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Nano-Therapeutics for the Lung: State-of-the-Art and Future Perspectives

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Abstract

Inhalation of aerosolized compounds is a popular, non-invasive route for the targeted delivery of therapeutic molecules to the lung. Various types of nanoparticles have been used as carriers to facilitate drug uptake and intracellular action in order to treat lung diseases and/or to facilitate lung repair and growth. These include polymeric nanoparticles, liposomes, and dendrimers, among many others. In addition, nanoparticles are sometimes used in combination with small molecules, cytokines, growth factors, and/or pluripotent stem cells. Here we review the rationale and state-of-the-art nanotechnology for pulmonary drug delivery, with particular attention to new technological developments and approaches as well as the challenges associated with them, the emerging advances, and opportunities for future development in this field.

Keywords

Nanoparticles; pulmonary delivery; inhalation; aerosolization; lung disease; lung repair; growth factors; stem cells

1. INTRODUCTION

Nanotechnology provides powerful, customizable tools for biomarker detection, imaging, and selective delivery of therapeutic agents to detect and treat diseases. In addition to cancer therapy, nanotechnology is widely used in cardiovascular, orthopedic, and neurological applications as engineered nanoscaffolds show promise for promoting cell growth and tissue regeneration. For instance, polyaniline blended gelatin nanofibers support the growth of rat cardiac myoblast cells [1]. Moreover, composite gelatin nanoparticle-fibrin hydrogels encapsulating bone morphogenic protein-2 (BMP-2) [2], hydroxyapatite-poly (lactic acid) (HAP-PLA) nanofibers [3], and gelatin nanofiber-HAP composite scaffolds [4], have been investigated for bone regeneration. Nanocomposites of silk-fibroin nanofibers and gold

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CONFLICT OF INTEREST

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nanoparticles [5] and nanofibers of poly(D,L-lactide-co-glycolide)/poly(ϵ -caprolactone) (PLGA-PCL) [6] have also been explored for nerve regeneration. These examples highlight the versatile roles of nanoparticles and nanomaterials in tissue engineering applications.

The lung, as an organ of gas exchange and a dynamic portal of entry for airborne particles and pathogens, is an established route for delivery of therapeutic reagents to treat pulmonary and systemic diseases. Pulmonary delivery of bronchodilators, antibiotics, antiinflammatory drugs, and various small molecules are well established in the management of cystic fibrosis [7], asthma [8], chronic obstructive pulmonary disease (COPD) [9], and acute respiratory distress syndrome (ARDS) [10]. Inhalational delivery of recombinant proteins and/or biomolecules such as insulin [11] and growth hormone [12] have also been established. While pulmonary delivery allows targeted and local delivery of therapeutic reagents like small molecules, proteins, DNA or siRNA, these reagents are often limited by rapid degeneration and/or clearance by the mucociliary system and alveolar macrophages [13, 14]. Nanocarriers for these therapeutic agents have shown promise at surmounting these shortcomings. Application of nanotechnology for lung growth and repair is an emerging research area (Table 1, Fig. 1). Here we review the current advances and challenges in this field.

2. RATIONALES FOR USE OF NANOTECHNOLOGY IN PULMONARY DELIVERY

Pulmonary drug delivery is often the route of choice for treatment of lung diseases. In contrast to systemic administration, targeted drug delivery to the lung takes advantage of an extremely large alveolar surface area, a dense capillary network and a very thin barrier for efficient drug absorption while avoiding gastrointestinal proteolysis and hepatic first-pass metabolism, resulting in enhanced drug bioavailability within the lung and reduced systemic off-target effects [15–17]. Nanocarriers assist in several aspects of pulmonary drug delivery, as described below:

2.1. Achieve Therapeutic Effects in the Lung at a Lower Drug Dose

Several studies comparing oral to inhalational drug delivery using aerosolized PLGA and alginate nanoparticles loaded with anti-tubercular drugs have shown superior experimental outcome of pulmonary drug delivery over oral and intravenous (IV) delivery. Inhaled PLGA nanoparticles encapsulating anti-tubercular drugs exhibit higher bioavailability (about 13-fold for rifampicin, 33-fold for isoniazid, and 15-fold for pyrazinamide compared to the corresponding oral administration) [18]. This study also observed equivalent therapeutic benefits after fewer doses via nebulization of drug-laden nanoparticles against *M. tuberculosis* in infected guinea pigs (5 inhaled vs. 46 oral). Similarly, alginate nanoparticles carrying anti-tubercular drugs demonstrate comparable therapeutic effects after 3 nebulized doses as compared to that of 45 oral doses [19].

2.2. Enhance Delivery of Hydrophobic Molecules

Nanoparticles have been used for inhalational delivery of poorly soluble drugs, *e.g.*, corticosteroids, hormones, and antifungal agents, that are otherwise difficult to deposit

within the mucus and absorbed slowly in the lung. Dilauroylphosphatidylcholine (DLPC) liposomes facilitate pulmonary absorption and retention (120 minutes) of hydrophobic drugs like cyclosporine A [20]. Inhaled large porous estradiol particles also exhibit higher estradiol bioavailability compared to that of smaller nonporous particles [21]. Intratracheal instillation of porous chitosan microparticles and microspheres loaded with the glucocorticosteroid budesonide in a rat asthma model demonstrate extended pharmacokinetic half-life and bioavailability compared to “conventional” formulation containing lactose dry powder for inhalation [22]. These studies support the effectiveness of nanoparticles in enhancing pulmonary delivery of hydrophobic drugs.

