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Review

Pulmonary Drug Delivery through Responsive Materials

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Abstract: Drug delivery is essential to provide correct treatments in many ways. The critical points in any drug delivery method are patient compliance, maximum efficacy in therapy, minimum toxicity, and enabling new medical treatments. Pulmonary drug delivery is one way of delivering therapeutics locally and systemically. The lung microenvironment and mechanical and biological barriers must be surpassed for successful drug delivery. This makes the delivery challenging. Formulations that can be delivered through the lung and have a responsive character are of great interest since they can hold the key to the successful delivery of therapeutics. This review has gathered fundamental studies related to materials (polymeric, lipidic, inorganic, and biomolecules) that are responsive to pH, enzymes, ROS, magnetism, and other variables, and it shows the advances and applications in pulmonary drug delivery for different diseases in vitro as well as in vivo.

Keywords: drug delivery; responsiveness; lungs; pulmonary delivery; inhaled formulations; polymers; enzymes; diseases

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1. Introduction

1.1. Pulmonary Delivery

The delivery of therapeutics and active compounds is critical in remedying a disease. There are many ways to deliver an active compound or drug to a specific area in the human body. The most common ways of providing a drug are (i) nasal, (ii) pulmonary, (iii) oral, (iv) transdermal, and (v) intravenous. Depending on the targeting of the specific disease, the drug's mechanism of action, and the nature of the compound, the most suitable way of delivery is chosen. The delivery of drugs is currently systemic in most cases [1]. This technique has proven efficient depending on the specific targeted disease. However, it also has some drawbacks, such as not providing sufficient quantities of drugs at the desired location, especially for drugs with limited therapeutic windows, and, most importantly, the crossing of specific biological barriers [1,2]. In these terms, pulmonary delivery is exciting since it can deliver compounds for local and systemic use [1,2]. The lung offers a remarkable and demanding route for drug delivery with high absorption and surface area, ca. 100 m^2 [3], and abundant vessels of lung tissues [4].

Moreover, lungs offer a highly vascularized surface area for drug absorption, an epithelial barrier of low thickness, and the absence of the first-pass effect that plagues oral delivery methods [1,2]. Delivery through this technique has resulted in improved biodistribution and reduced systemic toxicity compared to conventional formulations administered intravenously [5]. In local treatments of tuberculosis, asthma, lung cancer, or chronic obstructive pulmonary diseases, the inhalation of the drug goes directly to the site of action [1,2]. Pulmonary delivery would allow enhanced bioavailability, decrease side effects, and limit accumulation in the liver, spleen, and kidney [1,2]. Throughout the lung system, systemic delivery into circulation is also possible due to the lung's natural permeability to small molecules, proteins, and peptides [1,2]. The approach of pulmonary delivery has been researched extensively in the last few years. It has been used positively for

preparing formulations for inhalable carrier systems in addition to pure drug formulations. Inhalable carriers protect the drugs by avoiding early degradation and fast clearance [6]. Research has also been carried out on inhalers to improve their effectiveness and delivery of the therapeutic by using different types such as metered doses, nebulizers, and dry powder inhalers [4].

1.2. Finding the Way to the Lung

Aerosols are inhaled through the mouth and pass into the respiratory tract. The route in order is oropharynx, larynx, trachea, bronchi, bronchioles, and alveoli. Successful deposit in the airways and alveoli requires that carriers have a specific diameter. According to the literature, carriers must have a size of <5 mm. Particles of >5 mm diameter are deposited mainly in the oropharynx by inertial impaction and are then swallowed into the gastrointestinal tract [7]. Due to impaction, large particles (>10 μm) are deposited in the oropharyngeal and larynx region. Small particles (0.5–2 μm) are retained in the alveoli and smaller conducting airways, resulting from gravitational sedimentation. Very small particles (<0.5 μm) are generally not deposited and are expelled upon exhalation [8]. Drug-loaded nanoparticles are usually deposited in the pulmonary region by sedimentation after being released from the device due to lung agglomeration. These agglomerated nanoparticles can reside longer in the tracheobronchial region, thereby providing effective targeting and improved drug therapeutic efficacy. Even though pulmonary delivery has a favorable route to the target (lungs), pulmonary clearance exists to protect the patient from foreign bodies. Mucociliary clearance is the primary clearance mechanism for removing foreign particles in the airways. The epithelial cilia mechanically transport the particles along the mucous layer toward the oropharynx, where the particles are subsequently swallowed or expectorated. Coughing is the usual mechanism for removing large particles that deposit. In the alveolar region, macrophages may engulf and destroy particulate materials [7].

1.3. Formulations and Materials in Pulmonary Delivery

Pulmonary delivery is an evolving field in therapeutics, but it is also quite challenging. The anatomy and physiology of the lung are demanding and challenging and must be taken care of before designing specific formulations for drug delivery. Carriers at the nanoscale can successfully load and deliver biological therapeutics, but they need to obtain sizes less than 300 nm for efficient cellular endocytosis and transport across the mucosal barrier [9]. One major problem is nanoparticles' effectiveness in efficiently aerosolizing and depositing within the lung via inhalation, since particles of less than 1 μm are predominantly exhaled. Particles ideally need sizes between 1 and 5 μm for efficient deposition in the deep lung. At the same time, alveolar macrophages rapidly phagocytose particles with geometric diameters within the 1–5 μm range, making the therapeutic delivery difficult. Aerosolization of the formulations can lead to two groups of treatments: (i) those intended for lung administration and (ii) those that sustainedly release volatile compounds into the air for inhalation [10].

Much research has been carried out to find the proper nanocarrier to host specific therapeutics. Some of the most researched candidates are:

1. Nanoparticles: Regarding pulmonary drug delivery, nanoparticles help target a specific site for delivering a therapeutic substance. This becomes a significant advantage, specifically with drastic reductions in the dose of the therapeutic substance required. In addition, the occurrence of side effects has been reduced relatively.
2. Microparticles: These are prepared by encapsulating, entrapping, or dissolving the active drug within a polymer matrix. They can be employed for targeted delivery, sustained release, and controlled release of therapeutic agents in the pulmonary region.
3. Liposomes: currently, they are used in sustained-release formulations for lung diseases and gene therapy.

4. Powders: in pulmonary drug delivery, powders are used in different inhalational product preparations, like dry powder inhalers.
5. Microemulsions: These are used for controlled drug release and specific tissue targeting. They are also used for reducing the rate of degradation of drugs [8].

Since the research is evolving, other types of formulations are being studied, such as phospholipids [5] and nanoscale metal-organic frameworks (NMOFs), which are a class of porous materials that have become promising candidates for drug encapsulation and delivery due to their high porosity, biodegradable structures, and controllable surface functionalities [11]. Specific mention must be made of liposomes, with excellent safety and biocompatibility, and drugs loaded with liposomes have prolonged lung residence time [12]. Liposomes can be prepared in different formulations, such as solid lipid nanoparticles, nanostructured lipid carriers, and liposomes [13].

Moreover, hydrogels, a unique class of materials, hold significant potential in pulmonary delivery. Their soft and low mechanical modulus properties, along with a size range of 0.5 to 5 mm, make them a promising option. These hydrogels can swell after deposition, reaching a larger size and avoiding macrophage uptake [2]. The added responsiveness to a stimulus further enhances their potential for medical applications. These formulations can change their shape or degrade controllably to deliver their payload. Currently, the major responsive characteristics for these formulations are based on pH, temperature, and enzymes [1]. Formulations such as hydrogels, complexes, liposomes, and composites with specific responsiveness (pH, temperature, magnetic, enzymatic, and others) have demonstrated successful delivery of therapeutics through the lungs, as depicted in Figure 1.

