the drug nanocrystals are directly inhaled into the lungs, these may be absorbed to the systemic circulation as fast as intravenous injection, resulting in a shorter pulmonary exposure[71]. In order to retain the advantage of nanocrystals and also extend their pulmonary exposure in the lungs, researchers used lipids to modify the nanocrystals[148]. The lipid-modified nanocrystals could not only take the advantage of improving the dissolution rate of poorly water-soluble drugs, but also prolong the residence time of the drugs in the lung tissues by modulating the drug release rate. The nanocrystals of fluticasone propionate modified with phospholipids exhibited reduced dissolution rate of drug as compared to the unmodified nanocrystals and extended its retention time in the lungs[148]. In another study, Hu and coworkers[11] formulated curcumin acetate nanocrystals, a highly lipophilic and poorly water soluble drug, into respirable particles *via* spray drying and delivered them into the lungs of rats. The results showed that the absorption time in the lungs was 7.2 times longer than that of curcumin, and a better pharmacological effect was observed in a pulmonary hypertension rat model due to prolonged drug retention in the lungs. In addition, the irregular

shape of nanocrystals as compared to the conventional carriers, might also affect their lung exposure time. The recent literature has demonstrated that the rod or fiber-like nanoparticles could usually confer a less-efficient clearance by the macrophages[149]. Therefore, the irregularly shaped nanocrystals might experience a prolonged retention in the lungs. However, the nanocytotoxicity caused due to the long-term pulmonary retention of this nanomedicine should be seriously considered.

So far, a wide range of active substances including small molecules, proteins, peptides and nucleic acids have been formulated in inhaled sustained-release formulations[150]. Some of them have been evaluated in preclinical animal models or in clinic trial, and there are also a few approved products for marketing, which have been listed in Table 2[126,151−161]. The treatment of local diseases in the lungs such as asthma, COPD, respiratory infection and pulmonary hypertension can obviously benefits from the extended pulmonary exposure by using inhaled sustained-release formulations[126,151,153,158]. For some therapeutic macromolecules used for the treatment of systemic diseases, for example insulin, prolonged pharmacological effects and improved patient compliance also could be expected when they are formulated in inhaled sustained release formulations as long as the formulations could slow down the absorption rate of macromolecules[162].

4. Safety concerns in designing of inhaled formulations with extended pulmonary exposure

Despite the advantages of inhaled formulations with extended pulmonary exposure, the safety of exploiting these inhaled formulations, especially when large proportions of excipients have to be used in the particulate delivery systems, is always one of the main concerns in the drug development and clinical translation. Currently, the number of excipients approved for inhalation is rather limited, which include some sugars, amino acids, lipids, salt, phospholipid and small size PEGs[163]. The use of unapproved excipients in any novel inhaled formulations could increase workload, cost, time, and risk of rejection by the regulatory agencies[11]. This could partially explain the reasons of existence of limited number of inhaled formulations with extended pulmonary exposure on the present market. With the existing limited numbers of US food and drug administration (FDA)-approved excipients, it is extremely challenging to make innovative inhaled formulations[122]. Nevertheless, the efforts to develop novel excipients and assess the existing excipients for PDD applications have never ceased[122]. Fumaryl diketopiperazine (FDKP) used in Technosphere® inhaled insulin (Afrezza®) is an example of novel excipient although it is not used for extending pulmonary exposure of the drugs. With continuous innovation, more suitable and safe excipients for inhalation can be developed. In the following sections, the toxicity concerns of the existing excipients, nanocytotoxicity and immunogecity associated to the inhaled formulations with extended pulmonary exposure are reviewed.

4.1. Toxicity of excipients used in the inhaled formulations

4.1.1. Polymers
The safety concerns of the polymers applied for the PDD mainly involve their degradation pathways and the toxicity of the degraded products in the lungs. The uses of non-biodegradable polymers in the inhaled formulations are prohibited even though they could provide sustained release profiles for the loaded drugs.

The polymers, which are degraded and/or eliminated slowly, may lead to local accumulation and their degradation products may cause potential pulmonary toxicity. Thus, these should be avoided in inhaled formulations[18]. In this context, PLGA is one of the most commonly studied polymers as it exhibits excellent biocompatibility and biodegradability, and provides extended pulmonary exposure after inhalation. However, some researchers[164] have proved that PLGA particles could retain in mouse lungs for more than 20 days. The chronic toxicological data of the inhaled PLGA particles for such a long period of time in the lungs is still not well investigated[165]. The potential irritation effects of the acidic degradation products of PLGA (e.g., lactic acid and glycolic acid) to the lungs are expected[122]. To date, the safety profiles of the post pulmonary administration of the formulations composed of CS has not fully been examined[103]. Some data suggest that CS could open epithelial tight junctions, which may cause emphysema, and thus, the pulmonary delivery using CS is not often recommended[166]. Apparently, there are more work to be done to assess the safety profiles of other polymeric excipients prior to successful translation of the inhaled sustained release formulations composed of polymers in clinic.