2.3. Protect Against Degradation

Nanocarriers have several potential advantages over conventional inhalation drug delivery including slower drug degradation and controlled drug release as mentioned in previous review articles [23–28]. Nanoparticles can protect sensitive molecules (e.g. siRNA or microRNA), from rapid degradation or immune activation, facilitate their movement across extracellular and/or intracellular barriers (e.g. the surfactant and mucus layers), and retard endolysosomal degradation. For example, polymeric nanoparticles made of either chitosan [29] or poly (ethylenimine)/poly (ethylene glycol)-poly(ethylenimine) (PEI/PEG-PEI) [30] and PLGA microparticles [31] increase the bioavailability and delivery efficiency of the encapsulated siRNA. Intratracheal administration of chitosan nanoparticles carrying siRNA specific for green fluorescent protein (EGFP) achieves greater gene silencing compared to those of mismatched control nanoparticles, naked siRNA, and untreated control groups in a EGFP-expressing murine model, indicating an advantage of chitosan-siRNA nanoparticles [32].

Drug release from nanoparticles can be tailored to a desired therapeutic window. Porous PLGA nanoparticles loaded with insulin exhibit sustained core release over 7 days *in vitro* and produce more than three-fold higher drug availability compared to that of free insulin following aerosolized delivery to rat lungs [33]. Incorporating mucoadhesives like chitosan can further alter the dynamics of core release from PLGA nanoparticles to achieve prolonged drug action [34]. Tailoring nanoparticle drug release profiles to suit the intended applications could also decrease the total drug dose required to attain the desired therapeutic effects.

2.4. Protect Therapeutic Reagents from Pulmonary Clearance Mechanisms

Nanoparticles by virtue of their small size (<200nm) may circumvent endogenous mucosal and immune defenses in the distal lung via rapid penetration through respiratory secretions and the surfactant lining layer, and escaping detection and phagocytosis by alveolar macrophages. Incorporation of mucolytics (e.g. chitosan [34] and mucokinetic ambroxol hydrochloride [35]) and inert polymers (e.g., PEG [36]) can boost nanoparticle transport across the mucosal and surfactant barrier. For instance, elcatonin (derivative of calcitonin)-loaded PLGA nanoparticles coated with chitosan display better mucoadhesion and slower elimination from guinea pig lungs following nebulization [34], leading to greater and prolonged elcatonin pharmacological action (i.e. lower blood calcium levels) compared to that of free elcatonin or elcatonin encapsulated in uncoated PLGA nanoparticles. Altering

the nanoparticle surface charge to anionic or neutral by coating with hydrophilic “stealthy” polymers such as PEG and poly-(vinylpyrrolidone) (PVP) provides a protective barrier against hydrophobicity and opsonization, thereby repelling macrophages and opsonin proteins [37, 38].

Polymer coatings of low molecular weight PEI (PEI2K) [39], chitosan [34], d- α -tocopheryl polyethylene glycol 1000 succinate (TPGS) [40] permit passage through surfactant layers and protect drugs against mucociliary and alveolar macrophage clearance. Other coating techniques (incorporating biomolecules like wheat germ agglutinin, one of the least immunogenic lectins, [41]) improves the bioavailability of antitubercular drugs. Surface modification of nanoparticles with lung surfactants including DPPC [42], mucodhesives such as hydroxypropylcellulose [43], and absorption enhancers like dimethyl- β -cyclodextrin [44] can reduce particle internalization by phagocytic cells, retard mucociliary clearance, and enhance pulmonary penetration, thereby protecting nanoparticles from pulmonary clearance mechanisms.

2.5. Enhance Nanoparticle Internalization

Integration of penetration enhancers such as cell penetrating peptides (CPP), surface receptor specific ligands - monoclonal antibodies, integrin-binding moieties, and arginine-rich low molecular weight protamines - onto nanoparticles enhances cell and cytosol specific drug and/or protein delivery (for a review of CPP see [45]). CPPs such as TAT [46] and proline-rich peptides [47], among others amplify intracellular penetration by nanoparticles and proteins. TAT-RGD in combination with lipofectamine produces a 5-fold enhancement in plasmid DNA transfection efficiency in lung epithelial cells *in vitro* [48]. Intratracheally instilled TAT conjugated PEG-PEI nanoparticles also enhance transfection efficiency of gene therapy in murine lungs [49]. The application of other CPPs for pulmonary drug delivery remains to be investigated.

3. CHALLENGES IN NANO-FORMULATIONS FOR PULMONARY DRUG DELIVERY

In spite of their potential and real advantages, the stratified airway system and the unique microenvironment of the distal lung impose various obstacles to the optimal delivery and therapeutic action of inhaled nano-formulations, summarized below.