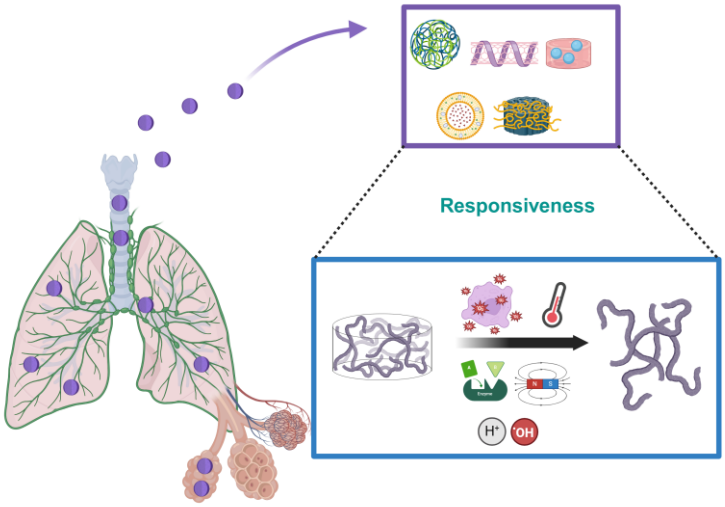

Figure 1. Schematic illustration of the pulmonary delivery via various responsive formulations.

Another critical parameter to consider when designing the formulation is how to deliver the specific compound. The three significant ways of delivering are nebulizers, where droplets from a bulk liquid using either compressed air (jet nebulizers) or ultrasonic waves (ultrasonic nebulizers) generate continuous aerosol streams; metered dose inhalers (MDIs), in which the drug(s) are suspended or dissolved in a liquefied propellant system, which may also contain excipients such as co-solvents or surfactants (portability and simplicity); and dry powder inhalers (DPIs), which provide single- or multi-doses via oral inhalation, depending on the design of the powder reservoir and metering components [7]. Among these, DPIs are considered the most promising delivery systems since they maintain high physicochemical stability in a vast range of temperatures, provide better sterility, and are easy to operate and cost-effective [6]. The most used techniques for preparing inhalable powder formulations are spray drying (SD) or spray freeze drying (SFD). Spray drying involves atomizing the compounds of the desired products into droplets by spraying,

followed by rapid evaporation into solid powder. Spray freeze drying also uses the atomization step and then solidification by cooling water and subsequently subliming them at low temperature and pressure interaction with the cryo liquid phase [14].

1.4. Barriers to Overcome in Pulmonary Delivery

Delivering the formulation-bearing therapeutics into the lung is quite a challenging procedure. The lung has specific limitations and barriers that must be overcome for a successful delivery. One of the most critical parameters is the aerodynamic diameter of the carrier. Very small or very large particles are eliminated naturally from humans. Suppose the carrier reaches and is deposited into the lung; it must avoid macrophage clearance [1,2]. The stiffness of the carrier is also noteworthy since stiffer particles are more likely to be eliminated from the macrophages [1,2]. This mechanical barrier is derived from the complex network of airway tissues. The carrier can always become stuck in numerous other sites irrelevant to the desired one. Finally, lung mucociliary clearance also offers a significant mechanical barrier by removing the deposited particles from the airways [8]. Current approaches for addressing these conflicting design issues include using porous polymers and swellable microparticles. By lowering the density of the microparticles, large porous particles provide an aerodynamic diameter ideal for the lung.

The chemical and immunological barrier is also crucial since proteolytic enzymes, surfactants, and alveolar macrophages constitute the chemical and immunological barriers in pulmonary drug delivery [8]. In the same line of limitations is the water solubility of drugs inside the lung since the total volume of the pulmonary lining fluid is small (ca. 150 mL) and distributed as a thin liquid film (not more than 30 μm) over the sizeable epithelial surface area (140–160 m^2) [15].

Furthermore, respiratory mucus in the upper airways and airway surface liquid (surfactant) in the lower airways pose significant physical and chemical barriers [16]. However, the most significant factor in therapy outcomes is the patient's behavioral attributes. Non-adherence to the therapeutic regimen, whether intentional or non-intentional, can have a profound impact. Poor inhaler technique and handling errors can lead to suboptimal and variable drug deposition in the lungs [8].

1.5. Disorders, Diseases, and Drugs for Therapy in Pulmonary Delivery

The lung is a human body organ with some major diseases and disorders currently treated with inhalable drugs or other therapies. The majority of them are idiopathic pulmonary fibrosis, acute lung injury, tuberculosis, chronic obstructive pulmonary disease, bronchial asthma, lung cancer, pneumonia, lung infection/inflammation, and cystic fibrosis. Subsequently, the major diseases/disorders and the current therapeutics under research can be seen and an overview is found in Table 1.

Table 1. Major diseases and disorders and current therapeutics under research.

Diseases–Disorders	Current Treatment and Research
Idiopathic pulmonary fibrosis	Liposomes-hyaluronic acid, Au nanoparticles functionalized, and interferon-g
Acute lung injury	siRNA technology and gene therapy
Tuberculosis	Mannose with a pH-sensitive prodrug, siRNA, and vaccine of Muramyl dipeptide
Chronic obstructive pulmonary disease	Bronchodilators, β-agonists, anticholinergics, corticosteroids, siRNA, and uridine triphosphate derivatives
Asthma	Bronchodilators, corticosteroids, leukotriene modifiers, mast cell stabilizers, immunomodulators, siRNA, anti-IgE Mab, interleukin-1 receptor, interleukin-4, lactoferrin, and vasoactive intestinal peptide
Cystic fibrosis	Antibiotics, K+-sparing diuretics (e.g., amiloride), bronchodilators, gene therapy, rhDNase (approved product: Pulmozyme), secretin, and targeted genetics adeno-associated virus
Lung cancer	mRNA and siRNA, gelatin NPs (cisplatin), PLGA NPs (amodiaquine), solid lipid NPs (cisplatin), NPs liquid crystals of lactoferrin/chondroitin (pemetrexed and resveratrol), and interleukin-2
Diabetes	Liposomes, co/spray-dried hyaluronic acid (recombinant human insulin), oligosaccharides microparticles (recombinant human insulin), ether/anhydride microparticles and nonporous particles with phospholipids, and dry powder formulations/liposome formulations with insulin

1.5.1. Idiopathic Pulmonary Fibrosis (IPF)

This disease is related to age, and the prognosis survival is no more than five years. No known cause exists for this disease. Scarring causes stiffness in the lungs and makes it difficult to breathe. Pulmonary therapy is targeted to provide drugs directly to lung lesions. Some inhaled drugs are currently in clinical trials [12]. In research, some work is related to liposomes with hyaluronic acid [17] and gold nanoparticles functionalized to deliver tyrosine kinase inhibitor [18].

1.5.2. Acute Lung Injury (ALI)

This disease is related to respiratory failure or acute hypoxic respiratory insufficiency due to injury (severe infections, shocks, traumas, and burns). Research is being conducted using siRNA technology to explore gene function and gene therapy. These formulations can directly target pulmonary cells and avoid first-pass elimination [11].

1.5.3. Tuberculosis (TB)

This infectious disease affects 1/3 of the global population [6]. The causes of TB are infection in the lungs from contagious bacilli. Current treatment includes a combination of drugs [8]. In research, mannose modified with a pH-sensitive prodrug is being investigated for intracellular therapy [19]. In research, siRNA is also considered a potential therapy [16].

1.5.4. Chronic Obstructive Pulmonary Disease (COPD)

COPD is a chronic lung disease characterized by bronchial inflammation, airflow limitation, hyper-inflammation, and respiratory muscle dysfunction. Current treatments involve bronchodilators, β-agonists, anticholinergics, and corticosteroids [8,20]. Pulmonary delivery of siRNA is another type of therapy under investigation [16].

1.5.5. Asthma

Asthma may be regarded as a type of COPD. It is characterized by episodes of obstruction to the airflow due to multiple stimuli, such as infection, allergies, and physical stimuli. For treatment, the drugs currently in use are bronchodilators, corticosteroids, leukotriene modifiers, mast cell stabilizers, and immunomodulators [8,20]. Research is also being conducted with siRNA [16].

