Airway epithelial-targeted nanoparticles for asthma therapy

Authors: Stanislav Kan [a,d], Dewi Melani Hariyadi [b], Christopher Grainge [c,d], Darryl Knight [a,d], Nathan Bartlett [a,d], Mingtao Liang [a,b,*]

[a] *School of Biomedical Sciences and Pharmacy, The University of Newcastle, Callaghan, New South Wales, Australia*
[b] *Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia*
[c] *School of Medicine and Public Health, The University of Newcastle, Callaghan, New South Wales, Australia*
[d] *Priority Research Centre for Healthy Lungs, Hunter Medical Research Institute, The University of Newcastle, New South Wales, Australia*
[*] *Corresponding author:* (email: roger.liang@newcastle.edu.au; tel: 612 4985 4959)

Abstract

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 therapeutical 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 pre-clinical models.

1. 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 hyper-responsiveness 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 beta-receptor agonists (LABA and SABA respectively) are considered the first-line control strategy for asthma (90). However, exacerbations 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 due to 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

humanised monoclonal antibodies (mAbs) 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 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 focused 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) occurs 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 feature of remodelled airways in asthma. Park et al. 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 pro-angiogenic mechanism mediated by exosomes (79). 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 and reducing chronic inflammation and reversing pathological structural changes are promising therapeutic strategies for asthma.

2. Airway epithelium as a target for asthma therapy

2.1 The role of the airway epithelium in asthma

The airway epithelium is considered an essential controller of host response to environmental challenges such allergens, viruses and pollutants that contribute to asthma pathogenesis and is also targetable by inhaled 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) and 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 extra-cellular 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).

2.2 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 to healthy patients (2).The increase of airway mucus production is associated with an increase of mucus-secreting goblet cell number (goblet cell hyperplasia) (7). There is an 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.

2.3 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 (TEER) and compromised tight junctions (97). Studies on junctional proteins from patients with asthma have demonstrated that the expression of ZO-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 pro-inflammatory activity and secretion of growth factors upon exposure to environmental stimuli (33). Additionally, compromised epithelial barrier in asthma may be due to incomplete repair and formation into fully differentiated and functionally intact airway epithelium (89).

2.4 Dysregulated differentiation of epithelial cells

The asthmatic epithelium appeared to exhibit dysregulated differentiation of epithelial cells with large number of basal cells compared to the epithelium of healthy subject (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 (p63), 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 signalling responses to normal cells.

2.5 Impaired immune response

The innate immune function of the human airway epithelium plays a critical role as it is responsible for orchestrating defence 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 susceptibility to asthma has been found expressed by airway epithelial cells (72).

3. Nanoparticles for drug delivery to the airways

3.1 Extracellular vesicles – nature's nanoparticles

Nanotechnology has great potential to revolutionise the diagnosis and treatment of human diseases. Extracellular vesicles (EV) are natural nano-sized (40–1000 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 patho-physiological state (9). The ability of the cargo transfer, high stability, long circulating half-life, as well as favorable safety profile makes them attractive candidates for the diagnosis and treatment of a variety of diseases including those of the respiratory system. Moreover, due to their endogenous origin, EVs have enhanced biocompatibility and intrinsic cell targeting properties (4). Kamerkar et al. showed that EVs secreted by primary fibroblast-like mesenchymal cells can bypass immune clearance by monocytes and macrophages resulting in prolonged drug release (52). 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. developed serum-derived EVs (tolerosomes) from OVA-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 (3). Several studies demonstrated that plain EVs derived from mesynchimal stromal cells (MSCs) might also have therapeutic effect for asthma (15, 21). Cruz et al. reported that plain EVs derived from murine MSCs inhibited airway hyperreactivity and reduced lung inflammation in a murine asthma model (15). In a more recent study, Du et al. investigated the immunomodulation effect of MSCs-derived EVs on peripheral blood mononuclear cells (PBMCs) of asthmatic patient. They found that EVs upregulated IL-10 and TGF-β1 from PBMCs, thus promoting proliferation and immune-suppression capacity of regulatory T cells (Table 1) (21). Taken together, the use of these natural particles holds great promise for the treatment of respiratory diseases.