3.1. Optimize Nanoparticle Deposition in Lung Tissues

Nanoparticle deposition following inhalation is influenced by particle size, shape, surface charge, and surface properties as discussed in previous review articles [50–52]. Nanoparticles suspended in air deposit poorly following inhalation. Nanoparticles suspended in liquid and aerosolized into droplets with an aerodynamic size less than $\sim 5\mu\text{m}$ effectively reach the distal lung but must overcome multiple barriers before and after uptake by lung cells. During passage through the surfactant and mucosal layers, nanoparticles face the attack of ionic fluids and/or endogenous nucleases, leading to potential premature breakdown and release of core reagents. Nevertheless, many studies have found that particles that are sufficiently small can avoid inertial deposition in the larger airways and

successfully pass in the smaller airways (for a review see [25]). Small nanoparticles (~100 nm) traverse the surfactant layer more efficiently than larger nanoparticles probably due to minimal steric hindrance [53]. Rod-shaped nanoparticles exhibit greater penetration through the lung surfactant layer than disk and barrel shapes [54]. Larger hydrophobic nanoparticles (~136nm) produce greater disruption of the surfactant layer than smaller hydrophobic nanoparticles (~12nm) [53] or hydrophilic nanoparticles [55]. Additionally, while hydrophilic nanoparticles can quickly traverse the surfactant layer, hydrophobic nanoparticles might get entangled in the surfactant layer. These nanoparticle properties should be tested and optimized for each formulation.

3.2. Maintain Integrity and Bioactivity of the Core Compound

This is of particular importance to the delivery of proteins, which can be subjected to physico-chemical degradation, aggregation, deamination, oxidation and glycation during the nanoparticle manufacturing process, resulting in a loss of bioactivity. A variety of approaches, including chemical modification, drying, and the addition of excipients such as buffers, albumin, salts, surfactants, mannitol, sugars and amino acids, may be used to improve protein stability, see review [56]. Similarly, the integrity of incorporated nucleic acids could be preserved by the use of cryoprotectants such as glucose, sucrose, and lactose [57].

3.3. Minimize Immunological Activation *In Vivo*

For effective drug delivery, nanoparticles must overcome the challenges of active immunological host defense of the lung towards foreign materials and pathogens. The active defense mechanisms against foreign material include the airway muco-ciliary clearance system, the alveolar macrophages and monocytes including mast cells, and the presence of proteases in the surface lining fluid. The alveolar macrophages readily phagocytose and eliminate foreign elements, and are activated by various cytokines, transcriptional factors and epigenetic changes in classical (M1) or alternative (M2) modes to differentially modulate inflammation, wound healing, T-cell responses and anti-tumor immunity. Mast cells participate in IgE-associated allergen responses by degranulation and release of chemokines, tryptase, chymase, and complements that mediate anaphylaxis and inflammation, but are also capable of responding to other ligands and producing mediators and growth factors that suppress inflammation and promote tissue remodeling and repair [58]. Nanoparticles that are recognized as foreign bodies by the immune cells and/or undergo opsonization could produce a cascade of inflammatory mediators and complement activation responses, escalate macrophage phagocytosis, and trigger allergic reactions and cytokine and antibody-mediated responses [59]. Certain aspects of the nanoparticle manufacturing procedure, e.g., grinding (milling), polymerization, heating or application of pressure, and use of organic solvents, emulsifiers and cross-linkers would add to immunogenicity and potential toxicity [59]. Various strategies have been attempted to minimize nanoparticle immunogenicity. Those include incorporating metal oxide based antioxidants such as platinum [60] and cerium oxide [61] that serve as anti-inflammatory agents by quenching reactive oxygen species (ROS) and reducing oxidative stress. Additionally, hemocompatibility of chitosan nanoparticles has been improved by the modification with acylation, PEGylation, N-carboxymethylation, incorporation of N-

succinyl, sulphate group or N-Phosphorylcholine, and inclusion of small molecules (e.g., phenyl alanine, tryptophan, arginine) or macromolecules (e.g., heparin) (for a review of chitosan modification, see [62]). Thiol modification of PLGA nanoparticles also reduces phagocytosis and improves hemocompatibility of the nanoparticles; this effect might be due to nitric oxide release as a result of trans-nitrosylation of the thiol groups on the nanoparticles surface in the presence of plasma proteins such as S-nitrosoalbumin [63].

3.4. Avoid Toxicity of Inhaled Nanoparticles

In addition to immunological responses, recent escalating usage of nanoparticles has raised concerns regarding their potential toxicity. Nanoparticles can trigger oxidative stress, direct inflammation, genotoxicity, and/or fibrosis [64]. For instance, inhaled titanium dioxide nanoparticles can promote ROS accumulation, leading to high levels of lipid peroxidation and/or pro-inflammatory cytokines [65]. Carbon black [66, 67], silicon dioxide [68], and multi-walled carbon nanotubes (MWCNTs) [69, 70], also cause lung inflammation, fibrosis, and genotoxicity. Long, thin nanoparticles and unmodified MWCNTs produce more granulomas, inflammation, and fibrosis compared to MWCNTs that are shorter and thicker [70]. PLGA is an FDA-approved polymer generally considered to be safe; however, certain surface modifications of PLGA nanoparticles have been reported to increase toxicity [71]; therefore, any modified formulation must be thoroughly tested for their long term effects prior to use in human subjects.