1.5.6. Cystic Fibrosis (CF)

CF is a genetic disease in which excessive mucus production leads to pulmonary obstruction, infections, and respiratory failure. Antibiotics, K+-sparing diuretics (e.g., amiloride), bronchodilators, and gene therapy could effectively treat CF [8,16,21].

1.5.7. Lung Cancer (LC)

Lung cancer is one of the most frequent tumors with high mortality. Typical treatments include immunotherapy, surgery, and radiation [8]. Regarding pulmonary therapy, mRNA and siRNA are potential candidates for pulmonary therapy [16,21]. Investigations are also being conducted with materials such as gelatin NPs to deliver cisplatin [22], PLGA NPs with amodiaquine [23], solid lipid NPs with hyaluronic acid to deliver cisplatin, and NPs liquid crystals of lactoferrin/chondroitin for delivering pemetrexed and resveratrol [24].

Special mention should be made of the pulmonary delivery of insulin. Delivering insulin to diabetic patients, not intravenously, opens the path to pulmonary delivery, a method currently under research. Some of the work involving different types of materials includes liposomes with agglomerates of insulin [25], co/spray-dried hyaluronic acid with recombinant human insulin [26], oligosaccharides microparticles with recombinant human insulin [27], ether/anhydride microparticles [28,29], and nonporous particles with phospholipids [30]. Furthermore, Aspen Aerogels, Inc. (Northborough, MA, USA) [31] and Aradigm Corp. (Hayward, CA, USA) [32] claim pulmonary dry powder formulations and liposome formulations with insulin, respectively.

Many pharmaceutical proteins and peptides are formulated as inhalation aerosols for local and systemic diseases. These formulations are at various stages of development, ranging from Phase I studies to approved products. Some examples of these products and types of local diseases are as follows:

- Asthma: anti-IgE Mab, interleukin-1 receptor, interleukin-4, lactoferrin, and vasoactive intestinal peptide.
- Tuberculosis: vaccine of Muramyl dipeptide.
- Cancer: interleukin-2.
- Chronic bronchitis: uridine triphosphate derivatives.
- Cystic fibrosis: rhDNase (approved product: Pulmozyme), secretin, and targeted genetics adeno-associated virus for cystic fibrosis.
- Idiopathic pulmonary fibrosis: interferon-g.

Also, drugs are available for systemic diseases such as anemia, anticoagulation, cancer, diabetes, endometriosis, growth deficiency, hemophilia, hypoglycemia, infertility, multiple sclerosis, neutropenia, obesity, osteoporosis, and viral infections.

2. Pulmonary Delivery of Compounds via Responsive Processes

The lungs are part of the lower respiratory tract and accommodate the bronchial airways when they branch from the trachea. The whole microenvironment of the lung has a fascinating structure with different types of cells, mucus, extracellular matrices, and defense mechanisms against foreign bodies. Someone could say that this part of the human body is complex and highly dynamic. Materials with superior properties are needed to study or deliver active compounds in the lung. In the past, materials could only change from an inert phase to a release phase. Now, materials are smart and responsive and can reach high levels of autonomy, where they can sense, respond/release, and adapt. To that end, new materials are needed to deliver drugs or active compounds for local diseases and through a systemic route for other disorders. The lung can be an essential passage to the human body. The materials currently under investigation are different not only in chemical or structural points but also in their level of responsiveness and type of stimuli. The most studied types of responsiveness currently under research for the lungs are pH and enzymatic. Some work has also been carried out on reactive oxygen species (ROS), magnetic, glucose, and temperature responsiveness.

More specifically, stimulus-responsive systems in medical applications are promising since they can change their shape or degrade and deliver the therapeutic at a desired site or moment in response to an external stimulus [1]. In pulmonary drug delivery, pH responsiveness can be significant since pH-responsive peptides have low toxicity and form complexes with siRNA. This responsiveness can help the carriers' endosomal escape, helping overcome one significant barrier in this type of delivery [33]. pH-responsive materials are significant for pulmonary delivery since these carriers can show a controllable delivery, which is very important for specific diseases and exploits tumors' low pH [4,5]. Various disease-specific enzymes in the lung are also essential in exploiting enzymatic degradation as part of responsiveness in specific carriers [1,34]. Like enzymatic degradation, researchers have targeted responsiveness towards reactive oxygen species (ROS), which is also considered one of the critical factors. ROS is produced by macrophages in specific lung diseases (e.g., fibrosis) [12] or tumor cells [35]. Carriers that ROS can degrade can be of great importance for specific drug release in the lung [12,36]. Another approach for exploiting another type of responsiveness is using a magnetic field. In that case, having nanocarriers with magnetic elements can enable guidance and concentration of the drugs to the desired lung region, leading to better efficiency and fewer side effects [6]. In pulmonary delivery, other types of responsiveness are also under investigation, targeting temperature [37] or glucose [38].

2.1. pH-Responsive Materials for Pulmonary Therapy

The novelty of nanocarriers demonstrating pH responsiveness in the context of pulmonary therapy is particularly intriguing, given the significant role of pH in the lung environment. Notably, the literature has explored various materials as pH-responsive carriers, including polynucleotides [4,14,33] and polymeric [21,39–41], lipidic [5], and inorganic [11,42] materials.

For the polynucleotides, an intriguing study can be found in the literature where the authors prepared small-sized DNA tetrahedrons. These systems are intended to be used as transmucosal delivery systems for pulmonary metastatic cancer—delivering immunomodulatory CpG oligonucleotide and PD-L1-targeting antagonistic DNA aptamer. The pH sensitivity was due to the hairpin containing an i-motif strand on TDN. The obtained aerosol was inhaled by mice intratracheally using a micro-nebulizer. These formulations effectively pass through the pulmonary mucosal barrier and enhance their intratumoral accumulation. Finally, in this study based on a lung melanoma metastasis model, it was found that these carriers could significantly inhibit tumor growth and prolong the survival time of mice compared to free immune agents [4].

In another study, the pH-responsive peptides LAH and LADap were evaluated on human epithelial cells (A549) with bronchoalveolar lavage fluid (BALF) (obtained from rats) in order to study the effect of ASL on transfection efficiency. The formulations were prepared as a dry powder of the pH-responsive peptides and plasmid DNA, with mannitol as a carrier, and were produced by either spray drying (SD) or spray freeze drying (SFD). The authors tested the physicochemical characteristics, aerodynamic performances, and in vitro biological activities. Their results showed that both powders had good aerodynamics and transfection efficiency characteristics. Ultimately, the study revealed that SD powders have advantages over SFD powders and have more potential for better nucleic acid delivery via inhalation [14].

Moreover, spray-dried powders (SD) have been prepared with antiviral siRNA, mannitol, and LAH4-L1, LADap6-L1, and LADap (Me) 6-L1 peptides as pH-sensitive compounds. These formulations were tested for pulmonary delivery and in vitro as antiviral agents against H1N1 viruses. This study showed that these carriers can be prepared as dry powders by SD without affecting their biological activity and physical characteristics. The transfection efficiency of LADap (Me) 6-L1 peptide SD powders was retained in the presence of BALF, making it a promising vector for pulmonary siRNA delivery. Furthermore, the antiviral activity was maintained after SD, and it can be concluded that these carriers

can be used as potential formulations for the prevention and treatment of influenza, paving the way for the multi-operational use of these carriers in other respiratory diseases [33].

Regarding carriers with a polymeric basis, some studies have been carried out on pH responsiveness. The first study refers to pH-responsive hydrogel nanoparticles incorporated into dry powder composites. The pH character is based on the polymer (methacrylic acid) with PEG-diacrylate. DLS tested the pH responsiveness, and TEM was used to measure morphological homogeneity and size. The authors propose preparing a multi-operational nanocarrier system for pulmonary delivery. For the preparation as a dry powder, mannitol was spray dried with the hydrogels, and the aerodynamic performance of the resulting powder was evaluated [39].

