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Nanomedicines for targeted pulmonary delivery: receptor-mediated strategy and alternatives

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Pulmonary drug delivery of nanomedicines is promising for the treatment of lung diseases; however, their lack of specificity required for targeted delivery limit their applications. Recently, a variety of pulmonary delivery targeting nanomedicines (PDTNs) has been developed for enhancing drug accumulation in lung lesions and reducing systemic side effects. Furthermore, with the increasing profound understanding of the specific microenvironment of different local lung diseases, multiple targeting strategies have been employed to promote drug delivery efficiency, which can be divided into the receptor-mediated strategy and alternatives. In this review, the current publication trend on PDTNs is analyzed and discussed, revealing that the research in this area has been attracting much attention. According to the different unique microenvironments of lung lesions, the reported PDTNs based on the receptor-mediated strategy for lung cancer, lung infection, lung inflammation and pulmonary fibrosis are listed and summarized. In addition, several other well-established strategies for the design of these PDTNs, such as charge regulation, mucus delivery enhancement, stimulus-responsive drug delivery and magnetic force-driven targeting, are introduced and discussed. Besides, bottlenecks in the development of PDTNs are discussed. Finally, we highlight the challenges and opportunities in the development of PDTNs. We hope that this review will provide an overview of the available PDTNs for guiding the treatment of lung diseases.

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1. Introduction

Pulmonary drug delivery systems (PDDSs) use the lungs as the route of administration, enabling the inhalation and absorption of therapeutic agents, such as active pharmaceutical ingredients (APIs), through the bronchial epithelium. PDDSs include nebulizers, metered-dose inhalers (MDIs), soft mist inhalers (SMIs) and dry powder inhalers (DPIs),[1] offering a non-invasive and patient-friendly approach for drug delivery that enhances patient compliance. In addition, they are versatile delivery systems, which are capable of facilitating both the local deposition and systemic delivery of therapeutic agents.[2,3] In comparison to other administration routes, PDDSs demonstrate remarkable efficiency in the treatment and management of localized lung diseases by achieving a high deposition of therapeutic agents in the lower respiratory tract, while minimizing systemic side effects. For instance, respiratory conditions such as asthma and chronic obstructive pulmonary disease (COPD) often require corticosteroid treatment, which can have adverse effects when distributed systemically. Therefore, inhaled corticosteroids are preferred over orally administered corticosteroids. Consequently, PDDSs such as inhaled therapies hold great promise for the targeted delivery of therapeutic agents to the lungs.

Further, with the development of nanotechnology, multiple pulmonary delivery nanomedicines (PDNs) have been reported for the functionalization of PDDSs,[4–6] where nanomedicines refer to nano-sized (10^0–10^2 nm) particulate drug delivery systems.[7] Accordingly, owing to their ultrasmall size and diverse materials, the solubility of APIs can be significantly enhanced and the release profile of diverse payloads can be accurately controlled, promoting the therapeutical efficiency for local lung diseases.[8,9]

Although nanomedicines are typically administered as injectables, research has demonstrated the potential of utilizing nanomedicines for the local delivery of therapeutic agents, such as mRNA and viruses, directly to the lungs.[8,9] Owing to their favorable safety profiles and nebulization potential, lipid-based and polymeric PDNs have gained significant attention,[10–12] *e.g.* liposomes,[4] solid lipid nanoparticles (SLN),[13] nanostructured lipid carriers (NLC),[5] polymeric micelles,[14] gelatin nanoparticles,[15] and poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles.[16] These nanoparticles have been

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demonstrated to facilitate the effective delivery of various entities, including small molecule drugs, siRNA, and genetic materials to manage lung diseases such as adenocarcinoma and acute lung injury. Furthermore, in 2018, the United States Food and Drug Administration (FDA) approved Arikayce®, a novel inhalable lipid nanoparticle formulation, for the treatment of refractory nontuberculous mycobacterial (NTM) lung infection caused by *Mycobacterium avium* complex (MAC),[1] suggesting that PDNs may be a promising approach for the treatment and management of rare or challenging-to-treat diseases. Thus, PDNs represent next-generation PDDSs and attracted tremendous attention from researchers worldwide. To date, numerous reviews have been published, summarizing the development of PDNs, which focused on diseases,[17–19] nanomaterials,[20–22] APIs[18,23] and physiologic barriers.[1]

However, despite their promising role in the treatment and management of lung diseases, PDNs have several shortcomings. Importantly, PDNs often lack the specificity required for the targeted delivery of therapeutic agents. In this case, with a deep understanding of the microenvironmental features of lung lesions, the targeted delivery of therapeutic agents to the lungs can be accomplished with pulmonary delivery targeting nanomedicines (PDTNs) for increased delivery efficiency. The most-widely employed targeting approach is receptor-mediated strategy. For example, studies have shown that a variety of receptors is overexpressed in lung cancer cells, including CD44, transferrin (Tf), and mannose receptor.[24–26] Moreover, the mannose receptor was reported to be overexpressed on the surface of macrophages in the event of a lung infection.[27,28] In SARS-CoV-2 infected lungs, the human angiotensin-converting enzyme 2 (ACE2) membrane receptor was identified as a specific receptor expressed on host cells, and therefore employed as the target in the design of PDTNs for COVID-19 treatment.[29] Conversely, receptors can also be used to target macrophages and dendritic cells to suppress lung injury. Neutrophil-derived nanovesicles with abundant chemokine receptors were reported to suppress inflammatory cell infiltration by down-regulating and neutralizing cytokines.[30] Besides membrane receptors, other novel strategies including charge regulation, mucus penetration enhancement, stimulus-responsive drug delivery and magnetic force-driven strategies have also been established. The diverse charge of the membrane of different cells was also considered a potential target for accurate drug delivery. The employment of cationic host defense peptides (HDP) is a typical example, which enhance the interaction with the anionic interface of biological membranes, especially that of bacteria.[31,32] This strategy successfully permeabilized the mycobacterial membrane for the delivery of anti-tuberculosis drugs.[31] Another innovative strategy for targeted delivery to the lung is the use of magnetic iron oxide nanoparticles. By formulating nanoparticles with magnetic iron oxide nanoparticles, drug release could be localized to a desired site *via* the application of an external magnetic field.[33] This technique successfully increased the drug concentration in the lung tissues with minimal drug distribution to the liver and kidneys, which tremendously improved the therapeutic efficacy and reduced the adverse effects commonly associated with off-target binding. Clearly, strategies to develop PDTNs such as surface engineering and the incorporation of magnetic iron oxide nanoparticles can further enhance their recognition ability, and by extension, specificity for the targeted delivery of therapeutic agents and reduce off-target effects.

