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Review

Pulmonary delivery of nanoparticle chemotherapy for the treatment of lung cancers: challenges and opportunities

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Abstract

Lung cancer is the second most prevalent and the deadliest among all cancer types. Chemotherapy is recommended for lung cancers to control tumor growth and to prolong patient survival. Systemic chemotherapy typically has very limited efficacy as well as severe systemic adverse effects, which are often attributed to the distribution of anticancer drugs to non-targeted sites. In contrast, inhalation routes permit the delivery of drugs directly to the lungs providing high local concentrations that may enhance the anti-tumor effect while alleviating systemic adverse effects. Preliminary studies in animals and humans have suggested that most inhaled chemotherapies are tolerable with manageable pulmonary adverse effects, including cough and bronchospasm. Promoting the deposition of anticancer drugs in tumorous cells and minimizing access to healthy lung cells can further augment the efficacy and reduce the risk of local toxicities caused by inhaled chemotherapy. Sustained release and tumor localization characteristics make nanoparticle formulations a promising candidate for the inhaled delivery of chemotherapeutic agents against lung cancers. However, the physiology of respiratory tracts and lung clearance mechanisms present key barriers for the effective deposition and retention of inhaled nanoparticle formulations in the lungs. Recent research has focused on the development of novel formulations to maximize lung deposition and to minimize pulmonary clearance of inhaled nanoparticles. This article systematically reviews the challenges and opportunities for the pulmonary delivery of nanoparticle formulations for the treatment of lung cancers.

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Introduction

Lung cancer is the second most common cancer worldwide, representing ~14% of newly reported cases. The majority (85%) of lung cancer cases is classified as non-small cell lung cancer (NSCLC), with the remaining classified as small cell lung cancer (SCLS)[1]. The American Cancer Society estimates that there were more than 200 000 new cases of lung cancer and approximately 150 000 deaths in 2017 in the United States alone, making it the deadliest among all types of cancer[2].

Unfortunately, an early diagnosis of lung cancer is challenging, and at the time a diagnosis most lung cancers are in advanced metastatic stage. The metastatic spread of cancer to distant organs is the dominant reason for the dismal survival rate of advanced-stage lung cancer patients, with a 5-year survival rate of only 10%[3–9]. The most common metastatic locations for lung cancer are typically the nervous system, bone, liver, respiratory system, and adrenal glands[10].

Surgical removal/resection is the main treatment for non-metastatic lung cancers. However, this technique can only be used in 10%–20% of patients with NSCLC and is limited by the number and the site of the lesions and the patient's respiratory and/or general status[11, 12]. Lung cancers for which surgery is not a feasible option generally require chemotherapy to prolong survival, control symptoms and improve the quality of life of patients[13–16].

Anticancer drugs must penetrate cancer tissues to attain a concentration necessary to exert effective tumor killing; indeed, suboptimal drug concentrations typically exhibit weak anti-tumor activity and additional concerns regarding drug resistance[17, 18]. Intravenous administration inevitably causes a considerable proportion of chemotherapeutics to be widely distributed in various organs, leading to substantially low drug concentrations at tumorous sites. This necessitates the administration of high doses to attain therapeutically effective drug concentrations at the diseased sites. Such high doses can cause severe adverse effects, especially at the sites of rapidly

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dividing cells such as hair, skin, spleen and liver, among others[18–20]. These toxicity concerns compromise the efficacy and compliance of systemic chemotherapy against lung cancer[21–24]. Furthermore, lung cancer sub-types may also be genetically diverse, making treatment even more difficult. Thus, there is an urgent need for new treatments with improved safety and efficacy.

Inhaled chemotherapy

Localized chemotherapy refers to the delivery of anticancer drugs directly to the affected organs, which can ensure higher concentrations in tumors compared to other non-target sites. Localized chemotherapy has been confirmed to be effective against various types of cancers, including ovarian and colorectal cancers[25–30]. Inhaled drug delivery facilitates the localized delivery of drugs directly to the lungs via the oral or nasal inhalation route. Inhalation is a non-invasive route of administration, and some inhaled dosage forms are easy to carry and use, making it a promising alternative to the parenteral routes of drug delivery for treating respiratory diseases. Inhalation therapies have been shown to be effective and are well accepted for the treatment of respiratory tract diseases such as asthma, chronic obstructive pulmonary disease (COPD) and respiratory tract infections.

Inhaled chemotherapy has been shown to be promising against lung cancers (Table 1)[31–36]. Inhalation can alter the bio-distribution of drugs and promote the accumulation of a larger fraction in the lungs compared to parenteral administration[37–41]. Furthermore, inhalation limits the systemic distribution of anticancer drugs and thus the associated toxicity[35, 36]. Most adverse effects associated with inhaled chemotherapy were shown to be localized, including cough and glottitis, which are common and treatable. In some cases, respiration-related complications, such as a drop in forced expiratory volume and hypoxia, have been reported. Most local adverse effects following inhaled chemotherapy have also been shown to be drug-, dose- and time-dependent[31–36, 42–44]. However, it is not clear whether these adverse events were associated with disease progression or inhaled chemotherapy[34, 35].

A high proportion of inhaled drug has commonly been detected in the lymph nodes[32]. Inhaled drugs can also be deposited in the lymphatic tissue via the lymphatic circulation[32]. Thus, inhaled chemotherapy may also be beneficial for the treatment of lung cancer that has metastasized to the lymph nodes[32]. Moreover, drugs that are absorbed into the lymphatic circulation can redistribute in peripheral airways, allowing access to otherwise poorly accessible areas of the lungs[42, 45]. Thus, inhaled chemotherapy may be extremely beneficial in cases of cancer that has metastasized to the lung, which are usually located away from the major airways but receive blood from the pulmonary arteries and veins[46–48]. Aerosolized delivery of liposomal interleukin-2 (IL-2) in dogs has been shown to be effective against pulmonary metastases from osteosarcoma[49]. A combination of intravenously injected human natural killer cells and inhaled interleukin-2 had a synergic effect and increased the survival of mice with osteosarcoma lung metastases[50]. Inhaled chemotherapy has also been used as an adjuvant with systemic chemotherapy; however, no improvement in tumor efficacy was observed compared to systemic chemotherapy alone[33].