4.1.2. Lipids
As aforementioned lipid-based particulate delivery systems such as liposomes, SLNs, NLCs have attracted considerable interests as there are comprised of some endogenous lipids and their safety profiles are more promising as compared to inhaled polymeric formulations[167]. The lung surfactants are composed of many different types of lipids including DPPC (saturated phosphatidylcholine), unsaturated phosphatidylcholine, phosphatidylinositol, phosphatidylglycerol, phosphatidylethanolamine, cholesterol, etc., which can potentially be used in the inhaled formulations to elicit better safety profiles than other lipids[163]. For examples, phosphatidylcholine was used as one of the components of inhaled solid lipid microparticles, which had been proven to be safe for the lung cells[168]. In addition, the cytotoxicity of liposomes composed of DPPC was examined by measuring the release of lactate dehydrogenase from lung tissues, and the results showed that liposome composed of DPPC did not damage lung tissues at the dose used in the study[169]. Other lipids such as phospholipid and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), have been used in TOBI® Podhaler® produced by Novartis Pharmaceuticals, and their long-term safety has been established at high daily doses in cystic fibrosis patients[170]. The DSPC could be catabolized and ultimately recycled by type II cells in the lungs[171]. Overall, the safety profiles of the lipids in the pulmonary formulations are more promising as compared to the polymeric excipients.

4.1.3. Metal ions
In some cases, metal ions could be introduced to increase the polarity of molecules such as fluoroquinolones to limit the absorption or form complexes with biologics such as insulin to provide extended lung retention. However, the cytotoxicity concerns of the metal ions to the lungs are inevitable. For example, the exposure of the ferric oxide nanoparticles in the lungs can cause oxidative stress and a variety of inflammatory reactions by recruiting inflammation and immune cells to the lungs[172]. Studies showed that Zn^{2+}, Al^{3+} and Cu^{2+} exhibit higher cytotoxicity than Mg^{2+} and Ca^{2+}[50]. In addition, the toxicity of the metal ions is largely related to their concentrations used, which provide some guidance of selection of the ions and dose.

Table 2 Examples of some inhaled sustained drug release formulations used to treat both local lung diseases and systemic diseases.

Disease	API	Main excipient used	Type of sustained release formulations	Therapeutic outcome	Stage	Ref.
Local lung diseases						
Asthma	Budesonide	PLGA, poly(vinylpyrrolidone)	Large porous particles	The formulation significantly reduced the total number of inflammatory cells and expression of IL-4 and IL-5 in BALF as compared to the physical mixture (budesonide/lactose).	Preclinical	151
	Fluticasone propionate	PLA, PLA-PEG	Mucus-penetrating nanoparticles	It inhibited the accumulation of BALF neutrophils for a longer period of time as compared to the free fluticasone propionate.	Preclinical	152
COPD	Fluticasone propionate	Glycerol tripalmitate, glyceryldistearate, CS oligosaccharide lactate	Mucoadhesive solid lipid microparticles	It was more effective than the pure fluticasone propionate in controlling oxidative stress.	Preclinical	153
Cystic fibrosis	Lumacaftor, ivacaftor	Squalene, PEG, Tween 80, Span 35	Nanostructured lipid carriers	It showed the superior ability to restore the expression and function of cystic fibrosis transmembrane receptor proteins as compared to the non-encapsulated drugs.	Preclinical	154
Lung infection	Amikacin	DPPC, cholesterol	Liposomes (Arikace®)	It demonstrated significant activity against *Mycobacterium avium* complex.	Approved by FDA	126
	Ciprofloxacin	Hydrogenated soy phosphatidylcholine, cholesterol	Liposomes (Pulmaquin®)	It showed antibacterial activity against *Pseudomonas aeruginosa*.	Phase III clinical trails	155
	Tobramycin	Precirol® ATO 5, Compritol® 888 ATO, Poloxamer 188, Tween 80	Nanostructured lipid carriers	It exhibited antibacterial potential against isolated *Pseudomonas aeruginosa*.	Preclinical	156
Lung cancer	9-Nitro-20(S)-camptothecin	Dilauroylphosphatidylc-holine, cholesterol	Liposomes	It was clinically effective in reducing tumor size with minimal toxicity profiles.	Phase II clinical trails	157

(continued on next page)

Table 2 (*continued*)