Hence, in the present review, the specific disease microenvironment of lung cancer, pulmonary infection, lung inflammation and pulmonary fibrosis is summarized, and the current PDTN targeting delivery strategies including receptor-mediated targeting and other strategies are listed and reviewed. In addition, the bottlenecks in the development of PDTNs are discussed. Finally, we highlight the challenges and opportunities in utilizing nanomedicines for targeted pulmonary delivery.

2. Current publication trend on PDTNs

To elucidate the development trend of the studies on PDTNs, a literature survey was conducted based on Web of Science Core Collection on April 4th 2023, revealing that approximately 1279 papers on PDTN research were published in the past 22 years, which have rapidly increased with time (Fig. 1A). Over half of the papers were published by researchers from the USA and China, and the other main publishing countries included India, Australia and Germany (Fig. 1B). The majority of these publications was research papers and reviews (Fig. 1C), suggesting that the research in this area has been gaining traction over time. The top 10 journals that published articles related to PDTN research include the *Journal of Controlled Release*, *International Journal of Pharmaceutics*, and *Expert Opinion on Drug Delivery*. Most of these journals are high-quality journals in the field of pharmaceutics or novel nanomaterials (Fig. 1D). Furthermore, the number of citations increased remarkably in recent years, with approximately 7700 citations recorded in 2022 (Fig. 1E). These bibliometric results indicate that PDTNs are an emerging research focus, attracting increasing interest worldwide. Thus, considering the boost in the publications on PDTNs, it is necessary to summarize and analyze these publications systematically to identify the gaps in the existing literature and highlight new opportunities to further develop this research area.

3. Nanomedicines for targeted pulmonary delivery and treatment of local diseases

One benefit of using the pulmonary system as the route of administration is that drug deposition and accumulation in the lung can be significantly enhanced, while the systemic distribution of the drug can be efficiently avoided. To date, nanomedicines have been successfully integrated in multiple pul-

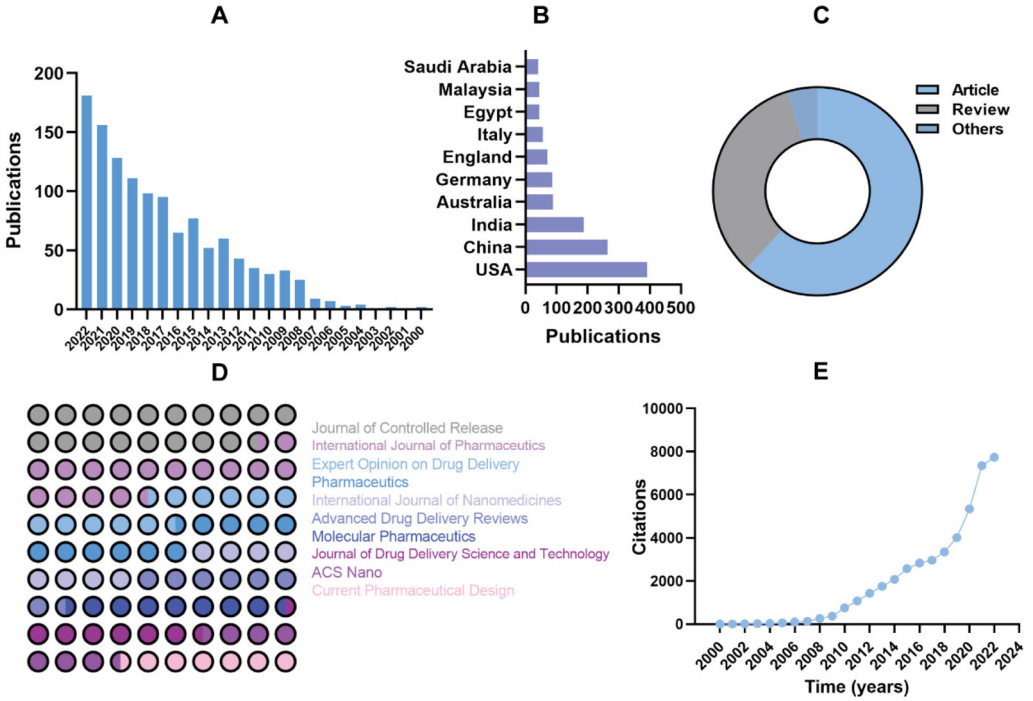

Fig. 1 Bibliometric analyses of PDTNs. (A) Number of publications *versus* year. (B) Number of publications from top-10 regions. (C) Types of publications. (D) Number of publications in top-10 periodicals. (E) Citations of these publications *vs.* time.

monary delivery formulations, such as nebulizers, MDIs and DPIs.[34–39] Nebulizers and MDIs are liquid-based formulations, whereas DPIs exist in the solid state. Nebulizers function by converting aqueous nanomedicine suspensions into aerosols through compressed air, ultrasound or vibrating mesh technology to facilitate deep lung delivery. Consequently, this method minimizes potential damage to delicate nanomedicines. However, accurately determining the dosage for nebulizer administration remains challenging, while the delivery process is time-consuming.[8,40] In addition, MDIs represent another form of pulmonary delivery formulations available in the liquid state. The metering valves integrated in the containers of MDIs effectively regulate the dosage delivered, ensuring a straightforward and prompt administration process. Upon activation of the valve, an abrupt decrease in external pressure triggers the rapid evaporation of the propellant, facilitating aerosolization, and subsequently drug delivery. However, it is worth noting that the non-polar solvent propellants primarily employed as dispersion media for APIs may not be suitable for most nanomedicines with polar surface characteristics,[41] which may cause the serious instability of nanomedicines. DPIs represent the only solid-state pulmonary delivery formulation in which drugs are loaded in solid-state particles to ensure a high drug loading and stability, enabling the delivery of micro-sized particles to lung lesions. However, the preparation of DPIs necessitates subjecting nanomedicine suspensions to either spray drying or freeze drying, potentially leading to unpredictable effects on nanostructures and drug encapsulation properties.[42] Consequently, designing an appropriate strategy to preserve the functionality of nanomedicines during translation into pulmonary delivery formulations poses a significant challenge.

Furthermore, even in the case of lung local diseases, most of the tissues in the lungs are normal, while only a small fraction is diseased. Thus, the ability to identify and differentiate normal cells from pathological cells is of great importance to enhance the efficacy of therapeutic agents delivered *via* the pulmonary route to achieve the desired therapeutic outcome. The sophisticated design of nanomedicines endows them with great potential to target deliver APIs to lung local diseases. Also, multiple strategies, such as receptor-mediated targeting, have been proposed to treat different lung local diseases. Here, the local disease microenvironments and targeting strategies are summarized and discussed in depth.

