Expert Opinion

Biodegradable polymeric nanocarriers for pulmonary drug delivery

Erik Rytting, Juliane Nguyen, Xiaoying Wang & Thomas Kissel†
Philipps-Universität Marburg, Institut für Pharmazeutische Technologie & Biopharmazie, Ketzerbach 63, D-35032 Marburg, Germany

Background: Pulmonary drug delivery is attractive for both local and systemic drug delivery as a non-invasive route that provides a large surface area, thin epithelial barrier, high blood flow and the avoidance of first-pass metabolism. *Objective*: Nanoparticles can be designed to have several advantages for controlled and targeted drug delivery, including controlled deposition, sustained release, reduced dosing frequency, as well as an appropriate size for avoiding alveolar macrophage clearance or promoting transepithelial transport. *Methods*: This review focuses on the development and application of biodegradable polymers to nanocarrier-based strategies for the delivery of drugs, peptides, proteins, genes, siRNA and vaccines by the pulmonary route. *Results/conclusion*: The selection of natural or synthetic materials is important in designing particles or nanoparticle clusters with the desired characteristics, such as biocompatibility, size, charge, drug release and polymer degradation rate.

Keywords: biodegradable polymers, gene and siRNA delivery, nanoparticles, peptides, proteins, pulmonary drug delivery

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1. Introduction

Pulmonary drug delivery is attractive for several reasons. It is the obvious choice for the local administration of drugs to treat disease locally within the lung, but there are several advantages of employing the pulmonary route to achieve systemic delivery of therapeutics.

Diseases that can be targeted with local pulmonary administration include chronic obstructive pulmonary disease (COPD), asthma, cystic fibrosis, infectious diseases, tuberculosis and lung cancer [1]. Delivering drugs via the lungs also provides a non-invasive route of delivery for targeting the systemic circulation, as the lungs provide a large surface area, a thin epithelial barrier, high blood flow, and less enzymatic activity compared to other areas in the body [2-4]. First-pass metabolism can be avoided by pulmonary administration, which can be especially useful for biopharmaceuticals, which are often extensively degraded following oral delivery [4,5].

1.1 Pulmonary physiology

After inhalation through the nose or mouth, air first enters the trachea, which divides into two main bronchi. Bifurcations continue through 23 stages before the alveolar sacs are reached. Several changes in the cellular milieu occur when moving from the bronchial regions to the alveolar regions deep in the lung, including differences in epithelial cell type, airway thickness and lining fluid [6].

The airway epithelium contains ciliated cells, secretory cells and basal cells; the alveolar epithelium is mainly comprised of Type I and Type II alveolar cells.

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Narrower airways result in narrower diffusion distances. The total distance spanning the air–blood barrier decreases from about 30 – 40 µm in the bronchial region to approximately 500 nm in the alveolar region [4,6]. The mucus layer lining the upper airways is about 5 – 10 µm thick, whereas the lung surfactant lining fluid secreted by the Type II alveolar cells has a layer thickness of only 50 – 80 nm [6].

Barriers to particle deposition include the physical defense of the oropharyngeal region and the bronchial tree. Other barriers to particle and drug transport in the airways include the mucus layer, the alveolar lining fluid, epithelial cells, basement membrane, the mucociliary escalator, macrophage clearance and proteolytic degradation [4,5]. Tight junctions in the distal airways are not as tight as the tight junctions in the bronchial and alveolar epithelia, suggesting that targeting these distal airways may result in higher bioavailabilities in the systemic circulation [7]. Alveolobronchial clearance is slower in the peripheral regions, and macrophage clearance is reported to be minimal for particles less than 260 nm [3,5]. Metabolism in the lung occurs by peptidases and Phase I enzymes; these metabolizing enzymes are found mostly in Clara cells, alveolar Type II cells and alveolar macrophages [3,6].

1.2 Nanocarriers for pulmonary delivery

Nanoparticles have gained increasing attention for pulmonary drug delivery, due to their advantages for targeted deposition, bioadhesion, sustained release and reduced dosing frequency for improving convenience to the patient [2]. Some incentives for using nanoparticles for the controlled delivery of drugs, peptides, proteins, genes, siRNA and vaccines in the lung include having an appropriate size for avoiding alveolar macrophage clearance and promoting transepithelial transport. Nanocarriers used for pulmonary applications also include liposomes, solid lipid nanoparticles and nanotubes, but since this review is limited to polymeric nanocarriers, the reader is referred to other excellent manuscripts of interest [8-10]. Lung targeting following intravenous nanoparticle administration also falls outside the scope of this review, but this has been discussed previously [11]. Due to their small size, most nanoparticles would be exhaled, but these multifunctional particles can be delivered to the lung by nebulization or by the incorporation of the nanoparticles into larger particles with an appropriate aerodynamic diameter by flocculation [12], spray drying, or other means.

