

REVIEW | Extracellular Vesicles in Lung Health, Disease, and Therapy

Airway epithelial-targeted nanoparticles for asthma therapy

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²Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia; ³School of Medicine and Public Health, The University of Newcastle, Callaghan, New South Wales, Australia; and ⁴Priority Research Centre for Healthy Lungs, Hunter Medical Research Institute, The University of Newcastle, New South Wales, Australia

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Kan S, Hariyadi DM, Grainge C, Knight DA, Bartlett NW, Liang M. Airway epithelial-targeted nanoparticles for asthma therapy. *Am J Physiol Lung Cell Mol Physiol* 318: L500–L509, 2020. First published January 8, 2020; doi:10.1152/ajplung.00237.2019.—Asthma is a common chronic inflammatory disease associated with intermittent airflow obstruction caused by airway inflammation, mucus overproduction, and bronchial hyperresponsiveness. Despite current treatment and management options, a large number of patients with asthma still have poorly controlled disease and are susceptible to acute exacerbations, usually caused by a respiratory virus infection. As a result, there remains a need for novel therapies to achieve better control and prevent/treat exacerbations. Nanoparticles (NPs), including extracellular vesicles (EV) and their synthetic counterparts, have been developed for drug delivery in respiratory diseases. In the case of asthma, where airway epithelium dysfunction, including dysregulated differentiation of epithelial cells, impaired barrier, and immune response, is a driver of disease, targeting airway epithelial cells with NPs may offer opportunities to repair or reverse these dysfunctions with therapeutic interventions. EVs possess multiple advantages for airway epithelial targeting, such as their natural intrinsic cell-targeting properties and low immunogenicity. Synthetic NPs can be coated with muco-inert polymers to overcome biological barriers such as mucus and the phagocytic response of immune cells. Targeting ligands could be also added to enhance targeting specificity to epithelial cells. The review presents current understanding and advances in NP-mediated drug delivery to airway epithelium for asthma therapy. Future perspectives in this therapeutic strategy will also be discussed, including the development of novel formulations and physiologically relevant preclinical models.

airway epithelial targeting; asthma therapy; extracellular vesicles; nanoparticles; pulmonary drug delivery

CURRENT AND EMERGING THERAPIES FOR ASTHMA

Asthma is the most common chronic inflammatory disease of the lungs, affecting over 300 million people worldwide, with that number expected to rise to 400 million by 2025 (69). The disease is typically associated with repetitive episodes of airway obstruction caused by airway hyperresponsiveness-induced bronchoconstriction, excessive production of mucus, and chronic inflammation (81). The majority of patients with asthma respond well to corticosteroid-based treatment regimens. The combinations of inhaled corticosteroids (ICS) with bronchodilators, including long- or short-acting β -receptor agonists (LABA and SABA, respectively), are considered the first-line control strategy for asthma (90). However, exacerba-

tions continue to occur, and asthma control is still poor in 10% of all patients with asthma, even with optimal combination therapy (76). Although a minority in terms of number of patients, this group accounts for 60% of health expenditure related to asthma because of the high rate of hospital admissions (47). Furthermore, in an effort to achieve a sustained bronchodilator response, asthma treatment typically requires frequent administration of medication (12). More recently, humanized monoclonal antibodies targeting specific disease effector molecules such as IgE and inflammatory cytokines IL-4, IL-13, and IL-5 have been approved as add-on therapies for severe poorly controlled asthma. However, these therapies are only approved for asthmatic patients with a specific biomarker-guided immunological profile that indicates increased activation of pathways associated with expression levels of the target molecule (10, 75). Many patients with severe disease are not able to access biologics and remain in need of alternative treatment options. Therefore, efforts have recently been fo-

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cused on the development of alternative strategies to fulfil the unmet medical need in asthma therapy.

Airway inflammation as well as airway structural changes represent pathological features of asthma. Airway inflammation is a complex process involving mainly eosinophils, neutrophils, mast cells, and CD4⁺ T lymphocytes (1). Inflammatory mediators, including cytokines, chemokines, and growth factors released by these cells, are the key effectors of airway inflammation. Structural changes of airways (airway remodeling) occur in almost all forms of asthma (30). These changes include a basement membrane thickening caused by a deposition of extracellular matrix components under the epithelium, an increase in the airway smooth muscle mass, and an increase of mucus-producing goblet cells (5). Angiogenesis and increased vascular density are also features of remodeled airways in asthma. Park et al. (79) showed that in vitro mechanically stressed airway epithelial cells (model of bronchoconstriction) released tissue factor (TF). Furthermore, bronchoalveolar lavage (BAL) from patients with asthma were enriched with TF, identifying a potential proangiogenic mechanism mediated by exosomes. Epithelial compression-induced production of TF was subsequently shown to be increased by IL-13, implicating interaction of type 2 inflammation and bronchoconstriction as driving airway vascular remodeling in asthma (71). Thus, restoring airway immune function, reducing chronic inflammation, and reversing pathological structural changes are promising therapeutic strategies for asthma.