Disease	API	Main excipient used	Type of sustained release formulations	Therapeutic outcome	Stage	Ref.
Pulmonary hypertension	Bosentan	PLGA, PVA	Polymeric nanoparticles	It exhibited a more persistent vasodilation effect on the pulmonary blood vessels.	Preclinical	158
	Fasudil	DPPC, cholesterol	Liposomes	It provided a sustained vasodilation effect on pulmonary blood vessels.	Preclinical	159
Systemic diseases						
Bone disorder	Calcitonin	PLGA, PVA, CS	Mucoadhesive polymeric nanospheres	It maintained the blood calcium at low level for a prolonged period of time.	Preclinical	160
Diabetes	Insulin	CS, mannitol, pentasodium tripolyphosphate	Nanocomposite microparticles	It exhibited a prolonged hypoglycemic effect as compared to the insulin solution.	Preclinical	161

API, active pharmaceutical ingredient; BALF, bronchoalveolar lavage fluid; COPD, chronic obstructive pulmonary diseases; CS, chitosan; DPPC, dipalmitoylphosphatidylcholine; FDA, US Food and Drug Administration; IL-4, interleukin-4; IL-5, interleukin-5; PEG, polyethylene glycol; PLA, polylactic acid; PLGA, poly(lactic-*co*-glycolic acid); PVA, polyvinyl alcohol.

4.2. Nanocytotoxicity

Nanoparticles could provide many benefits to the PDD as described in the previous sections. However, the cytotoxicity of the nano-sized materials is a serious concern for their clinical translation. One of nanoparticle-induced toxicological effects is associated to the production of reactive oxygen species, which can ultimately result in oxidative stress in the biological systems[173]. The increase in the reactive oxygen species caused by nanoparticles further leads to inflammation[174]. The toxicity of inhaled nanoparticles seems to be related to the physical and chemical properties of inhalable nanoparticles such as size, specific surface area and surface chemistry[175]. The clinical and experimental data suggested that the nanoparticles with smaller particle size and larger surface area could cause more lung injury[174]. This might be explained by the fact that the ultrafine nanoparticles might easily evade phagocytosis of macrophages than the larger nanoparticles. Consequently, these are not easily cleared from the alveolar area, leading to long-term accumulation and subsequent toxicity in the lungs[176]. For nanoparticles, the aggregation state in physiological situation also needs to be considered. A single gold nanoparticle with size 50 nm elicits a lesser acute hazard than its agglomerates or slightly larger particles when using pulmonary inflammation as a marker to assess the toxicity of nanoparticles[177].

The shapes of the nanoparticles also have impacts on their toxicity in the lungs. The spherical nanoparticles are considered to be safer and are more rapidly internalized by the macrophages than the rod- or fibrous-like nanoparticles[149], and the spherical nanoparticles seem to be safer delivery vehicles for PDD and also for other administration routes[178]. Moreover, the surface properties of the nanoparticles play a role in producing toxicity. For example, the nanoparticles with positive surface charges could exhibit a higher cytotoxicity than the negative or neutral charged nanoparticles[179]. In this aspect, the surface modification of the nanoparticles with PEG could often reduce the production of reactive oxygen species and eliminate cytotoxicity[180]. In addition, it was reported that the selection of muco-inert or muco-adhesive coating on the surfaces of the nanoparticles has an influences on the toxicity. Schneider et al.[73] assessed the preclinical safety of the MP and MPP following inhalation. They found the mice treated with MP showed acute inflammation, while the group receiving MPP showed almost no difference in the toxicity as compared to the mice treated with saline. Lastly, poorly water-soluble or more persistent nanoparticles in the lungs after inhalation could induce more oxidative stress, which might eventually lead to inflammation, fibrosis and cancer[173]. This implies the importance of the selection of biodegradable and biocompatible materials when designing inhaled formulations with extended pulmonary exposure.

4.3. Immunogenicity

The aforementioned toxicity is mainly referring to the inflammation caused the excipients in the inhaled formulations. Besides inflammation, the immunogenicity of inhaled formulations should not be overlooked, which can be caused not only by some biological active substances such as proteins but also by some excipients when being formulated with biologics. The immunogenicity refers to the ability of foreign substances to induce host immune response[181]. Immunogenic reactions may cause patients to suffer from cytokine storms or strong allergic reactions, such as anaphylaxis or pure red-cell aplasia representing life-threatening situations[182]. An immunogenic risk assessment

is an ongoing and continuous process throughout the clinical development with a goal of maximizing the safety to the patients[183].