Regional deposition of particles delivered to the lung depends on several factors, including particle properties such as aerodynamic diameter, charge, surface properties and hygroscopicity, as well as temperature, breathing pattern and the timing of the aerosol pulse injection within the breathing cycle [4]. The aerodynamic diameter (d_{ae}) is a function of size, shape and density. Porous particles will have a smaller d_{ae} than their physical diameter would suggest, and for non-spherical particles the d_{ae} is mostly dependent upon the short axis and the magnitude of the aspect ratio [5]. Shape does not only affect deposition by its influence on the d_{ae}, but other factors too, as particle fibers are not as easily cleared by alveolar macrophages compared to spherical particles [13].

The aerodynamic diameter (d_{ae}) has an important influence on particle destination. The optimal size for deposition in the deep lung for systemic delivery is approximately 1 – 3 µm [5]. Particles larger than 5 – 10 µm result in oropharyngeal deposition, and are more likely to be swallowed than to reach the lung. Particles smaller than 1 µm will likely be exhaled. For particles between 1 and 5 µm, the smaller particles generally reach the deeper parts of the lung, and the larger particles land in the upper airways [3]. Particles around 1 – 2 µm have a higher chance of crossing the air–blood barrier, and particles smaller than 150 nm encounter delayed lung clearance, increased protein interactions and more transepithelial transport compared to larger particles [13]. Particle size may also affect particle degradation and drug release rates.

Surface charge is another important property to consider in particle design. Low surface energy is needed to avoid particle agglomeration [5,13]. Electrostatic interactions are also possible between the alveolar wall and oppositely charged particles, but this depends on hydrophobicity and humidity [13].

2. Biodegradable nanocarrier materials

The design and synthesis of biodegradable polymeric materials that will provide the appropriate nanocarrier characteristics for temporal and spatial distribution of drug in the lung has been pursued extensively. Nanocarrier targeting to the lung tissue based on particle size and surface charge is an important aspect for material selection and design, but the release of the active agent will also depend on its distribution in the nanocarriers and the degradation rate of the polymer.

A number of synthetic and natural polymers have been utilized in formulating biodegradable nanoparticles [14]. Synthetic polymers have the advantage of sustaining the release of the encapsulated therapeutic agent over a period of days to several weeks. Natural polymers have a comparatively short duration of drug release. Polymers used for the formulation of nanoparticles include natural polymers such as albumin, gelatin, alginate, collagen, cyclodextrin and chitosan; synthetic polymers used for pulmonary applications include poly(lactide-co-glycolide) (PLGA) copolymers, polyacrylates and polyanhydrides. These polymers, together with novel polymers representing modifications to PLGA, are outlined below. The applications of these polymers to pulmonary drug delivery are then described in the following section.

2.1 PLA and PLGA

Polylactides (PLA) and poly (D,L-lactide-co-glycolide) (PLGA) have been the most extensively investigated for drug

delivery [14]. As polyesters in nature, these polymers undergo hydrolysis upon implantation into the body, forming biologically compatible moieties (lactic acid and glycolic acid) that are removed from the body by the citric acid cycle. The degradation products are formed slowly and do not affect normal cell function. These safe, non-toxic polymers are currently being widely used in drug delivery systems and tissue engineering research.

More details regarding the biocompatibility and biodegradation of PLA and PLGA in drug delivery applications are presented in a review by Anderson and Shive [15]. The release of drug in the PLGA matrix is controlled by diffusion of the drug through the matrix and by degradation of the polymer [15]. PLGA's degradation rate is affected by the copolymer composition and the molecular weight, and the release of drug can thus be varied from weeks to months [16]. Several methods such as solvent evaporation, nanoprecipitation and multiple emulsions allow formulations of small particles on an industrial scale [14,17].

2.2 Physical modification of PLGA

Modifications to the particle surface and size can be used to avoid particle clearance, increase circulation time, improve transport across biological barriers, or to prolong residence time at the site of absorption. Prolonging drug presence can be manipulated by using mucoadhesive materials, such as the biodegradable polysaccharide chitosan. Yamamoto *et al.* produced PLGA nanoparticles surface-modified with chitosan and encapsulating the peptide elcatonin [18]. The surface-modified nanoparticles delivered to the lungs of guinea pigs resulted in prolonged effects compared to the unmodified nanoparticles, and the chitosan-modified nanoparticles were eliminated more slowly than the unmodified version, suggesting that nanoparticle retention was responsible for the sustained effects.

