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Polymer-drug conjugates as inhalable drug delivery systems: A review

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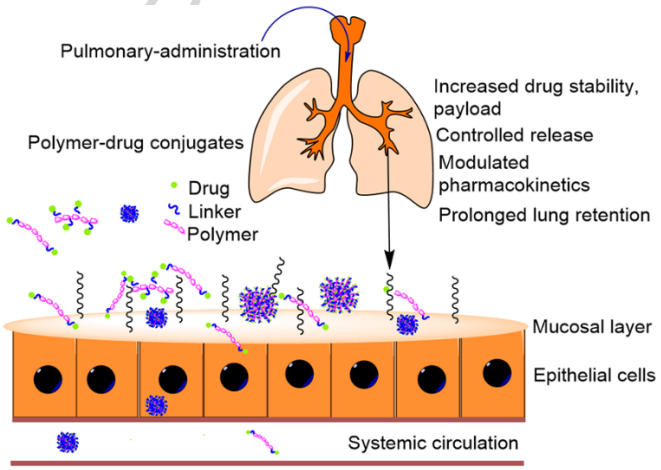
Abstract:

Accelerating interest by the pharmaceutical industry in the identification and development of less invasive routes of nanomedicine administration, coupled with defined efforts to improve the treatment of respiratory diseases through inhaled drug administration has fuelled growing interests in inhalable polymer-drug conjugates. Polymer-drug conjugates can alter the pharmacokinetics profile of the loaded drug after inhaled administration and enable the controlled and sustained exposure of the lungs to drugs when compared to the inhaled or oral administration of the drug alone. However, the major concern with the use of inhalable polymer-drug conjugates is their biocompatibility and long-term safety with the lungs, which is closely linked to their retention time in the lungs. A detailed understanding about the pharmacokinetics, lung disposition, clearance and safety of inhaled polymer-drug conjugates with significant translational potential is therefore required. This review therefore provides a comprehensive summary of the latest developments on several types of polymer-drug conjugates that are currently being explored as inhalable drug delivery systems. Finally, the current status and future perspective of the polymer-drug conjugates is also discussed with a focus on current knowledge gaps.

Graphical abstract:



Keywords: Polymer-drug conjugates; lung & pulmonary delivery; Inhalation; Drug delivery; pharmacokinetics

1. Introduction

Polymers and polymer-based drug delivery systems have undergone an enormous expansion in the past decade, with the clinical and pre-clinical development of polymer-based nanomedicines and other biomedical applications. The key feature of a polymer-drug conjugate is that rather than containing a drug that is non-covalently encapsulating within a polymeric structure, the drug is physically conjugated to the polymeric carrier [1]. In this regard, problems associated with 'burst drug release' can be largely overcome and the drug can be covalently linked to the polymer *via* linkages that are specifically designed to liberate drug within certain structures, or at a predicted rate *in vivo*.

The concept of polymer-drug conjugates was first introduced by Helmut Ringsdorf in 1975 [2]. According to this concept, an ideal polymer-drug conjugate is characterized by a hydrophilic polymer backbone as a vehicle and a bioactive agent that is usually bound to the polymeric scaffold *via* a biological response linker. Sometimes a targeting moiety or a solubility enhancer may also be introduced into the conjugate to improve pharmacokinetic behaviour and therapeutic efficiency (**Fig. 1**) [2, 3]. In general, polymer-drug conjugates offer several advantages as drug delivery moieties, including 1) the capability to achieve high drug payloads, 2) improved drug solubility, 3) modulation of drug pharmacokinetics (including prolonged plasma exposure and optimised biodistribution behaviour, resulting in enhanced therapeutic efficacy), 4) reduced systemic and local side-effects as a result of highly irritant or cytotoxic drugs, 5) enhanced *in vivo* drug stability, and 6) controlled rate and site of drug liberation. Despite these advantages, the full potential of polymer-drug conjugates as drug delivery platforms has yet to be fully harnessed, since the majority of current 'nanomedicinal' drug delivery systems still utilise the cheaper drug encapsulation approach.

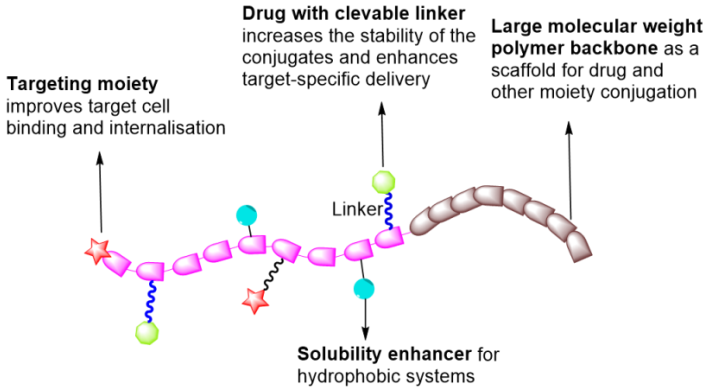


Fig 1. Example of a polymer-drug conjugate system, employing a polymer backbone, tissue targeting moiety, drug and solubility enhancer.

Polymer-drug conjugates are often synthesized using one of three strategies, including 1) conjugating the drug to an established polymers, 2) conjugating the drug to a monomer, followed by reversible addition fragmentation transfer (RAFT) polymerisation, ring-opening metathesis polymerisation (ROMP) or ring opening polymerisation (ROP), and 3) using an existing drug containing two or more functional groups as a monomer for poly-drug polymerization [4, 5]. The first strategy can lead to poor control over drug conjugation and limited drug loading, depending on the size and nature of the polymer structure. However, polymerisation of drug-monomer conjugates generally provides good control over drug loading and the final product [5, 6]. Drugs are often conjugated to the polymers *via* biodegradable linkers which can control the site and rate of drug liberation, although the linker has to be carefully selected to display optimal *in vivo* drug release rates for the intended therapeutic application [7]. It is important to note however, that the physicochemical properties of the polymer can have an impact on the *in vivo* liberation of drugs linked *via* 'biodegradable' linkers, particularly when access by an enzyme is required [1, 8, 9].

One of the most significant advantages that polymer-drug conjugates have, as alluded to above, is the ability to change the pharmacokinetic and biodistribution behaviour of the loaded drug [1]. In this regard, polymer-drug conjugates have traditionally been administered exclusively *via* the intravenous route as a result of their size and general hydrophilic nature limiting absorption after oral administration [3]. Subcutaneous or intramuscular delivery also provides a means to access the blood using a less invasive approach, but bioavailability can be limited in some cases. However, recent interest by big Pharma in non-invasive drug delivery approaches and targeted delivery to the lungs to improve the treatment of lung-resident illnesses that are traditionally treated with oral medications, has sparked a tremendous worldwide interest in the inhaled delivery of nanomedicines.

