TRANSLATIONAL REVIEWS

Nanoparticle Delivery Systems with Cell-Specific Targeting for Pulmonary Diseases

Zicheng Deng[1,2,3], Gregory T. Kalin[2,3], Donglu Shi[1]*, and Vladimir V. Kalinichenko[2,3,4]*

[1]The Materials Science and Engineering Program, College of Engineering and Applied Science, University of Cincinnati, Cincinnati, Ohio; and [2]Center for Lung Regenerative Medicine, [3]Division of Pulmonary Biology, and [4]Department of Pediatrics, College of Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

Abstract

Respiratory disorders are among the most important medical problems threatening human life. The conventional therapeutics for respiratory disorders are hindered by insufficient drug concentrations at pathological lesions, lack of cell-specific targeting, and various biobarriers in the conducting airways and alveoli. To address these critical issues, various nanoparticle delivery systems have been developed to serve as carriers of specific drugs, DNA expression vectors, and RNAs. The unique properties of nanoparticles, including controlled size and distribution, surface functional groups, high payload capacity, and drug release triggering capabilities, are tailored to specific requirements in drug/gene delivery to overcome major delivery barriers in pulmonary diseases. To avoid off-target effects and improve therapeutic efficacy, nanoparticles with high cell-targeting specificity are essential for successful nanoparticle therapies. Furthermore, low toxicity and high degradability of the nanoparticles are among the most important requirements in the nanoparticle designs. In this review, we provide the most up-to-date research and clinical outcomes in nanoparticle therapies for pulmonary diseases. We also address the current critical issues in key areas of pulmonary cell targeting, biosafety and compatibility, and molecular mechanisms for selective cellular uptake.

Keywords: nanomedicine; respiratory disorders; nanoparticle delivery systems; epithelium; endothelium

The respiratory system, a primary gate toward the external environment, is prone to potential threats from a variety of airborne species, including chemicals, pollutants, and microorganisms. The respiratory system is comprised of remarkably diverse cell types residing in unique cellular niches, including the trachea, conducting airways, pulmonary blood vessels, and highly vascularized peripheral alveoli, where the exchange of oxygen and carbon dioxide occurs between the air and blood. The lung is susceptible to various injuries by barotrauma, ionizing radiation, inhaled chemicals, high and low oxygen concentrations, airborne viruses, and bacteria that cause damage to the respiratory epithelium, endothelium, and stroma, leading to acute and chronic respiratory disorders.

Current Challenges in Gene and Drug Therapies

Recent studies have developed innovative gene therapy approaches for the treatment of chronic pulmonary disorders such as lung cancer and cystic fibrosis (1–4). However, efficient gene therapy demands specially designed delivery vehicles for high gene payloads, cell-specific targeting, and significant transfection efficiency (1). Viral vectors have been widely employed in gene delivery for their excellent cell invasion capabilities, but high biosafety risks and adverse immune responses have raised considerable concerns (4, 5). Drug solubility and selectivity are critical challenges in conventional deliveries of pharmacological agents (6). It is, therefore, critical to develop new approaches to deliver therapeutic agents to the

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*Co–senior authors.

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Correspondence and requests for reprints should be addressed to Vladimir V. Kalinichenko, M.D., Ph.D., Center for Lung Regenerative Medicine and Division of Pulmonary Biology, Cincinnati Children's Research Foundation, 3333 Burnett Avenue, MLC 7009, Cincinnati, OH 45229. E-mail: vladimir.kalinichenko@cchmc.org.

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pathological sites while ensuring biosafety and compatibility. In recent years, there have been extensive studies focusing on nanoparticle-based deliveries of nonintegrating DNA vectors, stabilized mRNAs, siRNAs, and small molecule compounds, which have shown a promise in treating pulmonary disorders.

Nanoparticle Delivery Systems and Their Advantages in Biomedical Applications

Nanomaterials have been developed and widely used in biomedical applications, such as cancer therapy (7–10), regenerative medicine (11–13), vaccine or vaccine adjuvants (14–17), infections (18–20), and tissue imaging (21–23). Nanoparticle-based gene therapies prevented lung tissue remodeling in the elastase-induced mouse model of emphysema (24), improved wound healing and regeneration in patients with diabetes (25), and increased angiogenesis in airway transplants (26). There has been extensive research on the development of nanoparticles composed of polymeric, metallic, and ceramic materials that enable efficient biodistribution (27), cell-specific targeting (28–30), and internalization (31, 32). Biological macromolecules, such as proteins and nucleic acids, have large sizes that hinder effective cellular uptake. Nanoparticles, even those larger than the biological macromolecules, can be internalized via endocytic pathways (33). Furthermore, nanoparticles can be engineered via surface functionalization to meet the key requirements of gene delivery, including cellular uptake. For example, endocytosis of nanoparticles can be enhanced by targeting caveolae, the bulb-shaped membrane invaginations. Shuvaev and colleagues developed nanoparticles with caveolae-specific antibodies for efficient delivery through the caveolae pathway (34, 35). Increased concentrations of nanoparticles in the targeted regions can be achieved through the enhanced permeability and retention (EPR) (36–38). Simply by the sheer size of nanoparticles, they tend to accumulate in tumor tissue because of leaky blood vessels that are formed via pathological angiogenesis. The surface charge of the nanoparticles is an important biophysical parameter that is often reversed between the nanoparticles and the targeted cells for electrostatic attraction at the nano–bio interfaces. In cancer diagnosis and therapeutics, the surface charge-driven targeting was proven to be effective for the detection of circulating tumor cells without any antibody-based biomarkers (30, 39). Furthermore, folic acid–modified nanoparticles have been shown to be effective in tumor cell targeting (40–42), leading to enhanced cellular uptake by tumor-associated macrophages (43). Surface functionalization of nanoparticles with polyethylene glycol (PEG) effectively increased the biomarker-based cell targeting by preventing nonspecific macrophage association (44, 45).

Recent advances in nanomaterials have shown a variety of novel "smart structures" capable of controlled release of the nanoparticle cargo via different triggering mechanisms (46, 47), endosomal escape (48–50), and magnetically guided nanoparticle delivery (51–53), making nano-based delivery more efficient. For drug delivery, hydrophobic small molecule compounds can be encapsulated into nanoparticles for improved stability and enhanced therapeutic effects (54, 55). Efficient gene deliveries have been achieved by the storage of DNAs or RNAs in polymeric carriers with surface charges that can be altered to improve cellular uptake and exogenous gene expression (56, 57). Recent nano delivery of CRISPR/Cas9 components to the lung tissue has been shown to be a breakthrough in gene delivery–based therapeutics (58). Specifically, zwitterionic amino lipids were employed to deliver Cas9 mRNA and single-guide RNAs, resulting in efficient gene targeting (59). Similarly, the selective organ targeting nanoparticles were successfully used for the delivery of CRISPR/Cas9 components in a tissue-specific manner (60).