A particularly inspiring study presents a combination of PEGylated cationic peptide-based mRNA delivery vectors [21]. In this study, hydrophilic PEGs were covalently linked to an amphiphilic pH-responsive peptide (LAH4-L1). These nano self/assemblies were characterized via DLS and TEM. The authors propose a promising alternative mRNA delivery candidate for mRNA vaccines that can be administered through inhalation. This innovative approach could revolutionize the treatment and prevention of various respiratory diseases. The nano-assemblies were tested in vitro and in vivo by formulating an intratracheal aerosol. Modification of the length of PEGs led to an improvement in the delivery of the mRNA, demonstrating the pivotal role of the PEG length chain in the transfection capacity. The successful delivery of respiratory mRNA without visible safety risks in mice could pave the way for exciting future developments.

For polymeric pH-responsive carriers, another significant study is based on a norbornene nanocarrier with a multi-drug compartment consisting of isoniazid (INZ), rifampicin (RIF), and a robust hydrophobic core. For a controlled drug release, the drugs are linked to the polymer backbone through an acylhydrazine linker to produce a unique nanocarrier from the statistical copolymer. The linkage is cleaved in acidic conditions, leading to the release of the drug. Despite the original design of these carriers for pulmonary tuberculosis, in vitro studies in A549 cells (MTT assay) have demonstrated high anticancer efficacy, hinting at the potential for a dual type of therapy [40]. This work underscores the practical implications of polymeric pH-responsive carriers in drug delivery.

Finally, regarding polymeric nanocarriers with pH responsiveness, another study is based on polysaccharides to selectively deliver rifampicin/pyrazinamide (RF/PZA) for tuberculosis. In this study, maleate gellan gum was formed by adding free radical polymerizable groups to form 3D networks via esterification. The pH responsiveness was controlled via natural silk added to the networks. These carriers showed high antimycobacterial activity and fast drug delivery [41].

In terms of lipid nanovesicles, one study [5] reports the development of pulmonary surfactant mimetic pH-responsive carriers as inhalable systems. Here, the authors use DPPC and DOPE to prepare lipid nanocarriers loaded with paclitaxel, a well-used anticancer drug. The nanovesicles were extensively characterized and evaluated for their pulmonary toxicology and compatibility. These pH-responsive nanoformulations were evaluated for therapeutic efficacy in metastatic lung cancer in mice. The in vitro lung deposition was performed through aerosolized nanocarriers using a jet nebulizer. Apart from the in vivo studies, in vitro experiments were performed in B16F10 murine melanoma and A549 human lung cancer cell lines. Their results showed an airway pattern similar to endogenous pulmonary surfactant and did not provoke an inflammatory response in alveolar macrophages. The biodistribution of the drug improved compared to i.v. Taxol showed a 75% higher inhibition of metastasis compared to i.v. Taxol and i.v. Abraxane.

Finally, in the area of inorganic materials, two studies in pulmonary delivery are explored [11,42]. The first study sought to alleviate early pulmonary fibrosis in acute lung injury (ALI). The nanocarrier used here was a nanoscale metal–organic framework (NMOF), and porous materials were promising in drug encapsulation and delivery. The metal–organic framework was designed through a Zr (IV)/based porphyrin with branched polyethyleneimine and poly (2-diethylamino) ethyl methacrylate. This system delivered

small interfering ZEB1/2 (siZEB1/2) for alleviating early pulmonary fibrosis during ALI. In Figure 2, the preparation and intratracheal therapeutic mechanism of the system can be seen. In vitro experiments were conducted on A549 cells, while in vivo experiments in mice were performed through intratracheal infusion. Their results showed that ZEB1/2 is a therapy target for ALI but can also be a promising siRNA delivery system for inflamed lungs to attenuate early pulmonary fibrosis progression.

The second study is a significant step forward in tuberculosis treatment, exploring the use of nanocarriers to target and deliver the drug (isoniazid (INH)) in high concentrations directly into the infected cells. These nanocarriers, based on mesoporous silica nanoparticles, are coated with poly (ethylene imine)–poly (ethylene glycol) (PEI–PEG) copolymer to enhance their dispersibility and stability. The drug is released naturally within acidified endolysosomes. This approach has shown improved compatibility towards infected cells and tolerance in a mouse model, suggesting the potential for more effective and targeted tuberculosis treatment compared to the use of free drugs [42].

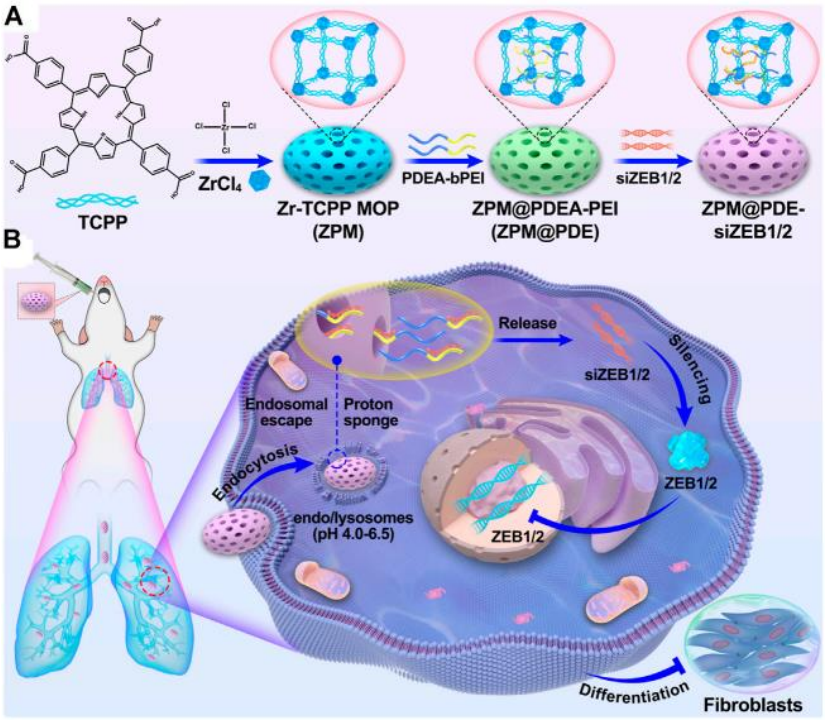

Figure 2. (**A**) The scheme for ZPM@PDE-siZEB1/2 preparation and (**B**) intratracheal therapeutic mechanism of ZPM@PDE-siZEB1/2 in ALI model mice. Reprinted with permission from [11].

2.2. Enzyme-Responsive Materials for Pulmonary Therapy

Apart from pH responsiveness, the second most used approach considered here is one where the release of the drug is triggered by an enzymatic response inside the lung's microenvironment. The studies found in the literature use formulations based on a polymeric nature [1,2,9,34,43].

The first study [1] revolves around the potential applications of PEG microparticles. These hydrogels, with their unique ability to change size in response to the environment, offer a promising solution to avoid macrophages. These formulations are designed to respond to matrix metalloproteinases, which are often overexpressed in pulmonary diseases. The authors meticulously characterized these particles, focusing on their physical properties and enzymatic degradation. The enzymatic response is triggered by a trimer peptide Gly-Leu-Lys (or GLK), which contains the enzyme-responsive glycine-leucine (GL) and is incorporated into a PEG's backbone. While the authors did not specifically target a disease,

their research hints at the potential of PEG microparticles in the treatment of various pulmonary diseases, including lung cancer, tuberculosis, and COPD.