The pulmonary route possesses several distinct advantages over conventional oral or injectable routes of administration, including lower enzymatic activity in the lungs than that found in the gut, the avoidance of first-pass metabolism as well as the thin alveolar membrane, high surface area for absorption and extensive vasculature that can facilitate the rapid systemic absorption of drugs after inhaled administration [10, 11]. However, despite the potential for very rapid drug absorption from the lungs for relatively low molecular weight materials (several thousand Da max), the tight intercellular junctions between alveolar cells

typically limits the passage and systemic access of larger constructs, such as polymers. This has the effect of slowing the systemic absorption of polymers and providing the opportunity for sustained lung exposure to polymer-drug conjugates (and therefore to the drug). This is particularly advantageous when treating lung-resident illnesses, since the exposure key disease-mediating cells to the drugs can be significantly increased when compared to oral drug administration, and systemic exposure (and therefore related side effects) can be reduced. It also means that lung clearance mechanisms other than systemic absorption must play a more significant role in removing the polymer from the lungs [12, 13]. To date however, there is limited knowledge about the contribution of each lung clearance pathway in the removal of inhaled polymers and nanoparticles from the lungs [14, 15], which is important for clinical translation and regulatory approval. Moreover, the majority of studies investigating the fate of inhaled nanomaterials have been based on examining the lung clearance kinetics of the drug, rather than the polymer or polymer-drug conjugate. The critical issue here is that the safety of inhaled polymer-drug conjugates has also been called into question, based on widespread literature suggesting that nanomaterials, albeit non-biodegradable/non-biocompatible nanoparticles, are 'toxic' in the lungs [16-20]. It is therefore important to understand the rate and mechanisms of polymer clearance from the lungs in order to design optimal dosing schedules that limit the long term retention of the polymer in the lungs and the potential for local adverse effects.

In the present review, we therefore provide a comprehensive overview of the state-of-the-art for inhalable polymer-drug conjugates (**Fig. 2.**). An additional focus of this review is to detail our current understanding about the *in vivo* behaviour and safety of inhaled polymer-drug conjugates. Finally, the need for further research and development of polymer-drug conjugates is also discussed with an emphasis on current knowledge gaps and future perspectives.

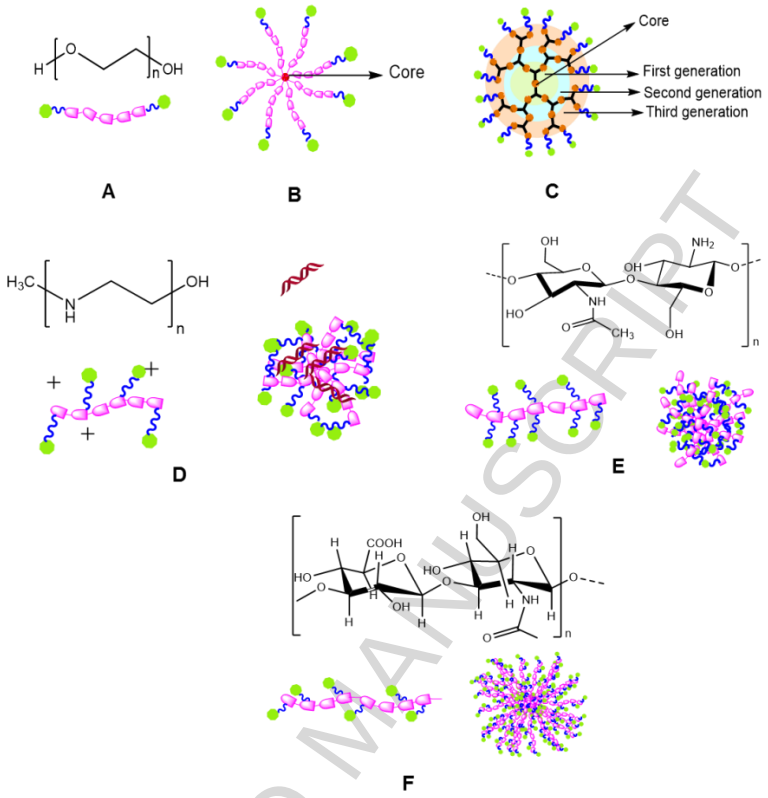


Fig 2. Chemical and schematic representations of polymers-drug conjugates that have been examined as inhalable drug delivery systems. **A**) Linear PEG-drug conjugates, **B**) PolyPEG star polymer-drug conjugates **C**) Dendrimer-drug conjugates (example of a generation 3 system is given), **D**) PEI-drug/DNA conjugates, **E**) chitosan-drug conjugates, and **F**) Hyaluronic acid-drug conjugates.

2. Polyethylene glycol (PEG)-drug conjugates

2.1 Linear PEG-drug conjugates

PEG is a polyether containing repeating units of ethylene glycol (**Fig. 2A.**). It is highly biocompatible, non-immunogenic, highly water soluble and FDA approved for use in medicine and other biomedical applications [21]. PEG exists as either linear or branched chains. Linear PEG is most commonly used as a drug carrier or surface coating on nanoparticles to improve biocompatibility and/or solubility, and conjugation processes are generally very straight forward [22-24]. While PEG-drug conjugates have most commonly been explored as delivery systems after IV administration, a few studies have explored the potential of these systems as inhalable drug vectors. The utility of PEG-based drug conjugates is that the PEG moiety can help to enhance penetrate through the mucus layer to gain access to the underlying epithelia, where most cells involved in lung disease reside. Furthermore, PEG can help to avoid prolonged mucosal exposure to drugs with 'mucus damaging' properties [25]. For example, alendronate has a low oral bioavailability (approx.

1-2%) and displays 'mucosal damaging' properties due to its structural similarity with phosphatidylcholine. Specifically, alendronate competitively displaces mucosal phosphatidylcholine which triggers mucosal damage [26]. Conjugation of alendronate onto low molecular weight PEG (510 Da) however, suppressed lung mucosal toxicity after pulmonary delivery, whereas administration of the free drug induced significant toxicity [26]. In another study, PEG was employed to prolong the residence time of steroidal drugs (e.g. prednisolone) within isolated lung preparations and increase the aqueous solubility of the drug [27]. The rate of absorption of mono substituted mPEG2000 conjugated at one end to prednisolone, and disubstituted PEG2000 (where prednisolone was conjugated to both ends of the PEG) across the lung epithelium in an isolated rat lung decreased by approximately 4 and 8 fold respectively compared to the free drug solution. It is unclear why mono- and di-substitution of PEG had this effect, but may be due to hydrophilic vs hydrophobic interactions between the different substituted PEGs [28]. To this end, a previous study evaluated PEG molecular weights that promoted efficient systemic absorption vs lung retention following intratracheal administration in rats [29]. This study showed that <2kDa PEGs were cleared from the lungs within 2 days, while >5kDa PEGs were retained in the lungs for up to 7 days.

In a separate study, Luo and colleagues compared two different molecular weight PEG-paclitaxel conjugates for inhalation chemotherapy against lung cancers and showed that the molecular weight of the PEG played a crucial role in chemotherapeutic efficacy (**Fig. 3 A-C.)** [30]. They reported that both PEG (20 kDa and 6 kDa)-paclitaxel conjugates showed superior anti-tumour efficacy compared with the commercial formulation of the drug (Taxol®) after both pulmonary and IV administration. Interestingly, equivalent anti-tumour efficacy and lower toxicity was observed for the high molecular weight PEG (20 kDa) construct at a 2.5 fold lower dose than the low molecular weight PEG (6 kDa) construct. This may have been due to the fact that the 20 kDa PEG-paclitaxel conjugate resided in the lungs for a more prolonged period of time than the smaller PEG construct.