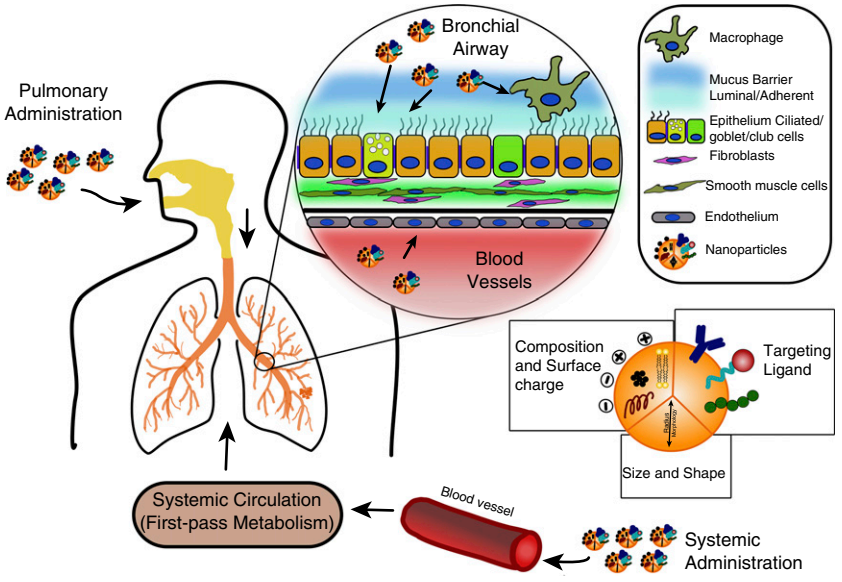

Figure 1. Systemic and pulmonary administrations of nanoparticles to target respiratory cell types. Nanoparticles can be delivered to the lung tissues through pulmonary administration (i.t. and i.n.) and systemic administration (i.v.). The ability of nanoparticles to target respiratory cell types are dependent on the size and shape of the nanoparticles, their composition and surface charge, and the presence of targeted ligands such as antibodies.

Nanoparticle Delivery Systems for Pulmonary Applications

To efficiently use the nanoparticles for biomedical applications, it is important to understand the role of respiratory cell types in pulmonary diseases. For example, an imbalance of ion transport in airway epithelial cells leads to various mucoobstructive diseases (61, 62). Dysfunction of airway ciliated cells is associated with ciliopathies, cystic fibrosis, and asthma (63–65). Lung repair after injury is dependent on multiple respiratory cell types, including lung epithelium, endothelium, and fibroblasts (66). Abnormalities in lung repair lead to

chronic respiratory diseases, such as idiopathic pulmonary fibrosis, pulmonary arterial hypertension, and chronic obstructive pulmonary disease (67–69). It is therefore critical to develop unique nanoparticle delivery systems capable of targeting the specific cell types in the human lung.

For selective targeting of respiratory cell types, new strategies will be needed because of the unique biological characteristics of the pulmonary system. As shown in Figure 1, nanoparticles can be delivered either intratracheally or intravenously, but both methods encounter different barriers. Intratracheal administration is commonly used as the first choice to target airway epithelial cells because nanoparticles can be directly delivered to the lung tissue without interference from the first-pass metabolism (70). However, the nanoparticles' movement toward the target cells can be obstructed by the mucus layer, bronchoalveolar fluid, and phagocytes within conducting airways and alveoli (71–73). Although the nanoparticles are capable of delivering the cargo to vascular endothelial cells without interference from the mucus barrier, the systemic delivery is not specific to the lung tissue. This problem can be alleviated by developing nanoparticles with cell- or tissue-specific properties and controlling the cargo release in the desirable regions (74). There are three major considerations in the nanoparticle design, as follows: *1*) physical properties (optimal size and shape for the EPR effect), *2*) chemical and surface properties (composition and surface charge for charge-driven cell binding), and *3*) bimolecular surface modifications (conjugation of antibodies on nanoparticle surfaces for specific cell targeting) (33, 72, 74, 75). Often, synergistic effects from multiple properties of the nanoparticles are used for specific therapeutics, especially in preclinical and clinical settings. Herein, we review the most recent advances in nanomedical research, with an emphasis on gene delivery for pulmonary therapeutics.

Desirable Features of Nanoparticles for Biomedical Delivery Systems

The overall properties of the nanoparticles play key roles in the drug and gene delivery. Nanoparticles need to be designed and synthesized to achieve special properties such as dispersion in physiological fluids, specific binding to targeted cells, encapsulation of drugs and genes, and spatial and temporal release of cargo upon smart triggering mechanisms. For example, the size and shape of iron oxide (Fe_3O_4) nanoparticles can be controlled by different synthetic methods and surface functionalization (76–78). The size, granularity, and nanostructure of the iron oxide nanoparticles can be changed by altering the concentration of poly-γ-glutamic acid during chemical synthesis. The hydrodynamic diameter and the surface charge of the nanoparticles are also dependent on their chemical composition. For example, the surface charge of polyplex nanoparticles can be changed by different percentages of polyacrylic acid and by the polyethylenimine (PEI):DNA ratio during chemical synthesis (79). Biocompatibility is another important feature of nanoparticles for biomedical delivery systems. For this purpose, nanoparticles are synthesized from biocompatible and biodegradable materials, such as poly lactide-*co*-glycolide (PLGA) (80), chitosan (81, 82), and fatty acids (79, 83). The surface function groups play an important role in nanoparticle biocompatibility. Recent studies reported that a replacement of 10% of surface active silanol groups by amine groups can reduce the cellular toxicity of silica nanoparticles (84).

Many different types of nanomaterials have been developed for biomedical applications. These typically include mesoporous nanoparticles (7, 9, 85), liposomes (86), and polyplexes (56). For gene delivery, plasmid DNAs or stabilized RNAs can be carried by cationic nanomaterials, such as PEI or polyamidoamine, via electrostatic interaction (87, 88). These cationic polymers can be added to organic or inorganic substrates to encapsulate the vectors. In drug delivery, amphiphilic materials such as lipids and block polymers are widely used to encapsulate different drugs. Some compounds, including RCM-1 or paclitaxel, are highly hydrophobic (89–92) and therefore easily self-assembled with amphiphilic materials into nanoparticles. In addition to drug loading, various strategies have been developed to control the release of the compounds in targeted tissues for improved efficiency. One common strategy is to use stimuli-sensitive materials, such as pH-responsive polymers (91), which can be degraded in the low pH endosomes or lysosomes to release the cargo (93). Another example of stimuli-sensitive materials is the chemically disassembled structures, such as disulfide bonds (46) and thioketal bonds (94), which can easily decouple in the presence of the particular chemicals in the cytoplasm.

An important consideration in the nanoparticle design is the ability to bind to specific cells or tissues. Targeting strategies can be classified into passive and active targeting. Passive targeting is usually related to the physiochemical properties based on the size and charge of the nanoparticles that enable the nanoparticles to bind on oppositely charged cells. Active targeting can be mainly achieved through conjugation of the nanoparticles with cell-specific ligands. The ligands usually vary and depend on the type of cells being targeted, and therefore, their specificity should change according to the nanoparticle design. Many types of nanoparticles have been developed to target respiratory cells *in vivo* (Figure 2). Polyplex nanoparticles, consisting of nucleic acids and cationic polymers, are often used in pulmonary gene delivery (79). Without nucleic acids, the polymers can self-assemble into polymeric nanoparticles (95). The biomolecules such as lipids, proteins, and DNAs are typically used in the nanoparticle development because of low toxicity and high efficiency of cargo delivery (96–98). Some inorganic nanomaterials, such as superparamagnetic iron oxide nanoparticles, were capable of accumulating in specific lung regions under a locally applied magnetic field (99).