Most lung cancers are in the metastatic stage at the time of diagnosis, and the treatment of lung cancer that has metastasized to other organs may further improve the efficacy of chemotherapy. There has been increasing interest in exploring the inhalation route for systemic drug delivery, such as insulin for diabetes or gene therapy[51, 52]. Thus, it is possible that delivery via inhalation may be used to deliver chemotherapeutic agents systemically and target lung cancer metastasis to other organs. However, the effect of inhaled chemotherapy on metastasized lung cancer has not been investigated.

Although inhaled delivery has a clear pharmacokinetic advantage over systemic delivery, ensuring the deposition of the drug in the resident tumor is key to achieving efficient anti-tumor activity. However, the efficacy of inhaled chemotherapy depends on multiple factors, including tumor size, disease stage, drug penetration at the tumor site, physico-chemical properties of drugs, local adverse effects, and patient condition. These factors play a dominant role in determining whether inhaled delivery is indeed a feasible and/or effective option for lung cancer therapy.

Respiratory tract obstruction due to lung cancer and other obstructive respiratory conditions such as cystic fibrosis and bronchiectasis can affect the deposition and distribution patterns of aerosols in the lungs. For example, a lung tumor can physically occlude the respiratory tract by reducing the cross-sectional area of the lung, which can divert the airflow to non-occluded areas and reduce the deposition of inhaled drugs to the tumor. The effects of tumors in terms of size and location on airflow, particle transport, and deposition patterns have been modeled[53]. It was shown that the particle deposition at tumor sites increases until the tumor blocks approximately half of the airway lumen and then decreases with further obstruction. It has also been proposed that the majority of the inhaled drug is deposited on the frontal surface of the tumor[53].

Despite the direct access to the lung tumor via inhalation, enhancing drug penetration to the lung tumor is also critical for achieving efficient anti-tumor activity. The depth of tumor penetration following topical deposition is usually limited and also depends on the physico-chemical properties of drugs, including the molecular weight, solubility, and apoptotic activity[17, 42, 54–58]. Furthermore, penetration of the drug to the tumor depends on the nature of the tumor, including the size, cellularity of the tumor and density of the interstitium[59]. It has been demonstrated that small nodules respond better to inhaled chemotherapy than larger nodules[40]. Thus, limited penetration and an inability to achieve an adequate drug concentration in the tumor tissue may limit the effectiveness of inhaled chemotherapy.

The uptake and direct toxicity of inhaled chemotherapy to healthy lung cells are relatively unknown. The deposition of high concentrations of anticancer drugs in healthy lung cells

Table 1. Clinical studies investigating the safety and efficacy of inhaled chemotherapy.

Drugs	Outcomes
Inhaled 5-Fluorouracil (5-FU)	Out of 6 patients receiving inhaled 5-FU, 4 showed an anticancer response, with 2 complete responders and 2 partial responders. *Safety* There were no side effects in the bronchial tree and pulmonary parenchyma but some patients showed glottitis. A very small fraction of 5-FU was detected in the systemic circulation[31].
Inhaled 5-FU	A satisfactory anti-tumor response was observed in 6 out of 10 patients who received inhaled 5-FU, with 2 complete responders, 4 partial responders and 4 with no response. A high concentration (5-15 times higher) of drug was detected in tumor tissue compared with surrounding normal tissues within the lungs. High levels of 5-FU were detected in regional lymph nodes following inhaled delivery, indicating absorption of 5-FU in the bronchial tree and into the lymphatic path. *Safety* Inhalation was well tolerated by the normal lung tissue. No systemic hazardous effects were observed[32].
Inhaled doxorubicin with intravenous docetaxel and cisplatin	An overall response rate >35% was observed. Out of 24 patients treated at a dose of 6.0 mg/m^2, 6 showed a partial response, 1 showed complete response, 13 had stable disease and 4 showed no response. *Safety* No toxicity due to inhaled chemotherapy. In some patients, >70% of the drug was delivered to one lung, indicating relative overdosing of that lung[33].
Inhaled doxorubicin as a solution at doses ranging from 0.4 to 9.4 mg/m^2	Inhaled doxorubicin showed dose-limiting toxicity. Patients showed high toxicity at a dose of 9.4 mg/m^2 of inhaled doxorubicin; however, lower doses of inhaled doxorubicin were shown to be safe. Cough, chest pain, dyspnea, sore throat and fatigue were common side effects. Most adverse effects were pulmonary, with no systemic side effects. One patient had grade 3 hypoxia at a dose of 3.8 mg/m^2, and one patient had a >20% drop in forced vital capacity at a dose of 7.5 mg/m^2 [34].
Inhaled carboplatin with or without intravenous carboplatin	Inhaled carboplatin was shown to prolong the survival of lung cancer patients. Inhaled carboplatin provided improved anticancer efficacy compared with intravenous carboplatin. The response in patients receiving intravenous carboplatin and docetaxel was reported as 5 partial responders, 8 stable disease and 7 non-responders. The response in patients receiving inhaled and intravenous carboplatin was reported as 2 complete responders, 6 partial responders, 3 stable diseases, and 9 non-responders. The response in patients receiving inhaled carboplatin only was reported as 1 complete responder, 4 partial responders, 5 stable disease, and 10 non-responders. *Safety* Inhaled carboplatin caused less incidence of neutropenia than intravenous carboplatin and docetaxel. Inhaled carboplatin caused remissive cough and a decline in forced expiratory volume in 1 s[35].
Inhaled gemcitabine (GCB) in a dose escalation study	Approximately half of inhaled gemcitabine was deposited in the lung, and the remaining drug was observed in the stomach and upper airways. Low plasma GCB levels were detected based on the pharmacokinetic data. The patient response was graded as 1 partial responder, 4 stable disease and 4 non-responders. *Safety* Limited systemic absorption and no severe systemic side effects were observed. One patient had bronchospasm. Other side effects included cough, nausea, fatigue and anorexia. The maximum tolerated dose of inhaled gemcitabine was 3 mg/kg[36].

may increase the risk of undesirable local toxicities. Overall, the effectiveness of inhaled chemotherapy against lung cancers is established, but there is considerable uncertainty regarding the toxicities of inhaled chemotherapy to healthy lung cells, making their safety a subject of constant debate. Hence, promoting uptake in cancer cells and minimizing accumulation in healthy cells may be a more effective approach to ensure the safety and efficacy of inhaled chemotherapy.