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

Type of NPs	Drug	Targeting ligand	Cell/ animal model	Outcomes	References
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 mono nuclear cells	Upregulation of IL-10 and TGF-β Proliferation and immune suppression of	(21)

				regulatory T cells	
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)
Poly-lysine 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–	No	Mouse model	Prevention of airway	(50)

	guanosine (CpG)-oligonucleotide (ODN)			hyperresponsiveness Reduction of inflammation	
Liposomes	Polyinosinic-polycytidylic acid	No	Human airway epithelial cells	Upregulation of IFN-β	(18)
Liposomes	Procaterol hydrochloride (PRO)	No	Guinea pig model	Sustained broncho protective 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)

3.2 Conventional polymer- and lipid-based nanoparticles

The use of conventional NP delivery systems, in particular polymeric NPs and liposomes, has shown a great promise in the area of pulmonary drug delivery. NPs made from natural and synthetic polymers have received great interest due to 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 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 of 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 (CK30PEG) can mediate thymulin analogue 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. had shown that inhalation of liposomes made of hydrogenated soy

phosphatidylcholine did not induce pathological effects on alveolar macrophages (73). A number of liposomes have been introduced as drug delivery vehicles for asthma and asthma exacerbations therapy. Dauletbayev et al. investigated the anti-viral 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 other study, Tahara et al. developed egg phosphatidylcholine (EPC)/cholesterol liposomes loaded with a short-acting pulmonary β2-agonist that demonstrated long-term bronchoprotective effect (over 120 min) in histamine-induced guinea pig model as compared to the drug alone (88).

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.

3.2.1 Barriers for nanoparticle 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 which 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 defence 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 into epithelial cells. Mucins are negatively charged due to the presence of glycans, which allow them to capture cationic particulates such as chitosan, polymethacrylate, and polyethylenimine-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 to the particle size (57). Therefore, understanding of 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. Owing to 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.

3.2.2 Coating with muco-inert polymers as a strategy to overcome respiratory barriers

Mucus layer hinders the transport of NPs and remain a critical barrier in the 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) (84). A recent study showed that the mucus-penetrating NPs provided uniform and long-lasting drug delivery to airways epithelium of asthmatic following inhalation (84).

Successful NP uptake into airway epithelial cells may also be limited due to 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 (PVP), when coated onto NP surface, can form a protective brush, shielding 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.

3.3 Airway epithelial-targeted nanoparticles

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 onto the particle surface to guide NP to epithelial cells and bypassing unwanted internalisation 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. utilised an intercellular adhesion molecule-1 (ICAM-1) targeted nanocomplex vector system to mediate gene transfection of the airway epithelium in vitro and in vivo (87). These lipid-based NPs could selectively internalise into airway epithelial cells leading to efficient transfection and restoration of gene expression. Additionally, such epithelial-targeted NPs were compatible with delivery by nebulisation. 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 anti-viral 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 pro-inflammatory cytokines such as IL-33, TSLP and IL-25 secreted by airway epithelial cells. Biologic drugs including recombinant proteins, antibodies that block the expression of pro-

inflammatory cytokines can be encapsulated into NP core whereas the attached epithelial targeting ligand should process the successful transport of the drug into 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 increased number basal cells (38). It has been demonstrated that CD151 and tissue factor (TF) are basal cell specific receptors which 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 luminar 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, therefore repair airway remodeling.

4. Future perspectives for airway epithelial cell targeted nanoparticles

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 behaviour. To facilitate clinical translation of nanomedicines for respiratory diseases, it is also essential to utilise low or non-immunogenic drug delivery systems.

4.1 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. had recently reported a device that utilized 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. 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 that offers several advantages including preservation of the EV structure, characteristics and high yield (over 99%) (96).

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. 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.

4.2 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 pre-existing 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 utilised to facilitate NP diffusion through mucus. These alternatives include poly(2-alkyl-2-oxazolines), polysarcosine, poly(vinyl alcohol), other hydroxyl-containing non-ionic 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.

4.3 Aerosol nanocomposites 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 airways, which permits their passage into lower airways. Nanocomposite microparticles has recently emerged to combine the advantages of nano- and micro-particles for pulmonary drug delivery (85). These delivery systems are composed of drug-encapsulated NPs dispersed in microstructures matrix (Figure 1). Upon administration, the inhaled microparticles can penetrate and be deposited deeply in the lung due to their favourable 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.