3.1 Receptor-mediated targeting strategy

By engineering the surface of nanoparticles with targeting moieties, the pulmonary route can be used to deliver nanomedicines directly to lung lesions to promote their cellular uptake. Therefore, pulmonary delivery nanomedicines can be tailored to improve their efficient delivery to lesions through the profound understanding the specific microenvironment of different lung local diseases. Here, the microenvironments and the current state of the art on nanomedicines for pulmonary drug delivery for the treatment of multiple lung diseases, including lung cancer, lung infection, lung inflammation, and pulmonary fibrosis, will be discussed. The successfully

Table 1 The current receptor-mediated targeting strategies for PDTNs

Diseases	Targeting receptors	Targeting moiety	Ref.
Lung cancer	Mannose receptors	Concanavalin A	27
	Tf receptor	Tf	25
		Lactoferrin	24
	CD44	Hyaluronic acid (HA)	6, 43 and 44
		Chondroitin	24
	LHRH receptor	LHRH peptide	45
	Biotin and RAR receptors	Biotin and all-*trans* retinoic acid (ATRA)	46
Tuberculosis mycobacterial infections	Mannose receptor of alveolar macrophages (AM)	Mannose	47–51
	CD44 of AM	HA	48
Nontuberculous mycobacterial infections	C-type lectin receptor (CLR) of AM	Fucose	52
COVID-19	ACE2 membrane receptor of host cells	ACL-modification	29
Lung inflammation	CD44 receptor of AM	HA	53
	Fc receptor of bronchial epithelium cells	Neonatal Fc receptor-targeting ligand (FcBP)	54
Pulmonary fibrosis	CD44	HA	55
		anti-CD44	56

employed receptor-mediated targeting strategies of PDTNs for local lung disease are summarized in Table 1.

3.1.1 Lung cancer. In 2020, lung cancer was reported to be the second most commonly diagnosed cancer globally.[57] Non-small cell lung cancer (NSCLC) is a subset of lung cancer with the highest incidence and mortality rate, accounting for about 85% of all lung cancer diagnoses.[58] Generally, lung cancer patients are diagnosed at the advanced stages, which is not suitable for surgical treatment. Accordingly, chemotherapy is usually the first-line treatment for these patients.[59] However, due to the high cytotoxicity of most anti-tumor drugs used in chemotherapy, the ability to distinguish between normal cells and tumor cells is of great significance but remains a major challenge for in the design of PDNs. Thus, it is a challenge to develop PTDNs to deliver anti-tumor drugs more efficiently by inhalation. To date, multiple receptors including mannose, Tf, CD44 (hyaluronate), retinoic acid (RAR), and biotin receptors have been used for the targeted delivery of nanomedicines. These receptors were deemed to be suitable targets because they were reported to be overexpressed on lung cancer lesions.

Vaghasiya *et al.* reported the preparation of inhalable concanavalin A (con-A)-decorated gelatin nanoparticles (CCG-NP) to deliver cisplatin to lung tissues by targeting mannose receptors.[27] The results showed that compared to the blank gelatin nanoparticles without con-A modification, CCG-NP exhibited a 2.2-fold higher cellular uptake in the lung cancer A549 cell model, indicating mannose receptor targeting cellular uptake. Consequently, the viability of the A549 cells treated with CCG-NP was significantly lower than that with non-surface-decorated cisplatin-loaded gelatin nanoparticles. This study highlights the feasibility of mannose receptor-targeting nanocarriers for lung cancer treatment.

Besides the mannose receptor, the Tf receptor family is also widely reported in tumor targeting, which aids in the transport of iron through blood plasma to cells. Parvathaneni *et al.* proposed the use of Tf ligand-conjugated inhalable amodiaquine (AQ)-loaded PLGA nanoparticles (Tf-AQ NPs) for the treatment of NSCLC.[25] Two cell models, A549 and H1299, were employed to evaluate the cellular uptake efficiency. Using coumarin-6 as a substitute for AQ, it was reported that the cellular uptake of the Tf-modified nanoparticles was ~6.7- and ~2.8-times higher by the 1549 and H1299 cells, respectively, compared to coumarin-6 in plain solution. The Tf-AQ NPs showed a high fine particle fraction (FPF) value (83.2% ± 3.0%) and an aerodynamic diameter of 4.4 ± 0.1 μm, indicating desirable aerodynamic properties for lung deposition. The pharmacodynamic study also showed an enhanced anti-tumor effect with Tf-modification both *in vitro* and *in vivo*.

CD44, a hyaluronate receptor and transmembrane glycoprotein found to be overexpressed on the surface of NSCLC cells, was reported as a well-established lung tumor targeting moiety.[43,44] Hyaluronic acid (HA) is a typical specific CD44-targeting material with high safety and easy functionalization potential. Wang *et al.* formulated HA-modified solid lipid nanoparticles (SLN@HA) for the co-delivery of cisplatin and β-phenethyl isothiocyanate (PEITC) for lung cancer ferroptosis therapy[6] (Fig. 2). The cellular uptake results showed about 2-times higher internalization of SLN@HA than SLN. More importantly, pretreatment with free HA significantly reduced the cellular uptake of SLN@HA, further indicating its CD44-mediated targeting ability. The *in vivo* results demonstrated the high co-localization distribution between nanoparticles and tumor tissues in the lungs, strongly supporting the specific delivery of SLN@HA. Besides CD44, the luteinizing hormone-releasing hormone (LHRH) decapeptide was

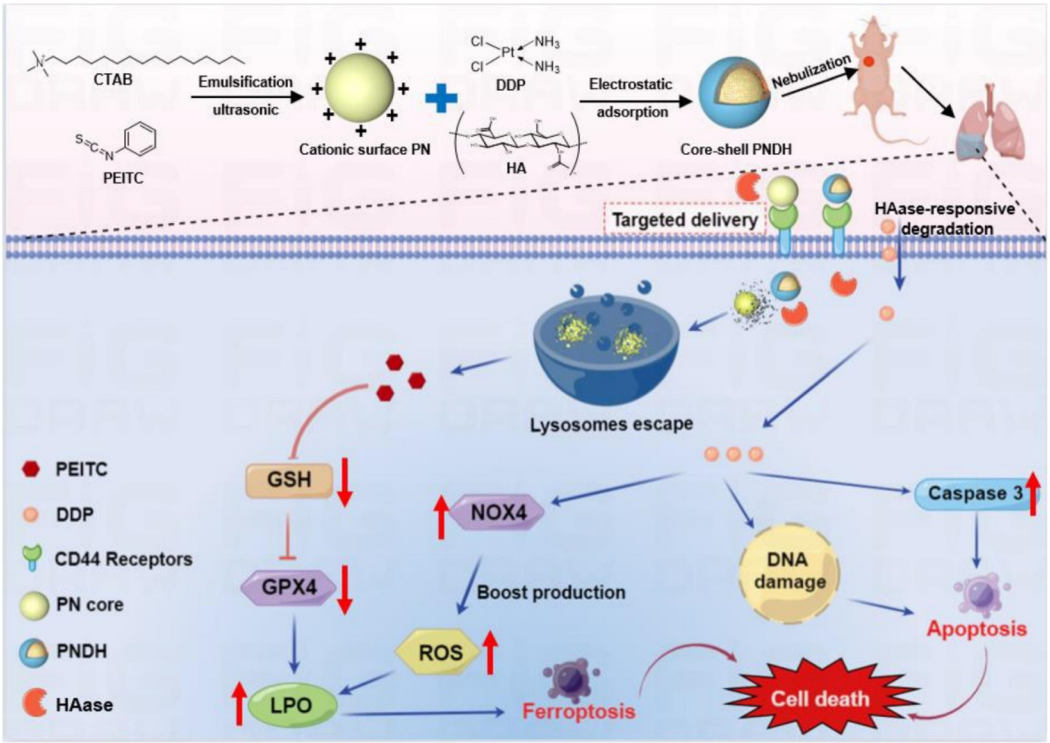

Fig. 2 Schematic illustration of SLN@HA for lung cancer targeting by pulmonary delivery. Reproduced from ref. 6 with permission from Elsevier.