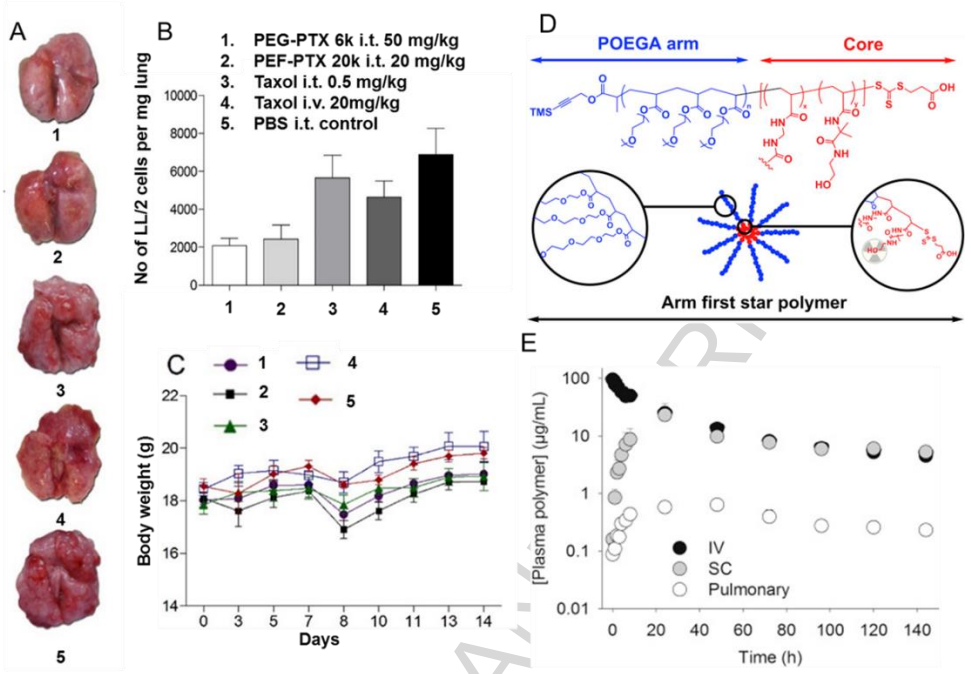


Fig 3. (Left images). Anticancer efficacy and tolerability of PEG-paclitaxel conjugates vs commercial Taxol® after intratracheal instillation or intravenous delivery in a murine model of Lewis lung carcinoma (Figures A-C). (A) Representative images of mouse lungs following each treatment. (B) Number of LL/2 cancer cells per mg of lung tissue. (C) Body weights of mice in each dose group over time (data represent mean ± SEM, n = 6–7; images adapted with permission from reference [30]). **(Right images).** Chemical structure of arm-first star polymer consisting of POEGA arm and core. The radiation symbol depicts the approximate location of the core confined ^{3}H radiolabel. (D) POEGA represents poly(oligoethylene glycol) acrylate. **(E)** Plasma concentration versus time profile of the star polymer after intravenous, subcutaneous, and pulmonary administration (5 mg/kg). (Data represent mean ± SD, n = 3-5; Reproduced with permission from reference [31, 32]).

2.2 PEG-based Star polymers

The general availability of only two reactive groups on a linear PEG chain limits the drug loading capacities of these materials [33]. This limitation can be overcome by using star-shaped, or 'hyper-branched polymers' with dendron-like structures that can contain a large number of reactive functional groups within a PEG-based scaffold [33-35].

Star-shaped PEG polymers can be easily synthesized by cross-linking linear polymer chains with two or more linear arms that radiate from a central core **(Fig. 2B and 3D.)** [34]. The pulmonary pharmacokinetics of a 64 kDa PolyPEG star polymer was recently evaluated in rats after intratracheal administration, and the pharmacokinetics compared to the polymer

after IV and subcutaneous delivery (**Fig. 3E.)** [31]. By virtue of its large size, the star polymer showed limited systemic bioavailability (approx. 3%) after intratracheal instillation, but high bioavailability after subcutaneous administration (approx. 80%). After 6 days, a significant proportion of the polymer dose was recovered in the lungs, faces, and urine, as a result of the polymers extensive retention within the lungs (~25%), clearance via the mucociliary escalator ~20%) and biodegradation in the lungs to low molecular weight products that were absorbed and excreted via the urine (~12%). In general, the pulmonary pharmacokinetics of the PEG-star polymer was reported to be similar to that of a similar sized polylysine dendrimer containing a fully PEGylated surface.

3. Dendrimer-drug conjugates

Dendrimers are fundamentally hyperbranched polymers, but contain a more well-defined scaffold structure. Specifically, dendrimers are constructed in a radial manner from monomers that contain at least 3 functional groups (with 2 functional groups being identical to build the branched structure). They contain three major domains 1) a central core on which the polymer scaffold is built, 2) the scaffold which is formed through a series of additions of layers or 'generations' of monomers, and 3) the outermost surface which contains multiple functional groups that can be used to allow the attachment of drugs, targeting ligands, imaging agents etc. (**Fig. 3C.**) [36]. In this way, they typically display much lower polydispersity than hyperbranched or star polymers, but are also more expensive and timely to manufacture due to their step-wise synthesis [37].

Drugs may be associated with dendrimers via either non-covalent entrapment in the hydrophobic scaffold, or via covalent conjugation to the surface. For a review on the advantages and disadvantages of covalent vs non-covalent association of drugs with dendrimers, the reader is directed to the following review [38]. In this review however, we focus specifically on covalent drug-polymer conjugates.

Dendrimers have been examined as potential drug delivery systems following intravenous administration for several decades now, and at present one dendrimer based drug conjugate is in Phase I clinical trials in Australia for the treatment of advanced breast cancer (DEP-docetaxel; Starpharma Pty Ltd). More recently, they have also begun to be explored as

inhalable drug delivery systems, most notably for the improved treatment of lung-resident cancers.