An interesting idea from another study [9] is formulating a nanoparticle-inside-microgel system for targeting chronic inflammatory lung diseases such as asthma, COPD, and cystic fibrosis. The key to this approach lies in the size of the particles and their ability to travel and deposit in the lung while avoiding macrophages. The authors have an interplay with the emulsion method for fabricating sizes. The microgels were fabricated by using Michael addition during emulsion. A di-sulfhydryl peptide and a 4-arm PEG maleimide were used to prepare the final formulation. The nanoparticles were physically entrapped inside the PEG network during cross-linking. The enzymatic response is based on the protease-triggered release of encapsulated nanoparticles. These carriers were aerosolized through a micro sprayer aerosolizer and were oropharyngeal aspirated from the mice. This work studied the physical properties of these formulations, the cellular biodistribution, phagocytosis, and clearance in vivo.

The same group has also prepared these formulations in previous work [34] as an enzyme-responsive nanoparticle-in-microgel delivery system. These carriers are designed for optimal aerodynamic size for deep lung delivery, improved lung resistance, avoiding macrophages, and reduced side effects and toxicity. The peptide sequence, CGRGGC (cysteine–glycine–arginine–glycine–glycine–cysteine), was explicitly designed to have high specificity for trypsin. These microgels showed a high internal porous structure and exhibited triggered release in the presence of physiological levels of the enzyme and little uptake by macrophages. The potential for clearance by alveolar macrophages was evaluated through macrophage uptake studies.

An interesting work [43] involves the preparation of nano gels that can encapsulate and transport anti-PD-1 antibody (aPD1). The nanogels were synthesized by crosslinking a four-arm PEG (containing MMP) via copper-free click chemistry with another PEG-DBCO. The specific disease under investigation is lung cancer, and the formulations were nebulized for pulmonary delivery. Once inside the tumors, the PEG nanogel is activated by the overexpressed matrix metalloproteinase-9 (MMP-9) in the tumor microenvironment, leading to the release of the encapsulated aPD1. In vitro experiments were conducted using 4T1 and B16F10 cells, while in vivo, the nano gel was deposited in mice through intratracheal injection. These formulations demonstrated improved efficacy of the aPD1 and reduced the potential toxicity associated with intravenously injected aPD1 in a mouse model (Figure 3). The authors propose that these responsive formulations, with their ability to encapsulate hydrophilic macromolecules, can be adapted to encapsulate different biologics (antibodies, proteins, or peptides) and efficiently deliver them to the lungs for the treatment of various pulmonary diseases.

Formulations based on PEG microparticles with potential MMP sensitivity have been thoroughly explored as drug carriers [2]. In this comprehensive study, the authors encapsulated and studied their delivery for three types of drugs: dexamethasone, methylene blue, and biotinylated horseradish peroxidase. The MMP sensitivity, a key feature, is attributed to the peptide Gly-Pro-Gln-Gly-Ile-Phe-Gly-Gln-Lys (GPQGIFGQK), which MMP hydrolyzes. The biocompatibility of these particles was not just demonstrated, but proven, via cell toxicity assays in vitro with IMR-90 and A549 cell lines. This robust biocompatibility, combined with the potential for enzyme-triggered release, makes these microparticles a reliable and promising tool for drug delivery. The authors also propose that these formulations can be adapted for various pulmonary diseases by modifying the peptide chain.

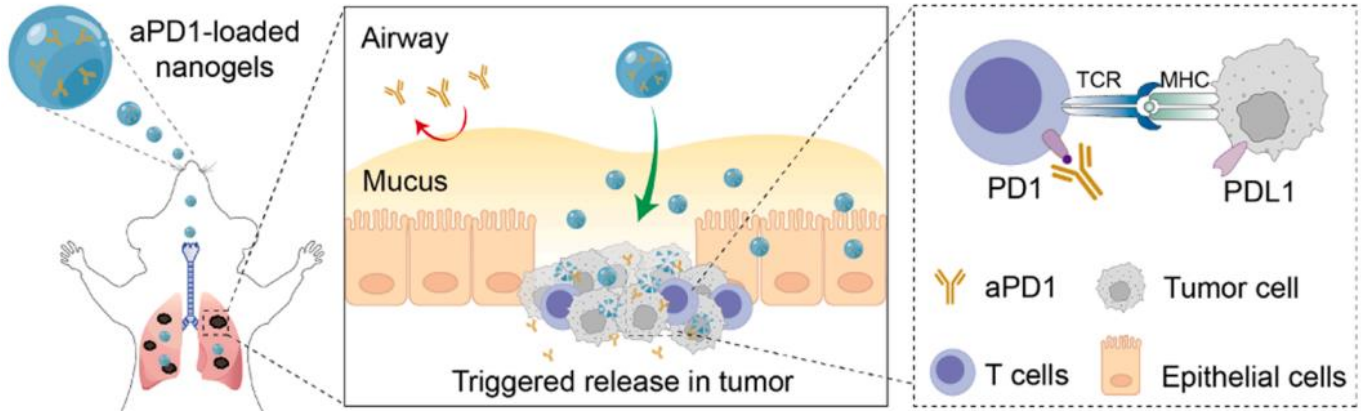

Figure 3. Schematic illustration of the PEG nanogel that transports aPD1 through mucus layer after pulmonary administration. The PEG nanogels dissociate and release encapsulated aPD1 in response to MMP-9 in the tumor microenvironment, leading to effective tumor inhibition. Reprinted with permission from [43].

2.3. Reactive Oxygen Species (ROS)-Responsive Materials for Pulmonary Therapy

Enzymatic and pH responsiveness are among the most critical factors in the formulations for pulmonary delivery. Nevertheless, responsiveness based on ROS (reactive oxygen species) also has some important work [12,35,36,44,45].

A study with liposomes as model carriers [12] is presented for delivering dimethyl fumarate. In this study, ROS-responsive liposomes were designed to deliver dimethyl fumarate in the lungs of pulmonary fibrosis. The nanocarriers were prepared from DSPE and PEG with ROS/sensitive linker thioketal. These liposomes were introduced to mice via intratracheal instillation; inside the lungs, these liposomes can attenuate lung fibrosis development by activating Nrf2-HO-1 signaling in macrophages to suppress TGF-β and ROS production. For in vitro experiments, RAW264.7 cells, a commonly used cell line for studying lung diseases, were used to study cellular endocytosis. The authors showed that these formulations can release the drug in a ROS-enriched microenvironment. These NPs can reduce macrophage accumulation and suppress the production of TGF-β and ROS to reduce fibroblast-to-myofibroblast transition and ECM deposition, attenuating fibrosis progression.

In the same type of responsiveness, a study on redox-responsive dimeric NPs for treating metastatic lung cancer is presented [35]. These NPs, based on conjugation through variable lengths of diacid linkers containing disulfide bonds (−SS−), were initially synthesized and self-assembled into uniform nanosized particles in the presence of vitamin E TPGS. The drug to be delivered, paclitaxel, was part of the initial dimer formulations. These carriers' responsiveness is based on the high level of glutathione (GSH) and reactive oxygen species (ROS) in cancer cells. In vitro experiments using mouse melanoma cells (B16F10), human pulmonary carcinoma cells (A549), and normal human bronchial epithelial (BEAS-2B) cells demonstrated a discriminating cytotoxicity between cancer and normal cells. The pulmonary delivery significantly inhibited tumor growth at lower drug doses. However, the drug-release behavior of dimeric NPs in vivo in mice is an area that requires further investigation, highlighting the ongoing research in this field.

Another system with ROS sensitivity is based on a nanosystem comprised of Janus Au/mesoporous silica core/shell nanoparticles for idiopathic pulmonary fibrosis (IPF) [36]. The core of the Au particles consists of pirfenidone and is loaded in mesoporous materials with two targeting moieties. One is reactive oxygen species (ROS)-sensitive thioketal grafted methoxy poly (ethylene glycol) (mPEG-TK), and the other is 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE). The design aims to anchor the Janus NPs on cell membranes via DSPE modification on the hemispherical surfaces and inhibit their cellular endocytosis by the passivation of PEG modifying on the other hemisphere of the

particle surfaces. By creating such dual-functionalized Janus NPs, ROS-responsive release of PFD in the lung is achieved to provide a favorable microenvironment for the injected MSCs, thereby enhancing the treatment outcome of MSCs on IPF. In vivo experiments were conducted in mice by introducing the carriers via tracheal injection. Moreover, the Janus-NPs could be effectively endocytosed by MSCs after drug release, achieving long-term tracking by CT for up to 60 days. The multifunctional Janus-PFD NPs developed in this work offer an innovative theranostic strategy for MSC-based IPF therapy.