AIRWAY EPITHELIUM AS A TARGET FOR ASTHMA THERAPY

Role of the Airway Epithelium in Asthma

The airway epithelium is considered an essential controller of the host response to environmental challenges such as allergens, viruses, and pollutants that contribute to asthma pathogenesis and is also targetable by inhaled nanoparticle (NP)-based therapeutics (61). The airway epithelium is an integral part of innate immunity and the inflammatory response, and it is capable of producing numerous mediators that activate the immune system (42); this response can be dysregulated in asthma (see below). Under normal circumstances, the airway epithelium consisting of basal, ciliated, and mucus-producing goblet cells forms a highly regulated and impermeable barrier enabled by the formation of tight junctions localized in the apical aspect of epithelial barrier (31). Structural integrity of the airway epithelium is further supported via cell-cell and cell-extracellular matrix interactions involving desmosomes, hemidesmosomes, and adherens junctions (36). In asthma, the airway epithelium displays structural abnormalities associated with airway smooth muscle (ASM) hypertrophy, mucus production, and impaired barrier underpinned by dysregulated differentiation and function of epithelial cells (44). In the case of ASM hypertrophy, there is accumulating evidence that bronchoconstriction reinforces this process by modifying epithelial cell function, where epithelial cell compression promoted proliferation and contraction of ASM cells via production of endothelin-1 (62).

Excessive Mucus Production

Excessive production of mucus is a prominent feature of asthma. It has been reported that the number of goblet cells was

30-fold higher in patients who died from asthma attack compared with healthy patients (2). The increase of airway mucus production is associated with an increase of mucus-secreting goblet cell number (goblet cell hyperplasia; see Ref. 7). There is ample evidence suggesting that there is an increased number of goblet cells in the epithelium of patients with asthma (28). The gel-forming mucins MUC5AC and MUC5B are the major components of airway mucus (7). Mucus overproduction by goblet cells in airways can lead to mucus plugging and airway obstruction. Thus, goblet cell hyperplasia is associated not only with excessive production of mucus but also with asthma mortality.

Impaired Epithelial Barrier

The integrity of the airway epithelium depends on apical tight junctions and adherent junctions that keep epithelial cells together. A number of studies suggest that the cells from patients diagnosed with asthma show decreased transepithelial electrical resistance and compromised tight junctions (97). Studies on junctional proteins from patients with asthma have demonstrated that the expression of zonula occludens-1, E-cadherin, and occludin is downregulated both in vitro and in vivo (86). Indeed, airway epithelial damage is associated with more severe airway hyperresponsiveness (11). The disrupted epithelial barrier may result in enhanced epithelial proinflammatory activity and secretion of growth factors upon exposure to environmental stimuli (33). Additionally, compromised epithelial barrier in asthma may be the result of incomplete repair and formation into fully differentiated and functionally intact airway epithelium (89).

Dysregulated Differentiation of Epithelial Cells

The asthmatic epithelium appeared to exhibit dysregulated differentiation of epithelial cells with a large number of basal cells compared with the epithelium of healthy subjects (38). Basal cells are progenitors capable of self-renewal and differentiation into different epithelial cell types and are characterized by expression of cytokeratin 5, tumor protein 63, and CD151 (37). It has been reported that airway remodeling is triggered by aberrant repair of the epithelium resulted from dysregulated differentiation of epithelial cells (100). These findings may suggest that asthmatic cells have a different cell phenotype and display altered signaling responses to normal cells.

Impaired Immune Response

The innate immune function of the human airway epithelium plays a critical role, since it is responsible for orchestrating defense against inhaled viruses, allergen, bacteria, and other environmental insults (43). Activation of innate immune receptors such as Toll-like receptors (TLR) in airway epithelial cells triggers production of interferons, cytokines, and chemokines, which affect adaptive immune responses. Epithelial responses to infection responses can be aberrant in asthma and may contribute to disease progression and exacerbations (95). Several studies have demonstrated that epithelial cells from patients with asthma exhibit deficient IFN- β and IFN- λ responses to rhinovirus infection, a main trigger of asthma exacerbations (95). In line with this role, polymorphisms of several genes associated with an increased sus-

ceptibility to asthma have been found expressed by airway epithelial cells (72).