Nanoparticles Targeting Pulmonary Epithelial Cells

Compared with systemic drug delivery by oral or intravenous routes, pulmonary administration (intratracheal or intranasal routes) delivers the nanoparticles directly to the lung via the trachea. Nanoparticle uptake by respiratory epithelial cells can avoid the first-pass metabolism, therefore increasing the local concentrations of therapeutic agents in the lung tissue (100–105). However, the airway diameter in different lung regions varies, causing an uneven distribution of the inhaled nanoparticles. Therefore, the nanoparticle design has to be tailored to the structure of

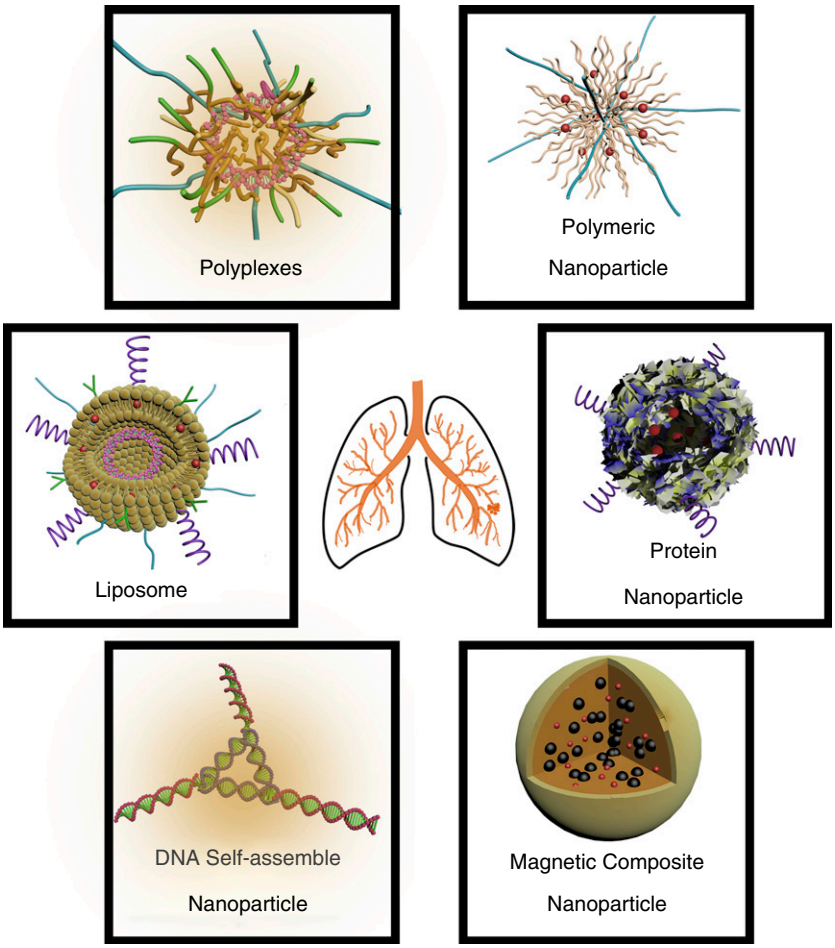

Figure 2. Typical nanoparticles that are used for delivery of therapeutic agents. These include polymer-based nanoparticles, which can be classified into polyplexes and polymeric nanoparticles on the basis of their cargo and assemble driven force. Liposomes, protein nanoparticles, DNA nanoparticles, and magnetic composite nanoparticles are also used for the delivery of therapeutics into the lung tissue.

the respiratory tract for optimal delivery. Several studies investigated the relationship between nanoparticle size and local uptake in the respiratory system. The larger particles with diameters >5 μm are mainly trapped in the upper airways (106–109), whereas the smaller particles with diameters of 1–5 μm are suitable for the lower airways (100, 108, 110). Particles with an average diameter of <500 nm can reach the alveoli via Brownian diffusion (111). Nanoparticles <200 nm in size have been reported to efficiently target respiratory epithelial cells while avoiding the clearance by macrophages (73, 108, 112).

Overcoming the Mucus Barrier

Aerosolized nanoparticle delivery is a promising route to reduce systemic toxicity and achieve specific targeting of pulmonary tissues. However, the airway mucus layer presents a considerable barrier for the use of aerosols. Mucus is produced by airway goblet cells and covers the epithelial surfaces in the trachea and conducting airways, acting as a barrier to protect the epithelium from foreign substances such as pathogens and pollutants (113). In the respiratory system, inhaled nanoparticles are rapidly removed by mucociliary clearance, which sets a limited time window for pulmonary delivery (114–116). To overcome this barrier, the nanoparticles have to penetrate an external (luminal) layer of mucus (Figure 1). To avoid being cleared, they must quickly enter the internal (adherent) mucus layer (114, 117). Mucociliary clearance usually removes nanoparticles of large sizes as well as those directly interacting with mucus. For mucus penetration, it is important to develop small nanoparticles that do not bind to the mucus. In a recent report, nanoparticles with diameters >500 nm showed low diffusion rates and were rapidly removed through mucociliary clearance, whereas those with diameters <200 nm had a better chance of mucus penetration (114). Based on these results, the mucus-penetrating property of nanoparticles has a size dependence, and the small nanoparticles have a higher ability to overcome the mucus barrier (118). To reduce interaction with mucus, the nanoparticles should be hydrophilic with a neutral surface (118–123). Nanoparticles containing PEG have been widely used specifically for this purpose (120, 124, 125). Beck-Broichsitter and colleagues developed a brush-like triblock polymer based on a PEG and polypropylene glycol coating and demonstrated that interactions between the nanoparticles and the mucus and the lung surfactant are dependent on the thickness and density of the PEG coating (126). In addition to PEG, several alternative modifications have been recently reported. Leal and colleagues designed the nanoparticles coated by mucus-penetrating peptides, which were more effective in mucus penetration and epithelial cell uptake compared with control nanoparticles without the peptide coating (127). Another strategy to penetrate the mucus is through the use of mucolytics in the nanoparticle design. This strategy has been shown to be successful by the use of disulfide breaking agents (N-acetyl-cysteine) and mucolytic enzymes (papain and bromelain), which were incorporated into the nanoparticle formulations (114, 119, 128–131). A "nano-into-micro" dry powder was recently designed for the delivery of ivacaftor (also known as Kalydeco or VX-770) to airway epithelial cells (132). In addition to ivacaftor, these nanoparticles contained mannitol and cysteamine, which reduced the viscosity and increased diffusion through the mucus.

