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Advances in therapeutic nano-drug delivery systems in infectious lung diseases: a review

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ABSTRACT

Infectious lung diseases are primary or secondary lung inflammatory diseases caused by infectious agents such as bacteria, viruses, or fungi. Oral or intravenous administration of antibiotics is the most common treatment method, but some drugs suffer from poor release stability, high systemic toxicity, and induction of drug resistance. Nano drug delivery systems (DDS) are promising alternatives for the treatment of pulmonary infectious diseases, with particular advantage in enhancing drug delivery stability and solubility, improving pulmonary accumulation, reducing systemic toxicity, and combating drug resistance. This review provides a brief overview of the mechanisms and classification of pulmonary infectious diseases, outline new approaches and ideas for representative pulmonary drug delivery methods in recent years, and briefly summarized the toxicity of the present pulmonary nano-drug delivery systems. We believe that nano-based therapeutic strategies will provide great potential to broaden the scope of treatment of infectious lung diseases and enhance the therapeutic efficacy.

Keywords: infectious lung diseases; nano-drug delivery; treatment

1. INTRODUCTION

Infectious lung diseases have become a global public health problem and a threat to public health. Infectious lung diseases are usually caused by bacteria, viruses, or fungi, causing inflammation and structural changes in the lung parenchyma or interstitial disease. It can be divided into primary and secondary infections, which are usually caused by the invasion of external pathogens. Patients with secondary infections often suffer from pulmonary or systemic underlying diseases such as cystic fibrosis (CF), primary ciliary dyskinesia (PCD), bronchiectasis, HIV etc. These diseases often lead to impairment of the physical or biological barriers of the lung and predispose the child to opportunistic infections of exogenous or endogenous origin[1]. Current treatments of infectious lung diseases are mainly antibiotics and vaccines[2, 3]. The mainstream drug delivery method is still oral administration or intravenous

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administration. As an important route of local administration, the inhalation route has attracted more attention. However, these three modes of administration have obvious advantages and certain limitations in the treatment of lung diseases. Patients who take oral medicine have high compliance and are easy to implement, but the medicine needs to be absorbed through the gastrointestinal mucosa, which is a challenge to the mucosal penetration and stability of the medicine, especially for poorly soluble drugs[4, 5]. The development of many new drugs was failed due to their low solubility[4]. Intravenous administration avoids crossing the gastrointestinal barrier, but as a systemic drug administration route, it needs to be administered in large doses to achieve a better therapeutic effect, which will inevitably cause toxicity to the normal organs (such as spleen, liver or kidney). Also, patients with intravenous drug administration have poor compliance and high treatment cost. Long-term intravenous drug injection often causes adverse reactions such as phlebitis and bacterial drug resistance. Inhalation is a local drug delivery route for the treatment of lung diseases. Through a special atomization device, such as pressurized metered-dose inhalers (pMDIs) and dry powder inhaler (DPI), drug particles can be directly inhaled into the lungs, reducing the side effects of systemic drug delivery, reducing the dosage and improving the bioavailability of drugs[6]. However, some drugs can also be deposited in the trachea, oral cavity, and other non-infected sites, causing potential damage to normal tissues[7].

To overcome the limitations of the above administration routes, scientists make traditional drugs into direct nano-scale drug particles or combine appropriate carrier materials with APIs to form nano-scale (Sizes about from 1nm to 1000 nm) particles to improve various characteristics of drugs, which is called nano-drugs. Nano-drugs have incomparable advantages over traditional drugs[8]. By using nano-materials with different carriers, the solubility, stability and tissue penetration of drugs can be greatly improved. By modifying the surface of nano-carriers, nano-carriers are endowed with the ability to target disease occurrence sites. A variety of drugs with different functions and mechanisms can also be encapsulated in nanocarriers at the same time to enhance the synergy among different drugs. In addition, nano-drugs have a good prospect of reducing the drug resistance rate for the extensive application of antibiotics in infectious diseases.

In this review, we aim to summarize new strategies for the treatment of pulmonary infectious diseases based on nano-drug delivery systems in recent years, and we focus on four carrier materials: (1) polymers (2) liposomes (3) solid liposomes (4) inorganic nanoparticles.

2. The risk factor for lung infectious diseases

2.1 Lung characteristics

One factor that makes the lungs susceptible to infection is the open airway. The trachea can be further divided into left and right bronchi, and it can repeatedly branch into 23-25 grades in the lung, forming a bronchial tree and the end of bronchiole formed cystic structure—alveoli. The surface area of alveoli is so vast that it can

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reach 100 times that of a normal skin surface[9]. This is conducive to efficient gas exchange between the body and the outside environment, but the large surface area also increases the risk of infection.

2.2 Immune factors

The immune function of the lung is maintained by natural immunity and acquired immunity. The natural immune system is highly conserved in all kinds of organisms from invertebrates to primates. It belongs to nonspecific immunity and is composed of immune cells (such as macrophages and neutrophils) and non-immune cells (such as epithelial cells). Natural immune cells recognize the main pathogenic-associated molecular patterns (PAMP) of viral nucleic acid through pattern recognition receptors (PRRS), such as toll-like receptors (TLR), to activate the downstream inflammatory pathway to anti-infection. Acquired immunity consists of cellular immunity mediated by T cells and humoral immunity mediated by B cells, dendritic cells and antigen-presenting cells (APC). APC can promote the activation of T cells and B cells, which play a key role in driving antibody production[10]. However, When the immune function is damaged, such as CD4+T cells are destroyed, infection will occur because lung immunity drops sharply. Another key immune component in lung immunity is macrophage, which includes M1 and M2 types. Type M1 usually plays an anti-infection immune role, which cause tissue destruction by secreting TNF-α and IL-12, active nitrogen and oxygen intermediates (RNI, ROI). Type M2 mainly plays an immunosuppressive role, which can promote wound healing, inhibit cytokine release and inflammatory response (Fig 1). This delicate balance between M1 and M2 protects the host from being attacked by invaders and from being damaged by the body's excessive immune response. But in some cases, cells will lose their functions.

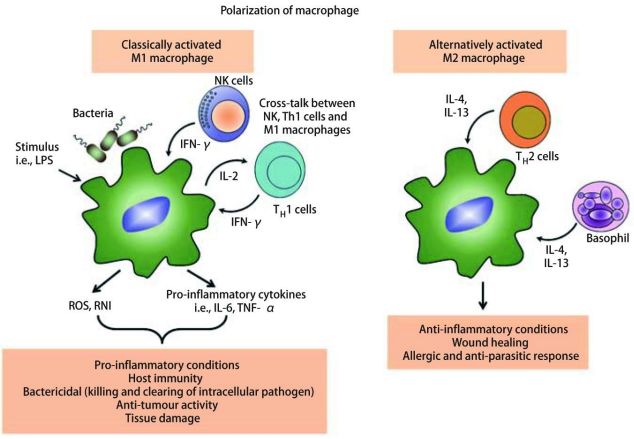

Figure1 | M1 (classical activation) and M2 (alternate activation) macrophages achieve the dynamic balance of the immune system through pro-inflammatory and anti-inflammatory effects, respectively. Copyright 2010, Thieme Medical Publishers [10].

2.3 Lung underlying diseases

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Common basic diseases that can cause lung infection such as prior pulmonary tuberculosis, cystic fibrosis and other forms of bronchiectasis, chronic obstructive pulmonary disease (COPD), silicosis and pneumoconiosis. Along with disease progression, these diseases usually cause structural and functional changes in the lungs. Also,Some bacterial and viral infections such as COVID and TB can lead to changes in the functional status of the body, in addition to metabolic diseases that cause changes in the immune environment of the body can also lead to lung infections, for example, a high glycemic environment in the lungs due to diabetes. [11, 12].

2.3.1Primary ciliary dyskinesia (PCD)

The bronchi of the lungs are lined with motile cilia that participate in mucociliary clearance. These cilia can swing excessively to remove foreign bodies such as bacteria and dust particles that enter the trachea and bronchi[13]. When the cilia can't swing due to the abnormality of the dynamin arm, bacteria may take advantage of it and causes opportunistic lung infections, such as Pseudomonas aeruginosa infection[14].