NPs FOR DRUG DELIVERY TO THE AIRWAYS

Extracellular Vesicles: Nature's NPs

Nanotechnology has great potential to revolutionize the diagnosis and treatment of human diseases. Extracellular vesicles (EV) are natural nanosized (40–1,000 nm) membrane particles that enable cell-to-cell communication by delivering various biomolecules, including proteins, lipids, and nucleic acids (92). Composition of EVs is highly heterogeneous depending on their cellular origin and pathophysiological state (9). The ability of the cargo transfer, high stability, long circulating half-life, and favorable safety profile make them attractive candidates for the diagnosis and treatment of a variety of diseases, including those of the respiratory system. Moreover, because of their endogenous origin, EVs have enhanced biocompatibility and intrinsic cell-targeting properties (4). Kamerkar et al. (52) showed that EVs secreted by primary fibroblast-like mesenchymal cells can bypass immune clearance by monocytes and macrophages, resulting in prolonged drug release. In addition, a number of studies suggest that plain EVs possess therapeutic potential to treat lung diseases such as asthma (3, 15, 19, 21). Almqvist et al. (3) developed serum-derived EVs (tolerosomes) from ovalbumin-fed donor mice to protect airways against inflammation. They showed that mice treated with tolerosomes display reduced levels of both the total number of cells and eosinophils in BAL fluid. Several studies demonstrated that plain EVs derived from mesenchymal stromal cells (MSCs) might also have therapeutic effect for asthma (15, 21). Cruz et al. (15) reported that plain EVs derived from murine MSCs inhibited airway hyperactivity and reduced lung inflammation in a murine asthma model. In a more recent study, Du et al. (21) investigated the immunomodulation effect of MSC-derived EVs on peripheral blood mononuclear cells (PBMCs) of asthmatic patients. They found that EVs upregulated IL-10 and TGF- β 1 from PBMCs, thus promoting proliferation and immune-suppression capacity of regulatory T cells (Table 1). Taken together, the use of these natural particles holds great promise for the treatment of respiratory diseases.

Conventional Polymer- and Lipid-Based NPs

The use of conventional NP delivery systems, in particular polymeric NPs and liposomes, has shown great promise in the area of pulmonary drug delivery. NPs made from natural and synthetic polymers have received great interest because of their high drug-loading efficiency and stability. High loading efficiency of these NPs contributes to sustained drug release, which leads to substantial reduction of drug dosage. Additionally, the polymer matrix improves poor water solubility and maintains the drug stability by protecting it from the environment. Furthermore, the usage of polymeric NPs enables multiple drug encapsulation (26). This can be crucial in the case of complex therapeutic regimens, such as those required to treat chronic pulmonary diseases (91). Numerous studies have demonstrated beneficial characteristics of polymeric NPs for asthma therapy (16, 50, 59, 64, 70). For example, thiolated chitosan NPs had been used to deliver theophylline, which

augmented its anti-inflammatory effects in a mouse model of allergic asthma (64). Addition of thiol groups to chitosan NPs was shown to enhance drug absorption by bronchial epithelial cells. Betamethasone phosphate-encapsulated polymeric NPs have also improved the therapeutic effect of standard asthma medications (70). Corticosteroid-loaded NPs accumulated at the site of airway inflammation and exhibited anti-inflammatory activity. Highly compacted DNA NPs composed of a block copolymer of poly-L-lysine and polyethylene glycol can mediate thymulin analog gene delivery and effectively reduced the inflammatory and remodeling process in a mouse model of allergic airways disease (16).

Liposomes have also been extensively investigated as a carrier vehicle for pulmonary delivery of anti-inflammatory drug. Liposomes are small vesicles formed by the entrapment of fluid by single or multiple lipid bilayers. These particles have demonstrated excellent biocompatibility/biodegradability and reduced toxicity for pulmonary administration. For example, Myers et al. (73) had shown that inhalation of liposomes made of hydrogenated soy phosphatidylcholine did not induce pathological effects on alveolar macrophages. A number of liposomes have been introduced as drug delivery vehicles for asthma and asthma exacerbation therapy. Daultebayev et al. (18) investigated the antiviral effect of polyinosinic-polycytidylic acid [poly(I:C)]-loaded liposomes on human airway epithelial primary cells. They found that poly(I:C)-encapsulated liposomes selectively upregulated antiviral cytokine IFN- β with low IL-8 costimulation in human airway epithelial cells (18). In another study, Tahara et al. (88) developed egg phosphatidylcholine/cholesterol liposomes loaded with a short-acting pulmonary β_2 -agonist that demonstrated a long-term bronchoprotective effect (over 120 min) in a histamine-induced guinea pig model compared with the drug alone.