employed for lung cancer targeting, and an enhanced therapeutic effect was also revealed.[45]

Although significant achievements have been made in the design and formulation of receptor-targeting PDTNs, the relatively slow turnover of receptors may result in the saturation of these receptors, thereby hampering the application of these PDTNs. Thus, a dual-targeting design was proposed to increase the selectivity and avoid potential off-target effects. Abdelaziz *et al.* reported the preparation of inhalable lactoferrin (Lf) and chondroitin dual-functionalized nanoparticulate liquid crystals (LCNPs) for the co-delivery of pemetrexed and resveratrol (Lf/CS-PEM-RES-LCNPs).[24] The Tf receptor-targeting ability of Lf and CD44-targeting ability of chondroitin were combined to promote the tumor-specific delivery. Enhanced cellular uptake and cytotoxicity effects were revealed compared to the non-targeting PEM-RES-LCNPs. The nanoparticles were further engineered as DPIs and had an FPF of about 61.6%, showing great promise as a pulmonary delivery system. Similarly, Kamel *et al.* prepared an inhalable dual-targeted hybrid lipid nanocore–protein shell composite consisting of biotin and ATRA.[46] Biotin is a ligand in multiple receptors such as streptavidin, neutravidin and avidin overexpressed on the cell surface, while ATRA is the typical target of RARs. The dual-targeting nanoparticles exhibited better cell internalization capacity compared to the single-targeting ones. These studies highlight the potential of dual-targeting PDTNs as a promising strategy to promote cellular uptake, improve tumor retention, and reduce off-target binding.

3.1.2. Lung infection. As the main gas-exchange organ of the body, the respiratory tract is susceptible to pathogen invasion, leading to lung infection. Induced by bacteria or viruses, lung infections are often debilitating and can sometimes be fatal.[47,60] Furthermore, the deep colonization nature of pathogen make it difficult for the traditional DDS to deliver drugs to infected lesions, resulting in unsatisfactory therapeutic outcomes.[61,62] Recently, the development of PDTNs shed light on the treatment of lung infection due to their versatility, showing great promise as a strategy for targeted therapy, and formulation strategies can be used to tune, modify, and control their payload release profile. Here, the progress of PDTNs in the treatment of different lung infections is summarized.

According to the data provided by the World Health Organization (**https://www.who.int/teams/global-tuberculosis-programme/**), the estimated tuberculosis incidence was 10.6 million in 2021. Tuberculosis, which is induced by *Mycobacterium tuberculosis* (MTB) infection, is one of the main causes of death, accounting for about 1.5 to 1.7 million deaths in 2021. Among the tuberculosis cases reported worldwide, about 80% was diagnosed as pulmonary tuberculosis, suggesting the importance of tuberculosis-treating PDTNs,[63] especially for the eradication of intracellular MTB.[64] When MTB invades the respiratory tract, it is captured by the mucus-secreting goblet cells and bypasses the mucociliary clearance system. Finally, MTB will be phagocytosed by AM due to the interaction between the MTB surface lipoarabinomannan and the surface mannose receptor of AM.[65,66] Thus, tuberculosis is challenging to treat because of MTB residing and surviving inside AM, and in this case, AM-targeting drug delivery is expected to exert a more efficient antibacterial effect.

AM-targeting strategies, including mannose receptor targeting and CD44 targeting, have been successfully employed.[28] Ma *et al.* designed a mannose-modified macrophage-targeting SLN loaded with a pH-sensitive prodrug of isoniazid (INH-CHO), denoted as MAN-IC-SLN, for intracellular anti-tuberculosis therapy.[47] The rationale for designing MAN-IC-SLN was two-fold, where firstly, the mannose on the surface of SLN could target and bind to the mannose receptor on AM for the cellular uptake and internalization of SLN. Secondly, the intracellular acidic environment would convert the prodrug (INH-CHO) back to isoniazid. The results revealed that the cellular uptake of AM was promoted from about 42.4% to 97.2% due to the mannose modification. Also, about 1.4-times higher drug release was recorded at pH 5.5 than that at pH 7.4. After inhalation, the MAN-IC-SLN group showed an 83% decrease in the number of colony-forming units (CFUs), which was much higher compared to the free INH group (about 60%). This mannose receptor targeting strategy was successfully employed in many other studies to deliver INH,[48] rifampicin,[49–51] and curcumin[49] for the treatment of intracellular tuberculosis infection, and most of them were based on SLN due to its desirable safety profile and good drug-loading capacity. In addition to the typical mannose receptor-targeting strategy, the CD44 receptor was also reported to enhance the AM-targeting ability of PDTNs.[67] Similar to lung cancer cells, the CD44 receptor was reported to be overexpressed on the surface of AM. Mukhtar *et al.* prepared a DPI formulation comprised of INH-loaded nanoparticles, which contained cross-linked mannosylated chitosan (MC) and HA to target the mannose and CD44 receptors simultaneously.[48] The prepared INH-MC/HA NPs were proven to enhance the internalization by AM to activate the immune response.

Besides tuberculosis, nontuberculous mycobacterial infections also manifest as intracellular infections. Huck *et al.* prepared a fucose-derivatized liposome for the treatment of non-tuberculous mycobacterial infections by targeting CLR over-expressed on AM[52] (Fig. 3). The non-fucosylated liposome showed about 20% lower AM uptake than the fucosylated liposome, and the free fucose pretreatment remarkably reduced the cellular uptake of the fucosylated liposomes, indicating that the targeting process was CLR mediated. The proposed fucosylated liposome was further engineered as DPI, and its FPF was determined to be >70% with preserved liposomal integrity and targeting capacity. This study provides a robust strategy for AM-targeting drug delivery in the design of PDTNs.