Acute lung injury therapies are quite problematic since these drugs cannot specifically target the lungs. An exciting work proposes the use of a novel active pharmacological carrier. These nanocarriers are explicitly designed to exploit the excessive ROS in this type of disease. The nanocarrier is based on ROS-sensitive cross-linked covalent cyclodextrin frameworks (OC-COF). This carrier is designed to incorporate H_2O_2-scavenging peroxalate ester linkages that could hydrolyze and eliminate ROS generated in inflammation areas (Figure 4). The drug used in this work is ligustrazine (LIG), an antioxidant and anti-inflammatory natural compound, which was loaded into OC-COF and evaluated as a dry powder inhaler. In vitro and in vivo tests showed favorable aerodynamic properties and prominent antioxidant and anti-inflammatory capacities [44].

Finally, a study involving ferroptosis therapy for lung cancer by boosting ROS production and lipid peroxidation has been published [45]. In this work, researchers prepared an inhalable biomineralized liposome (LDM) loaded with dihydroartemisinin and pH-responsive calcium phosphate. These carriers exhibit good nebulization properties and higher drug accumulation in lung lesions compared to intravenous injection. The proposed formulations showed an encouraging lung retention property and antitumor ability in a lung tumor murine model.

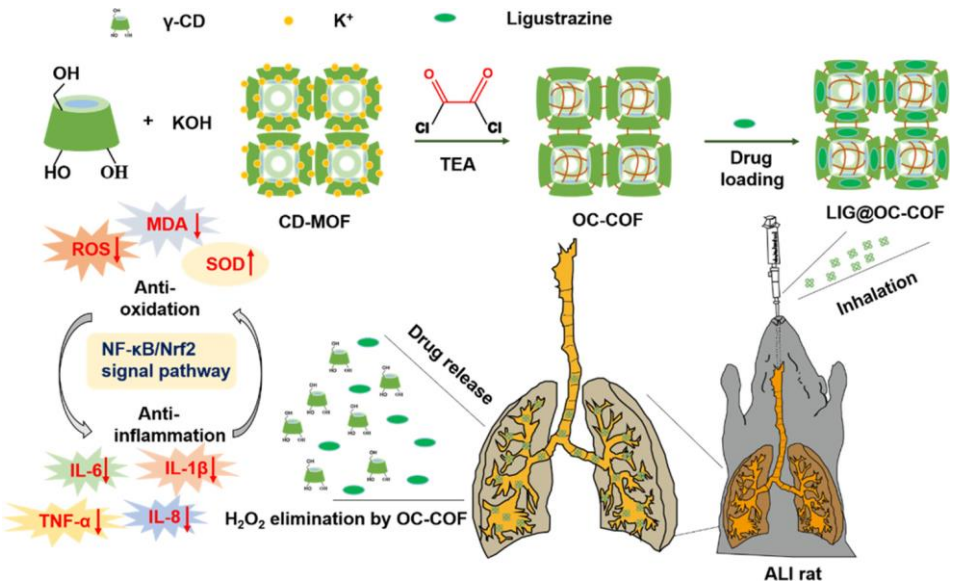

Figure 4. Schematic illustration of OC-COF synthesis and LIG@OC-COF preparation for inhalation therapy of acute lung injury. Reprinted with permission from [44].

2.4. Magnetic Responsive Materials for Pulmonary Therapy

Magnetic responsiveness is also reported as a method of pulmonary delivery. An interesting work on iron oxide NPs in microparticles targeting tuberculosis has been reported [6]. Microparticles (MPs) are developed based on a casting method using sacrificial templates and incorporate superparamagnetic iron oxide nanoparticles to concentrate MPs in alveoli and enable drug-on-demand release upon actuation of an external alternate magnetic field. The MPs are shown to be suitable for P3 (anti-tuberculosis drug candidate) delivery to the lower airways and for alveolar macrophage phagocytosis. The preparation of the system

and loading of the drug can be seen in Figure 5. In vitro experiments on cell lines of mouse fibroblasts were conducted for cytotoxicity, cell metabolic activity, and experiments with macrophages. Interestingly, the drug release can occur through pH changes, but when an external alternate magnetic field is used, the release is increased ten times.

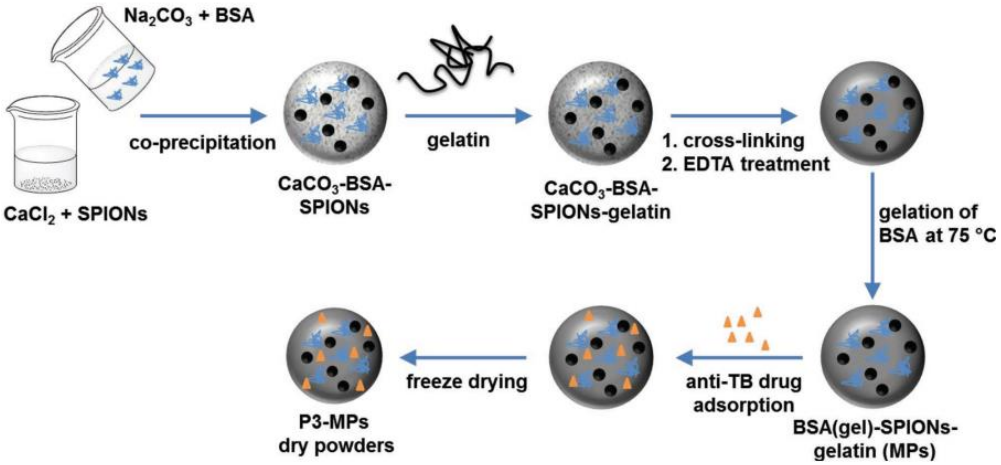

Figure 5. Production process of MPs and loading of anti-TB drug P3 within the produced MPs. Reprinted with permission from [6].

Finally, another study using magnetism as external stimulus has been published [3]. The nano-in-microparticles were prepared for pulmonary drug release of doxorubicin targeting lung cancer. The authors have prepared dry powder containing doxorubicin. The formulations contain lactose, the drug, and superparamagnetic iron oxide nanoparticles. The spray dry technique was used for the preparation of these powders. This study used the tracheal mimic experiment to show the in vitro targeting and retention of dry powders for a proof of concept idea, and the aerosol particles were assumed to be carried by a laminar airstream, consistent with other published literature.

2.5. Other Responsive Materials for Pulmonary Therapy

Other types of responsiveness in drug delivery through the lungs considered in studies include glucose [38] and temperature [37]. In the study with the glucose [38] response, different amphiphilic polymers (Soluplus®, Pluronic® F68, Pluronic® F108, and Pluronic® F127) were used to produce lyophilized formulations for the inhalation of insulin. The glucose sensitivity of the formulations was also attempted by incorporating phenylboronic acid. The suitability of these formulations as insulin delivery systems was rigorously assessed in vitro using pulmonary epithelial and macrophage cell lines. While the glucose-sensitive properties of the formulations could not be proven, the influence of phenyboronic acid on their properties, particularly in terms of the in vitro release of insulin from micelles, was evident. Importantly, these formulations did not significantly affect the in vitro toxicity of respiratory cell lines.