The main advantage of NP systems as a carrier vehicle is the capability of targeted drug delivery. This is accomplished by attaching NP with molecules (usually referred to as a targeting ligand) capable of specific recognition and binding to a receptor on target cells. These targeting ligands include antibodies, peptides, proteins, hormones, mono- and oligosaccharides, and charged molecules. Monoclonal antibodies are often used because of their high specificity and availability. Coating NPs with targeting ligands alters their distribution patterns, guiding the encapsulated drugs to a specific cell or organelle.

Barriers for NP Uptake by the Respiratory Tract

There are two critical biological barriers for NP-mediated pulmonary drug delivery: the mucus barrier and the immunological barrier in the airways. Mucus represents a viscoelastic layer that is secreted by specialized goblet cells. The major constituents of mucus are water (95–99.5%) and heavily glycosylated mucins (29). Mucins exert a key biological function in airway defense by protecting the epithelium against foreign pathogens (74). In diseases such as asthma, the average thickness of airway mucus can considerably increase due to hypersecretion of the mucus by goblet cells and impaired mucociliary clearance (27). The protective mucus layer can trap and eliminate drug carriers, thereby hindering their penetration in epithelial cells. Mucins are negatively charged due to the presence of glycans, which allow them to capture cationic particulates such as chitosan-, polymethacrylate-, and polyeth-

Table 1. *Therapeutic applications of extracellular vesicles and nanoparticles in asthma*

Type of NPs	Drug	Targeting Ligand	Cell/Animal Model	Outcomes	Ref. No.
EVs from serum (tolerosomes)	No	No	Mouse model	Reduction of total number of cells and airway eosinophils in BALF	3
EVs from murine mesenchymal stromal cells (MSCs)	No	No	Mouse model	Inhibition of airway hyperreactivity Inhibition of lung inflammation	15
EVs from mesenchymal stem cells	No	No	Peripheral blood mononuclear cells	Upregulation of IL-10 and TGF- β Proliferation and immune suppression of regulatory T cells	21
EVs from human adipose tissue-derived MSC	No	No	Mouse model	Reduction of eosinophils in lung tissue, collagen fiber content in airways Modulation of airway remodeling.	19
Chitosan polymeric NPs	Plasmid DNA	No	Mouse model	Inhibition of airway inflammation and hyperresponsiveness	59
Thiolated chitosan polymeric NPs	Theophylline	No	Mouse model	Decrease in eosinophils in BALF Inhibition of mucus hypersecretion Reduction of bronchial damage	64
PLA polymeric NPs	Betamethasone disodium phosphate	No	Mouse model	Attenuation of airway responsiveness Decrease in eosinophils in BALF fluid	70
Polylysine polymeric NPs	Methionine serum thymus factor Plasmid DNA	No	Mouse model	Prevention of lung inflammation, collagen deposition, and smooth muscle hypertrophy	16
PLGA NPs	Cytosine-phosphate-guanosine (CpG)-oligonucleotide (ODN)	No	Mouse model	Prevention of airway hyperresponsiveness. Reduction of inflammation	50
Liposomes	Polyinosinic-polycytidylic acid	No	Human airway epithelial cells	Upregulation of IFN- β	18
Liposomes	Procaterol hydrochloride (PRO)	No	Guinea pig model	Sustained bronchoprotective effect	88
Lipid nanocomplex	Plasmid DNA	ICAM-1 targeting peptide	Human airway epithelial cells/mouse model	Efficient transfection of airway epithelial cells LED-1 were stable for nebulization	87

BALF, bronchoalveolar lavage fluid; EVs, extracellular vesicles; NPs, nanoparticles; PLA, polylactic acid; PLGA, poly(lactic-co-glycolic acid); TGF, transforming growth factor.

ylenimine-based NPs. Furthermore, mucins contain hydrophobic globular domains that could capture NPs via hydrophobic interactions (54). Additionally, NP can be trapped in mucus due to the smaller size of mucus mesh spacing compared with the particle size (57). Therefore, understanding mucus composition and its clearance mechanisms is critical for the development of NPs to overcome mucus barrier.

Applications of NPs for pulmonary delivery are also limited by their rapid recognition and subsequent clearance by immune cells such as alveolar macrophages (48). Alveolar macrophages are phagocytic cells derived from monocytes and are abundant in the lungs. Because of the small size, NPs can reach the deep regions of the lungs and deposit in the alveoli where they can be engulfed by macrophages. In consequence, the half-life of drug-loaded NPs within the alveoli cannot exceed a few hours, which in turn results in low therapeutic efficacy and

an increasing dose frequency (24). Although the clearance by alveolar macrophages seems to be understood, there are still important gaps in our understanding of the exact mechanism behind NP uptake, transport, and their subsequent clearance by macrophages.