4. RECENT TECHNOLOGICAL DEVELOPMENTS IN PULMONARY NANO-THERAPEUTICS

Nanoparticle preparations that have been investigated for inhalation drug delivery are listed in Table 2. Our group recently screened a variety of nanoparticles made of natural (gelatin, chitosan, and alginate) and synthetic (PLGA, PLGA-chitosan, and PLGA-PEG) polymers for their efficiency in delivering protein and DNA to the distal lung following nebulization [72, 73]. Of these materials, PLGA nanoparticles stood out for their small size, excellent stability, sustained drug release over 21 days, high cytocompatibility, and rapid cellular uptake. In rat lungs, nebulization of PLGA nanoparticles bearing rhodamine-conjugated erythropoietin (Epo) or DNA encoding yellow fluorescence protein (YFP) results in persistent pulmonary Epo expression for up to 10 days and progressively increasing YFP expression for more than 7 days, respectively [72]. Widespread PLGA nanoparticle uptake and distribution within alveolar cells was documented using various tracers, magnetic resonance imaging and fluorescent and electron microscopy. A single inhalation of nebulized PLGA nanoparticles bearing Epo receptor (EpoR) cDNA upregulated the EpoR protein expression and the downstream signal transduction (phosphorylation of ERK1/2 and STAT5) for up to 21 days in rat lungs. Paracrine signaling via the Epo-EpoR pathway is cytoprotective in several organs. Inhalation of nebulized EpoR cDNA-loaded nanoparticles attenuates hyperoxia-induced acute lung injury assessed from tissue edema, apoptosis, oxidative damage to DNA protein carbonyl, and lipid, and preserved alveolar morphology compared to control animals treated with vector-loaded PLGA nanoparticles [73]. These results establish the time course and efficacy of nanocarrier-facilitated gene delivery to the

lung and the feasibility of the therapeutic approach of local EpoR upregulation in the protection against lung injury.

Besides polymeric nanoparticles, solid lipid nanoparticles (SLNs) have been used for lung repair and growth. An example is cholesterol SLNs loaded with ^{99m}Tc-labeled amikacin used in a cystic fibrosis model [74]. These SLNs show sustained drug delivery over 144 hours, and high drug bioavailability in the lungs (~38% by pulmonary delivery versus 2% by i.v. route at 0.5 hours after administration). The advantages of SLN's include prolonged drug release, faster degradation, and lower potential toxicity compared to polymeric nanoparticles (for a review of SLNs for controlled drug delivery, see [52, 75]). The disadvantages of SLNs include poor stability during storage resulting in drug leakage [76].

Liposomes are another class of nanoparticles that are capable of avoiding immune activation, protecting the encapsulated drugs, and extending drug availability. An example is the use of fluorescence labeled liposomes to load and deliver the bronchodilator salbutamol sulfate (SBS) [77]. Pulmonary delivery of SBS-loaded liposomes in a rat model demonstrated effective deposition in the lung with sustained SBS release for at least 48 hours. Guinea pigs with histamine-induced asthma and spray instilled with SBS-liposomes show up to 18 hours of anti-asthmatic effects compared to less than 8 hours when treated with free SBS. Thus, liposomes could be used to increase the concentration and retention of drugs in the lungs to prolong therapeutic effects. The limitations of liposomes include chemical instability due to rapid degradation of the lipid structure resulting from chemical reactions such as oxidation and hydrolysis during storage and upon sterilization, and physical instability because of aggregation of the vesicles and drug expulsion [78].

Another class of nanoparticles, the hyperbranched dendrimers such as poly (amidoamine) (PAMAM) and PEI, has been explored for pulmonary drug delivery as they possess abundant functional groups allowing drugs and biomolecules to be easily incorporated into or attached onto the nanoparticle surface. For instance, PEI-based dendrimers have been studied for gene therapy to treat cystic fibrosis [79]; mice receiving intratracheally administered PEI dendrimers carrying luciferase plasmid (pCMVLuc) exhibit more pronounced pulmonary luciferase gene expression compared to naked plasmid DNA. While unmodified dendrimers are highly toxic and unstable, surface modification such as incorporating PEG onto PAMAM dendrimers reduces their toxicity *in vitro*, and improves bioavailability *in vivo* [80].

A new form of nanoparticles for pulmonary delivery is the nano- and micron-sized porous nanoparticle-aggregate particles (PNAPs) consisting of spray dried nanoparticle aggregates suspended in a substrate of lung or artificial surfactants (e.g., DPPC [81]) and mucoadhesives (e.g., phospholipids [82], lactose [81, 83], mannitol [83], leucine [83], and hydroxypropylcellulose [81]). Large hollow nanoparticulates representing aggregates of polymer PMMA-MeOPEGMA nanoparticles (PMMA: poly(methyl methacrylate); MeOPEGMA: methoxy(polyethylene glycol)methacrylate) have been studied as carriers for salbutamol sulfate and aspirin to be used with dry powder inhalers (DPI) [84]. Drug release is depended on the hollowness of the nanoparticulate aggregates, and those with a thicker shell (less hollow) exhibit a lower drug release rate. Additionally, increasing nanoparticle

size increases the drug loading capacity, and inclusion of phospholipids (95% phosphatidylcholine) influences both the aggregate morphology and the drug release profiles. The efficacy of large nanoparticulates for pulmonary drug delivery *in vivo* has not been studied. The theoretical advantages of PNAPs over individual nanoparticles in DPI formulations include a high aerosolization efficiency (due to a small aerodynamic diameter of 1–3 μm) and controllable drug loading and release (via modification of nanoparticulate size and hollowness) that might contribute to better therapeutic efficacy [82, 84]. These advantages are balanced against the larger particulate size, which easily trigger macrophage clearance and immune activation mechanisms.