2.3.2Cystic fibrosis (CF)

Pulmonary cystic fibrosis is an autosomal recessive genetic disease caused by chloride ion transmembrane barrier caused by the expression defect of the transmembrane conductance regulator gene (CFTR), which will lead to the depletion of the mucus layer around cilia of the trachea and bronchial epithelium, and the protection of mucus layer will be lost, the colonized bacteria may become infected[15]. Pseudomonas aeruginosa (P. aeruginosa) is the most significant pathogen in CF[16]. Patients with CF have excessive mucus production in their lungs, and bacteria can produce adhesin to specifically bind with mucus, helping bacteria stay in mucus and escape the killing effect of drugs[17]. In addition, NTM is emerging as an important pathogen for secondary infections in CF patients, with Mycobacterium abscessus (Mab) receiving more attention from clinicians for its strong pathogenicity and long treatment period. Klebsiella pneumonia is not a typical pathogen of CF, but there is an increasing number of patients with secondary CF. Klebsiella pneumoniae and Pseudomonas aeruginosa share some similarities in the mechanism of infection. For example, impaired iron scavenging and iron uptake by the bacterium are the driving factors for proliferation and bio-touch formation in both bacteria, so lactoferrin, an iron scavenger, is a potential target for the treatment of infection[18].

2.3.3 Chronic Obstructive Pulmonary Disease (COPD)

The progression of COPD is often accompanied by chronic bronchitis and emphysema-like structural and functional changes. Later in the disease, bacterial and viral infections often occur. Conversely, the infections worsen COPD. Currently, the mechanism of viral infections secondary to COPD is unclear[19]. The common causative organisms in COPD patients are Haemophilus influenzae, Klebsiella pneumonia and Cataplasma, which are different from those in CF patients[20]. The boundary between colonization and infection is unclear in CF patients, and some

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colonized bacteria may transform into pathogenic bacteria and cause endogenous infections, so it is important to distinguish between colonized and infectious bacteria to clarify the source of infection. Pulmonary microbiome changes continuously in disease progression. During the development of COPD, the microbiome of the lung changes, and studies have shown that the microbial species in the sputum of COPD patients are reduced compared to healthy individuals[21, 22]. Wang *et al*.[23]suggested that chronic infection secondary to acute viral infection in some COPD patients may be caused by disruption of the host's antiviral immune response. Several studies suspected that COPD patients are more susceptible to COVID-19 infection. An article summarized and elaborated two possible mechanisms: (1)angiotensin-converting enzyme 2 is a receptor for SARS-Cov-2, and ACE2 expression is increased in COPD patients, which may increase susceptibility (2) inhaled glucocorticoid application suppresses the immune system and increases susceptibility[24].

3.Type of infectious lung diseases

3.1Pulmonary infectious diseases caused by bacterium

Bacterial infection is the main type of pulmonary infection, among which mycobacterium tuberculosis (MTB) and Pseudomonas aeruginosa are the main bacteria causing tuberculosis and Pseudomonas aeruginosa pneumonia respectively. The main pathogenic substances of bacteria are exotoxin released by gram-positive bacteria and endotoxin that the cell wall constituent of gram-negative bacteria. Bacterial toxins can damage the host cell by destroying the cell membrane, causing the ion exchange disorder of the cell or by delivering active enzyme components into the cell. Through these mechanisms, these toxins can directly damage pulmonary epithelial cells and pulmonary vessels and increase the permeability of the alveolar-capillary barrier[25].

3.1.1 Pseudomonas aeruginosa pneumonia

As mentioned above, CF patients are having a high risk in Pseudomonas aeruginosa infection, and clinical experimental data show that once colonization and infection of Pseudomonas aeruginosa in the lung occurs, it is almost impossible to eradicate it. P. aeruginosa infections of the lower respiratory tract can range in severity from colonization (without an immunological response) to severe necrotizing bronchopneumonia[26]. In the lower respiratory tract, the biofilm mode of growth of P. aeruginosa, leads to infection and chronic inflammation that can't be eliminated and causes lung function damage such as bronchiectasis and emphysema[27]. Pseudomonas aeruginosa isolated from CF patients showed significant phenotypic variations, such as mucoid phenotype and highly adherent small-colony variants (SCVs). SCVs strains showed different characteristics from wild-type (WT) parent strains, including enhanced adhesion, decreased mobility, increased biofilm formation, decreased production of pyridoxine and green pigment, and increased antibiotic resistance (β-lactams, quinolones, chloramphenicol, macrolides, penicillin, aminoglycosides, carbapenem)[28]. Some resistant strains of Pseudomonas

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aeruginosa have unique drug efflux systems, such as MexAB-OprM or MexEF-OprN[29].

3.1.2 Nontuberculous mycobacteria pulmonary disease (PNTM)

Non-tuberculous mycobacteria (NTM) are different from Mycobacterium tuberculosis originates from the natural environment which includes more than one hundred species, such as Mycobacterium tortugae, Mycobacterium avium, Mycobacterium kansasii, and Mycobacterium abscessus, is the more pathogenic and can cause disseminated abscesses in the lung and doesn't respond to conventional antibiotics. NTM infection is often secondary to HIV infection and some structural underlying lung disease can futher cause a variety of diseases, pulmonary infections and skin soft tissue infections are most common. Studies have shown that NTM was mainly isolated from respiratory tract specimens of patients with cystic fibrosis and primary ciliary dyskinesia, and these diseases induced lung structure pathological is a susceptible factor of PNTM[30]. The innate immune against PNTM includes Toll-like receptor-2, and the adaptive immune system as well as a multitude of proinflammatory cytokines most notably TNF-α IL-12 and IFN-γ[31]. NTM has a lipid-rich outer membrane with strong adhesion, and NTM has a tendency to form biofilm once adhesion occurs. Therefore, traditional antibiotics cannot penetrate its thick outer membrane and biofilm leading to therapeutic failure. Some studies have shown that hydrophobic modification of certain drugs is beneficial to improve the therapeutic effect on NTM[32]. However, direct modification of drugs may reduce drug efficacy and increase potential organism toxicity, so nanomaterials with good bio-penetration and modifiability are excellent choices for drug delivery.

3.1.3 Tuberculosis（TB）

Tuberculosis is caused by Mycobacterium tuberculosis (MTB) infecting the lungs. It can be divided into primary tuberculosis, secondary tuberculosis, hematogenous disseminated tuberculosis and tuberculous pleurisy. A certain of patients with TB are secondary to HIV infection, the immune deficiency caused by HIV is a most important reason[33]. Secondary pulmonary tuberculosis refers to the previous infection with Mycobacterium tuberculosis and reinfection. The typical change of secondary pulmonary tuberculosis is interstitial consolidation, patchy or fused[34]. The main natural immune cells involved in inherent immunity against tuberculosis are macrophages, dendritic cells, NK cells and neutrophils[35]. Chronic progression and bacterial immunity are typical features of TB. MTB can achieve immune evasion by modulating the host's intrinsic immunity to survive in the host for long periods of time. MTB can disrupt the cell membrane of immune cells and obstruct cell membrane repair by inhibiting mitochondrial function. In addition, MTB can induce iron death and necrotizing apoptosis in immune cells, itcan also inhibit the LAP pathway and suppress the phagocytic function of immune cells, especially macrophages. With the continuous development of the disease, a cavity with moderate thickness and smooth cystic wall may be formed in the lung. The fibrocystic wall of fibro-cavernous tuberculosis will resist the entry of drugs, and the load of tuberculosis in the cavity is higher and the infection is stronger. The data shows most cases of MDR-TB and

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almost all cases of XDR-TB are fibro-cavernous tuberculosis[36]. Drug-resistant TB is a major challenge in TB treatment and has become a major threat to global health today. The current first-line drugs for treating TB include rifampicin, isoniazid, ethambutol, streptomycin and pyrazinamide. However, due to irregularities in the treatment process and drug administration, MTB has evolved drug resistance mechanism to these drugs that include drug target gene mutations and drug efflux pumps. Currently, common drug resistance genes are *rpoB, KatG, inhA, rpsL, pncA*. Nano-drugs have promising applications for treatmening MDR-TB or XDR-TB infection. Tech *et al.* summarized the nano-drug delivery systems used for anti-tuberculosis drug delivery in recent years. In general, it seems that polymer and liposomal carriers are predominantly used and have potential in reducing drug side effects and resisting drug resistance[37].

3.2 Pulmonary infectious diseases caused by viruses

3.2.1 COVID-19

Alveolar epithelial cells highly express angiotensin converting enzyme 2(ACE2) and transmembrane serine protease 2 (TMPRS2), while TMPRS2 can cleave ACE2 and activate the virus structural spike (S) protein in SARS-CoV-2 to mediate SARS-CoV-2 to enter alveolar epithelial cells. In addition to infecting alveolar epithelial cells, SARS-CoV-2 also infects pulmonary capillary endothelial cells, causing inflammatory reaction and significantly increasing neutrophils. Monocytes and macrophages infiltrated into alveolar cavity, and alveolar wall showed diffuse thickening[38].In the process of infection, in addition to the pro-inflammatory stage and immune system activation, followed by the immunosuppression stage, which is characterized by the decrease of lymphocytes and $CD4^+$ and $CD8^+$T cells, which increases the risk of bacterial infection[39]. Two strategies are included in the innate immunity against COVID-19, one is recognizing the viral genome by TLR7 before infecting cells, and the other is that after viral infection of cells, cell membrane RNA sensors recognize dsRNA intermediates that further mediate the transcription of pro-inflammatory cytokines and chemokines. Antibodies produced by adaptive immunity can neutralize the virus by blocking the viral S protein with the receptor ACE2[40].