The inhaled delivery of dendrimers can improve metabolic stability when compared to oral administration, promote systemic access via a non-invasive route of delivery (note, dendrimers are not, or poorly orally bioavailable) or provide a long term depot for drug release in the lungs [39, 40]. Poly amino acids-based dendrimers such as polylysine also have the advantage of allowing biodegradation of the dendrimer scaffold, which provides a novel route of lung clearance [39, 41]. The potential biomedical application of inhaled dendrimers was first realised almost 2 decades ago by Baker and colleagues who examine the ability to delivery DNA to the lungs using a PAMAM dendrimer carrier and to use sialic acid conjugated dendrimers to treat influenza pneumonitis [42, 43]. Following on from these earlier studies, subsequent studies aimed to use PAMAM dendrimers as carriers for methylprednisolone (to treat lung inflammation) [44], peptide phage clones to enhance dendrimer absorption from the lungs [45] and to improve the systemic absorption of peptides and proteins, such as heparin [46, 47], calcitonin and insulin [48]. To this point however, only PAMAM dendrimers had been used, and none of the studies had systematically characterised the effect of dendrimer size or charge on pulmonary pharmacokinetics

Since then, several studies have examined the pulmonary pharmacokinetics of PEGylated polylysine dendrimers after aerosol and intratracheal administration to the lungs of rats and sheep. An initial study showed that after intratracheal instillation to the lungs of rats, the extent of systemic absorption for a series of PEGylated G4 polylysine dendrimers decreased with increasing construct molecular weight. In this case, construct molecular weight was controlled by changing the molecular weight of surface conjugated PEG, from 200 Da to 2300 Da (to give constructs with a molecular weight of 11 to 78 kDa) [41]. In general, the smallest 11 kDa dendrimer was rapidly absorbed from the lungs, but was also rapidly degraded to low molecular weight PEGylated fragments that were systemically absorbed and excreted via the urine. The 22 kDa dendrimer however, showed slower biodegradation *in vivo*, and approximately 30% bioavailability, while the 78 kDa dendrimer was largely retained in the lungs and was cleared mainly via the mucociliary escalator (**Fig. 4[I] A-D.**) [41]. These results suggested that intermediate sized dendrimers (around 22 kDa) may provide an ideal balance between lung retention and systemic access, while larger molecular

weight dendrimers may provide ideal lung deports for the sustained release of drugs in the lungs.

A subsequent study, however, compared the pulmonary pharmacokinetics of the 22 kDa dendrimer after aerosol administration to the lungs of rats and sheep, which provide a good model of human respiratory dimensions and physiology. In general, systemic bioavailability was lower after aerosol administration when compared to intratracheal administration in rats by approximately 50%. After aerosol administration to the lungs of rats and sheep, the dendrimer showed similar bioavailability in both species, albeit slightly, but not significantly, lower systemic bioavailability in sheep [40]. In sheep however, the dendrimer appeared to be cleared more rapidly from the lungs compared to in rats, suggesting that nanoparticle clearance is likely to be more efficient in larger species such as humans than in rodents. While pulmonary lymphatic exposure was also examined in sheep, the study showed that less than 0.5% of the dose appeared in lung lymph, despite showing extensive lymphatic exposure in rats after subcutaneous administration. Subsequent studies have also examined the pulmonary pharmacokinetics of PAMAM dendrimers in rodents [49-51]. For instance, Zhong *et al.* compared the pulmonary and intravenous pharmacokinetics of G3 amine-terminated and PEGylated PAMAM dendrimers in mice [51]. When compared to intravenous administration, both dendrimers showed higher and more prolonged lung exposure after pulmonary administration, as expected. Interestingly however, PEGylation appeared to enhanced systemic exposure to the dendrimer after pulmonary administration, but it is not known whether this was due to an increase in bioavailability (not reported) or reduced plasma clearance. Differential internalisation of both dendrimers with endothelial and epithelial cells in the lungs was also shown.

Following on from initial pulmonary pharmacokinetic evaluations in rats, a 56 kDa PEGylated polylysine dendrimer conjugated with doxorubicin via an acid labile chemical linker was examined as an inhalable chemotherapeutic nanomedicine in rats [39]. Upon intratracheal instillation, the conjugate was initially rapidly cleared within 24 h and only 15% of the administered dose was retained in the lungs after 7 days. Up to 13% of the pulmonary dose was systemically absorbed. However, twice weekly pulmonary administration of the conjugate was sufficient to reduce lung tumour burden by almost 95%, while intravenous administration of the doxorubicin solution formulation reduced lung tumour burden by only 30-50% (**Fig. 4[II] A-I.)**. Interestingly, intravenous administration of the dendrimer did not

have an impact on tumour growth. In addition, the doxorubicin solution formulation induced significant pulmonary toxicity after a single dose, whereas the doxorubicin conjugate was well tolerated after twice weekly dosing over 2 weeks.

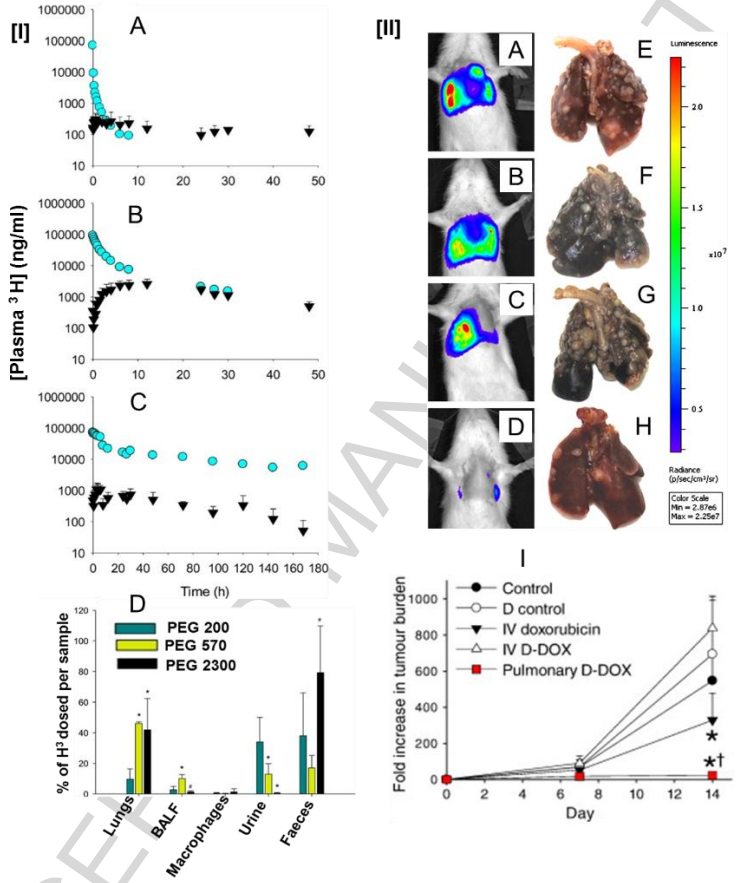


Fig 4. (Left images) [I]. Plasma concentration–time profiles of a ^{3}H-scaffold labelled 11 kDa PEGylated (PEG200) polylysine dendrimer (Panel A), 22 kDa PEGylated (PEG570) polylysine dendrimer (Panel B), and 78 kDa PEGylated (PEG2300) polylysine dendrimer (Panel C) after intravenous (cyan circles) and pulmonary (black triangles) administration at 5 mg/kg. Values represent mean ± SD (n = 3–6 rats). Distribution of intratracheally administered ^{3}H-dendrimer in lungs after sacrifice and in total pooled excreta (Panel D). Data are represented as mean ± SD (n = 4–6 rats). (Reproduced and modified from references [41, 52]). **(Right images) [II] (A-H)** Representative images showing changes in lung tumour burden following various treatments in syngeneic F344 rats bearing lung metastases of firefly luciferase-expressing MAT 13762 IIIB carcinoma. Panels A to D depict bioluminescent images of the lungs immediately before termination (18 to 21 days after injection of cells) and Panels E to H depict images of excised lung tissue showing the extent and size of

metastatic foci. Rats were treated with intratracheal saline alone (Panels A and E), intravenous doxorubicin (Panels B and F), intravenous doxorubicin-conjugated dendrimer (Panels C and G) and intratracheal doxorubicin-conjugated dendrimer (Panels D and H). The scale for bioluminescent images is depicted on the right. (Panel I) Fold increase in lung tumour burden determined by measuring total flux emitted from the lungs of rats 7 and 11–14 days after the first dose. Data is represented as mean ± s.e.m (n = 6–9). *Indicates $p < 0.05$ *cf.* IV D-DOX. † Indicates $p < 0.05$ cf. D control. (Reproduced with permission from reference [39])

Similarly, PAMAM dendrimers have also been examined for their ability to improve the treatment of lung-resident cancers after pulmonary administration of a doxorubicin-conjugated G4 PAMAM dendrimer [50]. This complex showed pH-dependent drug release with maximal drug liberation (80%) at an acidic pH (pH 4.5). Pulmonary administration of the PAMAM-doxorubicin conjugate showed more prolonged drug retention in lungs, lower systemic toxicity and improved cancer survival rates in mice when compared to pulmonary administration of the solution formulation of the drug.