5. OPPORTUNITIES AND FUTURE DIRECTIONS

The sheer variety and versatility of nanocarriers for customization promise many opportunities for the exploration of novel pulmonary therapeutic applications, some of which are described below in broad categories:

5.1 Delivery of Anti-Inflammatory and Anti-Infective Drugs

Anti-inflammatory and immunosuppressant drugs are often used to modulate the production of pro-inflammation cytokines, decrease oxidative stress markers, increase antioxidant enzyme activities, inhibit several leukocyte functions, and decrease chemotactic responses of neutrophils. Some of these drugs, however, are poorly water soluble. Nanocarriers can be used to improve the solubility and delivery efficiency of these drugs. For example, poly(ethylene glycol)–phosphatidyl-ethanolamine micelles have been developed as a pulmonary delivery system to improve the solubility of encapsulated beclomethasone dipropionate up to 1,300 times, and provide extended anti-inflammatory activity compared to the administration of solubilized drug at the same dose [85]. PLGA nanoparticles have been used to improve pulmonary delivery of the anti-fibrotic drug pirfenidone [86]. In a bleomycin-induced pulmonary fibrosis mouse model, intratracheal administration of pirfenidone-loaded PLGA nanoparticles provides sustained bioavailability in the lungs for over one week and mitigates inflammatory response in the bronchioalveolar lavage fluid significantly more than that of the pirfenidone solution [86]. Liposomes have been used to improve pulmonary delivery, controlled uptake, and therapeutic benefits of the antibiotic ciproflaxacin compared to that of inhaled free ciproflaxacin [87, 88]. Similarly, large porous PLGA nanoparticles (200–600 nm in diameter) increase the loading efficiency of the antifungal agent voriconazole to more than 30% and enhance sustained drug release over 15 days compared to nonporous nanoparticles [89]. These porous nanoparticles also show higher drug deposition and retention in distal murine lungs after inhalation due to the large particle surface area available for drug diffusion.

5.2. Delivery of Growth Promoters – Protein and DNA

Cytokine growth factors such as fibroblast growth factor (FGF) and hepatocyte growth factor (HGF) mobilize and amplify epithelial and endothelial progenitor cells, augment alveologenesis, and promote alveolar morphogenesis [90–93]. However, these biomolecules often have a short half-life and rapid clearance that limit their bioavailability; a drawback that may be improved by encapsulation in nanocarriers. For example, HVJ-complex

liposomes were used for trans-pulmonary arterial transfer of HGF cDNA to induce angiogenesis in rat lung [94], resulting in greater relative pulmonary perfusion and capillary density and flow-related decrease of vascular resistance in the transfected lungs. Poly(glycolic acid) (PGA) scaffolds impregnated with HGF-secreting adipose tissue derived stromal cells (ASCs) were implanted in a rat model of emphysema following lung volume reduction surgery (LVRS) [95]. A week later, alveolar and vascular growth were significantly enhanced compared to LVRS alone; improvements in gas exchange and exercise tolerance were maintained for more than a month. These results suggest that pulmonary delivery of HGF nanoparticles improves lung repair. Similar approaches could be used to deliver other growth-promoting proteins and DNA's individually or in combination.

5.3. Delivery of Stem Cells and their Products

Another emerging area for nanotechnology lies in stem cell based therapies. Clinical infusions of mesenchymal stem cells (MSCs) have demonstrated safety in COPD, associated with a decrease in serum C-reactive protein but without improvement in lung functions [96]. Type II alveolar epithelial cells (AEC2s), which are well recognized alveolar progenitors [97], demonstrate characteristic morphological differentiation and the capability to produce surfactant proteins when cultured at an air-liquid interface [98] or in a three-dimensional (3-D) collagen gel model [99]. Other candidate postnatal resident progenitor cells include basal, luminal, and Clara (club) cells, which have shown promise in assisting lung repair and growth [97, 100]. Recently developed chitosan microparticles functionalized with biotinylated anti-CD31 and anti-CD90 antibodies are able to isolate human umbilical vein endothelial cells (HUVECs) and ASCs from a mixture of cells, respectively [101]. Such strategies can in theory be explored to form *in situ* tissue scaffolds, capture certain stem cells via specific antibodies, and expand lung stem cell populations *ex vivo* via growth factor loading and delivery. The therapeutic potential of these stem cell-nanoparticulate systems remains to be explored.

Nano-carriers can also deliver biomolecules to facilitate the recruitment of mesenchymal and hematopoietic stem cells and endothelial progenitor cells (Table 3). For instance, stromal cell-derived factor-1 (SDF-1) has been encapsulated in chitosan (CS)-based nanoparticles as an attractant for MSCs [102]. These CS nanoparticles were incorporated with sodium tripolyphosphate (TPP, for crosslinking) and fucoidan (F, for mobilizing SDF-1, mimicking biological activities of heparin, and facilitating angiogenesis). Fucoidan enhances SDF-1 protection and maintains its mitogenicity and MSC attractant capacity; the CS/TPP/F nanoparticles are highly effective carriers for controlled release of SDF-1 and mobilization of stem cells. Sustained SDF-1 α release from poly(lactide ethylene oxide fumarate)-poly(lactic acid) (PLEOFPLA) hydrogels promotes bone marrow stem cell migration [103]. In addition, SDF-1 α -containing PLGA scaffolds implanted subcutaneously in mice not only promotes homing of mesenchymal and hematopoietic stem cells and facilitates angiogenesis but also reduces mast cell recruitment, inflammation and fibrosis at the implantation site [104]. These observations suggest that SDF-1 nanoparticles might be worthy of investigation as a candidate carrier in pulmonary applications to recruit progenitor cells and facilitate lung growth and repair.