3.2.2Human immunodeficiency virus (HIV)

Acquired immunodeficiency syndrome (AIDS) is caused by human immunodeficiency virus (HIV)infection. HIV infection can destroy the $CD4^+$T cells of the host, and undermine immune defense ability of the body will be weakened, and cause serious opportunistic infection leading to pneumocystis pneumonia (PCP)[41], community-acquired pneumonia[42], lung cancer[43] and other serious complications will occur in the lung. HIV infection may change the active substances on the surface of alveolar, among which surfactant protein A(SP-A) and surfactant protein D(SP-D) play an important role. SP-A can block the binding site between virus and CD4+T cells to inhibit its infectivity, but it can enhance the uptake of virus particles by DC cells and promote their transfer to CD4+T cells[44]. SP-D can inhibit the binding of gp120 to CD4 in a dose-dependent manner to anti-HIV infection[45]. Herein,

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surfactant proteins A and D may be a potential target for treating HIV-related lung diseases.

3.3 Pulmonary infectious diseases caused by fungus

Pulmonary fungal infections are invasive diseases caused by pathogens such as Aspergillus, Cryptococcus, Pneumocystis and endemic fungi[46]. Pulmonary fungal infections are often secondary to immunocompromised patients, such as intensive care unit (ICU) patients, patients receiving anti-tumor therapy and AIDS patients, but also in healthy people. Some studies showed that moulds and yeasts also exist in the respiratory tract of healthy people, and the occurrence of pulmonary mycosis destroys the diversity of fungi in the pulmonary, making a certain kind of fungi dominate[47].

3.3.1 Pulmonary cryptococcosis

Cryptococcus neoformans and Cryptococcus gatti are belong to Cryptococcus complex which can cause human disease[48]. Cryptococcus pulmonary infection is an important type of fungal infection after kidney transplantation[49]. After Cryptococcus infects the host's lungs, alveolar macrophages engulf Cryptococcus form Titan cells because of cell cycle stagnation or increased DNA content, which can inhibit the phagocytosis of macrophages and lead to Cryptococcus infection and Strengthen drug resistance[50]. The physical barrier of lung, such as cilia and mucus, and airflow can't effectively prevent the spread of Cryptococcus to the far end of lung. The host immune response involved in pulmonary Cryptococcosis is mediated by innate immune response and acquired immune response. Alveolar surfactant protein D(SP-D) may bind to Cryptococcus and attract eosinophils to resist Cryptococcus infection[51]. Neutrophils also participate in the host immune response of patients with pulmonary cryptococcosis. Neutrophils also participate in the host immune response of patients with pulmonary cryptococcosis. Wang *et al.* found that in the early stage of Cryptococcus neoformans, neutrophils depleted mice had fewer Cryptococcus infected cells, which was beneficial to prolonging the survival time of mice. At the same time, the decrease of neutrophils induced Th1-type immune response produced inflammatory protection and inhibited the lung injury. This is different from most studies on pathogen infection, and the anti-inflammatory effect of neutropenia may be related to specific pathogens[52].

3.3.2 Pulmonary aspergillosis

The common pathogens of pulmonary aspergillosis are Aspergillus fumigatus and Aspergillus flavus, and they can cause chronic infection in the cavities formed by chronic lung diseases such as tuberculosis, lung cancer, bronchopulmonary cysts and cystic fibrosis. Pulmonary aspergillosis is closely related to the immune status of the host, and when immunocompromised, invasive pulmonary aspergillosis (IPA) is triggered usually with a protective Th1 response, and the Th2 response associated with a higher fungal load and inflammatory response[53]. SP-A and SP-D proteins are involved in the development of IPA by interacting directly with pathogenic allergens and mediating the distribution of cytokines and chemokines as well as immune cells. Conjugates and amphotericin B, represented by itraconazole and voriconazole, are the

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main drugs used in the treatment of pulmonary aspergillosis, but according to related reports, itraconazole and voriconazole may induce significant hepatic and gastrointestinal toxicity, and itraconazole has a relatively low oral utilization rate and requires continuous dosing. Voriconazole requires high doses to achieve the desired therapeutic effect[54]. Posaconazole is a triazole drug that has been shown to have a better safety and tolerability in children with CF suffering from IPA infection and to be effective in containing Aspergillus infection[55].

4. Advantages of the Pulmonary Nano-drug delivery System

4.1 Improving drug stability and solubility, achieving controlled release

Some drugs require sustained releasing during clinical application to maintain therapeutic effects. However, ordinary drug particles are generally exposed directly to the internal environment, the drug rapidly disintegrates and eliminated after diffusion. Nanomedicines use the core-shell structure to wrap the drug inside the cavity or sandwich, forming a protective layer, the drug diffuses outward at a constant rate across the nano-shell, thus achieving controlled release of the drug and prolonged half-life of drug. At the same time, the drug is protected by the nanocarrier from direct contact with plasma proteins, digestive enzymes and other drug degradation and removal components, and the stability of the drug is enhanced. Nanocarriers can also enhance the solubility of drugs. Most of the drugs used in the lung are lipid-soluble drugs, because lipid-soluble drugs are more penetrating and can enter into the cells . However, some antibiotics such as aminoglycosides and β-lactam antibiotics have poor lipid solubility. If we directly change the drug structure or modify the drug, the drug efficiency would decrease and potential organism toxicity would increase. Nanomaterials with good bio-penetration and modifiability are an fantastic choice for delivering drugs. The use of nanocarriers can change the lipid solubility of these drugs, increase their solubility in target organs and improve the therapeutic effect[56]. While for some hydrophobic drugs, they can be physically modified by polymeric carriers to increase their solubility and delivery efficiency[57].

4.2 Weaken first-pass elimination effects, enhancement of drug lung accumulation

Drugs used in infectious lung diseases are usually given orally or intravenously. Oral medications own the best patient compliance and are easy to administer. However, drugs often need to cross the gastrointestinal mucosal barrier or need to reach the body via the blood circulation, and most drugs cannot effectively deposited in the lungs[58, 59]. To achieve the treatment concentration of drugs in the lungs, patients often need to take large doses of drugs, but inevitably bring serious systemic toxicity. Nano carriers are different from prodrugs in that researchers can modify them arbitrarily by attaching or encapsulating molecules with lung-targeting properties,

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such as chemical groups, nucleic acid fragments, peptides, etc. on the surface of the carriers to guide the specific deposition of drugs and increase the concentration of drugs in the lungs to achieve better therapeutic effects[60, 61]. In addition, cell membrane biomimetic nanoparticle is a new DDS which wrap the cell membranes with different biological activities on the nanoparticles, so that the organism could recognizes them as "self-components" and avoids removing them. The "nano ghost" model has become a new star in the field of DDS[62].

4.3 Fighting against drug resistance

Drug resistance is an adaptive process of natural selection that occurs mostly in patients requiring a long-term antibiotic application. These patients often have persistent low doses and irregular application of antibiotics or application of immunosuppressive drugs[63, 64]. When drug resistance occurs, clinicians are forced to change antibiotics or increase the dose taken, which places a heavy financial burden on patients and potentially more severe drug toxicity[65]. The mechanisms of bacterial drug resistance have been widely explored and are generally considered to be related to the following mechanisms: 1) catabolic inactivation of antibiotics; 2) alteration of the drug's target of action 3) altered permeability or other properties of bacterial cell membranes; 4) specific drug efflux pump systems; 5) bio-permeable membrane formation. Nano drug carriers put on a camouflage for the drug, evade the recognition of the drug by the pathogen, and avoid the drug removal outside the bacteriophage[66, 67]. In vitro studies have shown that nanodrugs, especially liposomal drugs, could enter bacteria or cells and release drugs in large quantities and continue to exert powerful antibacterial effects. Biofilm is a biological colony formed by microorganisms on the surface of attached tissues, where bacteria are highly aggregated and closely connected, and conventional antibiotics with relatively large particle size are difficult to traverse the tight bacterial interstices, leading to the development of drug resistance[68]. The particle size of nano-drug carriers is usually only 1-1000nm, which can pass through the gaps between bacteria in the biofilm and inhibition of biofilm formation process to overcome the drug resistance caused by biofilm[66].