2.3 Chemical modification of PLGA

Despite its many advantages, PLGA also has some inherent shortcomings. The lack of hydrophilic and functional groups leads to challenges regarding drug encapsulation and stability during storage. Other challenges include polyphasic release patterns, low encapsulation efficiency and high burst release [19,20]. A major challenge of nanoparticle delivery to the lungs is formulation instability due to particle–particle interactions.

A strategy to overcome some of the issues associated with the use of PLGA for pulmonary delivery of nanocarriers was to create a polymer that is more hydrophilic in nature than PLGA and to introduce functional groups for improved drug–polymer interactions within the nanoparticles. For example, block copolymers of hydrophilic poly(ethylene glycol) (PEG) with PLGA have been reported to show accelerated drug release [21,22]. Star and comb-shaped PLA or PLGA could be synthesized with multifunctional initiators, such as glycerol, pentaerythritol, amino-propanediol, poly(vinyl alcohol) and dextran [23-28]. A primary feature of these materials is that they have high molecular weights but relatively short PLA or PLGA chains, and more hydroxyl end groups, which leads to increased hydrophilicity and faster degradation rates compared to linear PLA or PLGA of similar molecular weight.

Polyelectrolytes with functional groups in the backbone, such as amine and sulfonic acid groups, were introduced into brush-like graft PLGA. These modifications affect the colloidal stability of carrier systems by imparting positive or negative surface charges and increasing protein or drug loading of carriers by electrostatic interactions [29-32]. These functional groups also accelerate the degradation rate by enhancing the hydrophilic character of the polyester [29,31,32].

Compared with amphiphilic block copolymers, the amphiphilic graft copolymers have multi-grafted hydrophobic/hydrophilic branches along a hydrophilic/hydrophobic polymer backbone. Therefore, the properties of nanoparticles can be easily varied by simply adjusting the graft density and side chain length of the branches.

2.3.1 *PVA-PLGA*

Dailey *et al.* reported the synthesis of a series of poly(vinyl alcohol) (PVA)-based branched polyesters with PLGA side chains (PVA-g-PLGA, **Figure 1A**) [33]. The PVA provides a hydrophilic basis for the copolymer, while the degree of the copolymer hydrophobicity could be varied according to the length of the PLGA side chains grafted onto the PVA. These copolymers exhibited a lower burst effect coupled with a linear infusion-like release profile of proteins, which could be controlled by the structure and molecular weight of the copolymer. Also, in contrast to the bulk erosion observed for PLGA, the PVA-g-PLGA copolymers exhibited a surface erosion biodegradation mechanism [34,35]. Further developments of this type of copolymer could satisfy the requirements of different drugs and proteins delivered by the pulmonary route.

PVAs have shown good protein compatibility, mucoadhesive properties and better temperature stability during bulk polymerization with lactide and glycolide. PVAs with molecular weights less than 15,000 g/mol will be eliminated from the body by renal excretion [36]. Biodegradation occurs by surface erosion, and the biocompatibility is comparable to that of linear PLGA [37,38].

Uncharged and charged PVA-PLGA having side chain lengths higher than 10 have the potential for the formation of microparticles and nanoparticles, and water soluble polyesters with PLGA side chain lengths smaller than three are capable of forming nanocomplexes with oppositely charged proteins [15,27,35].

2.3.2 *SB-PVA-PLGA*

Varying amounts of sulfobutyl (SB) groups were attached to the backbone to create SB-PVA-g-PLGA polymers with an increasingly negative surface charge (**Figure 1B**) [39]. The SB-PVA-g-PLGA polymer allows the preparation of nanoparticles that exhibit a core-corona structure with the