4. Polyethyleneimine (PEI)-drug conjugates

PEI has been successfully used as an inhalable non-viral gene and drug delivery vector [53-55]. Linear PEI contains repeating units of secondary amine-(NH) and aliphatic groups (-CH2-CH2-) (**Fig. 3D.**), while branch PEI contains primary, secondary and tertiary amines [56]. PEI has high transfection efficiency due to its high density of cationic amine functional groups that condense negatively charged DNA and RNA via electrostatic interactions [57, 58]. PEI can also interact with negatively charged cell membrane (glycoproteins and phospholipids) to promote the cellular uptake of conjugated drugs. Additionally, at the low pH encountered in lysosomes and late endosomes, the amine groups of PEI become protonated and act as proton sponge to rupture endosomes and promote the exposure of endosomal contents into the cytosol [59]. This unique and efficient endosomal escaping feature also allows for the selective delivery of cytotoxic drugs into the cytosol of tumour cells, thereby minimizing adverse effects on healthy tissues.

Pulmonary administration of PEI-RNA and WT1 (Wilms' tumor gene) complex was successfully delivered as an aerosol to the lungs of mice bearing B16-F10 melanoma metastases [60]. The PEI-RNA-WT1 complex promoted the apoptosis of B16-F10 cancer

cells in the lungs, which led to reductions in tumour size and angiogenesis. In addition, mean survival time in mice was significantly increased compared to untreated or WT1-2 RNA alone treated control groups [60]. However, the nucleotide/polymer complexation process may be limited by the uncontrolled formation of aggregates that lack transfection capability [61]. Formation of these aggregates during complexation can usually be overcome by correctly identifying nitrogen-to-phosphate ratios (the ratio of PEI amine groups per RNA phosphate group) and via the covalent conjugation of PEI with PEG linkers that can mask high density cationic charges and improve physiological stability [62]. Additionally, PEGylated PEI can also be coupled with cell penetrating peptides to enhance the intracellular delivery of nucleotides compared to PEGylated PEI alone. For instance, PEG-PEI conjugates coupled with TAT-peptide derived from human immune deficiency virus (HIV) enhanced cell penetration and transfection efficiency in human lung A549 and Calu-3 cells, and in mouse lungs when compared to PEI alone [63]. Kleemann et al. compared the transfection efficacy of PEI/DNA and TAT-PEG-PEI/DNA polyplexes after intratracheal instillation in rats [55]. TAT-PEG-PEI/DNA polyplexes targeted both bronchial and alveolar cells with 600% higher transfection efficiencies than the PEI/DNA polyplexes alone. Further, PEI/DNA polyplexes were only capable of partial targeting to bronchial epithelial cells. The low transfection efficacy of the PEI/DNA polyplex is due to their excessive cationic surface charge which promotes their binding and retention in upper respiratory tract. In contrast, PEGylation can shield the positive charge and prevent the formation of large aggregates of TAT-PEG-PEI/DNA complex so that they can more readily access alveolar regions of the lungs, where they may be more available for systemic absorption and transfection.

To this point however, PEI-based polymers have mostly been used to exclusively deliver genetic material and only a few studies have analysed the potential of PEI-drug conjugates and PEI-gene complexes for delivery via inhalation. Combination therapy (by combining the inhaled delivery of drugs and genetic material) maximizes the potential for anticancer activity in the lungs due to additive or synergistic effects [64, 65]. For instance, PEI-doxorubicin and Bcl2 siRNA complexes were investigated as potential treatments for metastatic lung cancer in mice [53]. The PEI-doxorubicin/SiRNA complex showed accelerated drug release profiles in an acidic pH, and more efficient cell killing via apoptosis in isolated B16F10 cells when compared to naked siRNA and doxorubicin alone. Furthermore, pulmonary administration of the PEI-doxorubicin/SiRNA complex enhanced antitumor efficacy and prolonged drug

retention in the lung tumour tissue of mice beyond 7 days. In general though, PEI has not been examined in detail as an inhalable carrier material.

5. Chitosan-drug conjugates

Chitosan is a popular inhalable delivery system and nanoparticle coating as a result of its capability to deliver drugs locally and systemically *via* mucosal routes [66, 67]. It is both biocompatible and biodegradable and is an FDA approved polymer for wound dressings [68]. Recently, chitosan was used to physically encapsulate or covalently conjugate drugs into its polymeric backbone to improve the efficiency of drug delivery to lungs [69-72]. Chitosan contains linear polysaccharides that are derived from chitin which is composed of D-glucosamine and N-acetyl-d-glucosamine (**Fig. 3E.**) [67].Chitosan is cationic in nature due to the presence of repeated glucosamine units with primary amino functionality. The amino groups in chitosan can be conjugated with various functional groups to enabling the further conjugation of drugs and other ligands. However, the major limitation of chitosan is its pH dependent solubility. Since the pKa of amino groups in chitosan lie between 5.5 and 6.5, it losses its surface charge and aggregates at physiological pH, whereas at mildly acidic environments it is partially protonated and displays higher aqueous solubility. While this can represent a solubility limit *in vivo*, the selective ionization/deionization events that occur at physiological pH and in the more acidic microenvironment of tumours can be used to facilitate efficient drug release from chitosan conjugates [73, 74]. As an example, a thermoresponsive hydrogel was prepared from chitosan-doxorubicin conjugates to achieve sustained release of doxorubicin and superior antitumor effects after intratumoural injection in nude mice bearing solid human lung adenocarcinomas [75]. The hydrogel containing doxorubicin-chitosan conjugates displayed sustained release profiles of drug compared to the hydrogel without chitosan-drug conjugation**)**. Furthermore, the hydrogel composed of chitosan-doxorubicin conjugates showed superior *in vivo* antitumor effect as compared to free drug or hydrogel containing drug solution.