Figure 1

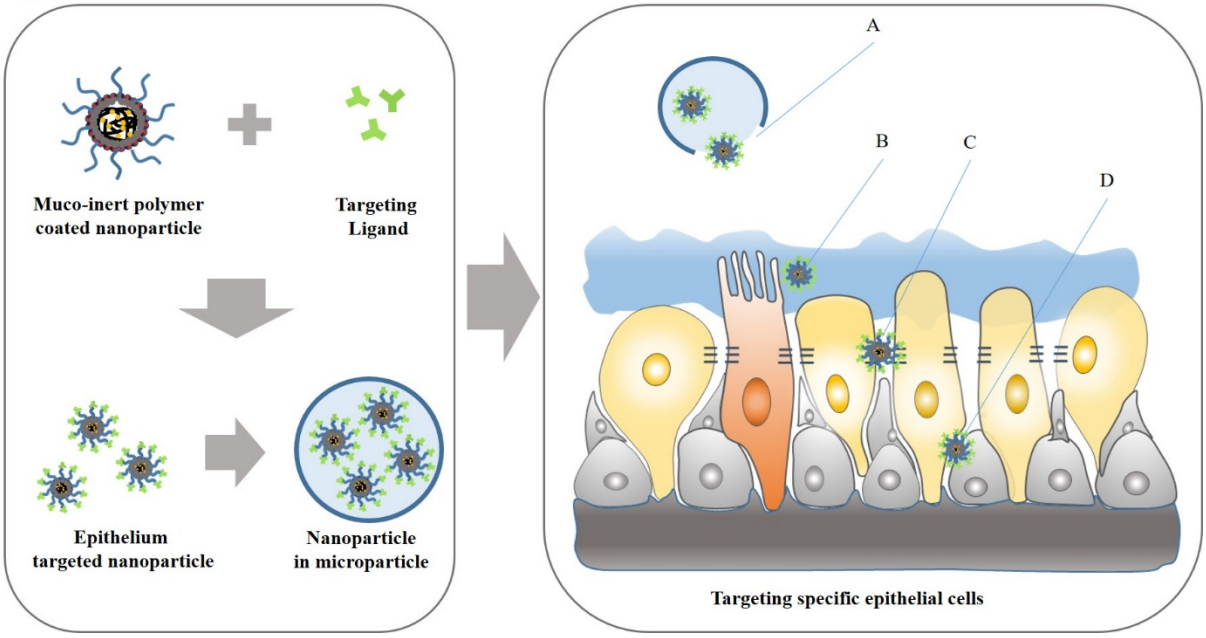

Fig. 1. Nanoparticle-in-microparticle fabrication and mechanism of targeting specific airway epithelial cells. Nanoparticles, coated with muco-inert polymer, are linked with epithelial cell-specific ligand and are loaded into microparticles. Targeting of specific epithelial cells occurs in the following 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).

4.4 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 dose (56). Lehofer et al. investigated the impact of nebulization on the drug release from liposomes by applying three types of nebulizers. They showed that vibrating mesh nebulizers has the highest drug release as compared to 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 (94). 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.

4.5 Physiologically relevant in vitro model

Due to the ethical concern regarding in vivo studies in animals or human, 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 air-liquid interface (ALI), making them more physiologically relevant and 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 towards a mucociliary phenotype. Additionally, the ALI model is characterised by the development of sealed barrier function, with cells expressing the tight junction proteins (ZO-1, E-Cadherin) (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 (ECM) 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. 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 the time (20).

4.6 Nanoparticle-physiological components interaction in vivo

The validation of NP performance in vivo remains a challenge. This 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 absorbed on the surface of NPs may facilitate their non-specific 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 (93). 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.

Conclusion

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 natural course of the disease. EVs, due to the endogenous origin of their components, are less immunogenic compared to 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.

Author contributions

S.K. prepared a figure, drafted, edited and revised the manuscript. D.M.H. drafted, edited and revised the Aerosol nanocomposites microparticles section. D.K., C.G., N.B., and M.L. edited, revised, and approved the final manuscript.

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

Author notes
Address for reprint requests and other correspondence: Mingtao Liang, School of Biomedical Sciences and Pharmacy, the University of Newcastle, University Dr, Callaghan 2308, New South Wales, Australia (email: roger.liang@newcastle.edu.au)

References

1. **Agrawal DK, and Shao Z**. Pathogenesis of allergic airway inflammation. *Current allergy and asthma reports* 10: 39-48, 2010.
2. **Aikawa T, Shimura S, Sasaki H, Ebina M, and Takishima T**. Marked Goblet Cell Hyperplasia with Mucus Accumulation in the Airways of Patients Who Died of Severe Acute Asthma Attack. *CHEST* 101: 916-921, 1992.
3. **Almqvist N, Lönnqvist A, Hultkrantz S, Rask C, and Telemo E**. Serum-derived exosomes from antigen-fed mice prevent allergic sensitization in a model of allergic asthma. *Immunology* 125: 21-27, 2008.
4. **Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, and Wood MJA**. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nature Biotechnology* 29: 341, 2011.
5. **Bergeron C, Tulic MK, and Hamid Q**. Airway remodelling in asthma: from benchside to clinical practice. *Canadian respiratory journal* 17: e85-e93, 2010.
6. **Boddupalli BM, Mohammed ZN, Nath RA, and Banji D**. Mucoadhesive drug delivery system: An overview. *J Adv Pharm Technol Res* 1: 381-387, 2010.
7. **Bonser LR, and Erle DJ**. Airway Mucus and Asthma: The Role of MUC5AC and MUC5B. *Journal of clinical medicine* 6: 112, 2017.
8. **Buckley AG, Looi K, Iosifidis T, Ling K-M, Sutanto EN, Martinovich KM, Kicic-Starcevich E, Garratt LW, Shaw NC, Lannigan FJ, Larcombe AN, Zosky G, Knight DA, Rigby PJ, Kicic A, and Stick SM**. Visualisation of Multiple Tight Junctional Complexes in Human Airway Epithelial Cells. *Biological Procedures Online* 20: 3, 2018.
9. **Cañas JA, Sastre B, Rodrigo-Muñoz JM, and del Pozo V**. Exosomes: A new approach to asthma pathology. *Clinica Chimica Acta* 495: 139-147, 2019.
10. **Castro M, Wenzel SE, Bleecker ER, Pizzichini E, Kuna P, Busse WW, Gossage DL, Ward CK, Wu Y, Wang B, Khatry DB, van der Merwe R, Kolbeck R, Molfino NA, and Raible DG**. Benralizumab, an anti-interleukin 5 receptor alpha monoclonal antibody, versus placebo for uncontrolled eosinophilic asthma: a phase 2b randomised dose-ranging study. *Lancet Respir Med* 2: 879-890, 2014.
11. **Chapman DG, and Irvin CG**. Mechanisms of airway hyper-responsiveness in asthma: the past, present and yet to come. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology* 45: 706-719, 2015.
12. **Chen X, Huang W, Wong BC, Yin L, Wong YF, Xu M, and Yang Z**. Liposomes prolong the therapeutic effect of anti-asthmatic medication via pulmonary delivery. *International journal of nanomedicine* 7: 1139-1148, 2012.
13. **Chithrani BD, Ghazani AA, and Chan WCW**. Determining the Size and Shape Dependence of Gold Nanoparticle Uptake into Mammalian Cells. *Nano Letters* 6: 662-668, 2006.