Finally, the last work refers to temperature-responsive formulations based on polysaccharides (κ-carrageenan) [37]. The temperature-responsive polysaccharide particles are prepared via emulsion and a subsequent sol–gel transition. This study showed that the κ-carrageenan particles exhibited temperature responsiveness to collapse at 52 °C. This leads to the conclusion that, for the time being, it cannot be used in pulmonary drug delivery. Furthermore, due to the in vitro aerosol dispersion performance of the κ-carrageenan particles with the cascade impactor, the characteristic delivery behavior of κ-carrageenan particles can be seen. Despite their high density, the particles were delivered more to the alveoli than to the pharynx and bronchi.

An overview of the different types of materials and their responsiveness as well as the targeted disease/disorder can be found in Table 2.

Table 2. Overview of the type of materials, type of responsiveness, and diseases targeted currently.

Type of Materials	Responsiveness	Disease Target	Ref.
DNA tetrahedrons	pH	Pulmonary metastatic cancer	[4]
Peptide/DNA complexes	pH	Multicarrier	[14]
siRNA-based powders	pH	H1N1 influenza virus	[33]
Nano self-assemblies	pH	Different respiratory diseases	[21]
Hydrogels	pH	Multicarrier	[39]
Nanoparticles	pH	Tuberculosis	[40]
Nanovesicles	pH	Pulmonary metastatic cancer	[5]
MOFs	pH	Fibrosis	[11]
Hydrogels	pH	Tuberculosis	[41]
Inorganic composites	pH	Tuberculosis	[42]
Hydrogel microparticles	Enzymatic	Lung cancer, tuberculosis and COPD	[1]
Hydrogel microparticles	Enzymatic	Multicarrier	[2]
Nanoparticle-in-microgel	Enzymatic	Chronic inflammatory lung disease	[9]
Nanoparticle-in-microgel	Enzymatic	Multicarrier	[34]
Nanogel	Enzymatic	Lung cancer	[43]
Liposomes	ROS	Idiopathic pulmonary fibrosis	[12]
Dimeric nanoparticles	ROS	Metastatic lung cancer	[35]
Core/shell nanoparticles	ROS	Fibrosis	[36]
Covalent organic frameworks	ROS	Acute lung injury	[44]
Liposomes	ROS	Lung cancer	[45]
Nanoparticles-in-microparticles	Magnetic	Tuberculosis	[6]
Nanoparticles-in-microparticles	Magnetic	Lung cancer	[3]
Polymeric micelles	Glucose	Diabetes	[38]
Polysaccharide particles	Temperature	Multicarrier	[37]

3. Future Perspectives and Challenges

Pulmonary delivery is considered a more user-friendly way of administrating drugs locally or systemically. Research on pulmonary delivery is evolving day by day, particularly in terms of the materials and drugs used. For instance, the use of biocompatible polymers and nanoparticles has significantly improved the efficiency and safety of drug delivery. Furthermore, the development of novel drugs, such as biologics and gene therapies, has opened up new possibilities for targeted therapy and response of the proposed formulations. The lung and its microenvironment are a quite complex system in terms of the way it is designed but also in its biology and its functions. The lungs are a complex system with many parts that function differently and are thus affected by various diseases and disorders. Depending on the disease, many therapeutics strategies exist in terms of drugs or methods of administration. Regardless, pulmonary delivery has gained ground in the last decades with products appearing in commercialization and research. Regardless, much work must be carried out in many aspects.

The basic challenges in delivering compounds into the lung are considered to be as follows:

1. Administration, deposition, and distribution (Figure 6a).
2. The physical characteristics and properties of the materials used (Figure 6b).
3. Overcoming the biological barriers of the lungs (Figure 6b).
4. The preparation of formulations with active properties that can respond and act accordingly (Figure 6b).

The formulations used are essential because they are the active compound carriers; they must protect the compound, efficiently reach the specific area of interest, deposit and surpass the biological barriers (especially the macrophages), and effectively deliver the compound. The physical characteristics and properties of the formulations are moving

towards that direction (size, hydrophilicity-solubility, charge, porosity, and softness [4,20]) since they affect the method of administration and how much of the formulations (subsequently the drug) reach the area of interest. By now, it is well established that the aerodynamic performance, especially the aerodynamic size of the formulations, must be efficient upon inhalation into the deep lung to avoid mucociliary clearance, reach the lung, and avoid macrophages [13,20]. The high degree of branching in the respiratory tract can be a potential barrier for inhaled particles [14]. Thus, appropriate apparatus and formulations should be used [13]. The formulations used need to be well studied in terms of morphological changes and drug leakage during the delivery process [13] and how their aggregation–agglomeration occurs upon delivery through a system of inhalation [20]. Regarding biological barriers, mucus exists in the respiratory tract, and the alveolar macrophages are the most important, as work must be carried out to avoid the macrophages and surpass the mucus with mucopenetrating formulations. A specific note has to be made for the delivery of biologics through the airway surface liquid covering the epithelial cells that consist mainly of phospholipids and surfactant proteins that may affect the stability of biologics such as DNA [14].

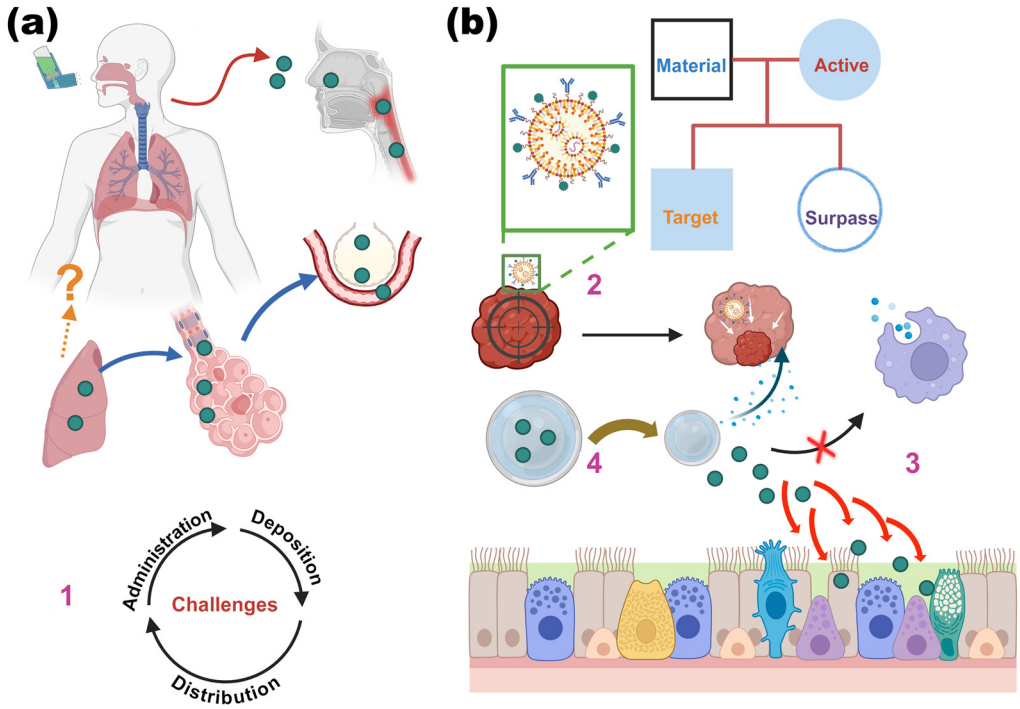

Figure 6. Primary challenges in delivering compounds through the lung. (**a**) Administration, deposition, and distribution and (**b**) physical properties of the materials with high targetability, overcoming the biological barriers of the lung and active materials that can act accordingly.

A gap exists between basic theory and clinical translation regarding research, a specific formulation, and reaching clinical phases [13]. It is believed that the biological safety of the formulations of the materials, thus going from academia to industry, needs to be carefully examined [13]. Reaching the targeted site, there is a need to prolong the drug retention, thus achieving controlled release [20]. At that point, it is also crucial to have a formulation capable of avoiding the defense mechanisms (e.g., macrophages); in that direction, stealth ability is a critical factor in research and something that will be important in future directions. The next point is targeting specific cells or areas, thus achieving selectivity. This is a very important point since it can evolve the formulations into a more multifunctional one targeting characteristic receptors of the cells for specific diseases [13].