5. The nano-drug delivery system of lung

5.1 Polymeric Microparticles and Nanoparticles

At present, the polymers used in the medical field can be divided into natural polymers and synthetic polymers. Most natural polymers come from animals, plants or microbiology such as chitosan, alginate, gelatin, albumin etc. Natural polymers are considered to be available carriers for drug delivery because of their biodegradability, biocompatibility and low toxicity[69]. With the demand for higher drug delivery performance and more stable delivery, synthetic polymers have been widely studied. Compared with natural polymers, synthetic polymers have the advantages of adjustable degradation kinetics and mechanical properties, so they have predictable

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properties and functions. We will introduce two promising polymers recent years. They are chitosan and poly(lactide-co-glycolide) (PLGA).

5.1.1 Chitosan (CS)

Chitosan is a natural occurring, linear polysaccharide could be produced by the exoskeleton of crustaceans such as shrimps, lobsters, and crabs, also can be found in some microorganisms such as fungi and yeast. CS is the most widely used natural polymer in DDS. The structure of chitosan is similar to cellulose[70], only the group attached to the C2 position is different, so chitosan has good fibrillogenic and moisturizing properties. Chitosan is the product of partial acetylation of the natural polysaccharide chitin which has good cell-affinity, biodegradability and low cytotoxicity[71]. Many studies have shown that chitosan can play the role of immune enhancement, anti-tumor, anti-inflammatory, anti-bacterial and anti-pulmonary fibrosis in lung[72-74].Chitosan particles can improve the bioavailability and reduce the side effects of drugs by prolonging the residence time of local administration or by opening the tight connection between epithelial cells[75]. Such features not only a proper delivery of the carriers, but also avoid the uptake of macrophage [76]. Chitosan contains a large number of free amino groups with cations, which can cross-link with multivalent anions. Herein,it is easy to combine with positively charged substances through electrostatic action, such as drugs, other polymers or modifying chemical groups. Chitosan has low solubility in water but become better in hydrochloric acid and acetic acid. In addition, inhalable chitosan drug formulations should comprise aerodynamic sizes ranging from 1 to 5 mm (dry) to reach the lungs successfully, swell upon deposition in the moist lung, and provide a sustained controlled drug release through the polymeric matrix. Researchers have made chitosan derivatives such as hydroxypropyl chitosan, carboxymethyl chitosan, and quaternized chitosan to improve water solubility and tissue penetration[74].Si *et al*.[77] developed a cationic antibacterial polymer named 2,6-diamino chitosan (2,6-DAC), which has better biodegradability and biocompatibility. 2,6-DAC enhances the ability to break plasma membrane and proton sponge effect of chitosan, showing excellent antibacterial activity. In some studies, chitosan nanoparticles(CSNP) are coupled with bioactive components such as functional modified peptides[78], cell membranes, cell receptors[79]and metal elements[76, 80] to make them have targeted killing or stronger drug protection ability. Ding et.al.[81] prepared RBC-hitchhiking (RBC-MPSS-CSNP) by using ionotropic gelation technique to load methylprednisolone sodium succinate (MASS) into CSNP, and mixed it with red blood cells from rat abdominal aorta were prepared [Figure2a]. The result shows RBC-MPSS-CSNP have a longer time in vivo, and preferentially accumulate in the lungs, which is conducive to the sustained release of drugs to the lungs and overcomes the shortcoming of short action time of MASS in vivo [Figure2b].

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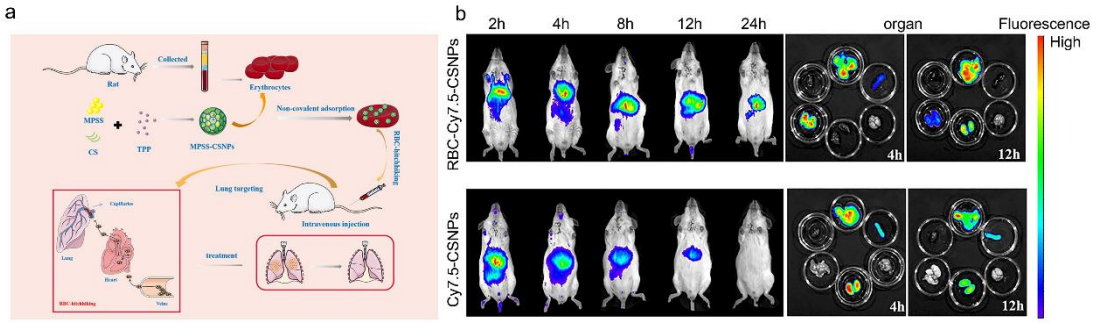

Figure2 | (a)The fundamental principle of RBC-hitchhiking, RBC-hitchhiking enter the lungs and weaken the clearance of the liver. (b) Cy7.5-CSNPs and RBC-Cy7.5-CSNPs were injected intravenously into rats, in vivo imaging and post-dissection organ imaging were performed at specific times. Copyright 2022, Science Direct [81].

Chitosan has a large number of amino groups, so it can be with mucous membranes with acidic groups, which can enhance the deposition of drugs in mucous membranes. Therefore, chitosan modification has a good application potential in local mucosal drug delivery. Scolari et.al[82]functionalized alginate nanoparticles loaded with rifampicin and the antioxidant ascorbic acid (RIF/ASC) with chitosan and nonionic surfactant T80, and provided a hydrophilic protective layer through "sugar cluster effect"[Fig3a]. Experimental data shows that NPs loaded with RIF/ASCs was mucus-inert, possibly penetrating the mucus layer of respiratory tract [Fig 3b], and then internalized. And NPS had no influence on cell metabolism in this process.

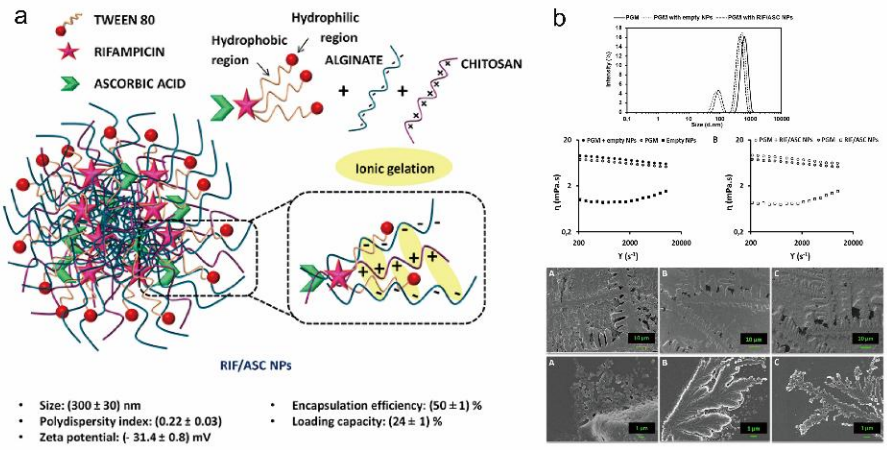

Figure3 | (a) General schematic description and main characteristics of RIF/ASC NPs. (b) PGM: porcine gastric mucin, using PGM as an in vitro model, to clarify the respiratory mucus adsorption of NPs loaded with RIF/ASC. Copyright 2021, American Chemical Society [82].

Researchers try to treat chronic lung diseases with chitosan nanoparticles, such as pulmonary cystic fibrosis; Tuberculosis and COPD[83]. The mutation of CTFR gene leads to pulmonary cystic fibrosis, followed by various pulmonary infections, especially Pseudomonas aeruginosa infection. Gene therapy is a feasible method to