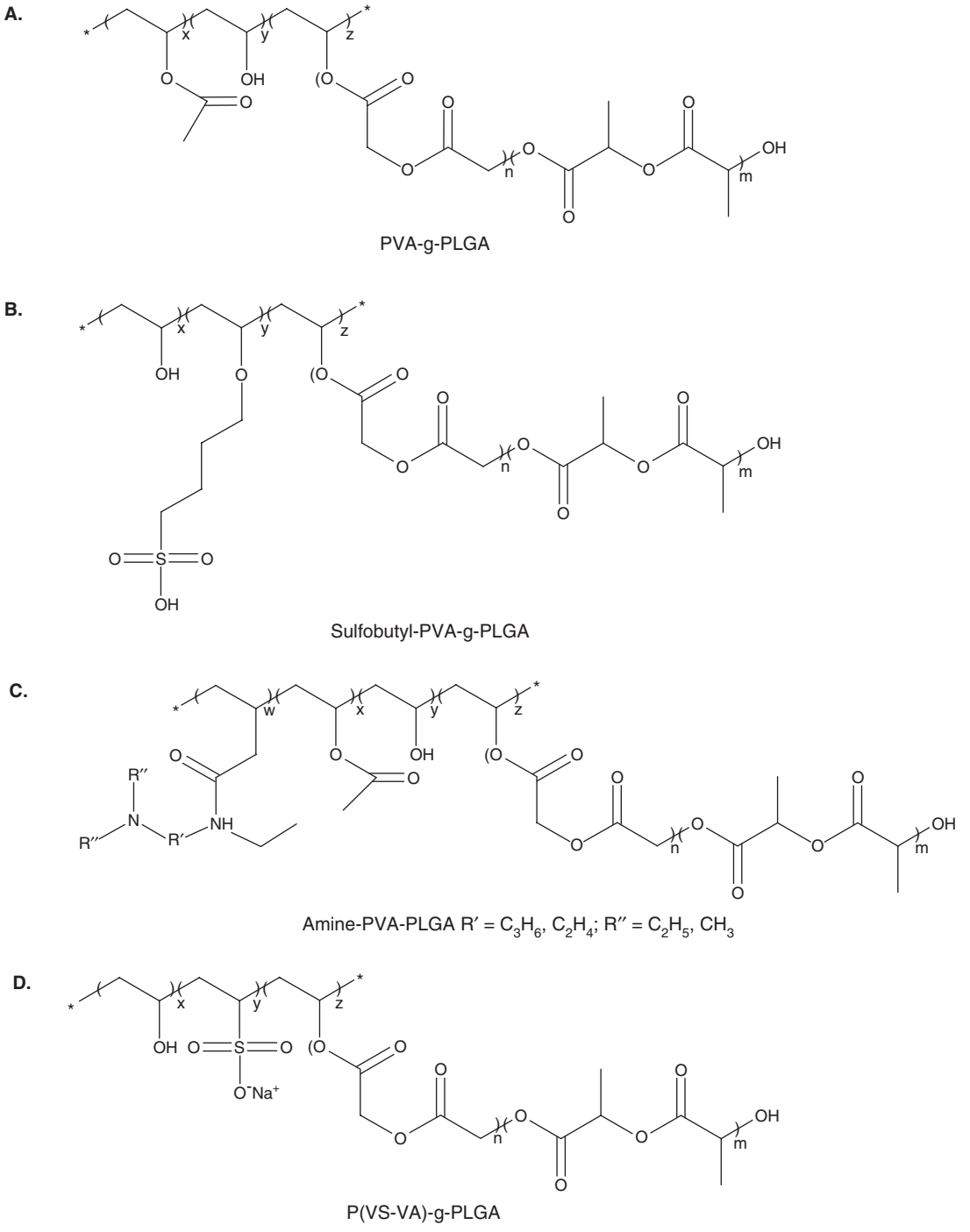

Figure 1. The chemical structure of a series of PVA-based branched PLGAs.

negatively charged hydrophilic sulfobutyl groups oriented towards the outer aqueous phase, providing for the rearrangement of the hydrophilic backbone to the particle surface. Jung *et al.* demonstrated the preparation of particles with this polymer having diameters between 100 and 500 nm [40]. These colloidal carriers can be prepared without the use of additional surfactants. This is of extreme importance for pulmonary application, as the inhalation of synthetic surfactants may disturb the surface tension of the pulmonary lining fluid and lead to impaired lung function or inflammation.

2.3.3 *Amine-modified PVA-PLGA*

Different amino groups, such as dimethylaminopropylamine (DMAPA), diethylaminoethylamine (DEAEA) and

diethylaminopropylamine (DEAPA) were attached to the backbone to create polymers with increasingly positive surface charges (**Figure 1C**) [41]. Wittmar *et al.* developed this class of biodegradable amine-substituted PVA polymers, onto which hydrophobic PLGA chains were grafted. The amphiphilic properties of diethylaminopropyl amine-poly(vinyl alcohol)-grafted-poly(lactide-co-glycolide) (DEAPA-PVA-g-PLGA) make it a versatile polymer for pulmonary drug delivery [29,42]. Using a modified solvent displacement method, nanoparticles can be generated from DEAPA-PVA-PLGA without high shear forces. Furthermore, degradation times can be tailored by the degree of amine substitution to range from a few days to several weeks, which is a vital aspect for long-term pulmonary application. In contrast, the long degradation rates of commercially available PLGA are a critical factor for its use in lung delivery [43].