Current research is not just about improving materials and targeting new diseases, but also about making a tangible difference in patient outcomes. The use of lungs as a

gateway for other types of disorders, for instance, could revolutionize drug administration. Evolving materials with specific physical properties to reach the lungs, avoid biological barriers, and ensure biocompatibility is a crucial step in this direction. The ongoing work on stealth and active targeting for specific diseases, as well as the responsiveness of these carriers to deliver drugs in a controlled manner with minimal side effects, holds immense potential to transform healthcare.

Significant work is being carried out on materials used, particularly polymer-based and liposomes, exploiting their biocompatibility, versatility, chemical modification, ease of preparation, and good mechanical–biological properties [15].

Finally, for pulmonary drug transport, research must be undertaken on the deposition mechanics, the nature of the therapeutic agent, the properties of the delivery system, the molecular basis of pulmonary diseases, and barriers to drug delivery (mechanical and biological) [8].

4. Conclusions

Pulmonary delivery is a way of administering drugs locally and even systematically to the human body. The lungs are a unique functional system with their microenvironment and thus its diseases and disorders. Using inhaled formulations delivers functional compounds such as drugs, proteins, peptides, antibodies, and small molecules to the affected area quickly and precisely. Specific points need to be considered for successful delivery during the delivery. The mechanics of the respiratory tract and the biological barriers must be overcome alongside the distinct targeting of specific receptors in the lung cells for each disease. The formulations must be prepared versatilely and subjected to specific changes inside the lung for a controllable release in a predetermined time. Size, porosity, elasticity, chemical structure, charge, and hydrophilicity play an essential role in preparing the formulations to have the highest deposition inside the lung, avoiding the macrophages, passing the mucus, and delivering the therapeutic compound. The latest cases in drug delivery, not only in the lung but generally, are being developed to have formulations with precise targeting, good biocompatibility, stability, and response to stimulus. The latter is crucial since the carriers now need to adapt to different conditions, depending on the body's functional system and the environment that changes because of the specific disease. Adapting also means responding or being responsive to specific triggers inside the lung, such as changes in the pH, enzymatic conditions, ROS or temperature. Also, these nanocarriers can be triggered by an external stimulus for releasing the compound, such as temperature, magnetism, or other stimuli.

The studies considered in this review focus on different formulations of responsive materials. Most studies (Figure 7a) use pH and ROS responsiveness, comprising 40% and 24% of the studies, respectively. Moreover, other types of responsiveness are covered in the remaining 1/3 of studies, including enzymatic (20%), magnetic (8%), and other (glucose and temperature, 8%) forms of responsiveness. In terms of the diseases (Figure 7b) that are targeted, the lion's share of research goes to lung cancer (28%). Also, many works target a multicarrier (20%) for many types of diseases but nothing specific. Important works also refer to combatting fibrotic tissues (idiopathic and cystic fibrosis) and tuberculosis, equally at 20%. Some works also target COPD and asthma more theoretically, as their formulations can potentially target these diseases. Finally, one work deals with the H1N1 virus and diabetes with insulin to be delivered systemically. Finally, the drugs or drastic compounds delivered (Figure 7c) are divided into biologics with 39% DNA, RNA, nucleotides, antibodies, and insulin, and drugs for various diseases with 54%. There are also two studies where the formulations were prepared, but no compounds were loaded.

Finally, from the materials point of view (Figure 7d), it is interesting to note the variety of materials used. A significant portion of them are polymeric in nature (42%), with half of them being PEG-related. The second most abundant type of material used is inorganic with 23%. The other materials are types of biomolecules (DNA, peptides, polysaccharides), at 19%, lipids 12%, and organic nature 4%. All these materials are formulated in different

types (Figure 7e), where they can be easily transferred into the lungs. Most are hydrogels (36%), with complexes around 20%. An exciting formulation is also the nanoparticle-in-microparticle (16%), where the authors exploit the nanoparticles' properties and the microgels' sizes to deliver bioactive compounds. A few works involve liposomes, 12%, and inorganic composites, 16%. Another essential parameter concerning the materials and the formulations used is the sizes of the formulations used in these works (Figure 7f). The size of the formulation is a crucial factor in drug delivery, as it can affect the drug's bioavailability and its ability to reach the target site. There are studies with nanocarriers less than 0.1 µm (23%), and the majority of the studies use carriers of 0.1–0.5 µm (31%) and microcarriers of 1–5 µm (26%). Interestingly, some studies use microcarriers larger than 5 µm (20%). Here, it is vital to notice that many studies use formulations with specific aerodynamic sizes, and, in humid environments (such as the lung), these sizes expand and become more than 5 µm to avoid the macrophages.

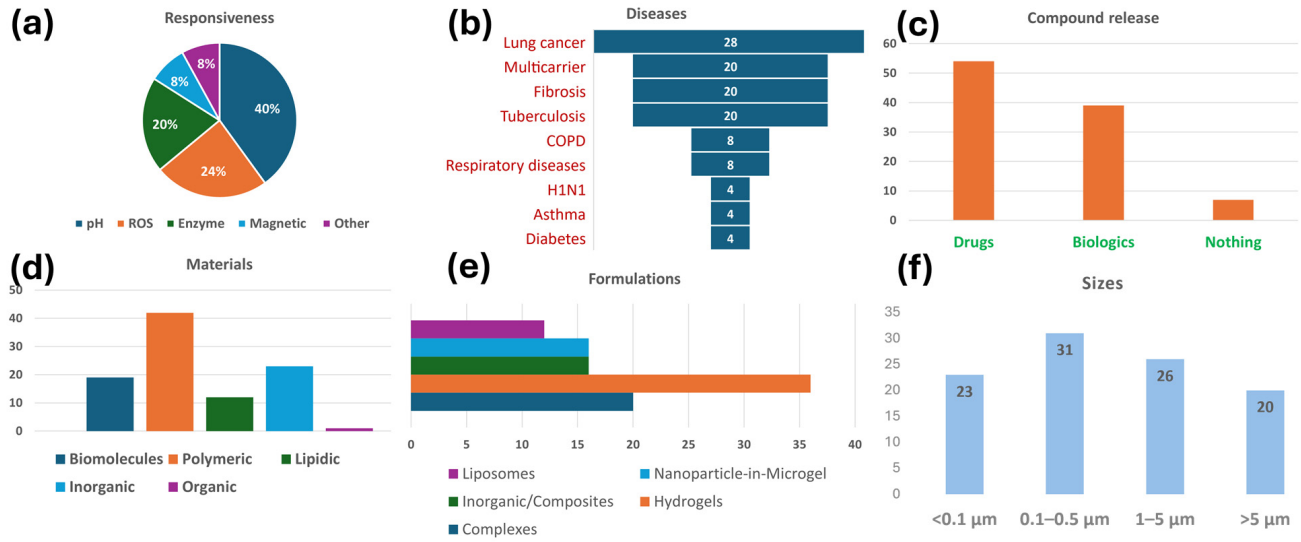

Figure 7. Statistical data (percentages) of scientific works in this review for pulmonary responsive drug delivery for (**a**) type of responsiveness, (**b**) targeted diseases, (**c**) compound release, (**d**) chemical nature of the materials, (**e**) formulations, and (**f**) sizes of the formulations.

Pulmonary delivery, a unique method of administering bioactive compounds or drugs, necessitates a deep understanding of the disease's molecular level. This understanding is crucial as it guides the development of inhaled formulations that can effectively deliver their therapeutic effects. The roles of therapeutics and responsiveness are equally important, varying depending on the nature of each therapeutic and the lung microenvironment's specific responsiveness. The materials used in this process are the backbone of the delivery system, responsible for loading, depositing, and delivering therapeutic to targeted cells. The formulations' chemical nature, preparations, and sizes are key to ensuring a successful delivery.

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