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treat CF. Viruses are usually transfected with target genes, but there is a potential danger of inducing immune response. Therefore, how to safely and effectively deliver target genes to target cells is still a difficult problem[84]. Fernández *et al.*[85] designed a non-viral gene transfection system (CS-pEGFP-siRNA complex) based on chitosan nanoparticles and used to deliver pEGFP and siRNA simultaneously. Successful transfection efficiency was achieved when cells were transfected either with CS-pEGFP or co-transfected with CS-pEGFP-siRNA at N/P charge ratio of 12. In another study, chitosan nanoparticles were used to deliver wild-type CTFR-mRNA(wtCTFR-mRNA), and capsaicin-less epithelial cell sodium channel permeability was used to improve transfection efficiency, which successfully restored the CTFR gene activity of transfected cells[86]. Besides gene therapy, chitosan also plays a role in dealing with Pseudomonas aeruginosa infection caused by CF. In order to overcome the formation of biofilm, researchers used DNase-1 to functionalize chitosan nanoparticles, which could effectively inhibit the formation of biofilm. DNase‐I functionalization of ciprofloxacin‐loaded chitosan nanoparticles overcomes the biofilm‐mediated resistance of Pseudomonas aeruginosa. The functional combination of chitosan nanoparticles and secretory leukocyte protease inhibitor (SLPI) can not only inhibit the reproduction of Pseudomonas aeruginosa, but also inhibit the inflammation caused by its infection[87]. In a study to discuss the antibacterial activity of chitosan coated antibiotic nanoparticles against Pseudomonas aeruginosa, 10 antimicrobial agents (antibiotics) were combined with different degree of deacetylated Chitosan and Chitosan oligosaccharide. All the chitosan showed synergistic activity with sulfamethoxazole, a sulfonamide antimicrobial agent[88]. Raafat et. al.[89] pointed out that chitosan has bactericidal effect, but it might not act on a single classical target, but an important link of its bactericidal effect is the combination of chitosan and phytic acid, and the extraction of potential membrane lipids (mainly lipophytic acid). Besides the infection caused by pulmonary fibrosis, chitosan was also applied in tuberculosis. The effect of the single drug in the treatment of pulmonary tuberculosis is limited, and it is easy to lead to drug-resistant pulmonary tuberculosis. Therefore, it is usually combined with drugs to treat pulmonary tuberculosis. Nano-carriers are used to deliver multiple drugs simultaneously, which has potential in the treatment of pulmonary tuberculosis. Cunha *et al.*[90]prepared chitosan microparticles (Aerodynamic diameter of 4μm) loaded with isoniazid and rifabutin, which had higher drug loading rate (isoniazid: 93%, rifabutin: 99%), stronger macrophage activation ability and better antibacterial ability (96%). Chitosan was further studied in COVID-19. Wang *et al*.[91]used chitosan to coat bovine serum albumin-integrated Silymarin/curcumin. Because of the presence of chitosan, the dispersion and deposition of drugs in the lungs were effectively improved. After treatment, the levels of serum CPR and IL-6 in COVID mouse model decreased significantly, and the pathological changes of mouse lungs were improved.

5.1.2 Poly(lactide-co-glycolide) (PLGA)

As the representative of synthetic polymer, poly(lactide-co-glycolide) (PLGA) has been widely used in slow releasing DDS because of its excellent biodegradability and

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biocompatibility[92]. PLGA can be randomly polymerized from lactic acid and glycolic acid, and reorsents different biological trait including molecular weight, degradability, size etc. with the mixed proportion of the two monomers[93, 94]. PLGA was able to increase the stability of hydrophobic drugs due to having a hydrophobic core[95]. And coupling it with PEG can increase the water solubility of the drug for enhancing its mucosal adhesion. PEG-modified PLGA (PEG-PLGA) particles have made some progress in lung drug delivery[96]. Li and co-workers[97]further explored the drug release in vitro, mucus penetration and macrophage uptake capacity of PEG-PLGA with different PEG ratios and molar masses, and investigated the activity of microspheres in rats. PEG2000-DSPE/PLGA1:1 group showed enhanced mucus permeability, lower macrophage uptake and longer lung retention time than 0.25:1 group. PLGA has free end groups that can be modified into hydroxyl end groups (PLGA-OH), carboxyl end groups (PLGA-COOH) and ester end groups (PLGA-COOR), while the encapsulation efficiency and sustained drug release characteristics of PLGA derivatives also be changed with different modified end groups[98].

In recent years, the focus of research on PLGA-based DDS has gradually shifted from complex modifications to the core drugs in system. In addition to the traditional anti-infection and anti-tumor chemical drugs, some bioactive drugs, such as polypeptide drugs, have gradually attracted clinical attention. Due to the special properties (appropriate molecular weight, poor mucosal permeability and are easily degraded in vivo) of peptides compared with traditional drugs[99], it is urgent to explore polypeptide drugs delivery carrier. Laura. et.al.[100] reported a nano-structures that CSE4 (a polypeptide drug that can enhance the telomerase activity of cells) was encapsulated in PLGA-PEI. PLGA-PEI nanoparticles loaded with CSE4 can protect ProSP-C cells from bleomycin-induced damage by reducing DNA damage, and promoting the repair of alveolar structure, thus playing a therapeutic role in Idiopathic pulmonary fibrosis patients. A report incorporated fluorocarbon fragments with hydrophobic and lipophobic properties into the peptide drug, and the fluorinated modification prevented the binding of the peptide drug to mucin glycoproteins, increasing the mucus penetration of the peptide drug by approximately 240-fold[101]. Wang *et al*.[102] encapsulated calcitonin gene-related peptide (CGRP) in PLGA-PEG nanoparticles to improve the action time and stability of CGRP. The results showed that compared with the free CGRP group, the experimental group of CGRP coated with PLGA-PEG has thinner pulmonary vascular wall and less inflammatory cell infiltration, which shows the potential of PLGA-PEG nano-carrier to improve the therapeutic effect of CGRP to treat lung inflammatory damage. Jannuzzi *et al*.[103] loaded a variable single chain antibody fragment (scFv) against Picchia pastoris (P. pastoris) into PLGA nanoparticles, and evaluated its anti-Paracoccidioidomycosis (PCM) effect on animals. ScFv was released at a controlled rate in vivo, and the fungal load of experimental animals was reduced significantly. The combination of traditional nanomaterials with new drugs could enrich their characteristic, and bring hope for the treatment of lung diseases. Recent studies have shown that PLGA can carry a variety of drugs and enhance its efficacy,

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exerting strong antibacterial and anti-inflammatory effects. PLGA can also be used as carries of some other drugs, such as Sparfloxacin[104] and Thymoquinone[105],levofloxacin[106] and luteolin[107], thus improving the therapeutic effects and reduce its systemic toxicity. PLGA-based DDS can realize the controlled release of drugs, which shows a bright future in the treatment of pulmonary mycosis.

Besides chitosan and PLGA, other natural polymers such as hyaluronic acid, alginate and synthetic polymers are also used in pulmonary infectious diseases. Hyaluronic acid (HA) modification can promote chitosan nanoparticles loaded with model plasmid (pCMV-β-Gal) to enter alveolar epithelial cells and improve the transfection efficiency of the plasmid[108]. Polymer-Lipid Nanoparticle formed by the conjunction of poly (β-amino esters) and polyethylene glycol-lipid can be administered in the whole body, thus facilitated mRNA transportation to the lungs which rarely intercepted by the liver[109]. Furthermore, polymer micelles (PCD/PPC/PPE) constructed by Polycaprolactone-polyethylene glycol carrier (PCL-PEG-COOH, PPC), Polycaprolactone-polyethylmethacrylate cationic carrier (PCL-PDMAEMA, PCD), and polycaprolactone-polyethylene glycol carrier connected with high-affinity targeting peptide (Esbp) targeting inflammatory endothelial cells (PCL-PEG-Esbp, PPE) have good biocompatibility and lung targeting ability in animal models of acute lung injury (ALI), and effectively enhance the anti-ALI effect of dexamethasone (DTX)[110].

5.2 Liposome

Liposomes are carriers contain phospholipids, which are made up of naturally occurring phospholipids such as phosphatidylcholine, phosphatidylserine, soybean lecithin, or egg yolk lecithin, sometimes complemented with other lipids and are easy prepared[111]. Also, the surface charge, particle size and drug encapsulation efficiency of liposomes can be easily adjusted.[112] Hydrophilic drugs are encapsulated in the capsule lumen formed by liposomes, and lipophilic drugs are entrapped in the lipid bilayer[113]. Liposomes are widely known for their versatility as DDS for many diseases, including cancer and infections[114]. Liposomal drugs are currently the only nano-drugs that primary used in clinic because of their excellent biocompatibility and slow releasing properties. The ingredient of liposome is similar to cell membrane, so the DDS based on liposome can easily penetrate the blood barrier and reach the target organs[115]. Most of the currently applied liposomal drugs are administered intravenously. Liposomes have been developed into most potential drug carriers over the last several decades partly because they are biocompatible as lipids derived from nature and have strong non-targeted tissue penetration and tissue adhesion[116]. Another advantage of liposomes as drug delivery is the ability to slow releasing antibiotics and reduces patients' organ burden[117].

However, conventional liposomes, for instance lipofectamine 2000, despite their high efficacy, the lack of on-demand release of their contents limited their therapeutic

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utility [118]. Therefore, researchers pay more attention to the liposome nano-drug delivery systems' organ targeting, controllable and sustained drug release in recent years. By surface modification or coupling different functional groups such as enzymes, aptamers, nucleic acid molecules, cell membranes, small molecules, chemical groups, can realize the above-mentioned functions. Acute respiratory distress syndrome (ARDS) is considered as a serious complication of COVID-19 infection. Pooladanda et.al. [119]prepared a by thin-film hydration method, where BRD4 and siRNA complexed with cationic lipid (BRD4-siRNA-LP). BRD4-siRNA-LP could significantly suppress LPS-induced lung inflammation. Weng et.al.[120]reported a DDS based on liposomes for delivering methylprednisolone (MPS) to lung (MPS-NSSLs-SPANb) to treat idiopathic pulmonary fibrosis (AE-IPF). Alveolar surfactant protein A (SP-A) is highly expressed in human type Ⅱ alveolar epithelial cells, but hardly expressed in other organs, thus it is an ideal target of lung tissue. Therefore, the DDS can be targeted to lung tissue through the SP-A nanobodies (SP-ANbs). IHC showed that MPS-NSSLs-SPANb specifically combined with lung tissue, but not with liver and kidney. Animal vivo imaging also showed that MPS-NSSLs-SPANb had high accumulation in 15min to 6h. The model mice treated with MPS-NSSLs-SPANb were obviously improved (Fig4).