14. **Conzemius R, Ganjian H, Blaas D, and Fuchs R**. ICAM-1 Binding Rhinoviruses A89 and B14 Uncoat in Different Endosomal Compartments. *Journal of Virology* 90: 7934, 2016.
15. **Cruz FF, Borg ZD, Goodwin M, Sokocevic D, Wagner DE, Coffey A, Antunes M, Robinson KL, Mitsialis SA, Kourembanas S, Thane K, Hoffman AM, McKenna DH, Rocco PRM, and Weiss DJ**. Systemic Administration of Human Bone Marrow-Derived Mesenchymal Stromal Cell Extracellular Vesicles Ameliorates Aspergillus Hyphal Extract-Induced Allergic Airway Inflammation in Immunocompetent Mice. *STEM CELLS Translational Medicine* 4: 1302-1316, 2015.
16. **da Silva AL, Martini SV, Abreu SC, Samary CdS, Diaz BL, Fernezlian S, de Sá VK, Capelozzi VL, Boylan NJ, Goya RG, Suk JS, Rocco PRM, Hanes J, and Morales MM**. DNA Nanoparticle-Mediated Thymulin Gene Therapy Prevents Airway Remodeling in Experimental Allergic Asthma. *Journal of controlled release : official journal of the Controlled Release Society* 180: 125-133, 2014.
17. **das Neves J, and Sarmento B**. Technological strategies to overcome the mucus barrier in mucosal drug delivery. *Advanced Drug Delivery Reviews* 124: 1-2, 2018.
18. **Dauletbaev N, Cammisano M, Herscovitch K, and Lands LC**. Stimulation of the RIG-I/MAVS Pathway by Polyinosinic:Polycytidylic Acid Upregulates IFN-beta in Airway Epithelial Cells with Minimal Costimulation of IL-8. *J Immunol* 195: 2829-2841, 2015.
19. **de Castro LL, Xisto DG, Kitoko JZ, Cruz FF, Olsen PC, Redondo PAG, Ferreira TPT, Weiss DJ, Martins MA, Morales MM, and Rocco PRM**. Human adipose tissue mesenchymal stromal cells and their extracellular vesicles act differentially on lung mechanics and inflammation in experimental allergic asthma. *Stem Cell Research & Therapy* 8: 151, 2017.
20. **Ding P, Wu H, Fang L, Wu M, and Liu R**. Transmigration and Phagocytosis of Macrophages in an Airway Infection Model Using Four-dimensional Techniques. *American Journal of Respiratory Cell and Molecular Biology* 51: 1-10, 2014.
21. **Du Y-m, Zhuansun Y-x, Chen R, Lin L, Lin Y, and Li J-g**. Mesenchymal stem cell exosomes promote immunosuppression of regulatory T cells in asthma. *Experimental Cell Research* 363: 114-120, 2018.
22. **Edmondson R, Broglie JJ, Adcock AF, and Yang L**. Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. *Assay and drug development technologies* 12: 207-218, 2014.
23. **Ekman A-K, Adner M, and Cardell L-O**. Toll-like receptor 7 activation reduces the contractile response of airway smooth muscle. *European Journal of Pharmacology* 652: 145-151, 2011.
24. **El-Sherbiny IM, El-Baz NM, and Yacoub MH**. Inhaled nano- and microparticles for drug delivery. *Global cardiology science & practice* 2015: 2-2, 2015.
25. **Ensign LM, Tang BC, Wang YY, Tse TA, Hoen T, Cone R, and Hanes J**. Mucus-penetrating nanoparticles for vaginal drug delivery protect against herpes simplex virus. *Sci Transl Med* 4: 138ra179, 2012.
26. **Español L, Larrea A, Andreu V, Mendoza G, Arruebo M, Sebastian V, Aurora-Prado MS, Kedor-Hackmann ERM, Santoro MIRM, and Santamaria J**. Dual encapsulation of hydrophobic and hydrophilic drugs in PLGA nanoparticles by a single-step method: drug delivery and cytotoxicity assays. *RSC Advances* 6: 111060-111069, 2016.
27. **Evans CM, Kim K, Tuvim MJ, and Dickey BF**. Mucus hypersecretion in asthma: causes and effects. *Curr Opin Pulm Med* 15: 4-11, 2009.
28. **Fahy JV**. Goblet Cell and Mucin Gene Abnormalities in Asthma[*]. *CHEST* 122: 320S-326S, 2002.