Coating DEAPA-PVA-g-PLGA nanoparticles with carboxymethylcellulose can prevent aggregation of the particles during nebulization; Dailey *et al.* reported the preparation of such particles in the size range of 76 – 256 nm [42]. Such aggregation occurs with more hydrophobic polymers. The degradation rate decreased with increasing amounts of CMC. DEAPA-PVA-g-PLGA nanoparticles were also shown to be taken up into alveolar epithelial cells (A549) to a low extent [42].

2.3.4 *P(VS-VA)-PLGA*

To control precisely the number of functional groups, P(VS-VA)-g-PLGA was recently developed (**Figure 1D**). The polyelectrolyte backbones were obtained by the radical copolymerization between vinyl acetate and vinyl sulfonic acid sodium salt and the subsequent hydrolysis. The obtained poly(vinyl sulfonic-co-vinyl alcohol) (P[VS-VA]) backbones were grafted with PLGA by ring-opening melt polymerization using $SnOct_2$ as catalyst through the hydroxyl group [44]. It was demonstrated that the degree of sulfonic acid substitution and the side length of PLGA can be easily controlled by the feed ratio. Surface characterization studies showed that, as observed for SB-PVA-g-PLGA, nanoparticles prepared from these polymers exhibited a core-corona structure with the negatively charged, hydrophilic sulfonic groups oriented towards the outer aqueous phase [45]. Nanoparticles prepared from this novel polymer class were reported to range in size from 120 – 151 nm [45].

2.4 Other polymers

Polyanhydrides and polyacrylates have also been recently investigated for nanocarrier-based pulmonary drug delivery applications. Fliegel *et al.* describe a novel class of biodegradable poly(ether-anhydride) polymers designed for pulmonary drug delivery [46]. These polymers are composed of sebacic acid and poly(ethylene glycol) (PEG) in various ratios. By the addition of 10% PEG, the fraction of particles deposited in the lower stages of a model lung could be increased, most likely due to minimized aggregation from surface roughness. Zhang *et al.* have used polybutylcyanoacrylate to prepare nanoparticles for intratracheal delivery [47].

In summary, chemical modifications to PLGA add several benefits in the selection of a suitable material for nanocarriers in the lung. The introduction of positive or negative charges can enhance the encapsulation efficiency and release profile of oppositely-charged drugs, proteins, or genetic material. Adjustments to the polymer structure can alter the balance between hydrophobic and hydrophilic groups, which can in turn affect drug loading, release, nanoparticle orientation, particle size and surface charge. Furthermore, certain functional groups will affect the polymer degradation rate, which not only affects the release of active agent but is also of concern when one considers the possible accumulation of polymer within the lung following repeated doses. The design of fast-degrading polymers, such as DEAPA-PVA-g-PLGA, overcomes some of the challenges associated with polymer accumulation due to slower degrading PLGA particles.

3. Pulmonary delivery of active agents

The previous section introduced several polymers that have been used and designed for pulmonary delivery of nanocarriers; this section will present some of the specific applications of these materials in the delivery of active agents, including drugs, peptides, proteins, DNA, siRNA and vaccines.

3.1 Drug delivery

As mentioned above, PLGA exhibits a triphasic drug and protein release kinetic with an initial burst effect, which is governed by diffusion kinetics, followed by a lag phase and a secondary burst phase [48,49]. By varying the PLGA chain lengths, the proportion of lactic to glycolic acid and the molecular weight, drug release profiles can be influenced [15].

Dutt *et al.* encapsulated isoniazid and rifampicin into PLGA microparticles and investigated their release profile from different formulations, demonstrating that isoniazid shows a sustained release of up to 3 days from porous microparticles and of up to 6 days from non-porous microparticles. By hardening the PLGA microparticles, a sustained release carrier system of up to 7 weeks *in vitro* and *in vivo* could be achieved. In a murine model one dose of PLGA microparticles was able to clear bacteria from the lungs and liver more effectively as compared to a daily administration of free drug [50,51].