In addition to bacteria, viruses are another main cause of lung infection. Especially, the COVID-19 pandemic has dramatically changed the world, putting a severe burden on human beings worldwide. However, although several novel anti-virus drugs have been approved by multiple countries for the treatment of COVID-19, it is worth noting that the development of SARS-CoV-2 host cells targeting PDTNs has the same importance. Inspired by the virus entry mechanism, the ACE2 membrane receptor was explored as a potential targeting

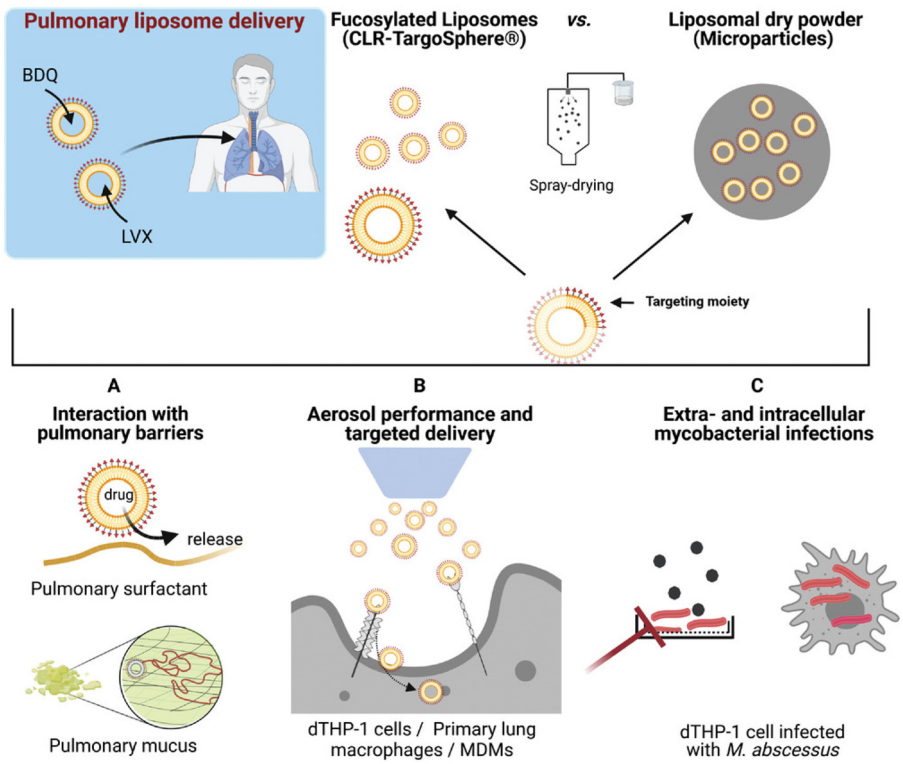

Fig. 3 Schematic illustration of the inhalable fucose-derivatized liposome for CLR-mediated targeting delivery to AM. Reproduced from ref. 52 with permission from John Wiley & Sons, Inc.

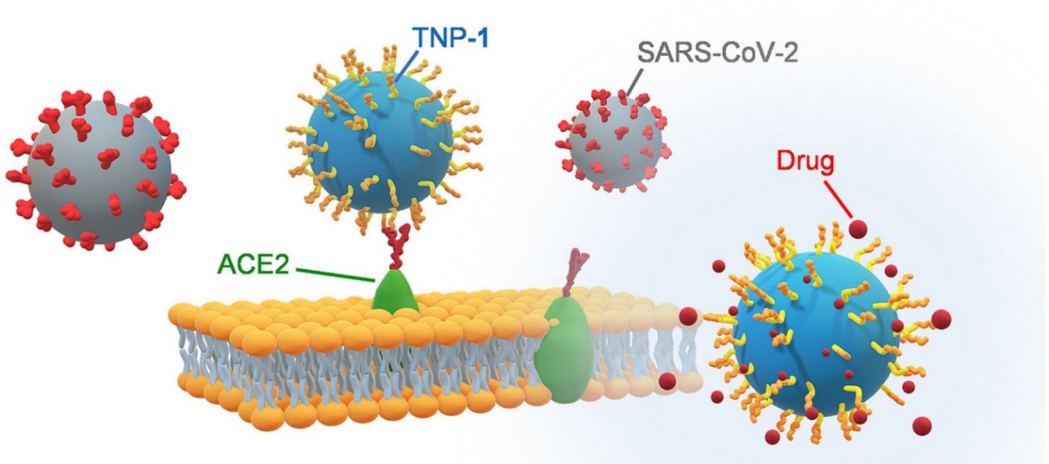

Fig. 4 Schematic representation of the design and development of various ligand-grafted polymeric nanoparticles for targeting ACE2. Reproduced from ref. 29 with permission from Elsevier.

option. Sanna *et al.* screened various ligand-grafted polymeric nanoparticles to optimize the ACE2 receptor binding capacity[29] (Fig. 4). Four targeting ligands, ACL, DCL, CPL, and TAPI-2, were used to modify the surface of remdesivir-loaded nanoparticles. According to the *in silico* simulation results and comparative binding analysis, the ACL-modified group was found to exhibit the best binding affinity to ACE2. The ACL-modified nanoparticles exhibited a significantly enhanced antiviral effect in comparison to free remdesivir. In addition, the blank ACL-modified nanoparticles without loaded drug also exhibited an antiviral effect, which can be ascribed to the competitive ACE2 binding affinity with the virus. This study demonstrate a perspective for developing ACE2 receptor-targeting PDTNs for the efficient delivery of antiviral drugs.

3.1.3. Lung inflammation. Lung inflammation, which is also defined as pneumonia, is accompanied by multiple lung diseases such as infection, COPD and fibrosis due to the overactivation of the immune system. When the respiratory tract is invaded by pathogens, immune cells such as AMs and neutrophils infiltrate the affected area due to the recognition of pattern receptors and subsequent chemotactic mediators. Following infiltration, the immune cells secrete antimicrobial factors, engage in phagocytosis, and generate reactive oxygen species, all aiding in clearing pathogens. However, once the immune cells are overactivated, severe tissue damage and pneumonia are caused by the overwhelming oxidative stress and metabolomic disruption. Furthermore, the excessive production of cytokines, and following inflammation signaling with this process result in a vicious circle, making it difficult to cure lung inflammation.

As crucial effectors in the inflammatory process, AMs and neutrophils are the desired target cells for treating lung inflammation. Thus, developing AM- or neutrophil-targeting PDTNs is a promising strategy for anti-inflammatory treatment. CD44, as mentioned previously, is a well-established receptor on AMs. Japiassu *et al.* designed an HA-decorated liposome for the delivery of dexamethasone to AMs, which is a classical anti-inflammatory drug.[53] The *in vitro* results showed that the TNF-α level secreted by M1-type AMs was reduced by about 57% with the treatment of dexamethasone-loaded HA-liposomes, which was significantly higher than the dexamethasone-loaded blank liposome group (about 30%). The *in vivo* LPS-induced lung inflammation model also showed a similar outcome, confirming the targeting capacity of the HA-decorated liposomes for AMs. Although other receptors overexpressed on AMs have not been explored for lung inflammation treatment, their potential utilization is anticipated in future research.