29. **Fahy JV, and Dickey BF**. Airway mucus function and dysfunction. *The New England journal of medicine* 363: 2233-2247, 2010.
30. **Fehrenbach H, Wagner C, and Wegmann M**. Airway remodeling in asthma: what really matters. *Cell and tissue research* 367: 551-569, 2017.
31. **Ganesan S, Comstock AT, and Sajjan US**. Barrier function of airway tract epithelium. *Tissue barriers* 1: e24997-e24997, 2013.
32. **Garay RP, El-Gewely R, Armstrong JK, Garratty G, and Richette P**. Antibodies against polyethylene glycol in healthy subjects and in patients treated with PEG-conjugated agents. *Expert Opinion on Drug Delivery* 9: 1319-1323, 2012.
33. **Georas SN, and Rezaee F**. Epithelial barrier function: at the front line of asthma immunology and allergic airway inflammation. *The Journal of allergy and clinical immunology* 134: 509-520, 2014.
34. **George S, Pokhrel S, Xia T, Gilbert B, Ji Z, Schowalter M, Rosenauer A, Damoiseaux R, Bradley KA, Mädler L, and Nel AE**. Use of a Rapid Cytotoxicity Screening Approach To Engineer a Safer Zinc Oxide Nanoparticle through Iron Doping. *ACS Nano* 4: 15-29, 2010.
35. **Goh J-Y, Weaver RJ, Dixon L, Platt NJ, and Roberts RA**. Development and use of in vitro alternatives to animal testing by the pharmaceutical industry 1980–2013. *Toxicology Research* 4: 1297-1307, 2015.
36. **Gopal S, Multhaupt HAB, Pocock R, and Couchman JR**. Cell-extracellular matrix and cell-cell adhesion are linked by syndecan-4. *Matrix Biology* 60-61: 57-69, 2017.
37. **Hackett NR, Shaykhiev R, Walters MS, Wang R, Zwick RK, Ferris B, Witover B, Salit J, and Crystal RG**. The human airway epithelial basal cell transcriptome. *PloS one* 6: e18378-e18378, 2011.
38. **Hackett TL, Singhera GK, Shaheen F, Hayden P, Jackson GR, Hegele RG, Van Eeden S, Bai TR, Dorscheid DR, and Knight DA**. Intrinsic phenotypic differences of asthmatic epithelium and its inflammatory responses to respiratory syncytial virus and air pollution. *Am J Respir Cell Mol Biol* 45: 1090-1100, 2011.
39. **Hajj R, Baranek T, Le Naour R, Lesimple P, Puchelle E, and Coraux C**. Basal Cells of the Human Adult Airway Surface Epithelium Retain Transit-Amplifying Cell Properties. *STEM CELLS* 25: 139-148, 2007.
40. **Hatakeyama H, Akita H, and Harashima H**. The polyethyleneglycol dilemma: advantage and disadvantage of PEGylation of liposomes for systemic genes and nucleic acids delivery to tumors. *Biol Pharm Bull* 36: 892-899, 2013.
41. **Hershfield MS, Ganson NJ, Kelly SJ, Scarlett EL, Jaggers DA, and Sundy JS**. Induced and pre-existing anti-polyethylene glycol antibody in a trial of every 3-week dosing of pegloticase for refractory gout, including in organ transplant recipients. *Arthritis Research & Therapy* 16: R63, 2014.
42. **Hiemstra PS, McCray PB, Jr., and Bals R**. The innate immune function of airway epithelial cells in inflammatory lung disease. *The European respiratory journal* 45: 1150-1162, 2015.
43. **Hirota JA, and Knight DA**. Human airway epithelial cell innate immunity: relevance to asthma. *Current Opinion in Immunology* 24: 740-746, 2012.
44. **Holgate ST**. The Airway Epithelium is Central to the Pathogenesis of Asthma. *Allergology International* 57: 1-10, 2008.
45. **Huang M-T, Chen Y-L, Lien C-I, Liu W-L, Hsu L-C, Yagita H, and Chiang B-L**. Notch Ligand DLL4 Alleviates Allergic Airway Inflammation via Induction of a Homeostatic Regulatory Pathway. *Scientific Reports* 7: 43535, 2017.