Reducing the Clearance of Nanoparticles by Alveolar Macrophages

The clearance of nanoparticles by airway and alveolar macrophages is a critical issue that has to be addressed before achieving epithelial cell targeting via the pulmonary route. The main factors that determine macrophage clearance include the size,

surface charge, and surface properties of the nanoparticles (72). The clearance of nanoparticles takes place mainly by phagocytosis for those between 500 nm and 6 μm. Therefore, size is a major consideration in nanoparticle design (27, 73, 108, 112). The surface potential (charge) of nanoparticles is also critical to avoid alveolar macrophages. The positively charged nanoparticles (cationic) are preferentially phagocytosed by alveolar macrophages, whereas the negatively charged nanoparticles (anionic) have a better chance of escaping macrophage clearance (72, 133, 134). Cationic nanoparticles can be internalized by pulmonary dendritic cells, stimulating dendritic cell recruitment and maturation in the lung tissue (135). In contrast, anionic nanoparticles have been found to be immunologically inert (135). Several polymeric materials, such as PEG (44, 45, 125), polyvinyl alcohol (136), and zwitterionic polymers (137, 138), have been reported to escape macrophage clearance. Therefore, these polymers are often applied on nanoparticle surfaces to reduce macrophage association. Shen and colleagues investigated the effect of PEG surface modifications on cellular uptake of the nanoparticles and microparticles by alveolar macrophages *in vitro* and *in vivo* and found lower particle uptake after PEG coating of the particles (139). Figure 3 summarizes two main strategies in nanoparticle synthesis to overcome the mucus barrier and nonspecific uptake by macrophages. Polyplexes, mesoporous nanoparticles, and liposomes are typically used to carry the therapeutic agents, such as drugs and DNA expression vectors, and deliver them to the respiratory epithelial cells (Figure 3A). Mucolytic agents enable the nanoparticle carriers to penetrate the mucus barrier and target the airway epithelium upon pulmonary administration (Figure 3B).

Enhancement of the Nanoparticle Uptake by the Epithelium

After overcoming the biobarriers of mucus and macrophage clearance, the nanoparticles reach the surfaces of epithelial cells. At this stage, epithelial endocytosis becomes the key issue in the nanoparticle design. The nanoparticles are normally conjugated with specific ligands to interact with epithelial receptors or adhesion molecules. Vitamin B_{12}-conjugated nanoparticles were shown to have higher cellular uptake by respiratory epithelial cells compared with the unconjugated counterparts because of the presence of the Vitamin B_{12} receptor on epithelial surfaces (140, 141). The cell-penetrating peptides provide alternative options to enhance the nanoparticle uptake by respiratory epithelium. Krishnamurthy and colleagues developed an amphiphilic shuttle peptide platform for protein delivery to the airway epithelial cells (142). Osman and colleagues designed a glycosaminoglycan-binding enhanced transduction peptide on the surface of the nanoparticles to enhance the cellular uptake (143). In addition to conjugation with specific ligands and peptides, the chemical composition of nanoparticles can also be optimized for epithelial cell uptake. Menon and colleagues compared epithelial cell uptake abilities among six different nanoparticles, including natural and synthetic polymer-based nanoparticles, in the delivery of DNA vectors to the alveolar type 1 epithelial cells *in vitro* and *in vivo*. Gelatin-containing nanoparticles exhibited higher cellular uptake compared with PLGA-containing nanoparticles (144). Using the noninvasive aerosol inhalation method, Patel and colleagues delivered stabilized mRNAs with hyperbranched poly-β-amino ester nanoparticles (PBAEs) to mouse lungs (145). PBAEs targeted ∼25% of total lung epithelial cells after a single dose, demonstrating that nebulized delivery of mRNAs facilitated by PBAE nanoparticles may provide clinically relevant delivery systems to the respiratory epithelium.

Although inhalation has been widely reported for targeting of the respiratory epithelium, intravenous administration can also be used if the nanoparticles have a specific modification to enhance the epithelial cell uptake. Li and colleagues developed liposome-based nanoparticles that contain nanobodies specific to surfactant-associated protein A to target type 2 alveolar epithelial cells. The nanobody-conjugated liposomes effectively delivered methylprednisolone into type 2 cells during bleomycin-induced lung injury in mice (146).

Nanoparticles Targeting Pulmonary Endothelial Cells

Although intratracheal administration is an attractive method to directly target lung tissue, reaching the endothelial cell layer from the air surface is more problematic because this requires crossing the epithelial barrier without unloading the therapeutic cargo (147). Recent studies showed that nanoparticles with transferrin were transported in epithelial cells through transcytosis, which is dependent on the transferrin intracellular transport pathway (148, 149), demonstrating a promising strategy to overcome the epithelial barrier on the way to reach endothelial cells. In another study, silver core and titanium dioxide nanoparticles were efficiently transported through alveolar epithelial cells (150). It was also shown that inhaled nanoparticles were capable of translocating from the air surface into pulmonary circulation (151). These studies provide the feasibility of transepithelial migration by surface-functionalized nanoparticles to target pulmonary endothelial cells *in vivo*.

Unlike respiratory epithelial cells, endothelial cells are not exposed to the air but line internal surfaces of arteries, veins, capillaries, and lymphatic vessels. Therefore, the endothelial cells can be directly reached by the nanoparticles through blood circulation. One of the advantages of intravenous nanoparticle delivery to endothelial cells is the absence of thick mucus layers. Nanoparticle uptake by endothelial cells can be controlled by mechanical forces that are generated by blood flow. Shear stress is critical for nanoparticle uptake by endothelial cells (152, 153). Optimizing the shape, size, and charge of the nanoparticles leads to successful targeting of endothelial cells under shear stress conditions. Nanoparticle uptake via endocytosis was increased by blood flow in a PECAM-1 epitope-specific manner (154, 155). Thus, the use of PECAM-1 (CD31)-specific antibodies in nanoparticle designs will increase endothelial targeting under shear stress conditions. However, as in any systemic delivery, the first-pass metabolism and the clearance by the kidney, intestine, and liver result in undesirable offsite distribution of the nanoparticles, leading to their reduced local pulmonary concentrations. Therefore, designing nanoparticles capable of accumulating in the lung tissue becomes an essential objective in intravascular delivery. Physicochemical properties of the nanoparticles, including charge and size, are important for passive targeting of endothelial cells, whereas specific ligands and antibodies on nanoparticle surfaces are

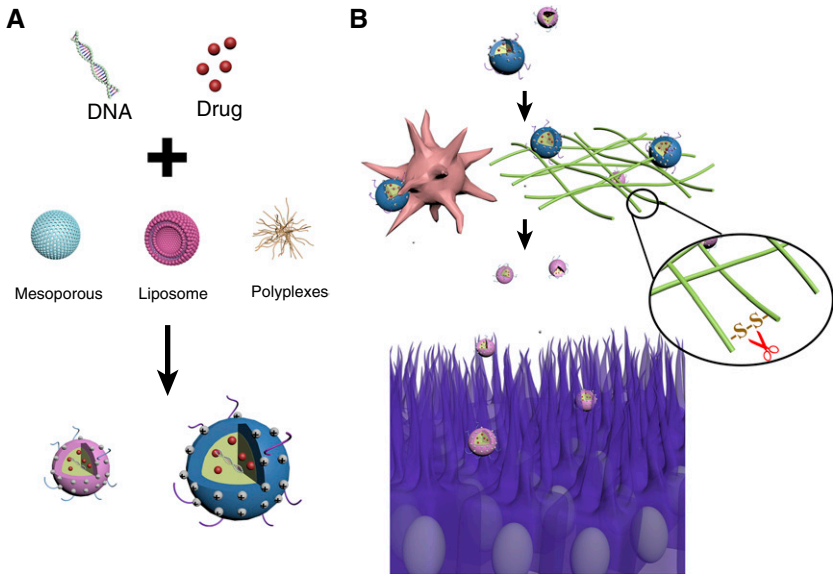

Figure 3. Nanoparticle design strategies to target respiratory epithelium. (*A*) Therapeutic agents, such as drugs and DNA vectors, are encapsulated into various nanomaterials to form the nanoparticles with a desirable size, charge, and specific surface ligands. (*B*) The mucus layer and macrophages are the main biobarriers eliminating nanoparticles with the large size, positive charge, and mucus-interactive surface. Controlling the size, charge, the surface composition of nanoparticles and using mucolytic agents are the main strategies to overcome these barriers and deliver the therapeutic cargo to respiratory epithelium.

used for active targeting. After internalization by endothelial cells, the nanoparticles escape from endosomes and release the payload of drugs, DNA expression vectors, or RNAs (Figure 4A).