Nano-carriers for inhaled drug delivery

The delivery of anticancer drugs via nanoparticles has been shown to be efficacious and safe in a variety of cancers[60–62]. Nanoparticles can encapsulate toxic anticancer drugs by biocompatible and biodegradable excipients and facilitate targeted and/or controlled delivery[63–67]. Anticancer drugs can also be formulated into drug nanocrystals with high drug loading and minimal use of excipients[68–70]. Thus, pulmonary administration of nanoparticles could also reduce the systemic toxicity of chemotherapeutic agents compared with free drugs. For example, Roa *et al* showed that inhaled doxorubicin nanoparticles exhibited lower cardiac toxicity compared with the same dose of free doxorubicin after intratracheal administration[71]. Zou *et al* showed that paclitaxel-polyglutamic acid conjugate was well tolerated by mice following intratracheal

administration[72]. Furthermore, the sustained release characteristics of nanoparticles may further aid the effectiveness of inhaled chemotherapy by maintaining drug concentrations at tumor sites for longer durations[73–75].

Due to their small size, nanoparticles inherently tend to penetrate and accumulate within the leaky tumor vasculature when a drug is delivered via systemic administration, which is termed the enhanced permeation and retention (EPR) effect[76–80]. The EPR effect may not play a role in tumor deposition when nanoparticles are administered via inhalation. However, delivery of the drug directly into the lungs enables passive targeting to the lung tumor. Furthermore, nano-carriers are taken up into the cancer cell via endocytosis, which typically does not occur in the case of solubilized drug[81, 82]. Thus, nanoparticles can increase penetration and accumulation of inhaled drugs in tumor tissues and cells, leading to improved anti-tumor activity compared with the free drug[42, 83–85].

A large fraction of nanoparticles are taken up by the reticuloendothelial system (RES), such as the liver, kidney and spleen, following intravenous administration[85–87], whereas the primary site of distribution of inhaled particles is the lungs. Thus, a relatively large fraction of nanoparticles is deposited in the lungs following inhalation compared to systemic delivery[73, 74, 83–85, 88, 89]. However, the accumulation efficiency of nanoparticles in lung tumors following inhaled and systemic administration have not been thoroughly compared. Interestingly, inhaled doxorubicin-conjugated dendrimer showed better anticancer activity compared to systemic administration, indicating that there is a limited EPR effect in some lung tumors[90].

Moreover, cellular uptake of particles is a particle size-dependent phenomenon and has been shown to increase with a decreasing particle size[91, 92]. Hence, the selection of nanoparticles for inhaled delivery is inherently advantageous in terms of penetration-enhancing ability, as compared with microparticles. Roa *et al* showed that nanoparticles embedded in an effervescent carrier matrix facilitated the rapid release of primary nanoparticles and enhanced anti-tumor activity compared with those embedded in a non-effervescent carrier matrix following inhaled delivery[71].

The ability of nanoparticles to release a chemotherapeutic agent in close proximity to the tumor is imperative to achieve selective and efficient tumor killing. However, premature release of encapsulated drug from nanoparticles may lead to non-specific toxicity to normal lung parenchyma. To circumvent this limitation, nanoparticles with site-specific and triggered release characteristics have been explored. Low extracellular and intracellular pH of tumor tissue/cells have been exploited to enable triggered release through the design of pH-sensitive fusogenic lipid nano-vesicles. These nano-vesicles fuse with the cell plasma membrane and lysosomal membrane at low pH, thus providing site-specific and triggered delivery of anticancer drugs to cancer cells[93–95]. It has been demonstrated that pulmonary surfactant mimetic pH-sensitive nanoparticles are cytotoxic to lung tumor cells while being compatible with healthy lung cells, indicating a selective toxicity of the developed formulation to lung cancer cells[96].

Nanoparticles can also be actively targeted to tumor cells by attaching tumor-specific ligands, which are thought to guide drug-loaded nanoparticles and facilitate specific interactions with lung cancer cells. Such targeting can inhibit the non-specific interaction between drug-loaded nanoparticles and healthy lung cells and reduce local toxicity[90]. Lung cancer cells overexpress several receptors, such as epidermal growth factor receptor (EGF receptor), folate receptor, and luteinizing hormone-releasing hormone (LHRH receptors[97–104]. Tseng *et al* showed that EGF receptor-targeted biotinylated gelatin nanoparticles deposited more selectively into cancer cells owing to receptor-mediated uptake and caused no injury to the lungs[105, 106]. EGF receptor-targeted inhalable magnetic nanoparticles demonstrated increased uptake in cancer cells compared with non-targeted particles and exhibited greater anti-tumor activities[107]. EGF receptor-targeted cisplatin-loaded gelatin nanoparticles demonstrated greater lung deposition and retention, resulting in enhanced anti-tumor efficacy compared with free cisplatin or non-targeted nanoparticles[108]. Taratula *et al* developed LHRH peptide-coated mesoporous silica nanoparticles (MSNs) to deliver anticancer drugs (doxorubicin and cisplatin) and antisense oligonucleotides targeted to MRP1 and BCL-2 against resistant lung cancer. Inhalation allowed the deposition of higher drug/siRNA concentrations in the lungs compared to intravenous administration. The targeted nanoparticles were effectively internalized into human lung cancer cells and demonstrated an enhanced anticancer activity[75]. Taratula *et al* also showed that lipid nanoparticle targeted to LHRH receptors can facilitate the selective deposition of doxorubicin and siRNA in lung tumor cells and minimize deposition in healthy lung tissues[109].