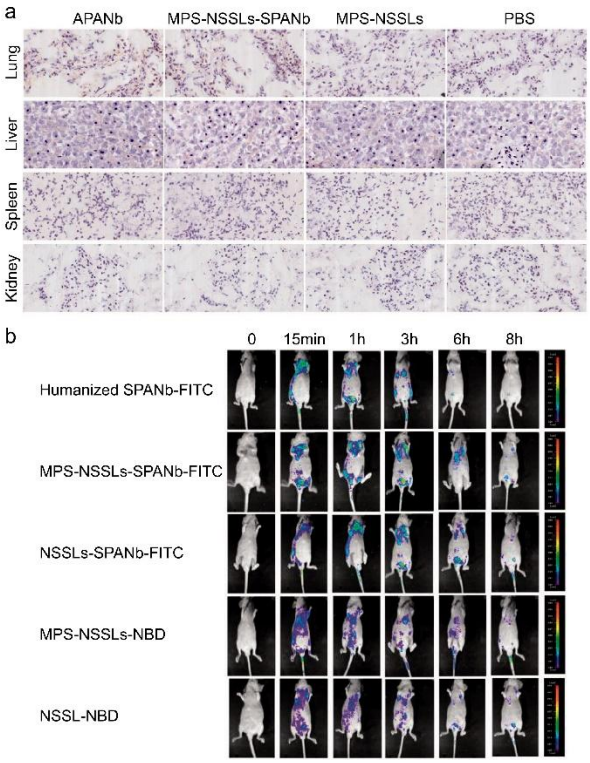

Figure4 | Images of immunohistochemical staining of human tissue specimens and in vitro binding to antigen SPA by MPS-NSSLs-SPANb. (a) The magnification was x20. Arrows are pointing to positive staining. (b) Real-time in vivo imaging of nude mice injected with different agents. (c) The 10-week survival rate of the regular-dose MPS-NSSLs-SPA, MPS-NSSLs, MPS (MPS 1 mg/kg), low-dose MPS-NSSLs-SPANb (MPS 0.5 mg/kg) and AE-IPF group. Copyright2021, Taylor&Francis Online [120].

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Angiotensin-converting enzyme 2 (ACE2) protects lung epithelial cells, but it is also a functional receptor for SARS-CoV-2, which can trigger lethal damage to the lung by SARS-CoV-2. The presence of excess soluble ACE2 in the lung may neutralize some viruses to resist pulmonary attack by the virus. Based on this idea, Arisoy *et al*.[121]prepared ACE2 loaded decoy liposomes that reduced SARS-CoV-2 infection leading to Vero E6 death fourfold. Gupta *et al.*[122] found that the combination of bacteriophage lysozyme (LYS) and levofloxacin liposome (LVX liposome) was effective against the biofilm formed by Staphylococcus aureus. The possible mechanism is that LYS can disrupt the bacterial cell wall and LVX plays a bactericidal role. This combination of enzymes and liposomal drugs provides a new pathway for the treatment of invasive Staphylococcus aureus lung disease.

Besides the loaded drug and targeted receptors affecting the therapeutic effect of drugs, the influence of surface charge of nanomaterial on the DDS has gradually received more attention. There was a relationship between the charge properties of liposomes and the biological barrier in the lung. The study showed that liposomes with neutral and negative charge had better permeability, retention time and stability in BALF containing alveolar surfactant[123]. A report used LVX liposomes for the treatment of CF-induced Pseudomonas aeruginosa infections, and the anionic liposomes had good encapsulation rates, slow releasing properties and low epithelial cytotoxicity. However, the study found that cationic liposomes were unable to encapsulate LVX, so it may not be a suitable nanocarrier for LVX[124]. A similar study prepared neutral and negatively charged liposomes by loading different proportions of cholesterol and gentamicin was encapsulated in them. The results showed that the minimum inhibitory concentration and minimum bactericidal concentration of neutral liposome to planktonic Pseudomonas aeruginosa and Klebsiella oxytoca were better than free gentamicin, while negative liposome is equivalent to that of free gentamicin. The negatively charged formulation exhibited the same bacteriostatic concentration as that of free gentamicin. However, the drug encapsulation efficiency of negatively charged formulation is better than neutral liposomes, which due to the stronger binding effect between negatively charged liposomes and positively charged gentamicin[125].

In 2018, ARIKAYCE® KIT (Amikacin Liposome Inhalation Suspension:ALIS) of Insmed Company was approved for marketing in the United States. It is the only drug specifically used to treat non-tuberculous mycobacterial lung disease (NTM-LD) caused by Mycobacterium avium-intracellulare complex(MAC) infection[126]. Amikacin is an aminoglycoside drug, which realizes an antibacterial effect by interfering with the synthesis of bacterial protein. It can penetrate the biofilm of MAC to play strong antibacterial activity in vitro[127, 128]. ALIS was prepared by mixing 70mg/ML amikacin dissolved in water with lipid (dipalmitoylphosphatidylcholine (DPPC) and cholesterol) dissolved in ethanol at a specific flow rate ratio. Compared with the prototype drug amikacin, the drug exposure in lung tissue and the intake of alveolar macrophages significantiy increased by inhaling ALIS, and the drug retention time in lung has been effectively prolonged[129, 130]. But, the application of ALIS will cause some adverse events, including (1) Respiratory toxicity (dyspnea, cough,

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hemoptysis and dysphonia) (2) Ototoxicity (mainly hearing loss). ALSI has low nephrotoxicity which makes it becomes a feasible choice for patients with renal insufficiency[130, 131]. A cohort study compared the therapeutic effects of ALSI was stronger than tobramycin inhalation (TSI) on CF patients with chronic Pseudomonas aeruginosa infection[132]. Besides ALIS, the researchers also evaluated the efficacy of liposomal ciprofloxacin on NTM caused by Mycobacterium avium subsp hominissuis and Mycobacterium abscesses sp. Ciprofloxacin (CIP) liposomes have enhanced biofilm penetration and better macrophage uptake, significantly reducing bacterial load in NTM patients[133].

Liposomal antibiotic nanoparticles own unique potential in fighting pulmonary infections caused by Pseudomonas aeruginosa. By using liposomal nanoparticles to ciprofloxacin and mucin could significantly enhance the adhesion ability of the lipo-drugs to lung epithelial cell monolayer and prolong retention time in the lungs[134]. Another study investigated the solubility of ciprofloxacin liposomes in artificial bronchodilator sputum medium (ABSM) in analogy to its status in bronchodilator-induced pulmonary infections. The study found that ciprofloxacin liposomes had slow-release properties and released antibiotics at higher concentrations over time than MIC. it could penetrate the mucus barrier to kill Pseudomonas aeruginosa[135]. Liposomal amphotericin B (LAMB) is another commercially available intravenous liposomal drug used primarily against fungal infectious diseases of the lung. Animal and clinical trials have shown that LAMB is effective in reducing fungal loads in animals. In addition to its therapeutic effects, LAMB has a preventive effect on pulmonary fungal diseases[136].

In addition to deliver antibiotics, liposomes are showing promise in the development of vaccines for infectious diseases. 2′,3′-cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) is a natural agonist of interferon gene stimulating factor (STING). Wang and his team[137]developed a potential "universal" mucosal adjuvant(PS-GAMP) for influenza vaccines by loading cGAMP into prepared liposomes based on lung surface active ingredients, which enter alveolar macrophages (AMs) via SP-A and SP-D-mediated endocytosis, followed by cGAMP ligation through cellular gaps into alveolar epithelial cells(AEC), further activated the STING pathway, inducing the production of immune type Ⅰ immune mediators, and promoting the recruitment and differentiation of $CD11b^+$ dendritic cells (DCs) and $CD8^+$ T cells as well as humoral immunity[Fig5a]. Due to the similarity to the composition of alveolar surface-active substances, PS-GAMP could enter AMs without being able to damage the cells. AMs exhibit high uptake of PS-GAMP. [Fig5b]. Bernasconi *et al*.[138] evaluated a novel influenza virus vaccine platform that achieves enhanced vaccine immunogenicity and host protection by combining liposomal nanoparticles (LNPs) with a vaccine adjuvant—CTA1-DD. The result showed that the constructed liposomal influenza vaccine, CTA1-3M2e-DD (FPM2e), remained extremely immunogenic and lung tissue-specific responses were observed after immunization of mice. And surprisingly, there was no significant pathological damage in the lungs of mice.