Chitosan solubility, degradation and toxicity can also be tailored via chemical modification with other hydrophilic moieties [76]. In one example, chitosan was conjugated to amino acids such as L-leucine to provide a potentially improved pulmonary delivery system for the model drug diltiazem [77]. The diltiazem-chitosan-leucine conjugate showed an initial rapid burst release of drug, which was followed by slower release with approximately 50% of the drug released over 16 days [69]. Additionally, amphiphilic L-leucine has surfactant-like properties

which can improve the aerosol performance of conjugated particles which are required for pressurized metered dose inhaler formulations. Very recently, water soluble conjugates of chitosan and the anti-tuberculosis drug isoniazid were therefore prepared and examined for their capacity to provide an inhalable platform for the lung delivery of the drug, which is currently administered as an oral tablet formulation [78]. Isoniazid was either conjugated to *N*-(2-carboxyethyl) chitosan (CEC) or to *N*-(3-chloro-2-hydroxypropyl) chitosan (CHPC). The antitubercular activity of isoniazid-CEC conjugates against *M. tuberculosis* H37Rv was similar to the parent drug, whereas the antitubercular activity of isoniazid-CHPC was poor. Additionally, both chitosan-based isoniazid complexes showed biodegradability *in vitro* and limited *in vivo* toxicity. It was suggested that the limited antitubercular activity of isoniazid-CHPC could be due to the lower surface presentation of isoniazid required to interact with the target bacteria, or due to limited breakdown of C-N bonds between isoniazid and the polymer.

Another interesting property of chitosan as an inhalable polymeric drug carrier is that it can transiently open transepithelial junctions between cells by dysregulating claudin-4 (Cldn4), thus allowing for enhanced systemic absorption compared to similar sized polymers [79]. Using this property, chitosan was modified with ethylene glycol to prepare a water soluble glycol chitosan conjugate (GC) [80]. The GC complex was then formulated with lipoid 100 to allow encapsulation of low molecular weight heparin (LMWH, approx. 18 kDa) into chitosan nanoparticles to facilitate the systemic delivery of LMWH following inhaled delivery. GC-based LMWH nanoparticles appeared to be safe in the lungs and did not display any significant signs of lung damage or inflammation. Both GC bearing nanoparticles and free heparin showed comparable blood coagulation times after 4h of administration. However, the pharmacokinetic behaviour of the nanoparticle was not examined and a conclusion as to whether heparin was liberated in the lungs, followed by being absorbed from the lungs, or whether the construct was absorbed intact cannot be made. Since the authors only evaluated the *in vivo* fate of the nanoparticle over a short period of time, it is also uncertain whether GC may have shown more prolonged systemic effects over heparin alone.

In another study, hydrophilic GC was modified with hydrophobic 5β-cholanic acid and used to form a nanoparticle that was examined for its potential as a pulmonary delivery vehicle [81]. Following intratracheal instillation of the nanoparticles, a large proportion of the dose was retained in the lungs of mice after 14 days. A mild inflammatory effect was also

observed in the lungs from 6 h to 3 days after dosing [81], but in general, this is consistent with the inhaled delivery of all nanosized material including endogenous proteins [82]. However, the pathways by which chitosan are cleared from the lungs and the time frame over which it is cleared is still unknown.

Finally, chitosan displays mucoadhesive properties that can be used to retain conjugated drugs in the respiratory mucosa for prolonged periods of time [83]. Thiolation however, can be used to increase the mucoadhesive effect by promoting the formation of disulphide bridges with thiol groups of cysteine rich proteins. This approach was therefore tested as a mean to enhance the mucus retention of calcitonin after pulmonary administration and prolong therapeutic effects in blood [84]. The thiolated GC nanoparticles were retained in the lungs two fold longer than the non-thiolated GC nanoparticles. The pulmonary 'bioavailability' of calcitonin relative to a subcutaneous dose (rather than to a conventional intravenous dose) was also reported to be moderately higher for the thiolated material (40%) than the non-thiolated material (27%), despite the more prolonged lung retention of the thiolated material. In both cases, the systemic access of calcitonin when formulated into a chitosan nanoparticle was higher than after pulmonary administration of calcitonin alone (10%). Importantly, the thiolated GC had a significantly more prolonged impact on reducing blood calcium levels in rats after pulmonary administration when compared to the non-thiolated nanoparticles and calcitonin alone.

6. Hyaluronic acid-drug conjugates

Hyaluronic acid (HA) - also known as hyaluronan - is a naturally occurring biopolymer composed of repeating disaccharides units of N-acetylglucosamine and D-glucuronic acid (**Fig. 3F.**) [85]. The pKa of the carboxylic group of HA is between 3 and 4 and thus at physiological pH, HA exists as a polyanion. HA can absorb significant amounts of water and expand up to 1,000-times compared to its original solid volume, forming hydrogels [86]. They are predominantly found in the extracellular matrix of connective tissues and in a small quantities within lungs [87]. Under haemostatic condition, HA usually does not activate immune cell infiltration, with the except of transient increases in alveolar macrophages [88]. Alveolar macrophages specifically bind to pneumocytes to incorporate themselves within the alveolar space or internalize HA to the lymphatic system [88]. Thus, HA is extensively explored as a drug delivery vector following various routes of administration, including

intravenous, intraperitoneal, oral and subcutaneous. For a review of these delivery routes, the reader is referred to the following reference [89].

Recently, a number of researchers have used HA as a polymer base for inhalable dry powder formulations for a range of lung-active therapeutics [90-92]. The lung clearance and pulmonary pharmacokinetics of HA was recently reported to be depended on its molecular weight. Upon pulmonary administration, low molecular weight HA (7 and 30 kDa) was rapidly absorbed into the blood, while higher molecular weight HA (67 and 215 kDa) showed prolonged lung retention and slow absorption into the systemic circulation (**Fig. 5[I].)** [93]. In comparison, a very high molecular weight HA construct (741 kDa) was rapidly cleared from the lungs via mucociliary clearance (**Fig. 5[II].)**.

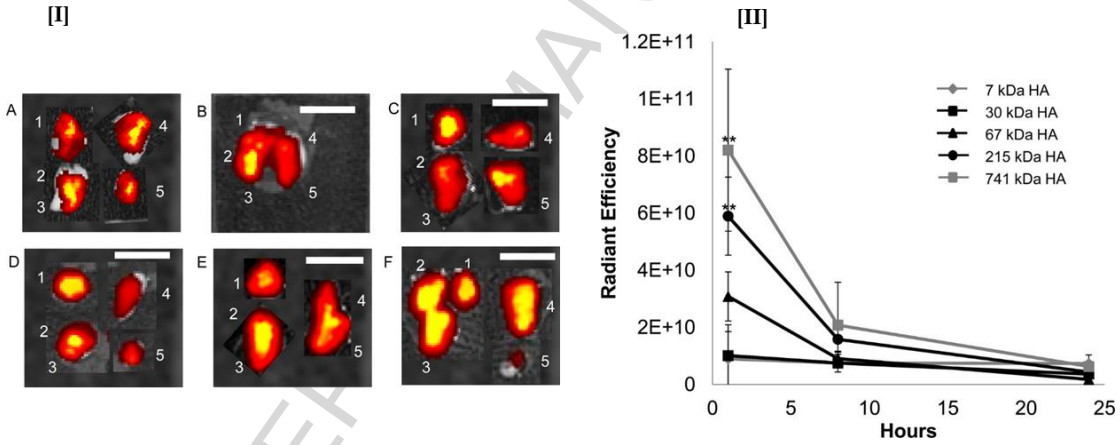


Fig 5. [I] Fluorescent images of excised lungs after pulmonary administration of IR-labelled HA of molecular weight 7 kDa (immediately after lung administration) (A), 7 kDa (8h after lung administration) (B), 30 kDa (after 8 h) (C), 67 kDa (after 8h) (D), 215 kDa (after 8h) (E), and 741 kDa (after 8h) (F). The white numbers represent the different lung lobes, with 1, 2, and 3 representing the 3 anatomical lung lobes on the right, and 4 and 5 representing anatomical lung lobes on the left. **[II]** Time course of lung clearance of IR-labelled HA after pulmonary administration. Each point is the average of at least three animals (Reproduced with permission from reference [93]).