For the treatment of tuberculosis, recombinant Mycobacterium tuberculosis antigen 85B (Ag85B) was encapsulated by spray-drying into PLGA-microspheres [52]. With a median diameter of 3 – 4 μm, these microspheres were suitable for targeting macrophages and for aerosol delivery to the lung. PLGA-rAg85B microspheres were able to stimulate an

antigen response that was two orders of magnitude higher than that observed with the pure rAG85B. In another study, PLGA microspheres, prepared using emulsion/solvent evaporation, were loaded with rifampicin and delivered to guinea pigs, which were infected with Mycobacterium tuberculosis [53]. Compared to nebulized rifampicin suspension, the aerosolized rifampicin-loaded PLGA microspheres were able to reduce most measures of tuberculosis infection. Encapsulation of three anti-tubercular drugs – rifampicin, isoniazid, and pyrazinamide – into PLGA nanoparticles achieved sustained therapeutic drug levels for 6 – 8 days in the plasma, and for up to 11 days in the lungs. The drug-loaded nanoparticles were prepared by the multiple emulsion technique and nebulized after vacuum drying. A significantly prolonged elimination half-life was observed compared to the orally administered drug and no tubercle bacilli could be detected in the lungs after five doses of treatment [54].

A second widely used class of biodegradable polymers for pulmonary delivery is chitosan and its derivatives. The degradation of chitosan has already been tested in several studies and it has been shown that chitosans are depolymerized enzymatically by lysozyme, albeit with a very slow rate [55-57]. Like lactoferrin or peroxide, lysozymes are present within the lung mucus and lysozyme is the most abundant antimicrobial polypeptide in respiratory tract secretions [58,59]. Learoyd *et al.* investigated the influence of chitosan molecular weight on the drug release of terbutaline sulfate spray powders using low, medium and high molecular weight chitosan. With increasing molecular weights, the drug release profile changed from a burst release to a sustained drug release profile over 2 – 4 h. The microparticles generated displayed a median diameter of 1 – 2.5 μm and were therefore suitable for inhalation [60].

Corrigan *et al.* investigated the influence of the preparation media on the morphology and characteristics of chitosan microparticles prepared by spray-drying. As the degree of acetylation of chitosans affects its physicochemical properties (i.e., viscosity, degradability and solubility), spray drying was performed in hydrochloric acid or acetic acid. It was observed that the presence of acetic acid leads to increased acetylation of chitosan during spray-drying. When loading chitosan microparticles with salbutamol by spray-drying, a high respirable fraction was achieved when aerosolized into a twin impinger. However, the burst release of the drug in less than 5 min requires further optimization for future pulmonary delivery [61].

3.2 Peptide and protein delivery

With its large alveolar surface area, thin epithelial barrier and low proteolytic activity compared to other administration routes, the lung represents an attractive route for the delivery of macromolecules, such as proteins. Due to their extreme sensitivity, the design of sophisticated drug carriers is required to overcome the many barriers of the lungs.

Amidi *et al.* generated insulin-loaded microparticles by spray-drying using *N*-trimethyl chitosan [62]. In all formulations the secondary and tertiary structure of insulin could be preserved. Even after 1-year storage at 4°C, the particle characteristics and insulin structure remained unchanged and intact. Grenha *et al.* developed a microparticulate carrier system for insulin-loaded chitosan nanoparticles. Using mannitol and lactose as excipients, the insulin-loaded chitosan nanoparticles were microencapsulated by spray-drying, yielding particles with Ferret diameters of 2 – 4 μm. *In vitro* studies showed that approximately 75 – 80% of the encapsulated insulin could be released from the nanoparticle-loaded microspheres within 15 min [63]. PLGA nanospheres coated with chitosan for pulmonary delivery of the peptide elcatonin have been mentioned previously as an example of the advantages of physical modifications to PLGA nanocarriers [18].

Kawashima *et al.* dosed PLGA nanoparticles prepared with insulin to guinea pig lungs and demonstrated a significant reduction in blood glucose level, with a prolonged effect over 48 h compared to insulin solution [64]. Insulin-loaded nanoparticles using a different polymer, poly(butyl cyanoacrylate), delivered to the lungs of rats, were shown by Zhang *et al.* to extend the duration of a hypoglycemic effect over 20 h [47].

3.3 Gene, siRNA and vaccine delivery

An ideal gene delivery system should show high transfection levels, be non-toxic and biodegradable for long-term application. Polyethylenimine (PEI) is one of the most effective cationic compounds for plasmid delivery into mammalian cells [65,66]. The cationic groups of carriers such as PEI can form complexes with oppositely-charged genetic cargo. However, the high toxicity and lack of biodegradability of PEI limits its potential for pulmonary application.