Passive Targeting of Pulmonary Endothelial Cells by Nanoparticles

Dunn and colleagues developed several formulations of cationic polyplexes containing PEI, fatty acids, cholesterol, and PEG (PEI/PEG) and demonstrated an efficient targeting of pulmonary endothelial cells (85–90%) after intravenous administration to adult mice (79). Nanoparticles were mostly detected in the alveolar microvasculature but not in nonendothelial cell types. The efficiency of endothelial cell targeting was dependent on the size and surface charge of PEI/PEG nanoparticles. The nanoparticles with diameters of 120 nm and ζ potential of +24 mV were found to be the most efficient (79). Reversing the charge on PEI/PEG nanoparticles abolished the endothelial cell targeting (Figures 4B and 4C), suggesting the critical role of positive charge in endothelial uptake (79). The same formulations of PEI/PEG nanoparticles effectively delivered DNA expression vectors to pulmonary endothelial cells of newborn and juvenile mice (156, 157). Interestingly, the PEI/PEG nanoparticles mostly targeted capillary endothelial cells but not endothelial cells of large blood vessels (79). The *passive targeting* may be compromised by a lack of specificity because of nanoparticles interacting with microvascular endothelial cells in many organs of the body. However, because the lung has dense microvascular networks, the nanoparticle uptake in the lung tissue is enhanced compared with other major organs.

Nanoparticle delivery of nonintegrating *CMV-STAT3* expression plasmid into systemic circulation increased endothelial proliferation and stimulated lung angiogenesis in the mouse model of alveolar capillary dysplasia with misalignment of pulmonary veins (141), a severe congenital disorder caused by loss-of-function mutations in the *FOXF1* (forkhead box F1) gene (158–160). FOXF1 is a proangiogenic transcription factor that is expressed in pulmonary endothelial cells and the stroma (161–164). Nanoparticle delivery of the *FOXF1* gene can be an attractive therapeutic option in respiratory disorders because FOXF1 is critical for lung angiogenesis (165, 166), tissue regeneration (167–169), and lung repair after injury (170–173). Consistent with this hypothesis, nanoparticle delivery of either *FOXF1* or its target gene, *FOXM1*, stimulated neonatal lung angiogenesis and improved lung function in the mouse model of bronchopulmonary dysplasia, a severe pulmonary disorder associated with premature birth (142). FOXM1 is proproliferative transcription factor (174–176) that stimulates organ regeneration and tissue repair after injury (177–180) by acting downstream of the RAS/ERK signaling pathway (181–183). Another formulation of PEI/PEG nanoparticles, which was stabilized by cholesterol and C15 epoxide-terminated lipids, has been shown to efficiently deliver siRNA to mouse pulmonary endothelial cells *in vivo*, disrupting the expression of the *Vegfr2* gene (also known as *KDR* and *Flk1*) and causing emphysema in the lung tissue (184).

In addition to size and surface charge, efficiency of passive endothelial targeting is dependent on the chemical composition of nanoparticles. PBAEs were shown to have an efficient endothelial uptake in the mouse lung (185). Endothelial siRNA delivery was recently achieved in nonhuman primates using 7C1, a low–molecular-weight ionizable polymer that forms nanoparticles (186). Altogether, these published studies demonstrated that the surface charge, size, and chemical composition of nanoparticles are critical for passive targeting of pulmonary endothelial cells after intravascular administration.

Active Targeting of Pulmonary Endothelial Cells by Nanoparticles

In addition to physicochemical properties of nanoparticles, the use of specific antibodies and surface ligands can further improve the endothelial-specific cell uptake (Table 1). It was reported that the GALA peptide specifically binds to the sialic acid–terminated sugar chains that are present on the surface of pulmonary endothelial cells (187, 188). This feature was used in a lipid-based nanoparticle design for siRNA delivery to the lung endothelium (189). Various molecules on endothelial surfaces, known as the "endothelial target determinants," can be targeted by nanoparticles (74). Some of these molecules are regulated during pathological processes, providing multiple options for endothelial targeting in pulmonary disorders (190). Using the