46. **Ishida T, and Kiwada H**. Accelerated blood clearance (ABC) phenomenon upon repeated injection of PEGylated liposomes. *International Journal of Pharmaceutics* 354: 56-62, 2008.
47. **Israel E, and Reddel HK**. Severe and Difficult-to-Treat Asthma in Adults. *New England Journal of Medicine* 377: 965-976, 2017.
48. **Jain RK**. Delivery of molecular medicine to solid tumors: lessons from in vivo imaging of gene expression and function. *J Control Release* 74: 7-25, 2001.
49. **Jo W, Kim J, Yoon J, Jeong D, Cho S, Jeong H, Yoon YJ, Kim SC, Gho YS, and Park J**. Large-scale generation of cell-derived nanovesicles. *Nanoscale* 6: 12056-12064, 2014.
50. **Joshi VB, Adamcakova-Dodd A, Jing X, Wongrakpanich A, Gibson-Corley KN, Thorne PS, and Salem AK**. Development of a Poly (lactic-co-glycolic acid) Particle Vaccine to Protect Against House Dust Mite Induced Allergy. *The AAPS Journal* 16: 975-985, 2014.
51. **Judge A, McClintock K, Phelps JR, and MacLachlan I**. Hypersensitivity and Loss of Disease Site Targeting Caused by Antibody Responses to PEGylated Liposomes. *Molecular Therapy* 13: 328-337, 2006.
52. **Kamerkar S, LeBleu VS, Sugimoto H, Yang S, Ruivo CF, Melo SA, Lee JJ, and Kalluri R**. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. *Nature* 546: 498, 2017.
53. **Karp PH, Moninger TO, Weber SP, Nesselhauf TS, Launspach JL, Zabner J, and Welsh MJ**. An in vitro model of differentiated human airway epithelia. Methods for establishing primary cultures. *Methods Mol Biol* 188: 115-137, 2002.
54. **Khanvilkar K, Donovan MD, and Flanagan DR**. Drug transfer through mucus. *Advanced Drug Delivery Reviews* 48: 173-193, 2001.
55. **Kleinstreuer C, Zhang Z, and Donohue JF**. Targeted Drug-Aerosol Delivery in the Human Respiratory System. *Annual Review of Biomedical Engineering* 10: 195-220, 2008.
56. **Knoch M, and Keller M**. The customised electronic nebuliser: a new category of liquid aerosol drug delivery systems. *Expert Opinion on Drug Delivery* 2: 377-390, 2005.
57. **Knowles MR, and Boucher RC**. Mucus clearance as a primary innate defense mechanism for mammalian airways. *The Journal of clinical investigation* 109: 571-577, 2002.
58. **Koczkur KM, Mourdikoudis S, Polavarapu L, and Skrabalak SE**. Polyvinylpyrrolidone (PVP) in nanoparticle synthesis. *Dalton Trans* 44: 17883-17905, 2015.
59. **Kumar M, Kong X, Behera AK, Hellermann GR, Lockey RF, and Mohapatra SS**. Chitosan IFN-γ-pDNA Nanoparticle (CIN) Therapy for Allergic Asthma. *Genetic Vaccines and Therapy* 1: 3, 2003.
60. **Lai SK, Wang YY, and Hanes J**. Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. *Adv Drug Deliv Rev* 61: 158-171, 2009.
61. **Lambrecht BN, and Hammad H**. The airway epithelium in asthma. *Nat Med* 18: 684-692, 2012.
62. **Lan B, Mitchel JA, O'Sullivan MJ, Park CY, Kim JH, Cole WC, Butler JP, and Park J-A**. Airway epithelial compression promotes airway smooth muscle proliferation and contraction. *American Journal of Physiology-Lung Cellular and Molecular Physiology* 315: L645-L652, 2018.
63. **Lane LA, Qian X, Smith AM, and Nie S**. Physical Chemistry of Nanomedicine: Understanding the Complex Behaviors of Nanoparticles in Vivo. *Annual Review of Physical Chemistry* 66: 521-547, 2015.