Given that severe pneumonia always starts from the bronchial epithelium, its cells not only secret pro-inflammatory and chemotactic mediators but are also fragile due to the overactivated immune response. Thus, targeting bronchial epithelium cells is another promising strategy for the treatment of lung inflammation. In the study by Yu *et al.*, they focused on the mucosal penetration process of inhalable nanomedicine by adjusting nanoparticle stiffness and utilizing FcBP.[54] The Fc receptor was found to be overexpressed on the bronchial epithelial cells, while the high-stiffness nanoparticles could penetrate the mucus-covered bronchial more efficiently. Interestingly, the results revealed that the high-stiffness nanoparticles showed higher internalization than their soft counterpart, while the FcBP surface modification further enhanced their uptake. The *in vivo* results also indicated that the FcBP-decorated nanoparticles with higher stiffness exhibited extended pulmonary retention and superior therapeutic efficacy. The mechanistic investigations revealed that the increased stiffness of the nanoparticles promoted the stronger aggregation of the actin filaments and enhanced Ca^{2+} signaling, thereby enhancing the neonatal Fc receptor-mediated targeting efficiency. Consequently, the manipulation of nanoparticle stiffness and the utilization of FcBPs are important considerations in the design of PDTNs for lung inflammation treatment.

3.1.4. Pulmonary fibrosis. Pulmonary fibrosis is a chronic and progressive lung disease characterized by fibroblast proliferation, excessive deposition of extracellular matrix, inflammatory damage, and structural destruction of lung tissue.[68] As an age-related progressive interstitial lung disease, pulmonary fibrosis is feature in an increasing number of patients, increasing the economic burden.[69] With a median survival of less than 5 years, pulmonary fibrosis is considered one of the most fatal diseases worldwide.[70] Furthermore, although several drugs such as nintedanib and pirfenidone have been approved for the treatment of pulmonary fibrosis, the survival and life quality have not been significantly improved. On the one hand, due to the complicated fibrosis development process, the single antifibrosis mechanism does not fulfill the requirement of efficient treatment. On the other hand, the main administration route of current drugs is oral administration, which has low delivery efficiency to lung lesions. Thus, it is urgent to develop novel PDTNs for the treatment of pulmonary fibrosis.[71]

Similar to lung cancer cells, fibrotic cells also exhibit the overexpression of the CD44 receptor, which facilitates HA-mediated motility. A recent study showed that primary lung fibroblasts isolated from the bronchoalveolar lavage of patients with collagen tissue disease-associated interstitial lung fibrosis showed elevated CD44 expression.[55] Thus, the CD44 receptor has emerged as a potential target for the design of PDTNs for the treatment of pulmonary fibrosis. Pandolf *et al.* developed HA-decorated liposomes to enhance the delivery efficiency to fibrotic cells.[72] Two different HA molecular weights (MWs), 4800 and 14 800 Da, were employed to assess the targeting performance. The results revealed that the higher HA MW was superior to be internalized by cells without inducing inflammation. In addition, the lung mucus penetration enhancement ability of the HA-decorated liposomes was also observed, implying their great potential to serve as inhaled nanomedicine. The same group also utilized anti-CD44 to functionalize gold nanoparticles (GNPs) to deliver the tyrosine kinase inhibitor imatinib.[56] The *in vitro* results demonstrated that the anti-CD44-functionalized GNPs successfully entered CD44-positive cells through specific binding. Moreover, the aerosolized CD44-targeted nanomedicine did not cause systematic toxicity, given that it showed negligible retention in other organs. These studies emphasize the feasibility of CD44-targeting PDTNs in enhancing the efficiency of pulmonary fibrosis management.

3.2 Alternative strategies

In addition to the receptor-mediated targeting strategy, numerous alternative approaches have been proposed to enhance drug delivery specifically to localized lung disease lesions. In this context, we provide a preliminary summary and discussion of the strategies involving charge regulation, mucus delivery enhancement, stimulus-responsive drug delivery, and magnetic force-driven targeting. Furthermore, we present an analysis of their applications and list successful instances in Table 2.

Table 2 Alternative strategies analyzed in this review (besides receptor-mediated strategy)

Strategies	Diseases	Ref.
Charge regulation	Lung infection	32
	Lung inflammation	73
Mucus delivery enhancement	Allergic asthma	74
	Platform study	75
Stimulus-responsive drug delivery	Idiopathic pulmonary fibrosis	76
Magnetic force-driven targeting	Platform study	77
	Pneumonia	33

3.2.1 Charge regulation. The biodistribution profile of nanomedicines is significantly influenced by their surface charge, given that it manipulates their interactions with various cells. Consequently, a charge regulation strategy has been proposed to construct PDTNs for the highly efficient treatment of localized lung diseases. Illustrative cases have demonstrated the efficacy of pathogen-targeting and immune cell-targeting approaches in treating lung infections or inflammations.

HDP, a therapeutical peptide characterized by a high density of positive charges and amphiphilic structure, has been extensively documented for its efficacy in pathogen eradication, particularly against bacteria.[78,79] Given that the bacterial membrane is primarily comprised of amphiphilic and negatively charged lipids, HDP can effectively disrupt the membrane through robust electronic interactions.[80] Therefore, it is also regarded as a desirable bacterial targeting moiety to enhance the drug delivery efficiency and optimize anti-infection outcomes.[81] For example, Sharma *et al.* reported a lung infection treatment PDTN system with the co-delivery of HDP IDR-1018 and anti-tuberculosis drugs.[32] The cationic and amphiphilic IDR-1018 could specifically deliver the anionic biofilm constructed by MTB. Consequently, a significantly reduced bacterial load in the lungs was revealed *in vivo* (about 3.02 log CFU mL^{-1}). These findings suggest that HDP holds great potential for targeted bacterial eradication. In addition, the effects of surface charge on the internalization of inhalable liposomes by immune cells were evaluated by Liu *et al.*[73] (Fig. 5). Three different liposomes with neutral, anionic and cationic surface charges were constructed, and the interactions between these liposomes and AMs or neutrophils were investigated. The findings indicated that the cationic liposomes exhibited a 3.2-fold higher cellular uptake in neutrophils compared to AMs, suggesting the potential selectivity of cationic liposomes for neutrophils. Furthermore, approximately 70.5% of anionic liposomes was taken up by AMs, highlighting the stronger interaction between AMs and anionic surfaces. This study highlights the targeting strategy in lung inflammation based on tunable surface charge.