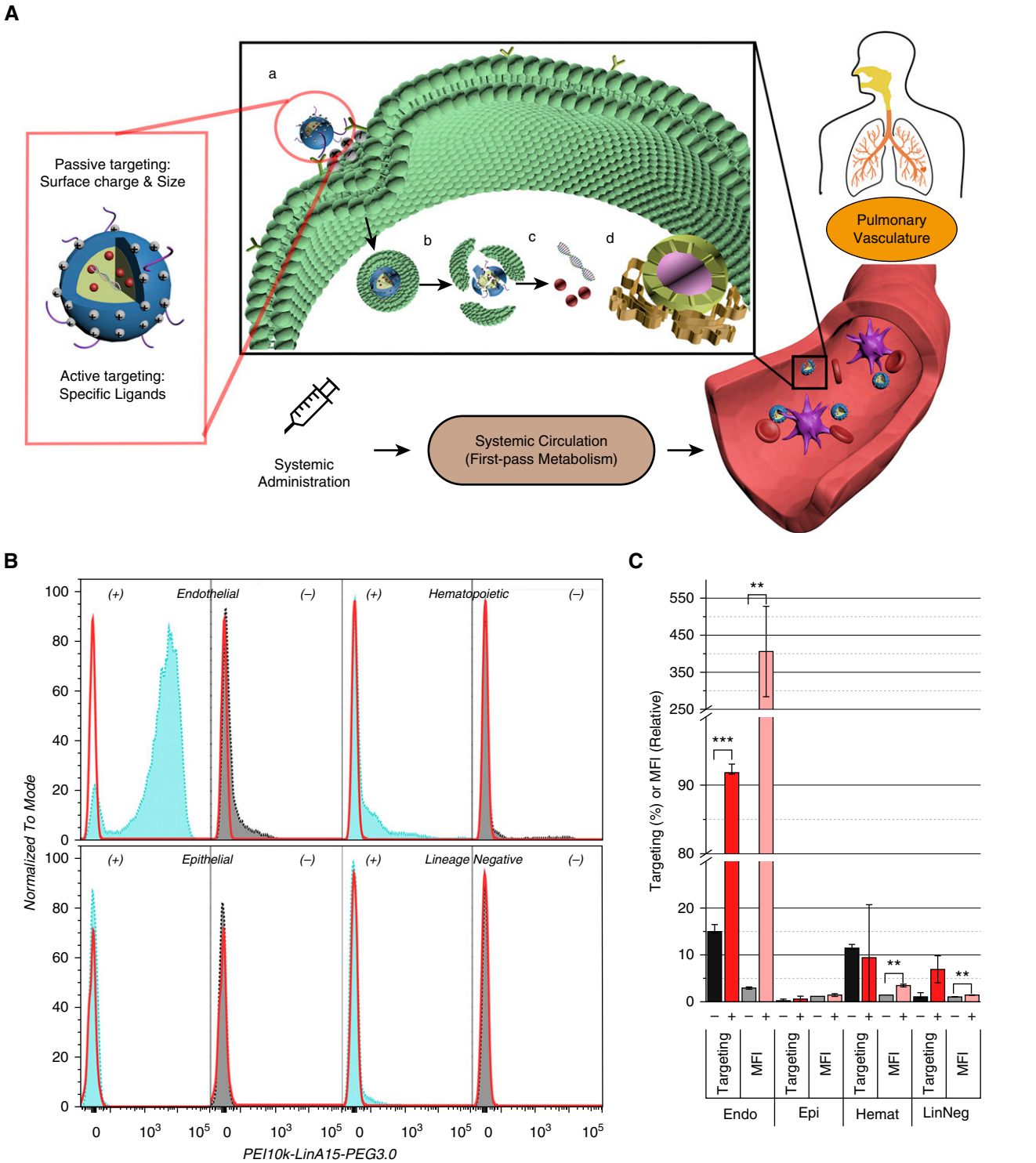

Figure 4. Targeting of pulmonary endothelial (endo) cells by nanoparticle delivery systems. (*A*) Schematic diagram shows the nanoparticle delivery to pulmonary endothelium through systemic circulation. The nanoparticles are internalized by pulmonary endo cells through the surface charge or specific ligands (*a*). After endocytosis (*b*), the nanoparticles escape from prelysosomes and lysosomes (*c*) and release therapeutic agents (*d*). (*B*) The targeting efficiency of polyethylenimine/polyethylene glycol nanoparticles with positive charge (blue) and negative charge (black) compared with untreated control (red line). Nanoparticles were injected into adult mice via the tail vein. Lungs were harvested, enzymatically digested, and used for FACS analysis to identify endo, epithelial, hematopoietic, and stromal (lineage-negative) cells. (*C*) FACS analysis shows the targeting percentage and median fluorescence intensity in different respiratory cell types. $^{**}P < 0.01$ and $^{***}P < 0.001$. Figures 4B and 4C are reprinted by permission from Reference 79. Epi = epithelial; Hemat = hematopoietic; LinNeg = lineage-negative; MFI = median fluorescence intensity; PEG = polyethylene glycol; PEI = polyethylenimine.

LPS-induced lung injury model in mice, Liu and colleagues reported that the nanoparticles containing the ESBP (E-selectin–binding peptide) are capable of targeting vascular endothelial cells activated by inflammatory stimuli (Table 1) (97). Compared with nanoparticles without ESBP, the accumulation of ESBP-modified nanoparticles in the lung tissue was significantly increased (97). EPHA2 (ephrin type-A receptor 2) is expressed in endothelial cells and is increased after lung injury (95). Patil and colleagues used the EPHA2 targeting ligand (the YSA peptide) to produce PLGA nanoparticles, demonstrating that the YSA peptide improved the nanoparticle uptake by human umbilical vein endothelial cells *in vitro* (95). Furthermore, the YSA-modified PLGA nanoparticles efficiently targeted pulmonary endothelial cells after bleomycin-induced lung injury (95). Expression of ICAM-1 (intercellular adhesion molecule 1) is increased in pulmonary endothelial cells after acute lung injury, and this property was used in nanoparticle designs (191–193). Jiang and colleagues used this strategy for nanoparticle delivery of simvastatin and the Ang-1 (angiopoietin-1) expression vector to pulmonary endothelial cells during acute lung injury induced by intratracheal administration of LPS (191). A multilayer nanostructure containing DNA strands and psoralen crosslink with the ICAM-1 antibody was recently manufactured, demonstrating excellent targeting of endothelial cells in the mouse lung (192).

Nanoparticle-based delivery methods were explored in pulmonary arterial hypertension (PAH), a progressive vascular disorder associated with loss of alveolar capillaries and aberrant proliferation of endothelial and smooth muscle cells in pulmonary blood vessels (194, 195). PEG-block-poly(ε-caprolactone) nanoparticles were generated and used to deliver rapamycin in a monocrotaline-induced rat model of PAH (196). The PEG-block-poly(ε-caprolactone) nanoparticles accumulated in the lung tissue and prevented PAH in rats (196). Active targeting of pulmonary blood vessels in monocrotaline-induced PAH has been also reported by Li and colleagues (197). The authors used glucuronic acid–modified liposomes for sildenafil delivery to smooth muscle and endothelial cells with increased expression of GLUT-1 (glucose transporter-1) (Table 1) (197). DNA triangular nanoparticles containing ATG101 single-stranded antisense RNA have been shown to efficiently transduce human pulmonary arterial endothelial cells *in vitro* (98). Although the molecular mechanism by which the DNA triangular nanoparticles targeted pulmonary endothelial cells has not yet been identified, this strategy was effective in the regulation of endothelial cell autophagy (98).

Nanoparticles Targeting Pulmonary Smooth Muscle Cells

Smooth muscle cells play important roles in pathogenesis of pulmonary disorders such as PAH and asthma. However, it is difficult to target these cells with nanoparticles. Glucuronic acid–modified liposomes were recently developed to deliver sildenafil to pulmonary artery smooth muscle cells (PASMCs) in monocrotaline-induced PAH in rats (197). Nanoparticle delivery of sildenafil to smooth muscle cells was achieved by targeting GLUT-1, effectively inhibiting the proliferation and remodeling of PASMCs in PAH. Gupta and colleagues developed liposomal nanoparticles for the delivery of fasudil to PASMCs via intratracheal instillation. These nanoparticles were taken up by PASMCs because of their small sizes, which enable the nanoparticles to escape macrophage clearance (198). Intratracheal administration of PLGA nanoparticles was used to deliver imatinib to PASMCs in lung tissue without significant accumulation of the drug in other organs (199). Although the PLGA nanoparticles with imatinib were shown to regulate the proliferation of smooth muscle cells *in vivo*, the molecular mechanism of the nanoparticle uptake by PASMCs has not been identified.

Nanoparticles Targeting Pulmonary Macrophages

Pulmonary macrophages play an important role in surfactant homeostasis, inflammation, and immune responses against bacteria and viruses, and therefore, nanoparticle systems targeting macrophages will facilitate the development of novel therapeutic strategies for respiratory disorders. Wang and colleagues designed gold nanoparticles with a hexapeptide coating to target alveolar macrophages on the basis of their phagocytic properties (200). Gold nanoparticles promoted macrophage polarization to the antiinflammatory M2 phenotype, improving lung repair after LPS-induced lung injury in mice (200).