64. **Lee D-W, Shirley SA, Lockey RF, and Mohapatra SS**. Thiolated chitosan nanoparticles enhance anti-inflammatory effects of intranasally delivered theophylline. *Respiratory research* 7: 112-112, 2006.
65. **Lee M-K, Yoo J-W, Lin H, Kim Y-S, Kim D-D, Choi Y-M, Park S-K, Lee C-H, and Roh H-J**. Air-Liquid Interface Culture of Serially Passaged Human Nasal Epithelial Cell Monolayer for In Vitro Drug Transport Studies. *Drug Delivery* 12: 305-311, 2005.
66. **Lehofer B, Bloder F, Jain PP, Marsh LM, Leitinger G, Olschewski H, Leber R, Olschewski A, and Prassl R**. Impact of atomization technique on the stability and transport efficiency of nebulized liposomes harboring different surface characteristics. *European Journal of Pharmaceutics and Biopharmaceutics* 88: 1076-1085, 2014.
67. **Li S-D, and Huang L**. Nanoparticles evading the reticuloendothelial system: role of the supported bilayer. *Biochimica et biophysica acta* 1788: 2259-2266, 2009.
68. **Liu XB, Ye JX, Quan LH, Liu CY, Deng XL, Yang M, and Liao YH**. Pulmonary delivery of scutellarin solution and mucoadhesive particles in rats. *Eur J Pharm Biopharm* 70: 845-852, 2008.
69. **Masoli M, Fabian D, Holt S, Beasley R, and Global Initiative for Asthma P**. The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy* 59: 469-478, 2004.
70. **Matsuo Y, Ishihara T, Ishizaki J, Miyamoto K-i, Higaki M, and Yamashita N**. Effect of betamethasone phosphate loaded polymeric nanoparticles on a murine asthma model. *Cell Immunol* 260: 33-38, 2009.
71. **Mitchel JA, Antoniak S, Lee J-H, Kim S-H, McGill M, Kasahara DI, Randell SH, Israel E, Shore SA, Mackman N, and Park J-A**. IL-13 Augments Compressive Stress–Induced Tissue Factor Expression in Human Airway Epithelial Cells. *American Journal of Respiratory Cell and Molecular Biology* 54: 524-531, 2015.
72. **Moheimani F, Hsu ACY, Reid AT, Williams T, Kicic A, Stick SM, Hansbro PM, Wark PAB, and Knight DA**. The genetic and epigenetic landscapes of the epithelium in asthma. *Respiratory Research* 17: 119, 2016.
73. **Myers MA, Thomas DA, Straub L, Soucy DW, Niven RW, Kaltenbach M, Hood CI, Schreier H, and Gonzalez-Rothi RJ**. Pulmonary Effects of Chronic Exposure to Liposome Aerosols in Mice. *Experimental Lung Research* 19: 1-19, 1993.
74. **Naughton J, Duggan G, Bourke B, and Clyne M**. Interaction of microbes with mucus and mucins: recent developments. *Gut microbes* 5: 48-52, 2014.
75. **Nowak RM, Parker JM, Silverman RA, Rowe BH, Smithline H, Khan F, Fiening JP, Kim K, and Molfino NA**. A randomized trial of benralizumab, an antiinterleukin 5 receptor α monoclonal antibody, after acute asthma. *The American Journal of Emergency Medicine* 33: 14-20, 2015.
76. **Pakhale S, Mulpuru S, and Boyd M**. Optimal management of severe/refractory asthma. *Clin Med Insights Circ Respir Pulm Med* 5: 37-47, 2011.
77. **Pampaloni F, Reynaud EG, and Stelzer EHK**. The third dimension bridges the gap between cell culture and live tissue. *Nature Reviews Molecular Cell Biology* 8: 839, 2007.
78. **Paranjpe M, and Müller-Goymann CC**. Nanoparticle-mediated pulmonary drug delivery: a review. *International journal of molecular sciences* 15: 5852-5873, 2014.
79. **Park J-A, Sharif AS, Tschumperlin DJ, Lau L, Limbrey R, Howarth P, and Drazen JM**. Tissue factor–bearing exosome secretion from human mechanically stimulated bronchial epithelial cells in vitro and in vivo. *Journal of Allergy and Clinical Immunology* 130: 1375-1383, 2012.
80. **Rock JR, Randell SH, and Hogan BLM**. Airway basal stem cells: a perspective on their roles in epithelial homeostasis and remodeling. *Disease models & mechanisms* 3: 545-556, 2010.