3.2.2 Mucus delivery enhancement. The trachea and bronchial tree are lined with a protective mucus layer, which exhibits remarkable viscoelastic properties that effectively prevent

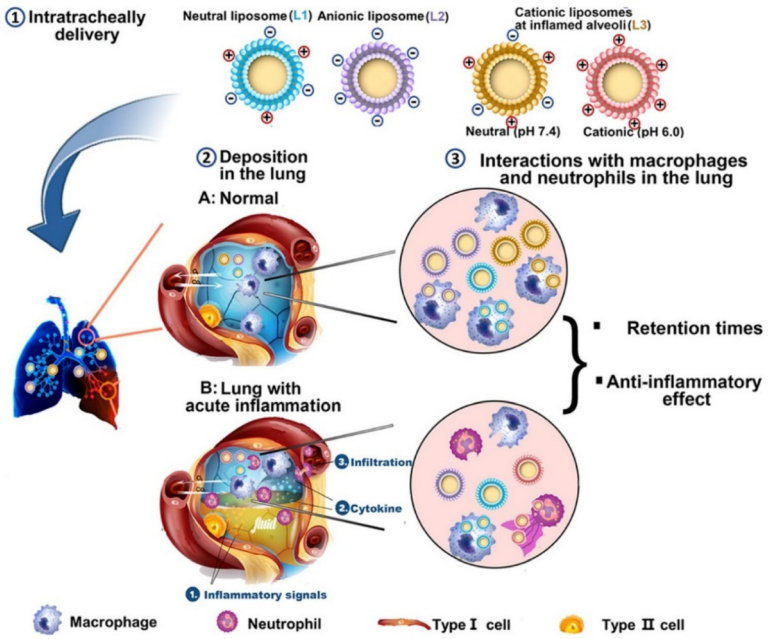

Fig. 5 Schematic representation of the tunable charged liposomes for target delivery in lung inflammation. Reproduced from ref. 73 with the permission from the American Chemical Society.

the infiltration of xenobiotics such as inhaled nanomedicines.[1] This physical barrier is primarily formed by a dense network of mucins, which are characterized by internal disulfide bonds and intricate noncovalent interactions. Moreover, the mucus possesses a negative charge due to the abundant carboxyl groups on the mucin glycan entanglement termini and phosphoric acid residues on DNA. Thus, due to these distinctive physicochemical attributes, mucus plays a pivotal role in impeding the transport of nanomedicines into lesions.[75] Therefore, various design strategies for PDTNs have been developed with the aim of enhancing their mucus delivery properties.[82] In this regard, two distinct approaches have been proposed to improve the performance of mucus delivery, namely, enhancing mucus penetration and promoting mucus adhesion.

Strategies to enhance mucus penetration have been proposed to overcome the barrier effect of mucus by reducing the interaction between nanomedicines and mucus. Our group developed engineered lipid nanoparticles with the surface modification of the zwitterionic material hexadecyl betaine (HB) and encapsulation of *N*-acetylcysteine (NAC) for enhancing mucus penetration.[75] The HB modification imparted a mucus-inert surface, inhibiting the interactions with mucins, while the encapsulated NAC effectively degraded mucins and reduced their viscosity. This engineering strategy resulted in an 8.25-fold increase in the mucus penetration efficiency. Besides mucus penetration enhancement, mucus adhesion promotion strategies have also been reported. Different from mucus penetration, the mucus adhesion strategy aims to prolong the drug residence time on the mucosal membranes, ultimately increase the drug distribution in lesions. To construct mucous adhesive PDTNs, materials with capabilities for hydrogen bonding and hydrophobic or electrostatic interactions have been widely employed to facilitate interactions with mucus components. For example, Zhao *et al.* prepared a positively charged nanogel by crosslinking arginine-grafted chitosan and tris(2-carboxyethyl)phosphine (TCEP) for mucolytic therapy in allergic asthma.[74] Upon nebulization administration, the robust electronic interactions between the arginine-grafted chitosan and mucus components endowed it with desirable mucus adhesive properties. Consequently, the released TCEP exhibited mucolytic abilities by inhibiting the formation of larger mucin aggregates *via* ionic interaction and breaking disulfide bonds. Thus, nebulization administration of the nanogel alleviated mucus obstruction and reduced airway inflammation. These findings underscore the significant importance of enhancing mucus delivery strategies in designing PDTNs for therapeutic applications.

3.2.3 Stimulus-responsive drug delivery. As previously mentioned, the microenvironments of local lung disease lesions differ from that of normal tissues. These distinctive characteristics not only offer multiple targeting receptors but also present opportunities for designing stimulus-responsive drug delivery systems. Various unique microenvironment parameters, such as overexpressed enzymes, pH levels, REDOX levels, and oxygen levels, have been successfully utilized and translated into stimulus-responsive nanomedicines for cancer therapy. These strategies have also been employed in the development of PDTNs. For instance, Zhang *et al.* developed a ribosomal protein-based mRNA nano-formulation for the inhalation therapy of idiopathic pulmonary fibrosis, incorporating a bifunctional peptide-modified corona that is responsive to

both overexpressed MMP-2 and low pH.[76] The dual-responsive peptide was designed with an MMP-2-sensitive sequence for the stimulus-triggered release of keratinocyte growth factor in fibrotic foci to suppress TGF-β1 production and enhance surfactant protein secretion, as well as a charge-reversible polymer AA-PLL-PEG-c(RGDfK) for facilitating the endosomal