Solid tumors are characterized by increased extracellular matrix deposition and tumor fibrosis[110, 111]. This matrix is mainly composed of collagen networks and leads to the compartmentalization of tumors, which enhances tumor cell survival and proliferation[111–113]. Such a dense collagen network can inhibit nanoparticle penetration and distribution into the tumor[110, 114–117]. Anti-fibrotic agents have been reported to decrease tumor interstitial fibrosis and promote the intratumoral distribution of nanoparticles[115, 118]. Inhaled anti-fibrotic agents, *ie*, losartan and telmisartan, have also been shown to improve the uptake and accumulation of nanoparticles in lung cancer models[119].

Overall, nanoparticles can improve the anti-tumor activity of loaded chemotherapeutics[120–123]. Nanoparticle-mediated inhaled chemotherapy has been shown to be safe and effective against lung cancer in pre-clinical and clinical studies (Table 2).

Drug resistance is another factor that can substantially compromise the therapeutic efficacy of chemotherapeutic agents against cancers. Lung cancers with acquired, *ie*, "pump" or "non-pump" resistance are less responsive to anticancer drugs[128]. Pump resistance is typically associated with the expression of proteins such as multidrug resistance-associated protein (MRP) and P-glycoprotein, which can actively pump

Table 2. Preclinical and clinical studies showing the safety and efficacy of nanoparticles in the pulmonary delivery of chemotherapeutic agents.

Drug	Carrier	Outcome
Paclitaxel	Polyethylene $glycol_{5000}$-distearoylphosphatidylethanolamine (PEG_{5000}-DSPE) micelles	The lung targeting efficiency via the pulmonary route was 132-fold higher than the intravenous route. Micelles substantially reduced the distribution of paclitaxel in non-targeted tissues compared with free paclitaxel following intratracheal administration. Micelles showed no sign of inflammation in lung tissues, highlighting the safety and suitability of the delivery vehicle for inhaled delivery[74].
Doxorubicin	Human serum albumin (HSA) nanoparticles adsorbed with Apoptotic TRAIL protein (TRAIL/Dox HSA-NP)	Inhaled TRAIL/Dox HSA-NP nanoparticles were distributed effectively throughout the lungs and provided sustained drug release. Inhaled TRAIL/Dox HSA-NP also expressed greater anti-tumor activity compared with TRAIL or Dox HSA-NP alone, with minimal side effects[124].
Losartan and telmisartan	Polystyrene nanoparticles	Losartan and Telmisartan showed significant *in vivo* anticancer activity against orthotopic and metastatic lung cancers. Animals receiving inhaled losartan and Telmisartan survived longer than untreated animals. The drugs were well tolerated by normal lung tissues[119].
Doxorubicin	56-kDa PEGylated-polylysine dendrimer	The dendrimer expressed improved anti-tumor activity following intratracheal administration compared with the drug solution administered intravenously. The drug-dendrimer complex was better tolerated by the lungs than free drug after intratracheal administration[90].
Epirubicin	Solid lipid nanoparticles	Epirubicin concentration in the lungs was higher than in plasma following inhaled nanoparticle therapy. The drug concentration in the lungs was higher with inhaled epirubicin nanoparticles compared with inhaled epirubicin solution[125].
9-Bromo-noscapine (9-Br-Nos)	Nanostructured lipid particles (NLPs)	The half-life of 9-Br-Nos-NLPs increased in the lungs compared with free drug powder after inhalation[73].
Doxorubicin and cisplatin, two types of siRNA targeted to MRP1 and BCL2 mRNAs	Luteinizing hormone-releasing hormone receptor-targeted mesoporous silica nanoparticles	Inhalation led to greater amounts of drugs and siRNA to be retained in the lungs than intravenous administration of the same formulation. Inhaled delivery also restricted the systemic uptake and accumulation of nanoparticles in other organs[75].
Paclitaxel	Lung surfactant mimetic and pH-responsive lipid nanovesicles	Fusogenicity of the nanoparticles enabled cytosolic delivery of paclitaxel to cancer cells but was non-toxic to normal cells. Inhaled delivery of drug-loaded nanoparticles led to lower drug concentrations in non-targeted sites (liver, spleen and plasma) compared with intravenous paclitaxel solution. Drug-loaded nanoparticles showed no lung toxicity[96].
Cisplatin	Sustained release lipid inhalation targeting (SLIT)	Inhaled cisplatin liposomes were well tolerated with no signs of systemic (nephrotoxicity, ototoxicity, or neurotoxicity) toxicity in lung cancer patients, which was attributed to a low systemic drug concentration. Side effects, including nausea, vomiting, dyspnea, fatigue and hoarseness, were observed[126].
9-Nitrocamptothecin	Liposomes	Inhaled 9-Nitrocamptothecin liposomes were safe and enabled disease stabilization in some lung cancer patients. The drug was also systemically absorbed following inhalation at high doses, leading to systemic side effects, including anemia, neutropenia and anorexia. A partial remission of liver metastasis was also observed in a patient with endometrial cancer, indicating the systemic potential of inhaled administration[127].

anticancer drugs out of cancer cells, reducing their intracellular concentration and consequently effectiveness[129]. Non-pump resistance is caused by the activation of anti-apoptotic cellular defense due to the upregulation of B-cell lymphoma-2 (BCL-2) protein, which prevents the release of cytochrome *c* and hence the execution of caspase-mediated cell apoptosis[130, 131]. Suppression of drug resistance-associated proteins such as BCL-2 protein and MRP could reduce the efflux of anticancer drug and promote apoptosis sensitivity against anti-tumor drugs. Nanoparticles can co-deliver anticancer drugs with genes and other adjuvants to effectively suppress these resistance mechanisms and increase the sensitivity of such resistant cancer cells against chemotherapies[75, 109, 132, 133]. Garbuzenko *et al* developed inhaled nanoparticles containing doxorubicin in combination with antisense oligonucleotides targeted to MRP1 mRNA as a suppressor of pump resistance and BCL-2 mRNA (as a suppressor of non-pump resistance) for lung cancer. This formulation has been shown to enhance the sensitivity of lung cancer to anticancer drugs, increasing the efficacy upon inhalation[132].