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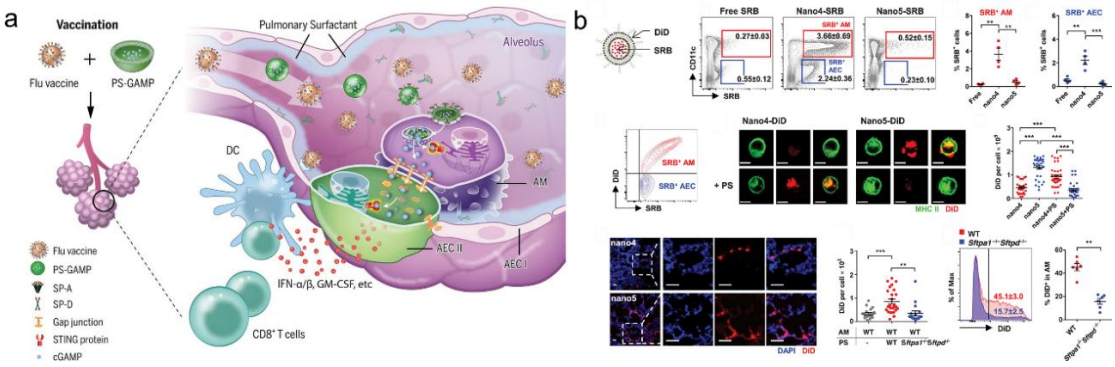

Figure5 | (a) Schematic diagram of the principle of PS-GAMP-mediated adjuvant. (b) Uptake of PS-GAMP by AMs. Copyright2020, HHS Public Access [137].

5.3 Solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) are prepared by solid lipids core(), surfactant(s) shell and water[139]. Since the early 1990s, the SLNs have been investigated as an alternative to polymeric nanoparticles[140]. Compared to particles made of PLA or PLGA, SLNs have several advantages , such as improved physical stability, controlled release, high drug targeting effect, faster degradation, lower cytotoxicity, higher tolerability and ease of production for scale-up[139, 141-143]. Chattopadhyay *et al.*[144]prepared different solid lipid nanoparticle suspensions containing ketoprofen and indomethacin as model compounds using supercritical fluid extraction of emulsions (SFEE). The feasibility of delivering different SLN nanosuspensions as aerosols for inhalation was investigated by nebulizing the prepared nanosuspension formulations using Aradigm's AERx® systems. The results showed that nanoparticles with an average volume diameter of <50 nm and exhibited excellent suitability for deep lung deposition. In addition, the drugs remained in a steady state within the SLN with drug loadings up to 10% and 20% w/w, well above their saturation limits in these lipids. Jaafar-Maalej *et al.*[145] developed lipid nanoparticles loaded with beclomethasone dipropionate (BDP) using a high-shear homogenization method and nebulized them in the form of aerosols. The lipid nanoparticles were displayed high entrapment efficiency values reaching 99% and an in vitro diffusion-controlled release from the lipidic matrix. Moreover, the nebulized SLN and NLC were proved to have suitable aerodynamic behavior for deep lung delivery and thus. LN can reasonably be considered as a promising drug carrier system that opens the way for lipophilic drug-targeting strategies by nebulization. Wang *et al.*[146]prepared SLNs consisting of lecithin, cholesterol, and a lipid-polyethylene glycol conjugate by solvent evaporation. Dry powders of the SLNs were prepared by thin-film freeze-drying (TFFD), spray drying, or conventional shelf freeze-drying method. The study demonstrated that the dry powder prepared by TFFD showed more ideal aerosol performance properties than that prepared by spray drying. In addition, the SLNs encapsulated with siRNA can also be successfully converted into aerosolized dry powder by TFFD, and there was no negative impact on the function of the siRNA. The resultant dry powder vaccines are expected to be delivered by non-invasive routes, such as intranasal or pulmonary administration. Ma and coworkers designed a

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mannose-modified macrophage-targeting SLN, MAN-IC-SLN, loaded with a prodrug of isoniazid (INH) and designed for the treatment of latent tuberculosis infection. The results showed that the cellular uptake of modified SLNs in macrophages (97.2%) was higher than that of unmodified SLNs (42.4%). In addition, to improve drug retention and efficiency, the antibiotic release was pH-sensitive, and a targeted drug release was obtained inside the macrophage endosomes. In the in vitro latent tuberculosis infection model and in vivo antibiotic efficacy test, the MAN-IC-SLNs showed a noteworthy increase in antibiotic efficiency compared to the free drug solution. The study suggested that macrophage-targeted and pH-sensitive SLNs could serve as a promising platform for the latent tuberculosis infection[147]. Li *et al.* [148]prepared curcumin solid lipid nanoparticles (Cur-SLNs) by solvent emulsification diffusion-low temperature curing method, and then they micronized Cur-SLNs micro powder by freeze-drying technology and mixed with flower-shaped lactose (FL) to obtain Cur-SLN-FL powder mist agent, which increased the drug loading of curcumin and significantly improved its drug release performance in artificial lung fluid. In addition, the cytotoxicity of Cur-SLN-FL powder in vitro was investigated by mouse fibroblast (L929) cells and human normal lung epithelial cells (BEAS-2B), respectively. The results confirmed that it had better safety performance for lung cells, which is expected to become a safe and efficient intrapulmonary drug delivery method for pulmonary inhalation drugs. Mehrabani *et al.* developed dry powder inhalers (DPIs) containing amphotericin B-loaded solid lipid nanoparticles (AMB-SLNs) to prevent invasive pulmonary aspergillosis (IPA) by lyophilization technique, accompanied by lactose as inhalation carrier. The statistical results showed that the optimized nanoparticles had the smallest size and the lowest Poly dispersity index PDI). Morphological study of the DPI formulations revealed formation of non-aggregated, uniformly sized spherical shape particles with smooth surfaces. In addition, the highest fine particle fraction (FPF)% was obtained using lactose10%, which exhibited the appropriate aerodynamic characteristics for pulmonary drug delivery. Therefore, this formulation was considered effective for drug delivery to the peripheral airways[149]. Chokshi *et al.*[150] prepared mannose appended rifampicin loaded solid lipid nanoparticles (Mn-RIF-SLNs) for the management of pulmonary tuberculosis (TB). The cytotoxicity studies using J774A.1 cell line depicted that the developed Mn-RIF-SLNs were non-toxic and biocompatible as compared to free drugs. Fluorescence microscopy and Fluorescence-Activated Cell Sorting (FACS) analysis demonstrated significantly higher intracellular uptake of Coumarin-6 (C6) loaded mannosylated SLNs (Mn-C6-SLNs) as compared to Un-C6-SLNs. An oral pharmacokinetic study in adult Sprague Dawley (SD) rats revealed that Mn-RIF-SLNs showed a remarkable enhancement in RIF bioavailability as compared to RIF solution. The biodistribution studies showed higher drug levels obtained in lungs of Mn-RIF-SLNs as compared to the Un-RIF-SLNs. Therefore, it can be concluded that the developed Mn-RIF-SLNs could serve as a potential tool for delivering antitubercular drugs to lung in the treatment of TB.

Active targeting to alveolar macrophages (AM) may improve the efficacy of ‘old’ drugs currently used clinically to treat pulmonary tuberculosis. Previous studies have

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shown that respirable solid lipid nanoparticle assemblies (SLNas) loaded with rifampicin (RIF) and surface-decorated with a mannose-based surfactant (MS) can effectively target AM through mannose receptor-mediated mechanism. Truzzi *et al.*[151] show the in vivo biodistribution of these mannosylated SLNas and compare their behavior with that of non-functionalized SLNas and bare RIF. The biodistribution of SLNas in mice following intratracheal instillation was assessed by whole-body real-time fluorescence imaging in living animals and quantification of RIF in excised organs and plasma. In addition, the uptake of SLNas was determined by using fluorescence microscopy on AM and alveolar epithelium. The results pointed out that RIF-loaded nanocarriers are suitable for effectively targeting AM via a mannose-receptor mediated pathway, which may provide a potentially more efficient inhaled therapy for the treatment of the pulmonary tuberculosis. A similar mannose-based targeting study was reported by Costa *et al.*[152] , showing a significant in vitro uptake of mannose-containing nanoparticles by macrophages, which suggests that these functionalized nanocarriers may represent a promising platform to deliver anti-infective drugs for treatment of pulmonary infectious disease. Rodenak-Kladniew *et al.*[153] developed SLN/Chi/Eu by incorporation of chitosan (Chi, a cationic biopolymer) and eugenol (Eu, essential oils) into a lipid matrix. This nanoparticle allowed for sustained release of encapsulated Ofloxacin (Ofx) for 24 h, and exhibited a strong bactericidal activity against Pseudomonas aeruginosa and Staphylococcus aureus. Moreover, it was shown that the nasal administration of hybrid SLN to mice reached therapeutic Ofx levels in lungs, which demonstrated that it can be considered as a promising system for pulmonary inhalation therapy. Gaspar *et al*.[154] prepared SLN containing rifabutin (RFB) for pulmonary delivery of drugs to treat tuberculosis. Then their antimycobacterial activity was evaluated in a mouse model of infection with H37Rv. The results showed that RFB-SLN enhanced the activity of RFB against M. tuberculosis infection, in comparison with untreated animals, suggesting that RFB-SLN microencapsulation could be a promising method for tuberculosis treatment. In another study, Pastor and coworkers manufactured lipid nanoparticles loaded with sodium colistimethate to enhance the antimicrobial therapy against Pseudomonas aeruginosa in patients with cystic fibrosis. The obtained nanoparticles presented antimicrobial activity against clinically isolated P. aeruginosa. Additionally, they appeared to be less toxic than free sodium colistimethate in vitro study[155].