5.4. Stem Cells as Delivery Vehicles for Drug-Laden Nanoparticles

Owing to their migratory and targeting efficiency and immunomodulatory capability, MSCs and lung stem cells have been employed to deliver drug-laden nanoparticles to damaged tissues. Systemic delivery of MSCs has a low engraftment rate in normal murine lung; however, engraftment is significantly boosted in bleomycin injured lungs [105]. Systemic injection of lung stem cells (LSCs) labeled with fluorescent nanodiamonds has been used to track LSC fate in injured murine lungs; the injected cells preferentially localize around terminal bronchioles and facilitate the repopulation of Club cells [106]. The released cellular contents could modulate the micro-environment of the targeted sites even if the intact cells do not persist. The use of stem cells as targeted carriers for pulmonary delivery of nanoparticles loaded with therapeutic reagents to facilitate lung repair and growth is a fertile area for innovation.

Blood-borne cells have also been tested as carriers for delivery of drug-loaded nanoparticles to the lung (Table 3). For instance, ^3H -labeled nanoparticles either non-covalently adsorbed onto erythrocytes (RBCs) or covalently conjugated with erythrocytes via ICAM-1 show seven- to ten-fold higher accumulation in the lung with reduced accumulation in liver and spleen compared to that of free ^3H nanoparticles [107]. Similarly, fluorescent-labeled PMMA nanoparticles hitchhiked onto monocytes preferentially target the sites of inflammation in a murine lipopolysaccharide lung inflammation model [108]. Compared to free nanoparticles, more than twice the amount of hitchhiker nanoparticles reached the inflamed site. These hitchhiking techniques while promising, must deal with their own challenges including standardization of the uptake, retention and release kinetics of loaded nanoparticles, toxicity of incorporated compounds to the carrier cells, and potential off-target effects on healthy tissues.

5.5. Combined Therapeutic Modalities

A combination of stem cells, growth factors, cell signaling molecules, and nanoparticle-based delivery strategies have potential pulmonary applications. For instance, dual stem cell and gene therapy using MSCs with modified HGF expression reduces radiation-induced lung injury possibly via modulating pro-inflammatory cytokines and pro-fibrotic factors [109]. Lipopolymeric nanohybrids made of DPPC and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol, and loaded with two antioxidants (D- α -tocopheryl polyethylene glycol 1000 succinate and dexamethasone disodium phosphate), have shown promise in attenuating neutrophil activation, pro-inflammatory cytokine production, airway blockage and pulmonary hemorrhage in lipopolysaccharide-mediated lung inflammation [110]. The possibilities for combined modalities are nearly endless; each combination needs to be evaluated to clarify its effectiveness and the interactions among the components.

5.6. Applications in 3D Models and Microfluidic Devices

Nanomaterials have been used in 3D models (Table 3) and microfluidic devices to study mechanisms of cell growth and remodeling. 3D scaffolds made of matrigel[®], gelfoam, collagen, PGA, polystyrene, pluronic F-127, or laminin, supplemented with mature or pluripotent cells, growth factors, and inflammatory mediators, and subjected to mechanical stimuli, contribute to segmental lung regeneration (extensively reviewed in [111, 112]). 3D

collagen-glycosaminoglycan scaffolds support differentiation and organization of pre-seeded fetal rat alveolar cells [113]. 3D matrigel supported by PLGA and poly (L-lactic acid) PLLA nanofibers also facilitate proliferation of seeded fetal pulmonary cells without differentiation [114]. Similar systems can be adopted to simulate native tissue responses to therapeutic modalities and could be used to reduce costs associated with *in vivo* experiments, including those of animal models.

Microfluidic systems and miniaturized “lung-on-chip” devices mimic the mechanical and biochemical stimulations experienced by lung cells at the air-tissue interface, allowing scalable and controllable parameters such as shear or diffusional forces and nutrient balances [115]. A 3D co-culture of lung epithelial cells, monocyte-derived macrophages, and dendritic cells at the air-liquid interface has been used to assess the toxicity of silver nanoparticles [116]. Microfluidic devices based on captive bubble surfactometry [117, 118] combined with cyclical compression and expansion of alveolar epithelial cell monolayer have been employed to simulate *in vivo* breathing and study nanoparticle-surfactant-epithelium interactions. Epithelial cells exposed to ultrafine silica nanoparticles (12nm) at the surfactant-epithelial interface and subjected to cyclical strain exhibit a 4-fold increase in ROS production, indicating increased oxidative stress [115]. Additionally, the study reports minimal ROS response on similar exposure to 16 nm Cd/Se quantum dots or 50 nm superparamagnetic iron oxide particles. Microfluidic systems represent a new age of miniaturized, highly controllable and potentially less expensive systems for toxicological and pharmacological screening of drugs and drug loaded nanoparticles to identify the most promising formulations for further *in vitro* and *in vivo* studies (Fig. 2).