Designing nanoparticles for active macrophage targeting is still challenging and requires further improvements. For instance, some pathogens can escape from immune surveillance by infecting macrophages, and antibiotic delivery into

Table 1. Nanoparticle Designs for Active Targeting of Endothelial Cells

Lung disease	Endothelial Cell Surface Molecules	Nanoprticle and Targeting Ligands	Reference
Normal lung	Sialic acid–terminated sugar chains	GALA peptide–modified lipid nanoparticle	(187–189)
Acute lung injury	E-selectin	ESBP-modified BSA Nanoparticle	(97)
Acute lung injury	EPHA2	YSA peptide–modified PLGA nanoparticle	(95)
Acute lung injury	ICAM-1	ICAM-1 antibody–modified lipid nanoparticle	(191)
Acute lung injury	ICAM-1	ICAM-1 antibody–modified DNA nanoparticle	(192)
Acute lung injury	ICAM-1	ICAM-1 antibody–modified polymeric nanoparticle	(193)
Pulmonary arterial hypertension	GLUT-1	GlcA-modified liposomes	(197)

Definition of abbreviations: EPHA2 = ephrin type-A receptor 2; ESBP= E-selectin–binding peptide; GALA = GALA peptide (WEAALAEALAEALAEHLAEALAEALEALAA); GlcA = glucuronic acid; GLUT-1 = glucose transporter-1; ICAM-1 = intercellular adhesion molecule 1; PLGA = poly lactide-*co*-glycolide; YSA = YSA peptide (YSAYPDSVPMMS).

macrophages can be deployed to eradicate intracellular pathogens. Wang and colleagues developed nanoparticles responsive to reactive oxygen species for antibiotic delivery (201). The nanoparticles were functionalized with the folic acid ligand, which interacts with the folate receptor on the surface of macrophages. Their approach showed efficient macrophage targeting *in vivo* using a mouse model of lung injury induced by *Pseudomonas aeruginosa* (201). In addition to macrophage targeting via folic acid ligand, the nanoparticles can be rendered sensitive to increased reactive oxygen species concentrations and triggered to release antibiotics on sites of active lung inflammation. An additional example of active macrophage targeting is the use of the mannose functionalization in nanoparticles. Mannose interacts with the mannose receptor (CD206) on the surfaces of macrophages (202, 203). Truzzi and colleagues developed the inhalable lipid nanoparticles with a mannose-based surfactant on their surfaces to deliver rifampicin, efficiently inhibiting *Mycobacterium tuberculosis* infection in alveolar macrophages (204). A similar mannose-based targeting study was reported by Costa and colleagues, showing a significant uptake of mannose-containing nanoparticles by macrophages *in vitro* (205).

Nanoparticles Targeting Pulmonary Fibroblasts

Idiopathic pulmonary fibrosis (IPF) is a chronic pulmonary disorder characterized by aberrant activation of fibroblasts and excessive production of a collagen-rich extracellular matrix in the lung tissue (206, 207). Nanoparticles targeting pulmonary fibroblasts were recently developed for potential IPF treatment. Glycol chitosan nanoparticles efficiently transduced primary human fibroblasts isolated from IPF lungs (208). The uptake of chitosan nanoparticles by fibroblasts was dependent on collagen concentrations in the extracellular matrix, suggesting that physical interactions between collagen and fibroblasts accelerate the nanoparticle uptake (208). To develop a highly efficient nanocarrier for protein delivery into pulmonary fibroblasts, Zhang and colleagues synthesized zwitterionic chitosan-based particles to deliver antifibrotic msFGFR2c protein into human lung fibroblasts *in vitro* and *in vivo* (209). After intratracheal administration to bleomycin-treated rats, the nanoparticles efficiently targeted pulmonary fibroblasts, decreasing α-SMA expression, inhibiting pulmonary inflammation, and decreasing lung fibrosis (209). Recently, Kim and colleagues developed a nanoparticle delivery system containing polymeric antisense oligonucleotides and DhBD23 (dimeric human β-defensin peptide) (210). These nanoparticles decreased *Tgfb* mRNA in mouse lung fibroblasts *in vitro* and demonstrated highly selective accumulation in lungs of mice treated with bleomycin (210). Although the identity of targeted cells in the lung tissue remains incompletely understood, both DhBD23 and polymeric antisense oligonucleotides were needed to stabilize the nanoparticles for lung-specific delivery. Hyaluronic acid (HA), which binds to CD44, a receptor critical for fibroblast activation, was used to target pulmonary fibroblasts (211). Liposomal nanoparticles were modified to include HA for the purpose of creating fibroblast-targeting nanoparticles. It was found that HA increased the internalization of nanoparticles by human lung fibroblasts isolated from patients with IPF and bronchiolitis obliterans syndrome (212).

Simultaneous Targeting of Several Respiratory Cell Types by Nanoparticles

In addition to the cell-specific nanoparticle delivery systems described above, several formulations of nanoparticles have been designed to increase their accumulation in the lung tissue without targeting a specific pulmonary cell type. A typical example of these nanoparticles is the phosphatiosomes, which were used in mouse and rat models of acute lung injury caused by intratracheal administration of LPS (213). The mechanism of lung-selective targeting via phosphatiosomes is based on the interaction between the phosphatidylcholine on the surface of the nanoparticles and the pulmonary surfactant, causing the aggregation of nanoparticles in the lung but not in other tissues (213). Nanoparticles guided by the magnetic field represent a promising strategy for organ- or region-selective targeting, including in lung regions with pathological changes identified by computed tomography or magnetic resonance imaging. Price and colleagues synthesized the superparamagnetic iron oxide nanoparticles that were guided by an external magnet after intratracheal administration in mice, successfully accumulating in the left lung lobe (Figure 5) (99). The use of magnetic-sensitive nanoparticles in pulmonary diseases was also supported by other studies. Reczyńska and colleagues applied the fatty acid–based iron oxide nanoparticles to successfully target lung cancer cells (214).

Nanoparticle Delivery Systems in Clinical Trials

Several nanoparticle delivery systems have been used in clinical trials to develop new therapeutic approaches for respiratory disorders. Clinical trials in patients with lung cancer have been recently reviewed (215, 216). Examples of lung cancer clinical trials include the polymeric nanoparticles

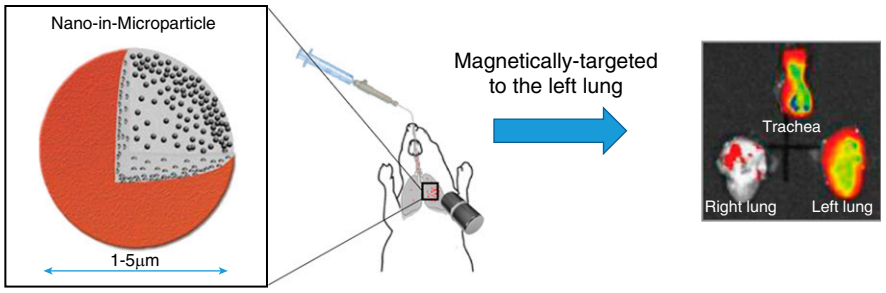

Figure 5. The magnetic field guide targeting. The nano-in-microparticles are accumulated in the left lung lobe after the magnetic field guide targeting (99). Reprinted by permission from Reference 99; further permissions related to the material excerpted should be directed to the American Chemical Society.