Physiological barriers to inhaled drug delivery

For inhaled chemotherapy, drugs should be deposited and retained in the lungs at therapeutically effective concentrations to elicit an efficient anti-tumor effect. However, the architecture of the respiratory tract and clearance mechanisms of the lungs pose a key challenge to the deposition and retention of inhaled nanoparticles in the lungs. To effectively address these issues, it is important to understand the barriers to deposition and retention of inhaled nanoparticles. The current understanding of the deposition and clearance behaviors of inhaled nanoparticle is largely derived from studies investigating the clearance of environmental nanoparticle pollutants, which can be extrapolated to drug nanoparticles to a certain extent[134, 135].

Deposition of inhaled particles

The lungs are composed of a series of branching airways, which can be classified into the conducting zone and the respiratory zone. The conductive zone or upper airway consists of the trachea, which divides into two bronchi and further subdivides into bronchioles, whereas the respiratory zone, or the deep lung, includes the respiratory bronchioles, the alveolar ducts and the alveolar sacs.

Inhaled particles are carried with tidal air through the respiratory tracts. Particulate properties such as geometric size, shape and density determine the inertia acting on particles during their travel through the airway and thereby determine their deposition along the respiratory tract[136–138]. This aerodynamic behavior is often characterized by the aerodynamic diameter, which represents the diameter of a sphere of unit density. Particles of the same aerodynamic diameter reach the same velocity in the air stream as the particle of interest of arbitrary density. Particle measurement techniques, such as light scattering, laser diffraction or image analysis, provide geometric diameters, which can be converted to the aerodynamic diameter using a widely accepted model that describes the relationship between the geometric diameter, density and aerodynamic diameter[139]:

$$D_a = D_g * \sqrt{\frac{\rho}{\chi \rho_0}}$$

Where D_a is the aerodynamic diameter, D_g is the geometric diameter, ρ_0 is the unit particle density, ρ is the particle density, and χ is the dynamic shape factor of the particle.

Based on the aerodynamic diameter, inhaled particles are believed to distribute along the airways via three main mechanisms: inertial impaction, gravitational sedimentation and diffusion[140, 141]. Particles with an aerodynamic diameter >5 μm lack the ability to change their trajectories with the tidal air, leading to impaction and deposition in the upper airways. The main mechanism of deposition is thus inertial impaction[142–144]. Particles with an aerodynamic diameter between 1 and 5 μm are believed to deposit mostly in the lower airways (bronchioles and alveoli) via the mechanism of gravitational sedimentation[145]. Particles with an aerodynamic diameter smaller than 1 μm remain suspended in the airstream and are likely exhaled after inhalation without being deposited in the airway. The main deposition mechanism for these particles is diffusion[145, 146]. Interestingly, as the particle size decreases to less than approximately 500 nm, lung deposition may increase[147-149].

For medications targeting the lower airways (*ie*, the deep lung), particles with an aerodynamic diameter of 1–5 μm are highly desirable. The performance of inhaled formulations is often described in terms of the fraction or dose of particles in the size range of 1-5 μm, which is termed as the fine particle fraction (FPF) or fine particle dose (FPD). Alternatively, the mass median aerodynamic diameter (MMDA), which is defined as the aerodynamic diameter at which 50% of the particles are smaller, can also be used as an indicator of the aerosol property of inhaled formulations[138].

Clearance of particles in the respiratory tract

Depending on the regional distribution and particle properties, inhaled particles are cleared primarily via three mechanisms: muco-ciliary clearance, phagocytosis, and systemic uptake[150].

Muco-ciliary clearance is the dominant clearance mechanism in the upper airway[151]. The ciliated columnar epithelium secretes mucus, which traps the particles deposited in the upper airways. These entrapped particles are propelled by the action of beating cilia in a proximal direction, causing them to be coughed out or swallowed. The majority of insoluble particles with a size >5 μm deposited in upper airways and are eliminated via muco-ciliary clearance[152]. Smaller particles are deposited in the deep lungs where muco-ciliary clearance is less functional and thus are retained longer than larger insoluble particles[135, 152–154]. Macrophages are also present in the upper airway, but phagocytosis is less dominant in this region[134, 155].

The clearance mechanisms in the deep lungs are relatively complex and depend on particle properties such as dissolu-

tion kinetics. Slowly dissolving or insoluble particles may interact with epithelial and immune cells in the lungs and be removed by muco-ciliary clearance, phagocytosis via alveolar macrophages, and endocytosis[156–158]. Phagocytosis by alveolar macrophages is believed to be the dominant clearance mechanism in the deep lungs[159, 160]. This process involves particle internalization by macrophages, followed by lysosomal digestion or removal of particle-loaded macrophages into the lymph or via muco-ciliary clearance[161–166]. Phagocytosis by macrophages is mainly responsible for clearance of particles between 1 and 5 μm in size[167–170]. Particles with a size <200 nm are not recognized by macrophages due to their small size[153, 171] and/or rapid uptake by epithelial cells[172]. The role of protein/receptor-mediated uptake has been highlighted in the translocation of a small fraction of inhaled nanoparticles to the systemic circulation[152, 153, 161, 173]. Intact nanoparticles may also enter the systemic circulation by endocytosis via alveolar caveolae[158].