Coating With Muco-Inert Polymers As a Strategy to Overcome Respiratory Barriers

Mucus layer hinders the transport of NPs and remains a critical barrier in pulmonary drug delivery. The surface coating or surface modification of NP determines their physical and chemical properties, including their ability to cross the mucus layer. Traditionally, to prolong drug residence in the lung, NPs coated with mucoadhesive polymers such as chitosan have been investigated (68). The dogma is that mucoadhesive NPs

may be maintained for a longer time in the lungs, resulting in less frequent application of dosage form (6). However, it has now been recognized that mucoadhesive NPs tend to become trapped in the most superficial mucus layer and are less likely to reach the underlying airway epithelium (84). Moreover, mucoadhesive NPs do not spread uniformly within the mucus, potentially leading to uneven delivery of inhaled drugs within the airways and reduction in drug efficacy (84). Another strategy to overcome the mucus barrier and enhance drug absorption is to develop mucus-penetrating NPs by mimicking the surface properties of viruses that allow them to avoid mucoadhesion (60). Mucus-penetrating NPs can rapidly navigate through the mucus barrier and reach the underlying epithelial cells, but only when the particles have diameters smaller than mucus mesh spacing (<300 nm) and the particle surface is densely covered with muco-inert polymers such as polyethylene glycol (PEG; see Ref. 84). A recent study showed that the mucus-penetrating NPs provided uniform and long-lasting drug delivery to airway epithelium of asthmatic subjects following inhalation (84).

Successful NP uptake in airway epithelial cells may also be limited because of the recognition and subsequent clearance by immune cells. Tuning the particle surface with muco-inert polymers can also decrease the recognition and clearance of NPs by macrophages (67). Inert polymers such as PEG and polyvinylpyrrolidone, when coated onto the NP surface, can form a protective brush, shielding a charged and hydrophobic surface, thus hampering innate immune capture (58). Numerous studies have shown that such “stealth” NPs exhibited considerably improved residence time and enhanced drug delivery in vivo (25, 98). Thus, surface modification with muco-inert polymers is a promising approach for developing NPs that can traverse the mucus barrier and avoid clearance by immune cells.

Airway Epithelial-Targeted NPs

Airway epithelial cells are a promising target for NP-based asthma therapies, and strategies aimed at targeting airway epithelial cells could prove beneficial in altering the course of the disease via modification of structural and immunological abnormalities.

Airway epithelial-targeting ligands, such as anti-intercellular adhesion molecule-1 (anti-ICAM-1) and/or anti-epithelial adhesion molecule (anti-EpCAM), can be covalently attached on the particle surface to guide NP to epithelial cells and bypassing unwanted internalization into immune cells. Despite extensive research into the use of NP systems for treatment of respiratory disease, there are very limited studies investigating the efficacy of epithelial-targeted NPs in asthma therapy. Recently, Tagalakis et al. (87) used an intercellular adhesion molecule-1 (ICAM-1) targeted nanocomplex vector system to mediate gene transfection of the airway epithelium in vitro and in vivo. These lipid-based NPs could selectively internalize into airway epithelial cells, leading to efficient transfection and restoration of gene expression. Additionally, such epithelial-targeted NPs were compatible with delivery by nebulization. These results provide evidence to support the potential application of airway epithelial-targeted NPs for therapeutic asthma interventions. For instance, targeted delivery of innate immune activating molecules, such as TLR7 agonist, to asthmatic

epithelium may prevent the aberrant inflammatory immune response by inducing IFN- β and IFN- λ responses. TLR7 is intracellularly expressed in bronchial epithelial cells, airway smooth muscle, and innate immune cells (23). The main function of TLR7 is recognition of viral single-stranded RNA and regulation of antiviral IFN production. Furthermore, most rhinoviruses, the major causes of asthma exacerbation, enter the airway epithelial cell via ICAM-1 receptor-mediated endocytosis (14). Thus, TLR7 agonist-loaded ICAM-1-targeted NPs may have a synergistic effect with potential to bind the receptor, thereby enhancing NP uptake and blocking viral entry.