CRLX101 containing camptothecin, (clinicaltrials.gov, identifier: NCT01380769), the BIND-014 nanoparticles with docetaxel (NCT01792479), the albumin-stabilized nanoparticles with paclitaxel (NCT00077246), and the NC-6004 micellar nanoparticles with cisplatin (NCT02240238). All nanoparticle formulations for patients with lung cancer were administered intravenously. In contrast, clinical trials for nonmalignant respiratory disorders mostly use the inhalation or intranasal route to deliver therapeutic agents encapsulated into nanoparticles (Table 2). Inhaled liposomal nanoparticles with amikacin (amikacin liposome inhalation suspension) were employed to develop novel therapeutic approaches for patients with bronchiectasis (NCT00775138), chronic *Pseudomonas aeruginosa* infections (NCT01315678), *Mycobacterium* abscesses (NCT03038178), and nontuberculosis mycobacteria infections (NCT01315236). In patients with mycobacteria infections, liposomal amikacin improved the sputum conversion and the lung function while showing limited systemic toxicity (217). Furthermore, liposomal nanoparticles with amikacin demonstrated tolerability, safety, and efficacy in patients with cystic fibrosis and a *Pseudomonas aeruginosa* infection (218).

Inhaled liposomal nanoparticles with amphotericin B (AmBisome) were tested in patients with allergic bronchopulmonary aspergillosis (NCT02273661) and invasive pulmonary aspergillosis (NCT00391014), whereas liposomal nanoparticles with cyclosporine A were used to develop new therapy for bronchiolitis obliterans syndrome in lung transplant patients (NCT01650545). The inhalation of liposomal cyclosporine A was well tolerated and improved the lung function in lung transplant recipients without evidence of systemic toxicity (219). Although inhaled cyclosporine did not change the rate of acute rejection after lung transplantation, the cyclosporine treatment significantly improved survival and extended periods of rejection-free survival in lung transplant recipients (220). Intranasal delivery of liposomal nanoparticles with the CFTR (cystic fibrosis transmembrane conductance regulator) expression vector has been performed in a phase 1 clinical trial in patients with cystic fibrosis (NCT00004806). However, phase 2 and 3 clinical trials are yet to be performed for CFTR gene therapy. Recently, CAL02 nanoparticles, which consist of liposomes that capture bacterial toxins, were used intravenously to develop a therapy for severe pneumococcal pneumonia, showing a promising safety profile, tolerability, and efficacy to neutralize bacterial toxins (221). On the basis of outcomes of clinical trials and preclinical studies in experimental animals, additional nanoparticle carriers are currently being designed and tested to deliver DNAs, RNAs, antibiotics, peptides, and various small molecule compounds, showing a promise for human respiratory disorders.

Future Perspectives

Multiple nanoparticle carriers are capable of delivering therapeutics to the lung tissue. However, there are critical issues to be addressed before clinical applications can be used. Although the passive targeting increases the accumulation of nanoparticles in the lung tissue, the uptake of the nanoparticles is not specific. Although active targeting is based on cell-specific markers, the availability and specificity of these biomarkers is limited. The molecular mechanisms through which the nanoparticles enter the cell and unpackage to release therapeutics should be carefully identified. This is particularly important for the design and engineering of nanoparticles with synergistic targeting strategies. Nanoparticles with multifunctionalities, which are based on "smart structures" using new biochemical and biophysical properties, are still rapidly evolving. Future research should be focused on the fundamental understanding of the bio–nano interfaces and on the unique functions of nanoparticle carriers in a clinical setting. For instance, a nanoparticle can function as a tool for both the diagnostic and the therapeutic at the same time if designed appropriately. High-quality medical imaging has already been achieved

Table 2. Liposomal Nanoparticles Used in Clinical Trials to Develop Therapies for Patients with Nonmalignant Respiratory Disorders

Administration route	Nanoparticle/Cargo	Disease/Condition	NCT Number	Phase	References
Inhalation	Liposomal amikacin (ARIKAYCE)	*Mycobacterium* infection	NCT03038178	2	(222)
Inhalation	Liposomal amikacin (ARIKAYCE)	*Mycobacterium* infection	NCT01315236	2	(217)
Inhalation	Liposomal amikacin (ARIKAYCE)	*Pseudomonas aeruginosa* infection	NCT01315678	3	(223)
Inhalation	Liposomal amikacin (ARIKAYCE)	Bronchiectasis	NCT00775138	2	clinicaltrials.gov
Inhalation	Liposomal amikacin (ARIKAYCE)	Cystic fibrosis	NCT03905642	2	clinicaltrials.gov
Inhalation	Liposomal amikacin (ARIKAYCE)	Cystic fibrosis	NCT01316276	3	clinicaltrials.gov
Inhalation	Liposomal amikacin (ARIKAYCE)	Cystic fibrosis	NCT00777296	1/2	(218)
Inhalation	Liposomal amikacin (ARIKAYCE)	Cystic fibrosis	NCT00558844	1/2	(218)
Inhalation	Liposomal amphotericin B (Ambisome)	Allergic bronchopulmonary aspergillosis	NCT02273661	2	clinicaltrials.gov
Inhalation	Liposomal cyclosporine A	Bronchiolitis obliterans	NCT01650545	1/2	clinicaltrials.gov
Inhalation	Liposomal amphotericin B (Ambisome)	Invasive pulmonary aspergillosis	NCT00391014	2	clinicaltrials.gov
Intravenous	CAL02 liposome	Pneumonia pneumococcal infections	NCT02583373	1	(221)
Intranasal	Liposome/pGT-1 gene	Cystic fibrosis	NCT00004471	1	clinicaltrials.gov
Intranasal	Liposome/CFTR gene	Cystic fibrosis	NCT00004806	1	clinicaltrials.gov

Definition of abbreviations: ARIKAYCE = amikacin liposome inhalation suspension; CFTR = cystic fibrosis transmembrane conductance regulator; NCT = National Clinical Trial.

using fluorescent and superparamagnetic nanoparticles, which can also carry therapeutic agents to the site of imaging. There have been a variety of "on-demand" triggering mechanisms developed for nanoparticle carriers. These include pH sensitivity, temperature control, light sensitivity, and chemical differences between the intracellular and extracellular environment, all of which can be used in future nanoparticle design to overcome the biobarriers. Synergic therapies, which are based on the simultaneous delivery of multiple therapeutic agents via nanoparticle delivery systems, provide an attractive future direction for clinical management of lung diseases. A codelivery system, consisting of the drug and the gene product encapsulated into the same nanoparticles, can address some complex therapeutic needs. In preclinical and clinical studies, biocompatibility and toxicity of the nanoparticles remain major challenges that will have to be addressed jointly by collaborations between engineers, chemists, and biomedical researchers.

Summary

The advancements in nanomaterials have provided vast therapeutic opportunities for life-threatening respiratory disorders. To overcome several key barriers in drug administration for pulmonary diseases, versatile nanoparticles have been designed and synthesized for a variety of therapeutic needs. Nanoparticle-based carriers can efficiently deliver antibiotics, nonintegrating DNA expression vectors, stabilized mRNAs, siRNAs, and small molecular compounds to the lung tissue in a cell-specific manner. The pulmonary administration of nanoparticles via the intratracheal route avoids systemic clearance and provides high onsite concentration and specific targeting of respiratory epithelial cells and macrophages. The intravenous administration of nanocarriers is extensively applied to target pulmonary endothelial cells and achieve lung tissue accumulation. Pulmonary and systemic delivery routes have been used to target other respiratory cell types, such as fibroblasts and smooth muscle cells. Specific receptors and adhesion molecules on the cell surface of nanoparticles provide additional possibilities for cell-specific targeting strategies in pulmonary disorders. A variety of nanoparticles have now been designed and synthesized to improve the delivery of therapeutic agents to pulmonary tissues, providing innovative therapeutic approaches for treatment of human respiratory disorders. ■

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