Nanoparticles that undergo quick dissolution after deposition in the deep lungs may rapidly release drug, which can be absorbed into the systemic circulation[162, 163, 174]. The rate of absorption of a drug molecule is closely associated with its lipophilicity and molecular weight, whereby low-molecular-weight lipophilic drugs are the most rapidly absorbed.

Improving lung deposition

Particulate properties such as particle size, density, and surface composition play a vital role in developing effective inhalable medicines by determining the site of deposition. Thus, developing formulations with appropriate particulate properties is key to the effectiveness of inhaled medicines. Individual nanoparticles with sizes <500 nm tend to agglomerate due to strong cohesive forces, resulting in aggregates of uncontrolled sizes[175–177]. These aggregates are difficult to disperse into individual nanoparticles after inhalation, leading to inconsistent, unpredictable and often poor aerosolization[84, 178]. Hence, nanoparticles are often administered as particles/droplets with 1–5 μm aerodynamic diameters. Nebulizer devices can convert nanoparticle suspensions into highly inhalable droplets. Alternatively, particle engineering can convert nanoparticles into uniformly sized inhalable particles.

Nanoparticles as inhalable droplets

Typically, nanoparticle suspensions are aerosolized into droplets with appropriate aerodynamic diameters using currently available inhalation devices. Nebulizers and pressurized Metered Dose Inhalers (pMDI) are employed to assist nanoparticle inhalation.

Nebulizers

The nebulizer is the most commonly used device for inhaled delivery of nanoparticle suspensions[179]. In general, nebulizers utilize compressed air to convert a suspension of nanoparticles into inhalable droplets[180]. For example, aerosolization of telmisartan and losartan bearing a solid lipid nanoparticle suspension using a jet nebulizer resulted in a FPF >70% and was deposited into the lungs in separate *in vivo* inhalation experiments[119]. Aerosols of nanoparticle suspensions exhibit a higher FPF than drug solutions after nebulization, indicating the suitability of nanoparticles for inhalation delivery[96, 125]. There have been concerns about the negative effects of nebulization on the structure of delivery vehicles, especially lipid-based particles as well as susceptible drugs and genes[181]. Mainelis *et al* demonstrated that the one-jet collision nebulizer facilitated the deposition of liposomes containing doxorubicin and siRNA into the deep lungs without compromising liposome integrity and the biological activity of susceptible antisense oligonucleotide[182]. The bulky traditional jet nebulizers are not convenient to use; more portable and efficient nebulizers, such as vibrating mesh nebulizers, have recently been developed[180, 183–186]. The mesh nebulizer was used to aerosolize a paclitaxel lipid nanocapsule suspension and showed an FPF >80% without altering the primary properties of the lipid nanocapsules[181].

Pressurized metered dose inhaler (pMDI)

The pressurized metered dose inhaler (pMDI) creates small inhalable droplets of drug suspended in compressed propellant (*ie*, hydrofluoroalkane [HFA]). The small size of pMDI devices thus offer greater portability and can be used for inhaled delivery of the nanoparticle suspension. Conti *et al* showed that pMDI can convert a dendrimer–siRNA complex suspension into highly respirable droplets, leading to an FPF of 77%. The integrity and biological activity of siRNA in dendriplexes formulated for pMDIs remained intact after long-term exposure to the propellant HFA[187]. However, the application of pMDI technology is limited due to the typically low efficiency, with only approximately 10% of the aerosol emitted from pMDIs being deposited in the deep lungs[188]. Usage error by patients who lack hand-mouth coordination may also lead to low delivered doses[189–191]. Furthermore, pMDIs are unable to deliver high-dose medications[180].

Nanoparticles as inhalable particles

Delivery of nanoparticles as a suspension often requires the nanoparticles to be stored in a liquid medium. Long-term storage as a liquid suspension may lead to physico-chemical instabilities such as aggregation, hydrolysis of polymer and drug leakage/degradation[192, 193]. Formulating nanoparticles as a dry powder offers greater long-term stability than as a suspension[192, 193]. Additionally, the majority of DPIs are breath actuated, avoiding the problem of coordinated inspiration and actuation. Controlling the size of nanoparticles is central for their formulation into reliable and efficient inhalable dry powders. Nanoparticles can be dried with/without excipients via spray-drying, freeze-drying and spray freeze-drying to generate stable and uniformly sized inhalable particles. A number of strategies have been explored to engineer nanoparticles into inhalable particles, which are discussed below.

Blending with carrier particles

Small particles with sizes <10 μm are highly cohesive and exhibit poor flow and inhalation performance[194, 195]. Such

cohesive particles are often formulated as "interactive mixtures" to improve their flow and dispersibility[196]. Interactive mixtures represent powders in which small particles are adhered to the surfaces of large carrier particles[197–199]. Kalantarian *et al* showed that mixing of 5-FU nanoparticles with lactose particles (Pharmatose® 80) led to a low FPF of ~20%[178]. Such a low efficiency of interactive mixtures is often attributed to inefficient de-agglomeration and poor detachment of drug particles from carrier particles upon inhalation[200].