It may also be possible to develop epithelial-targeted NPs to inhibit expression of proinflammatory cytokines such as IL-33, thymic stromal lymphopoietin, and IL-25 secreted by airway epithelial cells. Biologic drugs, including recombinant proteins, antibodies that block the expression of proinflammatory cytokines, can be encapsulated into the NP core, whereas the attached epithelial-targeting ligand should process the successful transport of the drug in the targeted cells. Another approach implies targeting specific epithelial cells. Basal cells are multipotent progenitor cells that can give rise to ciliated and goblet cells during repair following the epithelial damage (80). The epithelium of asthmatics is less differentiated and contains an increased number of basal cells (38). It has been demonstrated that CD151 and tissue factor (TF) are basal cell-specific receptors that are overexpressed on the surface of airway basal cells (39). Several studies have shown that tight junctions in epithelium of asthmatic subjects are severely disrupted compared with that of normal subjects (97). This compromised barrier function, presumably, may allow basal cell-targeted NPs to pass through epithelial cells and enter into underlying basal cells. A number of studies suggested that Notch signaling pathway promotes the luminal differentiation of airway basal cells. Particularly, there is evidence that the expression of Notch1 and Notch2 is upregulated in asthmatics (45). By delivering Notch antagonist or small-interfering RNA (siRNA), it may be possible to promote basal cell differentiation and therefore repair airway remodeling.

FUTURE PERSPECTIVES FOR AIRWAY EPITHELIAL CELL-TARGETED NPs

There are some fundamental barriers to effective NP-mediated pulmonary drug delivery, in particular, protective mucus layer and mucociliary clearance by immune cells and efficient NP deposition in the airways. These limitations could be addressed by diligent design of NPs, conferring them with features such as mucus-penetrating stealth functionalities and suitable aerodynamic behavior. To facilitate clinical translation of nanomedicines for respiratory diseases, it is also essential to use low or nonimmunogenic drug delivery systems.

EV-Enabled Delivery to Airway Epithelium

Naturally released EVs represent promising drug delivery vehicles to treat respiratory diseases.

However, live cells release only a finite number of EVs, and procedures for isolation, purification, and cargo incorporation are complicated. Scaling up cell EV production poses a major challenge for the field. This issue can be overcome by engineering synthetic EVs using bionanotechnology. Jo et al. (49) had recently reported a device that used a centrifugal force and

a filter with micro-sized pores to produce large numbers (250 times the quantity of naturally released EVs) of cell-derived EVs. These EVs (~100 nm), loaded with RNAs and membrane proteins, showed efficient penetration into NIH 3T3 fibroblasts (49). Wu et al. (96) developed a unique acoustofluidic platform that integrates acoustics and microfluidics to isolate EVs directly from biological fluids with high yield and purity. This automated exosome isolation method with short processing time offers several advantages, including preservation of the EV structure, characteristics, and high yield (over 99%).

Although a number of studies have reported on the intrinsic therapeutic effect of plain EVs against asthma, there are no published studies on drug-loaded synthetic EVs. This could be fully or partially because of the poor drug-loading efficiency. The alternative approaches, such as microfluidic systems, can assist to improve drug encapsulation efficiency. Recently, Yoon et al. (99) proposed a microfluidic system that generates EVs by slicing with 500-nm-thick silicon membrane resulting in formation of spherical EVs (100–300 nm). During self-assembly, the plasma membrane components efficiently encapsulated exogenous cargo (polystyrene latex beads) from the buffer solution. The resulting EVs could deliver the encapsulated beads, while bare beads failed to penetrate the plasma membrane of recipient cells (99). This approach allowed for the incorporation of exogenous material, whereas natural EVs can only deliver endogenous cargo. Nevertheless, further studies aimed at developing efficient strategies for scaling up synthesis of EVs and improving drug encapsulation are necessary to validate therapeutic efficacy of natural particles against asthma.

PEG Alternatives For Surface Coating

PEG-coated NP showed an enhanced permeability and retention effect for pulmonary drug delivery (40). However, because of the wide use of PEG-based commercial products, there is an emerging body of literature that highlights the presence of anti-PEG antibodies (Abs), including both preexisting and treatment-induced Abs produced by the human immune system (46, 51). The existence of anti-PEG Abs has been correlated with loss of therapeutic efficacy and a marked increase in risk of serious adverse effects of some PEGylated therapeutics (32, 41). Thus, research in academia and pharmaceutical industry has also been focused to find alternative less immunogenic systems that could be used to facilitate NP diffusion through mucus. These alternatives include poly(2-alkyl-2-oxazolines), polysarcosine, poly(vinyl alcohol), other hydroxyl-containing nonionic water-soluble polymers, zwitterionic polymers (polybetaines), and mucolytic enzymes (17). It should be noted that airway epithelial-targeted NPs need to simultaneously overcome mucus and the epithelium barrier, which may have different requirements for surface properties. Thus, further studies are necessary to validate these alternative approaches in the design of NPs for pulmonary drug delivery.