HA provides multiple sites for the covalent conjugation of drugs and ligands into its backbone. HA can therefore be used either as a surface coating on nanoparticles, or as a direct drug carrier via conjugation. HA can also be used as a tumour-targeting moiety since it binds to CD44, a transmembrane glycoprotein which is commonly overexpressed on the surface of cancer cells [94, 95]. However, in healthy cells CD44 is highly glycosylated which limits its capacity to bind to HA [96]. Thus, conjugation of anti-cancer drugs onto HA carriers can facilitate the improved retention of the drug in tumours and cancer cell

internalisation and avoid targeting and uptake by normal healthy cells [97]. Using this principle, Ishiguro et al. developed HA-cisplatin conjugates and freeze-dried the formulation with 2.5 % trehalose as cryprotectant for pulmonary administration of a dry powder [98]. The conjugate efficiently bound to CD44 expressing cancer cell lines (H1299 and H358) and a single intratracheal aerosolized bolus dose was effective in treating lung tumours in mice [98]. In another study, a HA (35 kDa)-cisplatin conjugate displayed prolonged *in vitro* release of cisplatin over time [99]. Pulmonary delivery of this HA-cisplatin conjugate when compared to intravenous administration, resulted in higher lung exposure in rats in the order of 5.7 and 1.2 fold after 24 h and 96 h respectively [99]. As a result, the pulmonary administration of HA-conjugated anticancer drugs can enhance the exposure of lung resident cancers to chemotherapeutic drugs when compared to after intravenous administration, provide efficient lung cancer killing when compared to intravenous administration of the drug solution formulation, reduce systemic and lung toxicity and enhance drug uptake by cancer cells.

Another prominent feature of HA is its ability to passively target the lymphatic system. Recent evidence showed that pulmonary delivery of HA-conjugated anticancer drugs also provides control over lymphatic cancer metastases [93, 98, 100]. Nanoparticles prepared using HA with a molecular weight of approx. 75 kDa and a particle size below 50 nm may access the lymphatic system [93]. High molecular weight HA also breaks into small molecular weight components by hyaluronidases that can also facilitate the entry of HA-conjugated drugs into the lymphatic circulation [101]. The potential lymphatic targeting features of HA following inhalation may also allow then to act as efficient polymeric nanocarriers for the vaccines delivered via mucosal routes, such as following inhalation.

7. Other Polymers

With the exception of the above mentioned polymers that are more commonly evaluated as inhalable drug carrier systems, several other polymers have also been evaluated as inhalable drug vectors. Carbopol® for instance, is a high molecular weight polyacrylic acid-based crosslinked mucoadhesive polymer that typically contains repeating carboxyvinyl units. Carbopol® is commonly used in oral tablets and mucosal formulations. In a recent study though, Carbopol was conjugated to wheat germ agglutinin (WGA) derived from *Triticum vulgare* to enhance binding of the polymer to N-acetyl-D-glucosamine residues located on surface of epithelial cells in alveoli [102]. Pulmonary administration of WGA-carbopol

modified liposomes containing calcitonin showed a prolonged therapeutic effect in rats and a high uptake by alveolar A549 cells when compared to unmodified liposomes containing calcitonin. Additionally, the WGA-carbopol modified liposomes did not increase the total protein and LDH levels in the BALF of rats, confirming the absence of any significant pulmonary toxicity.

Poly(lactic-co-glycolic acid) PLGA is also a safe and biodegradable polymer that is rapidly gaining attention for its potential as an inhalable drug carrier [103]. However, the majority of studies on PLGA as pulmonary carrier have focused on encapsulation/adsorption of drugs, proteins, and peptides into PLGA nanoparticle structures, rather than by direct conjugation of drugs onto PLGA [104]. PLGA can be modified to contain cell targeting moieties, but it has a limited availability of favourable functional groups for chemical conjugation [105]. Uncapped PLGA contains both hydroxyl and carboxyl groups that can be utilized to conjugate drugs or targeting agent [106]. However, the cleavage of the amide bond formed from the conjugation of carboxyl groups of PLGA with amine-containing drugs is not easy under physiological conditions, thus requiring drug 'liberation' *via* degradation of the PLGA backbone. In comparison, hydroxyl groups in PLGA can be functionalized with carboxyl group in drugs or targeting agents to yield an ester bond that is more readily cleavable under physiological conditions [107]. Mo et al. for instance, conjugated WGA lectin to PLGA to enhance the *in vitro* therapeutic efficacy of paclitaxel [108]. PLGA-WGA nanoparticles actively transported paclitaxel into the cancer cells *via* lectin-receptor-mediated endocytosis and showed an improved cytotoxicity activity against both A549 and H1299 cells.

Other polymer such as HPMA [N-(2-hydroxypropyl)methacrylamide] conjugated to pirarubicin *via* hydrazone bonds showed high therapeutic potential against lung cancers [109]. This conjugate was found to improve the treatment of metastases in patient suffering from stage IV lung cancer. Although the conjugate was administered via the intravenous route, it has the significant potentially to also be delivered via pulmonary route to enhance the local therapy of lung cancers. In a similar way, conjugates of several other polymers (such as dextrans, poly glutamic and poly aspartic acids) are yet to be assessed for their suitability as inhalable drug carriers [3, 5].

8. Current opinion on the future of inhalable polymer-drug conjugates

Polymer-drug conjugates have enormous potential as an inhalable drug delivery platforms for the treatment of lung diseases. They also have some potential as systemic delivery systems

using the inhaled route as an alternative to more invasive injectable routes of delivery They have several chemical and structural attributes which can be tailored to modulate drug release kinetics, cellular and subcellular interactions within the lungs and lung clearance pathways. Although a large number of polymer-drug conjugates have been reported so far, but they have almost exclusively been administered via the intravenous route in preclinical studies (including a few undergoing clinical trials) [3]. This is important because the physiochemical properties of polymer-drug conjugates need to be modulated according to the intended route of administration to define their *in vivo* pharmacokinetic and pharmacodynamic behaviour [3, 110]. Further, despite a number of preclinical studies having been undertaken, the clinical translation of polymer–drug conjugates has been slow (irrespective of the route of administration) due to limitations such as poor drug loading, low bioavailability and circulation times, inadequate information about polymer-related toxicity and inappropriate design (i.e. lack of sustained or controllable drug release, unsuitable choice of linkers to conjugate drugs or unsuitable methods of drug conjugation) [1, 3, 111].