The lipid excipients may not provoke an immune response as these are relatively small molecules. However, some protein drugs and some polysaccharides based excipients could display immunogenic properties, inducing humoral immune responses, especially when these are in different aggregation states[184]. The conjugation of lipids and proteins may increase the immunogenic potency too[185]. Various proteins and peptide therapeutics have occupied a considerable fraction of current pharmaceutical market, which have drastically changed the landscape of treatment of many diseases. The lungs are recognized as attractive administration route and alternative to injection routs for the therapeutic proteins and peptides[186]. Currently, several inhaled peptide and protein drugs, such as insulin, calcitonin, cyclosporin A and aldesleukin are under the development and in clinical trials[187]. The potential immune responses of the therapeutic proteins undermine their clinical utility[188]. An example is inhaled human insulin. Although inhaled human insulin has been launched on the market, its dry powder or liquid forms, are more immunogenic than subcutaneous insulin injection[189].

Protein aggregates have been considered as a potential risk factor for patient immunogenicity[190]. It would not be surprising to encounter immunogenic reactions while developing inhaled biologic formulations with extended pulmonary exposure, where excipients such as polymers and lipids involved. The aggregation of proteins and some unwanted interactions between proteins and excipients might occur during their preparation (e.g., at gas−liquid or solid−liquid interfaces and filling), storage, transportation, and administration (e.g., vigorous shaking before administration)[191], which could raise immunogenicity of the products. In order to avoid protein aggregation, non-ionic surfactants can be added, which could stabilize peptides and proteins and diminish their adsorption induced unfolding and aggregation[192]. In addition, the coupling of polymeric excipients with proteins and peptides could also produce biological drugs to reduce the immunogenicity. For example, the combination of PEG and albumin with peptides can reduce peptide immunogenicity in vivo[193]. Moreover, the duration of treatment, administration route, and frequency of administration impact the immunogenicity of the biological drugs[194]. Data have shown long-term treatment and frequent administration were often increase the immunogenicity of biological drug products[195], which should be critically evaluated during the development of the inhaled formulations with extended pulmonary exposure using sustained-release formulations. Besides protein and peptide drugs, the immunogenicity of the nucleic acid-based therapeutics such as DNA, RNA species or analogues and their vectors often hinder their translations into the clinical practice[196]. The extracellular DNA can be recognized by immune cell receptors as damage-related molecular patterns or pathogen-related molecular patterns, activating pro-inflammatory signaling pathways or immunosuppressive cell functions[197]. Some specific RNA sequences or structures can also be recognized by the corresponding immune receptors to trigger a series of immune events, including cytokine secretion, immune cell proliferation and survival, or activation of adaptive immunity[198].

5. Conclusions and perspectives

The administration of drugs to or via the lungs has been exploited to treat both local lung diseases and systemic diseases. Due to the unique physiological and anatomic features of the respiratory tract, most inhaled medicines can rapidly be eliminated from the lungs, resulting in transit pharmacological effects. The inhaled medicines with extended pulmonary exposure may not only improve the patient adherence and compliance by reducing the frequency of drug administration, but also provide clinical benefits to the patients. To extend pulmonary retention and prolong pharmacological effects of inhaled therapeutics, various physical and chemical principles have been adopted to overcome the inherent clearance mechanisms of the lungs, namely mucociliary escalator, phagocytosis of macrophages, and translocation to cells and systemic circulation. The modification of the inhaled drugs with respect to their size, polarity, permeability and mucoadhesiveness via molecular engineering or conjugation is considered as powerful means to sustain pulmonary retention of the products, as long as the modification would not significantly compromise their pharmacological activities and cause unwanted side effects. The pharmaceutical technologies such as formulating biomucoadhesive, swellable, and large porous particles intended for inhalation could also overcome the clearance mechanisms of the lungs. Design of inhaled sustained release formulations has become a commonly applied strategy to extend the pulmonary exposure and pharmacological effects. However, the number of excipients approved for inhalation is rather limited at this moment. The incorporation of various unapproved excipients in some innovative inhaled formulations and assessment of their safety profiles require additional workload, cost, and risk, which hinder their translations into the clinical practice. From our perspective, the right choice of excipients with satisfactory balance between efficacy and safety is critical for the successful translation of inhaled sustained release formulations. As we learned from the approved product of amikacin loaded inhalable liposomes, endogenous lipids-based formulations and drug-lipid conjugates hold the greatest promising for the clinical applications in the near future.

In conclusion, the future efforts should be placed on exploring innovative strategies to not only overcome the lung clearance mechanisms but also develop innovative and suitable excipients to expedite translation of the inhaled formulations with extended pulmonary exposure to the clinic.

Author contributions

Yi Guo, Dongmei Cun and Mingshi Yang contributed in conception, design, and drafting of the manuscript. Hriday Bera, Changzhi Shi and Li Zhang contributed in manuscript drafting. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

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