Aerosol Nanocomposite Microparticles

There has been a progressive evolution in the use of inhalable NPs for the treatment of respiratory diseases (55). However, the delivery of NPs to the lungs is challenging as their size is not suitable for deep lung deposition, and they are mostly exhaled from the respiratory tract (78). Successful deep lung deposition requires particles to be large enough to avoid being exhaled but small enough to avoid impaction in the upper

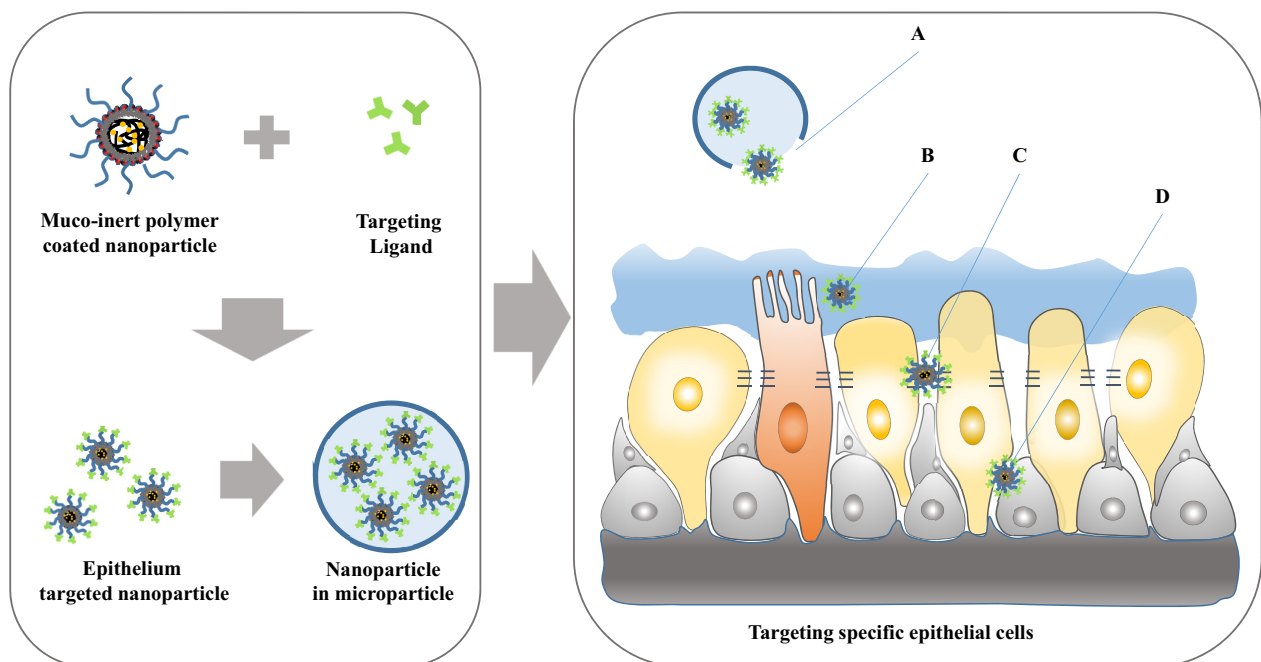


Fig. 1. Nanoparticle-in-microparticle fabrication and mechanism of targeting specific airway epithelial cells. Nanoparticles, coated with muco-inert polymer, are linked with epithelial cell-specific ligand and are loaded into microparticles. Targeting of specific epithelial cells occurs in the following steps: A) burst release of nanoparticles from microparticles, B) rapid mucus penetration due to muco-inert polymer coating, C) paracellular nanoparticle transport owing to disrupted tight junctions, and D) targeting specific epithelial cells (i.e., basal cells).

airways, which permits their passage into lower airways. Nano-composite microparticles have recently emerged to combine the advantages of nano- and microparticles for pulmonary drug delivery (85). These delivery systems are composed of drug-encapsulated NPs dispersed in a microstructure matrix (Fig. 1). Upon administration, the inhaled microparticles can penetrate and be deposited deeply in the lung due to their favorable aerodynamic particle size. Subsequently, the microparticles disintegrate to release the nanoparticles into the peripheral airways, hence evading the pulmonary clearance and offering a sustained drug release.