Enlargement by co-drying with carrier/excipient

Nanoparticle aggregates

Co-drying nanoparticles with excipients lead to the formation of inhalable nanoparticle aggregates in an excipient matrix[201–203]. Azarmi *et al* used spray-freeze-dried doxorubicin nanoparticles with lactose to produce particles with an aerodynamic diameter of ~3 μm[204]. FPF of the PLGA nanoparticle containing 6-3-hydroxyl-7H-indeno[2,1-c]quinolin-7-one dihydrochloride (TAS-103) improved from <1% to >10% after spray-drying with trehalose[84], although it still displayed low aerosol performance. Upon inhalation, TAS-103-loaded PLGA nanoparticles provided 300 times higher drug concentration in the lungs of rats than those in plasma. The drug lung concentrations in rats were also 13-fold higher with TAS-103-loaded PLGA nanoparticles compared with the free drug administered via the intravenous route[84]. Some studies have shown that the carrier excipients dissolve and release primary nanoparticles upon deposition and thus achieve the aerosolization properties of microparticles while maintaining the release benefit of nanoparticles[84, 204, 205]. L-leucine is a commonly used force-control agent that is known to reduce inter-particle cohesion and improve the dispersibility of small particles[206, 207]. El-Gendy *et al* showed that the particle sizes of paclitaxel-cisplatin nanoparticles and L-leucine freeze-dried nano-aggregates were ~1–5 μm, which demonstrated an excellent FPF of >70%. Furthermore, L-leucine showed no cytotoxic effect up to 5 mg/mL in A549 cells[208]. Varshosaz *et al* spray-dried doxorubicin-loaded bovine serum albumin nanoparticles with trehalose, mannitol and L-leucine in which mannitol enabled a higher FPF than trehalose; L-leucine was abandoned in this study due to the formation of irregularly shaped particles[209].

Effervescent particles

Ely *et al* introduced the effervescent technology, which involves spray-drying nanoparticles with effervescent excipients to enhance aerosolization and provide an effervescent effect for the quick release of nanoparticles upon dissolution of the excipients in aqueous media[210]. The effervescent effect is typically achieved by the combination of sodium bicarbonate and citric acid with ammonia. The pH of the feed solution is kept low to retard effervescing during the particle formation or drying process[210]. The effervescent technology has also been explored to facilitate inhaled delivery of nanoparticles against lung cancer. Azarmi *et al* showed that nanoparticles spray-dried with effervescent excipients achieved an MMAD of ~5 μm, and animals (BALB/c nude mice) receiving effervescent particles showed no change in body weight or morbidity, indicating the safety and tolerability of the inhaled carrier system[211]. It has been shown that an effervescent carrier containing doxorubicin-loaded NP nanoparticles distributed throughout the lungs and released primary nanoparticles in the lungs[212]. Mice receiving doxorubicin-loaded n-butylcyanoacrylate nanoparticles that were spray-freeze-dried with effervescent excipients survived longer compared with those receiving intravenous doxorubicin solution or inhaled free doxorubicin[71]. Jyoti *et al* demonstrated that effervescent carriers improved the aerosolization and also increased the release of anticancer agent (9-bromo-noscapine) from the nanoparticles, leading to greater anticancer activity compared with non-effervescent carriers[73].

Improving tumor targeting

Lung cancer cells are often located at specific sites in the lungs (*ie*, only in one lobe). However, inhaled chemotherapeutic agents may distribute uniformly throughout the lungs. Targeting inhaled nanoparticles specifically to the tumor cells is another approach to improve the safety and efficacy of inhaled chemotherapy.

Magnetic targeting

Drugs co-formulated with magnetically active particles can be guided to a specific location in the body using a strong external magnet[213–216]. As this process involves physical force to facilitate drug targeting, this concept of drug delivery is termed physical targeting. A range of pure metals and alloys can be used for this purpose, including iron oxide, cobalt, nickel, platinum and magnesium[217]. Magnetic nanoparticles have been shown to facilitate drug deposition in specific lung regions of mice with the help of a permanent magnet[218–221]. McBride *et al* spray-dried superparamagnetic iron oxide nanoparticles (SPIONs) with lactose and doxorubicin to form particles with an aerodynamic diameter of 3.27±1.69 μm. Such formulations showed more than twice the spatial deposition and retention in the regions under the influence of a strong magnetic gradient compared to a liquid suspension in an *in vitro* tracheal mimic study[222]. Verma *et al* showed that inhaled quercetin-loaded PLGA-coated magnetic (Fe_3O_4) nanoparticles showed marked *in vitro* anticancer activity and were well tolerated in mice with no signs of lung toxicity[223].

Reducing phagocytic clearance

Particle engineering provides efficient control over particle size to generate inhalable nanoparticles and minimize mucociliary clearance in the upper airways. Nevertheless, particles deposited in the deep lungs are still subjected to clearance by phagocytosis, which can reduce the efficacy of inhaled chemotherapy. Alveolar macrophages can engulf particles <5 μm, depending on their physico-chemical properties such as size and surface chemistry[224–226]. Thus, an ideal pulmonary delivery system should circumvent the clearance of drug from the lungs. Unfortunately, only a few investigations have studied

the effect of phagocytosis on the anti-tumor efficacy of inhaled nanoparticulate chemotherapeutics.

Large porous particles

Edward *et al* introduced the concept of large porous particles, which possess large geometric sizes ~10 μm but exhibit aerodynamic diameters <5 μm due to their low density[227–229]. The large sizes of these porous particles enable them to overcome interparticle forces, facilitating good aerosol performance and improving deposition in the deep lungs. Moreover, such large particles may escape phagocytosis by alveolar macrophages[227, 228, 230]. Spray-drying emulsions containing phospholipids and propellants have been developed to produce low-density hollow particles[227, 228, 230–232].

Recent studies have shown the feasibility of using porous particles to improve the inhalation of nanoparticles. Tsapsis *et al* reported that nanoparticles can form large porous/hollow 'Trojan' particles under specific spray-drying conditions with or without excipients, which can disintegrate into individual nanoparticles upon reconstitution[225]. It was proposed that spray drying conditions that generated high Péclet numbers could form large porous particles[225]. The Péclet number is dimensionless and describes the mass transport of solutes in drying droplets. It is defined by the following equation[225]:

$$P_e = \frac{R^2}{DT_d}$$

where P_e is the Péclet number, R is the radius of the droplet, D is the diffusion coefficient of the nanoparticle, and T_d is the time required for the droplet to dry.