Although lipid nanoparticles can be a promising carrier system for pulmonary delivery of drugs[156-158]. However, the selection of which compounds and through which carrier systems for pulmonary delivery need to be adjusted by practical therapeutic approaches to ensure stability and pulmonary selectivity of the formulation in vivo.

5.4 Inorganic nanoparticles

Lots of inorganic substances have been used to synthesize NPs, including silica, gold, iron oxide, alumina and titanium dioxide. Inorganic NP carriers offer high biocompatibility, high stability, high delivery efficiency and resistance to microbial

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degradation. Based on the plasmonic and magnetic properties of inorganic materials, they can be used for diagnosis and treatment of pulmonary diseases. For example, mesoporous silica nanoparticles (MSNs) are considered multifunctional nanocarriers that can offer various functions to improve pulmonary disease therapy. The mesoporous scaffold protects the drug until it reaches the target site. Additionally, the MSNs surface is easily functionalized, enabling nanoparticles to obtain desired characteristics in terms of biocompatibility, the presence of targeting fraction, and controlled drug release features. Within the last few years, there has been much progress and successful results in the use of MSNs for lung delivery. Although few studies are using MSNs for the treatment of COPD, ALI/ARDS, and some infectious respiratory diseases, the studies suggest a high potential for the use of these nanoparticles for the treatment of these respiratory diseases. More importantly, the intrinsic properties of MSNs can offer unique advantages in fighting inflammation and lung infections. Higher doses of especially insoluble drugs can be administered topically by using porous carrier matrices to reduce their adverse effects and improve their biodistribution. Gulin-Sarfraz *et al.*[159] manufactured two different sizes of mesoporous silica particles (MSPs) loaded with dexamethasone (DEX) and investigated their feasibility as delivery carriers in the treatment of airway inflammation in mice. Two different mice models of airway inflammation were administrated by inhalation with drug-loaded particles as an aerosol. The results demonstrated that the melphalan (MEL)-induced airway inflammation could be treated by aerosolized DEX-loaded MSPs, which indicated that MSPs are viable drug carriers of corticosteroids for pulmonary delivery in the anti-inflammatory treatment of chemical-induced lung injury. This study emphasizes the potential of MSPs as drug carriers in the treatment of respiratory diseases. Rathnayake *et al.*[160] prepared a targeted drug delivery nano-assembly containing liposome-coated colistin (Col)-loaded porous silica particles in which the liposomal coating is attached to a pseudomonas aeruginosa (PA)-targeting peptide LL-37 (Col@MSN@LL-(LL-37)), which is capable of delivering antibiotics to extracellular and intracellular bacteria in order to address the problems of antimicrobial resistance and off-target toxicity caused by overuse of antibiotics. The results showed that Col@MSN@LL-(LL-37) efficiently targeted the clinical strain of PA14 and effectively inhibited the growth of intracellular PA14 within the lung epithelial cells. Additionally, no significant cytotoxic effects on the mammalian cells were observed with Col@MSN@LL-(LL-37). Thus, this lipid-encapsulated targeted nano-assembly can be considered as an antibiotic delivery platform for the treatment of a wide range of intracellular infections. Tenland and coworkers [161] prepared MSNs containing a novel antimicrobial peptide NZX that can inhibit M. tuberculosis. The majority of NZX was adsorbed in the pores of MSNs by electrostatic interactions in this carrier. The results showed that NZX-MSPs were efficiently taken up by cells and preferentially by primary macrophages. In addition, NZX-MSPs showed significantly enhanced M. tuberculosis elimination compared to the free peptide in infected primary macrophages with M. tuberculosis H37Rv and a murine model of TB. At the same time, these MSPs were non-toxic to mammalian cells at therapeutic

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concentrations. In another study, Xu and co-workers synthesized silica (SiO_2) nanoparticles (NPs) by laser ablation method. The inhibition of carbonic anhydrase by SiO_2 NPs may protect alveolar epithelial cells from H_2O_2-induced oxidative stress and also acts as an antibacterial agent against Klebsiella pneumonia. This protective effect of SiO_2 NPs was confirmed in alveolar epithelial cells (A549) by measurement of MTT, ROS level, CAT and SOD activity and GSH content. At last, the antibacterial activity of the nanoparticles against K. pneumoniae was confirmed, which was mainly attributed to the interaction of nanoparticles with the cell wall and the disruption of bacterial membrane. In conclusion, this data may provide some useful information on the development of pneumonia treatment and management[162]. Following pneumonia treatment, Clemens *et al.*[163] evaluated the efficacy of moxifloxacin (MXF) via disulfide snap-top redox-operated MSNs (MSN-SS-MXF) delivery by different administration routes in a mouse model of tularemia. BALB/c mice were intranasally infected with F. tularensis LVS and one day later, treated by intravenous (i.v.), intramuscular (i.m) or subcutaneous (s.c.) routes with free MXF or MSN-SS-MXF, and the doses were given every 48 h for a total of 3 doses. The results showed that MSN-SS-MXF was significantly more effective than free drug in the reduction of bacterial load in the lung, regardless of the route of administration. Interestingly, the biodistribution analysis of MSN silica showed significant levels in lungs, liver, and spleen of i.v. treated animals and lower levels were found in i.m. or s.c. administered animals. These results are consistent with flow cytometry studies. In summary, the results showed the potential of MSN-SS-MXF in optimizing the therapeutic efficacy of infectious respiratory diseases. Iron oxide nanoparticles (IONPs), as typical inorganic NPs, are also frequently used as drug delivery carriers. Tewes *et al.*[164] formulated porous microparticles loaded with superparamagnetic iron oxide nanoparticles (SPIONs)−in combination with a target-directed magnetic gradient field to achieve targeted aerosol delivery to specific regions of the lung. Microparticles were characterized by various physicochemical methods. They concluded that if loaded with drugs, these particles may be useful in the treatment of localized lung diseases, such as tumor nodules or bacterial infectious lesions. Miranda and coworkers prepared a magnetically responsive microparticulate system for pulmonary delivery of an anti-TB drug candidate (P3). Microparticles (MPs) are developed using a casting method and the results showed that MPs were suitable for P3 entry into the lower airways and alveolar macrophage phagocytosis, which may improve TB treatment efficiency and patient compliance, as a predefined amount of drug can be released at a predetermined time and at a desired frequency according to treatment requirements[165].

In summary, inorganic nanoparticles offer some advantages such as excellent intrinsic physical properties and versatile surfaces, showing great potential for the construction of multifunctional nanoprobes for in vivo therapy. However, the studies on their behavior in vivo, especially pharmacokinetics, are still in their infancy and further research is needed.

7.CONCLUSION

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This review summarizes the underlying basic mechanisms of the infectious lung diseases development and provides a brief overview of their classification. New therapeutic strategies using polymers, liposomes, solid liposomes, and inorganic nanoparticles as drug delivery carriers are discussed in recent years. In general, surface modification of nano- materials and targeted drug delivery are still the current research hotspots. Pulmonary nano-drug delivery systems are highly promising therapeutic tools for the treatment of infectious diseases in the lung. However, except for some liposomal antibiotics, these delivery systems are still have some distance from true clinical application, which is related to their potential but unevaluated toxicity. Additionally, some studies have only been completed with in vitro cellular assays, so they need more systematic and sophisticated animal studies to assess their efficacy and toxicity. It is difficult to say whether a particular characteristic of a drug delivery system is good or bad. That's depend on the therapeutic goals and protocols.

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DECLARATION OF COMPETING INTERESTS

The authors declare no conflict of interest.

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