To overcome these drawbacks of PEI and at the same time maintain its high transfection efficiency, Thomas *et al.* developed biodegradable PEIs composed of a linear 423 Da PEI and a branched 1.8 kDa PEI [67]. These two low molecular weight PEIs, which have been shown to be less toxic than its high molecular weight counterpart [68], are crosslinked with bi- and oligo-functional acrylates to obtain biodegradable high molecular weight PEIs. Creating a combinatorial library of vectors, it was shown that the optimal vector *in vivo* was the mixed PEI crosslinked with propylene glycol glycerolate diacrylate. It combined the highest transfection efficiency (186 times higher than the physical mixture of the parental PEIs) with low toxicity, whereas the commercially available 22-kDa PEI caused 50% mortality.

Another group of biodegradable polymers used for gene delivery are chitosans, which are considered to be non-toxic. One of their major drawbacks, however, is the modest transfection efficiency *in vivo* and *in vitro* [69,70]. Further investigations are necessary to clarify the mechanisms of

uptake and transfection efficiency. Köping-Höggard *et al.* administered chitosan-pDNA polyplexes to the lungs of mice, showing that the high molecular weight chitosan is well tolerable to mice and is able to promote gene delivery into the lungs. However, PEI remained a superior pDNA vector for pulmonary gene delivery [71]. After further optimization, a comparable luciferase gene expression to that of PEI was achieved when administered to mouse lungs. The molecular weight of chitosans in this study ranged from 1.2 – 10 kDa. Physicochemical studies showed that low molecular weight chitosans are able to release pDNA in the presence of the model anion heparin, leading to a higher transfection efficiency compared to the more stable high molecular weight chitosan polyplexes [72].

Another approach used to improve the bioactivity of chitosan oligomer polyplexes was to introduce a trisaccharide branch that targets cell surface lectins [73]. Lectins are reported to be exposed on airway epithelial cells and have the ability to bind sugar residues [74,75]. The transfection efficiency of the trisaccharide-substituted chitosans was significantly higher in human liver hepatocytes (HepG2) and a human bronchial epithelial cell line (16HBE14o$^-$) than unmodified chitosan and PEI 25 kDa. Luciferase gene expression in mouse lungs was fourfold higher for trisaccharide-substituted chitosans than unmodified chitosan but unfortunately no comparison to PEI 25 kDa *in vivo* was shown.

Chitosan has also been used for siRNA delivery to human lung carcinoma cells (H1299). With a chitosan/siRNA formulation containing sucrose as lyoprotectant, a 70% knockdown was achieved [76]. Howard *et al.* prepared polyplexes between siRNA and chitosan [77]. Effective knockdown, both *in vitro* and *in vivo*, was observed. In the H1299 human lung carcinoma cell line and in murine peritoneal macrophages, knockdowns of 77.9 and 89.3%, respectively, were achieved. With a 40% reduction in EGFP fluorescence in bronchial epithelial cells of transgenic EGFP mice, chitosan/siRNA polyplexes showed successful RNA interference.

Chitosan has also been reported to display therapeutic potential in the case of respiratory syncytial virus (RSV). RSV causes bronchiolitis and pneumonia and is also a severe risk factor for asthma. Treatment of rats prior to RSV infection with chitosan/siRNA-polyplexes containing a siRNA that interferes against the RSV-NS1 gene (siNSI), reduced the virus titers in the lung. SiNS1-treated rats showed less inflammation and hyperresponsiveness compared to the control [78].

Pulmonary DNA vaccination represents a non-invasive and less painful administration route for immunization. The opportunity to combine the genetic information of various antigen epitopes and cytokines, easy production and the high stability of plasmid DNA compared to recombinant proteins and pathogens make it an attractive class of vaccines. Many pulmonary pathogens, such as *M. tuberculosis bacillus*, respiratory syncytial virus (RSV) and severe acute respiratory syndrome corona virus (SARS) could all be treated once a suitable vaccination has been developed [79-81]. Bivas-Benita *et al.* prepared poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles and coated them with polyethylenimine (PEI). These PLGA-PEI nanoparticles were loaded with DNA and uptake into the endo-lysosomal compartment of the human airway submucosal epithelial cell line, Calu-3, was detected [82].

N-trimethyl chitosan and dextran microparticles were investigated for pulmonary delivery of diphtheria toxoid. The microparticles were prepared by drying an aqueous solution of polymer and diphtheria toxoid using a supercritical fluid (SCF) spraying process. In contrast to dextran microparticles, only the *N*-trimethyl chitosan microparticles led to a detectable secretion of IgA when administered to the lungs [82].