Table 3 Excipients for pulmonary drug delivery approved by the FDA

Excipient name	Dosage form	CAS number
1,2-Distearoyl-*sn-glycero*-3-phosphocholine	Capsule	816944
Alcohol	Aerosol, metered, spray, liquid, solution	64175
Anhydrous citric acid	Solution, suspension	77929
Anhydrous trisodium citrate	Suspension	68042
Apaflurane	Aerosol, metered	431890
Ascorbic acid	Aerosol, metered, spray, solution	50817
Benzalkonium chloride	Solution, spray	8001545
Black ink	Capsule, powder	
Calcium carbonate	Solution	471341
Calcium chloride	Capsule	10035048
Carrageenan	Powder	9000071
Cetylpyridinium chloride	Aerosol, metered, spray	6004246
Chlorobutanol	Inhalant, liquid, solution	57158
Citric acid monohydrate	Inhalant, liquid, solution, suspension	5949291
Dichlorodifluoromethane	Aerosol, metered, spray	75718
Dichlorotetrafluoroethane	Aerosol, metered, spray	76142
Edetate disodium	Solution, spray, suspension	6381926
Ferric oxide yellow	Powder	51274001
Fluorochlorohydrocarbons	Aerosol, metered, spray	
Gelatin	Capsule, powder	9000708
Glycerin	Solution	56815
Glycine	Powder	56406
Hydrochloric acid	Aerosol, metered, spray, solution	7647010
Hypromellose 2906 (4 mPa s)	Powder	9004653
Lactose	Capsule, powder	
Lactose monohydrate	Powder	64044515
Lecithin, soybean	Aerosol, metered	8030760
Magnesium stearate	Powder	557040
Mannitol	Powder	69658
Menthol	Aerosol, metered, spray	
Methylparaben	Solution	99763
Nitric acid	Aerosol, metered, spray	7697372
Norflurane	Aerosol, metered	811972
n-Phenyl-1-napthylamine	Liquid	90302
Nutmeg oil	Liquid	8008455
Oleic acid	Aerosol, metered	112801
Petrolatum	Liquid	8009038
Phenylethyl alcohol	Inhalant	60128
Polysorbate 80	Suspension	9005656
Potassium chloride	Powder	7447407
Propylene glycol	Liquid, solution	57556
Propylparaben	Solution	94133
Saccharin	Aerosol, metered, spray	81072
Saccharin sodium	Aerosol, metered, solution	6155573
Silicon dioxide	Capsule, powder	7631869
Sodium bicarbonate	Solution	144558
Sodium bisulfate	Solution	7681381
Sodium bisulfite	Inhalant, liquid, solution	7631905
Sodium chloride	Inhalant, powder, solution, suspension	7647145
Sodium hydroxide	Aerosol, metered, solution, powder	1310732
Sodium lauryl sulfate	Capsule, powder	151213
Sodium metabisulfite	Solution	7681574
Sodium sulfate anhydrous	Solution	7757826
Sorbitan trioleate	Aerosol, metered, spray	26266580
Sulfuric acid	Capsule, solution	7664939
Thymol	Liquid	89838
Titanium dioxide	Capsule, powder	13463677
Trichloromonofluoromethane	Aerosol, metered, spray	75694
Trisodium citrate dihydrate	Powder, solution, suspension	6132043
Tromethamine	Solution	77861
Turpentine oil	Liquid	8006642
Zinc oxide	Solution	1314132

escape of mRNA. Consequently, the designed dual-peptide endowed the nano-formulation with remarkable capabilities to mitigate idiopathic pulmonary fibrosis progression, highlighting the potential of stimulus-responsive drug delivery in the design of PDTNs.

3.2.4 Magnetic force-driven targeting. Another strategy to localize nanoparticles to specific lung regions involves the use of magnetically-driven carriers. Studies have shown that a neodymium magnet can be used to orient and control the movement of magnetic nanoparticles such as superparamagnetic iron oxide nanoparticles (SPIOs). Given this property, the use of magnetic nanoparticles may be a good alternative strategy for the design of PDTNs to increase the drug distribution in lung lesions. Poh *et al.* prepared nanoparticle aggregates *via* the triple-encapsulation of Q203, bedaquiline, and SPIOs with a magnetic saturation of 28 emu g^{-1} for the management of tuberculosis.[77] Different force ratios ($F_{magnetic}/F_{Gravity}$) were compared to mimic the lung deposition process. The results showed that as the force ratio increased, the deposition efficiency of the particles improved significantly but the dispersion performance was compromised. The authors concluded that an optimal balance between deposition and dispersion occurred at the force ratio of 10. A similar study was carried out by Abdelaziz *et al.* to treat methicillin-resistant *Staphylococcus aureus* (MRSA) pneumonia, where magnetic nanoparticles were crosslinked with vancomycin, enabling the use of magnetic force to guide the nanoparticles to the desired target site.[33] These studies demonstrate a novel approach for targeting specific regions of the lung during pulmonary delivery. However, in the case of clinical trials, the potential health threats of high magnetic field gradients should be considered. The specific factors that should be considered in the evaluation of magnetic nanoparticles include their composition, size, surface composition and properties, given that these factors can influence the behavior of the nanoparticles in the body, such as their distribution, clearance, and interaction with cells and tissues.

4. Challenges and opportunities

Despite the significant efforts in the development of PDTNs, a substantial gap exists between basic theory and clinical translation. To date, no PDTNs have been translated into a clinical trial or market, implying that several challenges obstruct their development. In this context, the key challenges that have attracted significant attention are outlined, and the opportunities they present will be explored.

Firstly, there is an urgent need to identify new target sites based on a deeper understanding of the lesion microenvironment. The current selection of receptors for lung diseases is insufficient to meet the requirements for developing multifunctional PDTNs. Most of the reported research on PDTNs has focused on CD44 or mannose receptor targeting. However, the use of these receptors as targets for delivery constitutes a double-edged sword, where the CD44 and mannose receptors are two highly expressed receptors in many lung diseases, but this compromises their specific illness conditions.[45,83,84] In addition, the expression levels of these receptors may vary across different disease stages or patient conditions, necessitating the development of precise nanomedicines.[85] Addressing these challenges requires advanced and systematic pathological studies.

Another critical concern is the biological safety of the reported PDTNs. A diverse group of excipients has been used in laboratory studies, but some of these excipients are not FDA-approved or generally recognized as safe (GRAS). As shown in Table 3, only limited excipients were approved by the FDA for pulmonary delivery. Achieving the transition from academia to industrialization demands the development of novel materials with high safety profiles or the utilization of FDA-approved materials.

Furthermore, as pulmonary delivery systems, PDTNs must possess the desired aerodynamic performance to be efficiently inhaled into the deep lung.[39,86] However, many studies overlook the systemic investigation of aerodynamic performance, limiting their further applications. By employing the appropriate inhalation apparatus and combining suitable inhalation preparations (such as MDIs, SMIs and DPIs), the inhalation performance of PDTNs can be improved.

Finally, the potential morphological changes and drug leakage of soft PDTNs during the pulmonary delivery process should be carefully considered. Unlike traditional inhalable drug solutions or dry powders, the sophisticated and delicate structure of PDTNs may be compromised during inhalation.[87–89] Therefore, monitoring the drug encapsulation efficiency and the stability of targeting moieties during aerosolization is crucial when designing PDTNs.

In summary, a comprehensive approach encompassing pathological findings, excipient(s) development, and nanostructure design is essential for advancing the translation of PDTNs.

5. Conclusions

Considering the boosted incidence rate of lung diseases, the development of novel PDTNs to efficiently deliver APIs to lesions is urgent. The ultimate goal of PDTNs is to achieve improved treatment efficacy, while minimizing side effects. In this review, the current publication trends in PDTN research were analyzed and a significant increase in publications was observed, indicating the growing attention this field is receiving from the research community. Further, the lung disease lesion microenvironment was introduced, and the corresponding PDTN design was listed regarding lung cancer, pulmonary infection, lung inflammation, and pulmonary fibrosis. The specific target receptors and their binding moieties were highlighted. Finally, the challenges and opportunities in these areas were addressed, including the exploration of new targeting receptors, the selection of appropriate excipients, the enhancement of aerodynamic performance, and the rational

design of nanostructures, all aimed at improving the treatment outcomes of PDTNs. In summary, the collective efforts of scientists and engineers are of the utmost importance in advancing the development of PDTNs for the treatment of lung diseases.

Conflicts of interest

There are no conflicts to declare.

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