5.7. Applications in Bioengineered Lungs

Currently, lung transplantation is the only definitive treatment for end stage lung diseases. However, the lack of donor lungs, a high rate of complications, and stagnant long-term outcomes highlight the need for alternative lung replacement approaches. The major thrust has been the development of 3D acellular lung extracellular matrix (ECM) as transplantable scaffolds [119, 120]. Lung ECM scaffolds not only provide the necessary shape and architecture but also provide chemo-attractive abilities and molecular cues for cell attachment, viability, differentiation and proliferation, and can be subjected to the mechanical stimuli necessary for cellular repopulation.

Production of decellularized lung matrix scaffold involves several steps: First is the removal of cells using detergents (e.g. CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate), SDS (sodium dodecyl sulfate), sodium deoxycholate and triton X-100), acids (e.g. peracetic acid), alcohols/solvents (e.g. glycerol and acetone), biological reagents (e.g., doxyribonuclease, ribonuclease, trypsin and EDTA), or physical means (e.g. freezethaw and mechanical stimuli). Next, the scaffold is repopulated with the appropriate cells in a bioreactor that maintains cell growth, vascular perfusion, ventilation, and other physiological conditions such as pH, temperature, gas exchange, nutrient transfer, and metabolism similar to that of a native lung [121, 122]. Decellularized lungs seeded with human inducible pluripotent stem cells (iPSCs) followed by activin-A treatment and transforming growth factor- β (TGF- β) inhibition result in iPSC differentiation into lung-

specific endodermal cells [123]. Successful recellularization requires extensive proliferation, uniform cell distribution, and adequate nutrient and oxygen supply. Current obstacles include low cell retention and heterogeneous differentiation and proliferation. To surmount these difficulties, a recent study employed a synthetic pleural coating of inert calcium alginate hydrogel onto the excised lung segments to inflate these segments, increase the retention of inoculated cells, and protect recellularized lung tissue slides for high throughput studies [124]. Calcium alginate prevents non-specific cell attachment while allowing attachment and proliferation of pulmonary vascular endothelial cells that are inoculated into the scaffold. Another prospective development is the supplementation of growth-factor laden nanoparticles adsorbed onto stem cells to repopulate the engineered lung scaffolds. While nanoparticles have been incorporated as a part of engineered tissues in other applications such as cardiac patches [125], jugular veins for reconstructing pulmonary arteries and right ventricles [126], and heart valves [127] there are few reports of their use in lung repair and regeneration.

CONCLUSION

This non-exhaustive review focuses on the evolving nanotechnology relevant to the delivery of therapeutic agents to the lung, its current and potential future applications (Fig. 3), and the considerable challenges that lie ahead. The impressive variety of nanopreparations and delivery approaches, summarized above, attest to the emergent bioengineering opportunities for improving pulmonary therapeutics, taking advantage of novel nanomaterials and a unique noninvasive administration route with a large absorptive alveolar surface and thin barrier for efficient local delivery of bioactive molecules, genes, proteins, and progenitor/stem cells to lung tissues.

In spite of the rapid recent advances, nanotechnology applications for lung protection, repair and growth, remains at a nascent stage. Most of the new nanomaterials have only been tested *in vitro* or in short-term preclinical rodent models. Major technical and physiological challenges remain to be overcome before many of these preparations are ready for clinical testing. The important challenges include the need for biomaterials and formulations that a) improve the efficiency of drug encapsulation into nanoparticles, i.e., decrease drug waste during manufacturing, b) reduce the use of harsh and potentially toxic ingredients in the synthesis of nanomaterials, c) promote rapid targeting and specific uptake by alveolar epithelium, d) improve the precision of controlled intracellular nanoparticle degradation and the timed release and bioactivity of the core compound, e) minimize “leak” of inhaled nanoparticles into systemic circulation and any attendant off-target effects, and f) suppress pro-inflammatory and immunogenic host reactions against nanoparticles especially in chronic applications.

For polymeric nanoparticles, which are relatively inexpensive to produce and have high stability, the efficiency of payload incorporation could be improved and the manufacturing process could be optimized to maximize the bioactivity of the incorporated biomolecules. For liposomal and other lipid-based nanoparticles, the challenge will be to find ways of improving the stability of the preparation. For most of the nanomaterials reviewed herein, there is a paucity of data concerning their long-term efficacy or safety profiles in chronic

lung disease models. Most published *in vivo* data have been obtained in small animal models; there is a lack of data from large animal models where the profiles of nanoparticle distribution, deposition, and uptake, as well as the risk-benefit ratios and cost-benefit ratios may differ in significant ways from that in small lungs. The larger absolute quantity of drug-loaded nanoparticles required for uniform distribution throughout large lungs may accentuate nanomaterial related risks of pro-inflammation, cytotoxicity, and immunogenicity. These important translational issues remain to be tackled and resolved. In addition to the therapeutic applications, there remains a need for the development of biodegradable, non-toxic, imaging contrast and tracer agents to obviate the problems with existing materials such as iron oxide, titanium, gold, or other metals. Surmounting these daunting challenges will not be easy or quick, and require concerted multidisciplinary team efforts among bioengineers, physiologists, and clinicians. On the other hand, the rewards of success in building versatile and effective treatment modalities for lung diseases will be immense and well worth the effort.

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Fig. (1).
Nanotechnology assisted pulmonary therapeutics.

Fig. (2).
Nanotechnology based models for pulmonary therapeutics.

Fig. (3).
Key applications of nanotechnology in lung research and therapy.