81. **Rogers DF**. Airway mucus hypersecretion in asthma: an undervalued pathology? *Curr Opin Pharmacol* 4: 241-250, 2004.
82. **Ruge CA, Schaefer UF, Herrmann J, Kirch J, Cañadas O, Echaide M, Pérez-Gil J, Casals C, Müller R, and Lehr C-M**. The Interplay of Lung Surfactant Proteins and Lipids Assimilates the Macrophage Clearance of Nanoparticles. *PLOS ONE* 7: e40775, 2012.
83. **Schleh C, Rothen-Rutishauser B, and Kreyling WG**. The influence of pulmonary surfactant on nanoparticulate drug delivery systems. *European Journal of Pharmaceutics and Biopharmaceutics* 77: 350-352, 2011.
84. **Schneider CS, Xu Q, Boylan NJ, Chisholm J, Tang BC, Schuster BS, Henning A, Ensign LM, Lee E, Adstamongkonkul P, Simons BW, Wang S-YS, Gong X, Yu T, Boyle MP, Suk JS, and Hanes J**. Nanoparticles that do not adhere to mucus provide uniform and long-lasting drug delivery to airways following inhalation. *Science Advances* 3: 2017.
85. **Sham JOH, Zhang Y, Finlay WH, Roa WH, and Löbenberg R**. Formulation and characterization of spray-dried powders containing nanoparticles for aerosol delivery to the lung. *International Journal of Pharmaceutics* 269: 457-467, 2004.
86. **Smallcombe CC, Linfield DT, Harford TJ, Bokun V, Ivanov AI, Piedimonte G, and Rezaee F**. Disruption of the airway epithelial barrier in a murine model of respiratory syncytial virus infection. *American Journal of Physiology-Lung Cellular and Molecular Physiology* 316: L358-L368, 2018.
87. **Tagalakis AD, McAnulty RJ, Devaney J, Bottoms SE, Wong JB, Elbs M, Writer MJ, Hailes HC, Tabor AB, O'Callaghan C, Jaffe A, and Hart SL**. A Receptor-targeted Nanocomplex Vector System Optimized for Respiratory Gene Transfer. *Molecular Therapy* 16: 907-915, 2008.
88. **Tahara K, Tomida H, Ito Y, Tachikawa S, Onodera R, Tanaka H, Tozuka Y, and Takeuchi H**. Pulmonary liposomal formulations encapsulated procaterol hydrochloride by a remote loading method achieve sustained release and extended pharmacological effects. *International Journal of Pharmaceutics* 505: 139-146, 2016.
89. **Tam A, Wadsworth S, Dorscheid D, Man SFP, and Sin DD**. The airway epithelium: more than just a structural barrier. *Therapeutic Advances in Respiratory Disease* 5: 255-273, 2011.
90. **Tan LD, Bratt JM, Godor D, Louie S, and Kenyon NJ**. Benralizumab: a unique IL-5 inhibitor for severe asthma. *J Asthma Allergy* 9: 71-81, 2016.
91. **Ungaro F, d' Angelo I, Miro A, La Rotonda MI, and Quaglia F**. Engineered PLGA nano- and micro-carriers for pulmonary delivery: challenges and promises. *Journal of Pharmacy and Pharmacology* 64: 1217-1235, 2012.
92. **Vader P, Mol EA, Pasterkamp G, and Schiffelers RM**. Extracellular vesicles for drug delivery. *Advanced Drug Delivery Reviews* 106: 148-156, 2016.
93. **Walkey CD, and Chan WCW**. Understanding and controlling the interaction of nanomaterials with proteins in a physiological environment. *Chemical Society Reviews* 41: 2780-2799, 2012.
94. **Wanda Hagmolen of ten H, van de Berg NJ, Bindels PJE, van Aalderen WMC, and van der Palen J**. Assessment of Inhalation Technique in Children in General Practice: Increased Risk of Incorrect Performance with New Device. *Journal of Asthma* 45: 67-71, 2008.
95. **Wark PAB, Johnston SL, Bucchieri F, Powell R, Puddicombe S, Laza-Stanca V, Holgate ST, and Davies DE**. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *The Journal of Experimental Medicine* 201: 937, 2005.

96. **Wu M, Ouyang Y, Wang Z, Zhang R, Huang P-H, Chen C, Li H, Li P, Quinn D, Dao M, Suresh S, Sadovsky Y, and Huang TJ**. Isolation of exosomes from whole blood by integrating acoustics and microfluidics. *Proc Natl Acad Sci U S A* 114: 10584-10589, 2017.
97. **Xiao C, Puddicombe SM, Field S, Haywood J, Broughton-Head V, Puxeddu I, Haitchi HM, Vernon-Wilson E, Sammut D, Bedke N, Cremin C, Sones J, Djukanović R, Howarth PH, Collins JE, Holgate ST, Monk P, and Davies DE**. Defective epithelial barrier function in asthma. *Journal of Allergy and Clinical Immunology* 128: 549-556.e512, 2011.
98. **Yang M, Lai SK, Wang Y-Y, Zhong W, Happe C, Zhang M, Fu J, and Hanes J**. Biodegradable Nanoparticles Composed Entirely of Safe Materials that Rapidly Penetrate Human Mucus. *Angewandte Chemie International Edition* 50: 2597-2600, 2011.
99. **Yoon J, Jo W, Jeong D, Kim J, Jeong H, and Park J**. Generation of nanovesicles with sliced cellular membrane fragments for exogenous material delivery. *Biomaterials* 59: 12-20, 2015.
100. **Yu XM, Li CW, Chao SS, Li YY, Yan Y, Zhao XN, Yu FG, Liu J, Shen L, Pan XL, Shi L, and Wang de Y**. Reduced growth and proliferation dynamics of nasal epithelial stem/progenitor cells in nasal polyps in vitro. *Sci Rep* 4: 4619, 2014.