4. Conclusions

Pulmonary drug delivery is attractive for both local and systemic drug delivery as a non-invasive route that provides a large surface area, a thin epithelial barrier, high blood flow and the avoidance of first-pass metabolism. Nanoparticles have several advantages for controlled drug delivery by the pulmonary route, including sustained release, reduced dosing frequency, as well as being an appropriate size for avoiding alveolar macrophage clearance or promoting transepithelial transport. Particles or nanoparticle clusters with aerodynamic diameters between 1 and 5 μm have the highest probability of successful lung deposition. The selection of natural or synthetic biodegradable polymeric materials for nanocarriers is important in order to design particles with the desired characteristics. Biocompatibility, size, charge and drug release rates must all be considered, but in order to avoid accumulation of polymeric materials following repeated dosing, the polymer degradation rate is crucial.

5. Expert opinion

Although significant progress has been made in recent years relating to the design of biodegradable nanocarrier strategies for the delivery of drugs, peptides, proteins, genes, siRNA and vaccines, the future of pulmonary delivery strategies is expected to be influenced critically by the outcome of ongoing discussions.

An issue that remains surrounded by considerable debate is the question whether the lung should be used as an entry port for systemic drug administration. In this context, the safety of the nanocarriers and a lack of inflammatory and immunogenic potential need to be demonstrated under chronic treatment conditions. Such studies have not been presented with drug-loaded nanocarriers, but will be necessary during future clinical trials. Such issues are not limited to pulmonary drug delivery, but are also important in oral and intravenous administration.

A second area where a general lack of information can be recognized is the interface between nano-objects and lung tissue/cells. There are still many questions to be answered with regard to the fate of nanocarriers in the lung. For example, what physical and chemical characteristics affect clearance by alveolar macrophages? Which particle properties affect cellular internalization and transport across the pulmonary epithelium? Furthermore, predictive correlations between *in vitro*, *ex vivo* and *in vivo* models are necessary in preparation for clinical trials of nanocarrier-based drug delivery systems in the lung.

The third area where more fundamental information needs to be generated is related to the aforementioned topics but addresses the aspects of biomaterials used for pulmonary delivery systems. The residence time of nanocarriers in lung tissue, their degradation mechanisms and the clearance of degradation products will ultimately affect the safety and biocompatibility of such delivery devices. The accumulation of carrier materials within the lung, including polymer and its degradation products, may bring about long-term concerns that outweigh the benefits of therapy with polymeric carriers. More polymers with short half-lifes would clearly be desirable, as this would be more suitable for repeated administration. A consensus about testing strategies, including both *in vitro* and *ex vivo* models, has yet to be reached.

The fourth area where advances would be desirable relates to the design of nanocarriers, especially the incorporation of sensitive therapeutic agents, such as proteins, p-DNA and siRNA, which require particle production methods avoiding high shear stress. Important progress regarding particle stabilization is necessary to prolong the shelf life of nanoparticles intended for pulmonary delivery. Reconstitution of dried nanosuspensions for nebulization or packaging of nanoparticles into adequately-sized particle clusters for inhalation of a dry powder are important steps to deliver the desired doses to the desired regional targets within the lung. As diffusion distances in nanocarriers are shorter by definition, control over drug loading and release under *in vitro* as well as *in vivo* conditions remains a challenge. In addition to new technologies, polymer design may also help to address such problems, as we have shown in some of the examples presented above.

Pulmonary drug delivery is a fascinating area of research which needs input from various disciplines ranging from medical sciences to aerosol physics. As these interdisciplinary research activities continue in the area of biodegradable nanocarriers for pulmonary drug delivery, one can expect significant advancements in the future that will extend hope to healthcare professionals and patients alike.

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Affiliation

Erik Rytting[1] PhD, Juliane Nguyen[2], Xiaoying Wang[3] PhD & Thomas Kissel[†4] PhD
[†]Author for correspondence
[1]Postdoctoral Research Associate
Philipps-Universität Marburg,
Institut für Pharmazeutische
Technologie & Biopharmazie,
Ketzerbach 63, D-35032 Marburg, Germany
[2]PhD candidate
Philipps-Universität Marburg,
Institut für Pharmazeutische
Technologie & Biopharmazie,
Ketzerbach 63, D-35032 Marburg, Germany
[3]Postdoctoral Research Associate
Philipps-Universität Marburg,
Institut für Pharmazeutische
Technologie & Biopharmazie,
Ketzerbach 63, D-35032 Marburg, Germany
[4]Professor and Head
Philipps-Universität Marburg,
Institut für Pharmazeutische
Technologie & Biopharmazie,
Ketzerbach 63, D-35032 Marburg, Germany
Tel: +49 6421 2825881;
Fax: +49 6421 2827016;
E-mail: kissel@staff.uni-marburg.de