Aerosolization Devices For Pulmonary Delivery

Nebulizers, metered-dose inhalers (MDI), and dry powder inhalers (DPI) are the most widely used devices for generation of NP aerosols for pulmonary delivery. There are three types of nebulizers and, according to their mechanism to transform NP suspension into inhalable aerosol, they can be classified into air-jet, ultrasonic, and vibrating-mesh nebulizers. Nebulizers can provide a relatively high dosage to the lungs and are particularly beneficial for respiratory diseases that require high doses (56). Lehofer et al. (66) investigated the impact of nebulization on the drug release from liposomes by applying three types of nebulizers. They showed that vibrating mesh nebulizers have the highest drug release compared with air-jet and ultrasonic nebulizers (66). MDI is a pressurized inhaler that delivers NPs by using a propellant on actuation. However, actuation-inhalation coordination remains a challenge during clinical use, which may lead to exhalation or escape of the delivered NPs and result in a low pulmonary deposition (38a). Compared with nebulizer and MDI, DPI is more convenient to use and has advantages of improved drug stability and being free of propellant. In addition, the dried powder state would be ideal for the delivery of hydrophobic formulations. So far, comparative studies on the efficacy of different types of aerosolization devices has been very limited for pulmonary delivery of NPs. Such studies will no doubt lead to better understanding the effect of particle properties on overall device performance in terms of drug stability, delivered dosages to the lung, deposition rate, consistency of the delivered dosage, and drug loss.

Physiologically Relevant In Vitro Model

Because of the ethical concern regarding in vivo studies in animals or humans, the use of the in vitro cell models represents the primary method in the evaluation of therapeutic NPs for respiratory diseases (35). Media-submerged monolayer cell cultures, despite their wide use, are physiologically unrealistic because cells in such systems fail to undergo mucociliary differentiation (65). In comparison, both primary cells and cell lines show cell differentiation and mucus layer when cultured at the air-liquid interface (ALI), making them more physiologically relevant and a better choice for assessing the effects of potential therapies (53). This ALI model mimics the features found in the human airway and drives differentiation of basal cells toward a mucociliary phenotype. Additionally, the ALI model is characterized by the development of sealed barrier function, with cells expressing the tight junction proteins (zonula occludens-1, E-cadherin; see Ref. 8). Three-dimensional (3D) culture systems, which grow cells in a synthetic or

biological scaffold, have also gained increasing interest in drug discovery and tissue engineering (22). Such 3D composition mimics cell-cell and cell-extracellular matrix interaction, which is observed in the natural in vivo environment (77). In addition, the introduction of immune cells may contribute to better recapitulation of the in vivo physiology. For example, Ding et al. (20) had combined the 3D airway epithelium consisting of airway epithelium and macrophages with time-lapse confocal imaging to allow imaging of macrophages in 3D over time.

NP-Physiological Components Interaction In Vivo

The validation of NP performance in vivo remains a challenge. This is presumably due to limited understanding of the interaction of NPs with complex physiological components such as pulmonary surfactants, phospholipids, and proteins in lungs (63, 83). For instance, phospholipids and proteins in lungs may mask targeting ligand by forming corona on inhaled NPs (82). A number of studies reported that proteins adsorbed on the surface of NPs may facilitate their nonspecific uptake by receptor-mediated endocytosis (13, 34). In addition, opsonin proteins that present in the blood serum tend to bind to NP surface, making them “visible” to immune cells (94). These unwanted interactions can alter the size and the surface properties of NPs, affecting cell-NP interactions, leading to macrophage recognition and subsequent reduced uptake of NPs by target cells. Detailed studies investigating interaction between NP and physiological components such as pulmonary surfactants and opsonin proteins in vivo are therefore required to develop formulations that can evade immune response and demonstrate enhanced uptake by target cells.

CONCLUSIONS

In this review, current status and prospective strategies of EVs and conventional NP-mediated therapies for asthma treatment are discussed. Although there is currently little information available on NP-mediated targeted approaches for asthma treatment, targeting airway epithelium holds great promise to alter the natural course of the disease. EVs, due to the endogenous origin of their components, are less immunogenic compared with conventional NPs. Additionally, they possess natural targeting functionality and also could be artificially engineered by linking to a cell-specific ligand. Coating conventional NPs with PEG and targeting ligands may overcome pulmonary delivery barriers and facilitate targeted delivery to airway epithelial cells. However, formulation of NPs into microparticle systems to enable deep lung deposition, development of physiologically relevant cell culture systems, and better understanding of interaction of NP with physiological components are some of the key aspects that need to be considered and addressed for the future development of NP-mediated asthma therapy.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

S.K. prepared figures; S.K. drafted manuscript; D.M.H., C.G., D.A.K., N.W.B., and M.L. edited and revised manuscript; C.G., D.A.K., N.W.B., and M.L. approved final version of manuscript.

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