When $P_e \ll 1$, nanoparticles diffuse towards the center of the receding droplet by diffusion, yielding relatively dense dried particles. However, when $P_e \gg 1$, nanoparticles do not have enough time to redistribute to the center of the receding droplet, leading to their accumulation at the air-water interface. Further drying leads nanoparticles to be held together by physical forces (*eg*, van der Waals forces) or embedded in an excipient matrix forming a shell earlier in the drying phase. The increased vapor pressure ruptures the cell, and water vapors escape in the final phase of drying leading to formation of porous particles[225]. The physical properties, including the porosity and morphology of such large porous particles, were shown to depend on the nanoparticle size, chemical nature, excipients used, and nanoparticle concentration in the resultant particles[224, 225, 233]. Hadinoto *et al* investigated the effect of phospholipids on the formation of such large porous particles. The phospholipid concentration was shown to govern the degree of hollowness of the resultant particles[224]. Furthermore, the release of drugs was shown to depend on the degree of hollowness[233]. However, to date, no studies have employed the porous particle platform for the inhaled delivery of anticancer drugs, which could be potentially useful for inhaled chemotherapy.

Swellable hydrogel particles

El-Sherbiny *et al* developed swellable hydrogel particles as carriers to prevent macrophage uptake of nanoparticles[234, 235]. PEG-g-NPHCs self-assembled nanoparticles of a model protein, bovine serum albumin (BSA), were prepared and encapsulated in sodium alginate via spray-drying followed by ionotropic gelation using Ca^{2+} ions. The coated particles had aerodynamic diameters of ~3 μm and a relatively low FPF of ~30%. The microspheres showed swelling that was followed by enzymatic degradation[235]. The coated hydrogel particles demonstrated significantly delayed phagocytosis[234]. Such swellable hydrogel inhalable particle may be attractive for inhaled delivery of nanoparticulate chemotherapy against lung cancers.

Surface-modified particles

Surface coating and conjugation of actives with polyethylene glycol (PEG) have been shown to reduce clearance from the lungs. This phenomenon was attributed to the ability of PEG to facilitate muco-penetration and reduce uptake by alveolar macrophages[226, 236–239]. Luo *et al* demonstrated that the conjugation of paclitaxel with PEG not only improved its lung residence time but also enhanced the anticancer activity in a mouse model of lung cancer[240]. Paclitaxel conjugated with higher-molecular-weight PEG demonstrated greater *in vivo* anti-tumor activity compared with lower-molecular-weight PEG[240]. PEGylation was also shown to reduce the lung inflammation and enable a higher tolerable dose than using free paclitaxel alone[240].

Surface coating of particles with a lung surfactant (1,2-dipalmitoylphosphatidylcholine [DPPC]) has also been shown to reduce phagocytosis[241, 242]. In the presence of phospholipids, the adsorption of opsonic proteins on inhaled particles is inhibited, which allows inhaled particles to escape phagocytosis[241, 242]. Meenach *et al* generated inhalable lung surfactant-mimic phospholipid and PEGylated lipopolymer nanoparticles using advanced organic spray-drying process[243]. Spray-drying at optimal temperatures facilitates the formation of more inhalable particles[243]. Inhalable lung surfactant (DPPC/DPPG)-based carrier particles loaded with paclitaxel demonstrated an excellent FPF of >70% and enhanced anti-tumor activity compared with free paclitaxel[244]. However, *in vivo* studies investigating the efficacy of such inhalable surfactant modified nanoparticles against lung cancer are scarce.

Conclusion

Chemotherapy through pulmonary delivery is believed to achieve much higher drug concentrations in the lungs and reduce systemic drug exposure. This technology could offer a promising alternative to the oral and parenteral delivery of chemotherapies for the treatment of lung cancers. Nevertheless, effect of high concentrations of inhaled anticancer drugs in the lungs centers on local toxicity remain largely unknown. Moreover, the distributions of most inhaled free anticancer drugs in the lungs are not tumor-specific. Nanoparticle formulations are promising for the inhaled delivery of chemotherapeutics against lung cancer. Nanoparticles may encapsulate toxic drugs and release them in a more site-specific

and controlled manner. Additionally, nanoparticles can carry multiple drugs, DNAs and RNAs, as well as imaging agents.

Recent research efforts have focused on enhancing the lung tumor deposition of inhaled drug delivery systems as well as minimizing their clearance from the lungs to maximize the efficacy and control the side effects. There are a few challenges for the pulmonary delivery of nanoparticles, largely stemming from their extremely low mass and cohesive nature.

Only the fraction of drug liberated from nanoparticles is able to exert anticancer activity. Due to analytical limitations, it is difficult to quantify the fraction of drug liberated from the nanoparticles rather than the total bound and unbound fraction of drug, making it difficult to assess the true potential of nanoparticles for improving drug penetration/uptake. Furthermore, the drug is typically quantified in the whole lung rather than the lung tumor, which may further add to the uncertainty about the true targeting potential and hence the anti-tumor efficacy of nanoparticles. Moreover, physicians typically prefer systemic routes over the inhaled route due to greater predictability and reliability (drug deposition may vary due to different lung functions of the patients). Thus, further improvement of aerosolization technology to enhance control over the dose, reliability and predictability of the inhaled drug fraction is desirable.

It is possible to utilize particle engineering and ensure consistent and highly efficient delivery of nanoparticles to the lungs through nano-aggregates, large porous particles, and other formulation techniques. Furthermore, physical targeting by magnetic nanoparticles and active targeting by ligand anchoring have shown the potential to enhance tumor targeting and improve the efficacy of inhaled anticancer drugs. Nanoparticles have also been shown to facilitate the co-delivery of anticancer drug with anti-sense oligonucleotides, making them an attractive candidate against drug-resistant lung cancers. Particle size enlargement and surface modification (*eg*, with PEG and surfactants) have been suggested to be effective for reducing the phagocytic clearance of nanoparticle formulations. In conclusion, inhaled nano-particulate chemotherapy bears great potential for the treatment of lung cancer. Efforts are needed to further investigate the safety and efficacy of this technology in clinical settings.

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