Table 1

Generation of nanocarriers and their potential use in pulmonary therapeutics.

Generation	Payload	Potential Use
1 st	Core compounds Drugs Growth factors Genes	Protect against infection, inflammation and injury Facilitate repair and growth
2 nd	Surface enhancements Polymers: Poly(ethylene glycol), dextran, poly(ethyleimine) Cell penetrating peptides: TAT, proline rich peptides	Retain nanoparticles in the lung Prevent clearance by alveolar macrophages Enhance cell penetration
3 rd	Targeting motif for specific cells e.g., lung cells, stem cells	Recruit cells <i>in situ</i> for healing, repair and growth Facilitate cell reprogramming Stimulate cell differentiation
4 th	Cell or microparticle carriers of nanoparticles	Multi-functional therapy Multiple drug release Enhance cell specific interactions Facilitate site-specific targeting

Table 2

Nanoparticle formulations for improved pulmonary drug bioavailability and efficacy following delivery via airway.

Nano-/micro-carrier type	Therapeutic agent	Results
Polymer based <ul style="list-style-type: none"> – Porous PLGA microparticles (~ 26 µm) [33] – Porous chitosan microspheres and microparticles (~1–4 µm) [22] – Chitosan nanoparticles (~ 300 nm) [32] – PLGA nanoparticles (241.7 nm) [86] – PLGA and alginate nanoparticles (~186–290 nm) [18,19] – PLGA nanoparticles (~ 160 nm) [72] – PMMA-MeOPEGMa (~120–220 nm) [84] – PEG-phosphatidylethanolamine (~ 14.6 nm) [85] 	<ul style="list-style-type: none"> – Insulin – Budesonide – siRNA specific for EGFP – Pirfenidone – Rifampicin, isoniazid, pyrazinamide – Epo, cDNA for YFP – Salbutamol sulfate, aspirin – Beclomethasone dipropionate 	<ul style="list-style-type: none"> – Enhanced drug retention and hypoglycemic effect – Longer drug retention and half-life, reduced inflammation – Greater gene silencing compared to free siRNA – Sustained drug bioavailability, increased anti-inflammation compared to free drug – Improved bioavailability, reduced dosage compared to orally delivered free drug – Prolonged Epo and YFP expression <i>in vivo</i> – Enhanced drug loading and sustained drug release – Improved drug solubility and antiinflammatory activity
Lipid based <ul style="list-style-type: none"> – Solid lipid microparticles (2 µm) [74] – DLPC liposomes (~700 nm) [20] – Liposomes (~90 nm) [87,88] – Soybean phosphatidylcholine liposomes (~33–58 nm) [77] 	<ul style="list-style-type: none"> – Amikacin – Cyclosporine A – Ciproflaxacin – Salbutamol sulfate 	<ul style="list-style-type: none"> – Enhanced bioavailability – Prolonged drug retention – Sustained drug release, enhanced antimicrobial activity – Prolonged drug retention and antiasthmatic effect
Dendrimer based <ul style="list-style-type: none"> – PEI (Size not reported) [79] 	<ul style="list-style-type: none"> – Luciferase pDNA 	<ul style="list-style-type: none"> – Enhanced gene expression

cDNA: Complementary DNA, DLPC: Dilauroylphosphatidylcholine, EGFP: Enhanced green fluorescence protein, Epo: Erythropoeitin, MeOPEGMa: Monomethylether of poly (ethylene glycol), pDNA: Plasmid DNA, PEG: Poly (ethylene glycol), PEI: Poly (ethylenimine). PLGA: Poly (lactic-co-glycolic acid), PMMA: Poly (methylmethacrylate), SiRNA:Small interfering RNA, YFP: Yellow fluorescence protein

Table 3

Nanocarriers used in cell based therapy.

Carrier type	Cell types	Results
PGA scaffolds	ASCs secreting HGF [95]	Improves alveolization and vascularization in emphysema model following lung volume reduction surgery
SDF-1 loaded Chitosan nanoparticles	MSCs [102]	Facilitates MSC mobilization
SDF-1 loaded PLEOF-PLA hydrogels	BMSCs [103]	Enhances cell migration in response to SDF-1 release
SDF-1 loaded PLGA scaffolds	MSC and HSC [104]	Simulates stem cell homing, reduce inflammatory response
Tritium(³ H) radiolabeled PS “hitchhiker” nanoparticles	Red blood cells [107]	Boosts nanoparticle delivery
FITC labeled PMMA “hitchhiker” nanoparticles	Monocytes [108]	Enhances nanoparticle delivery to inflammation site
3D collagen-glycosaminoglycan scaffolds	Fetal lung cells [113]	Facilitates cell differentiation and organization
3D matrigel with PLGA and PLLA nanofibers	Fetal lung cells [114]	Supports cell proliferation

AEC2: Type 2 alveolar epithelial cells, ASC: Adipocyte stem cells, BMSC: Bone marrow stem cells, FITC: Fluorescein isothiocyanate, HGF: Hepatocyte growth factor, HSC: Hematopoietic stem cells, MSC: Mesenchymal stem cells, PLEOF: Poly (lactide ethylene oxide fumarate), PLGA: Poly (lactic-co-glycolic acid), PGA: Poly (glycolic acid), PMMA: Poly (methylmethacrylate), PS: Polystyrene, SDF-1: Stromal-cell derive factor-1