The success of polymer drug conjugates as a 'inhalable drug delivery platforms' will therefore be depend on defined advancements in their preclinical development, and refinements in their design as per the requirements for an ideal pulmonary drug delivery system. For instance, Shamay et al. recently developed a VEGFR-1 targeted N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer–doxorubicin conjugate to inhibit primary tumour growth and slow the development of cancer metastases in mice by actively targeting the tumour vasculature [112]. This approach is likely to emerge as an attractive technology for improving the therapeutic efficacy of cytotoxic drugs. Similarly, Lomkova et al. reported the synthesis of a smart micellar polymer-betulinic acid conjugates based on HPMA copolymers for passive tumour targeting. These conjugates enabled pH-dependent controlled release of drug within tumour cells followed by disassembly of the micellar structure to facilitate elimination of the water-soluble HPMA copolymer by renal filtration [113]. In another recent study, Camacho et al. demonstrated the high therapeutic potential of low molecular weight polymer–drug conjugates composed of 10 kDa HA or poly(vinyl alcohol) for the delivery of chemotherapeutics to the lungs and provided further insight into the development of polymer drug therapeutics based on low molecular weight polymers [114]. Apart from these polymers, dendrimers (lysine based) and star polymers have very narrow size ranges (≤20 nm), low polydispersity (< 0.1), display ease of surface functionalization and good biocompatibility for pulmonary drug delivery applications [31, 39,

41]. The clinical translation of these polymer-drug conjugates as inhalable therapeutics however, will depend upon better understanding their deposition, dissolution, absorption, lung clearance, interactions with different biological barriers of the lungs and safety/toxicity within the lung environment, particularly in large species with similar respiratory dimensions to that of humans [14].

Regulatory approval for the clinical use of polymer drug conjugates will also be dependent on in-depth assessments of their systemic and lung disposition, as well as the pathways and kinetics of lung clearance [14]. Furthermore, the lung clearance kinetics of polymer drug conjugates needs to be evaluated by tracking the polymers, and not just the conjugated drugs. This is because the kinetics of the drug is based in large part on its rate of cleavage from the conjugated polymers and it does not necessarily correlate well with the rate of polymer clearance [14]. This is important, since the long term retention and build-up of polymers or nanoparticles in the lungs may stimulate long term inflammatory and structural changes in the lungs, and may also exacerbate underlying respiratory diseases for which these systems are intended to treat. Although a considerable amount of literature is available on the fate of inhaled PEGylated polylysine dendrimers, at this stage it is difficult to predict the extent to which this information relates to the pulmonary clearance of other polymer-drug conjugates [31, 39, 41].

An important requirement of polymer-drug conjugates is also to define their overall safety in the lung microenvironment, in particular the impact of repeated inhaled exposure on lung retention and the and safety of the individual excipients. It has been widely demonstrated that environmental pollutants including particulate material, inorganic/metal nanoparticles and non-biodegradable polymeric nanoparticles are capable of inducing inflammation, oxidative stress, cytotoxic and genotoxic effects in the lungs after inhalation [16-20]. In contrast, however, very few studies have been carried out to determine the immunological, inflammatory and toxicological potential of inhalable polymer drug conjugates or biodegradable polymers which have potential for pulmonary application. In a recent study, the safety of an inhaled G5 fully PEGylated polylysine dendrimer and an alpha-carboxyl OtButylated methotrexate conjugated PEGylated dendrimer (substitution of 50% surface PEG groups with alpha-carboxyl OtButylated methotrexate) was evaluated in rats. The results showed that the dendrimers did not induce a significant local lung inflammatory response over 2 weeks after a single 5 mg/kg dose, but safety studies following repeated dosing were

not performed (unpublished data). Further, it has been observed that the cumulative lung administration of up to 80 mg of partly PEGylated dendrimers to rats had little impact on the lung tissue, with mild increases in alveolar macrophages consistent with the presence of a mild and reversible adaptive physiological response in the lungs, which normally occurs after the inhaled exposure of any nanomaterial, including proteins [39, 115, 116]. In contrast, the intratracheal instillation of a cationic PAMAM dendrimer led to pulmonary inflammation in mice due to accumulation of autophagosomes in the lung tissues and inhibition of Akt-mTOR signaling pathways [117].

Among the biodegradable polymers, a few studies have examined the pulmonary toxicity of chitosan, PEI, PLGA, albumin and hyaluronic acid. For instance, Grenha et al. showed that chitosan based formulations do not induce overt toxicity against Calu-3 or A549 cells (determined via MTT assay) and are compatible with bronchial and respiratory epithelial cells [118]. Muhsin et al. examined the safety of chitosan l-leucine conjugates *in vitro* and reported the conjugate to be relatively more toxic and pro-inflammatory than chitosan alone. The authors suggested that the level of toxicity and inflammatory effects can still enable its utilization for pulmonary drug delivery unless intra-lung concentrations are increased beyond that which they reported [77]. However, this was an entirely *in vitro* study, and one can only assume that the pro-inflammatory effects of the polymer would be increased *in vivo* this highlights the need for stringent *in vivo* testing of pharmacokinetics and safety prior to considering inhalation studies in humans, particularly those with already compromised respiratory disease.

PEI based polymers used for gene therapy or RNA interference have been found to cause well-known adverse effects especially high cytotoxicity in both *in vitro* and *in vivo* studies [119]. The safety study conducted on inhaled PLGA based systems have shown different results in bronchial (Calu3) and alveolar (A549) cell lines. PLGA based nanoformulations showed insignificant cytotoxicity and inflammation towards bronchial epithelial cells irrespective of their surface chemistry, charge (depending on their stabiliser) and dose [120]. However, a significantly higher inflammatory response was observed following exposure of A549 alveolar epithelial lung cells to a family of PLGA based systems covered with different polymeric stabilizers [121]. The safety study conducted on HA has confirmed so far that it plays an important role in inflammation due to its affinity for the CD44 receptor that is expressed on a variety of cell types [122]. However, the inflammatory potential of hyaluronic

acid has been found to depend upon its molecular weight. Relatively low molecular weight fragments (<250 kDa) have been found to stimulate the production of a variety of inflammatory cytokines [122, 123]. Conversely, high molecular weight hyaluronic acid structures (>250 kDa) have been found to possess anti-inflammatory effects [122, 124]. While biodegradation is generally considered to be an ideal mechanism by which inhalable polymers can be cleared from the lungs, the rate of biodegradation of different types of polymers is therefore likely to play a major role in defining their safety in the lungs. For instance, the slow degradation rate of polymers such as PLGA may lead to enhanced accumulation and toxicity in the lungs when compared to, for instance, proteins due to long term accumulation upon repeated administration [125]. In addition, polymer accumulation in the respiratory region of lungs for prolonged periods of time has been found to cause depletion of lung surfactants [14]. Besides this, there are quite a few excipients that have been approved by different regulatory agencies for potential use in pulmonary drug delivery applications. Only a few amino acids, sugars and some polyethylene glycols have been approved for use in inhalable products. This limited number of approved excipients can slow the development of inhalable polymer-drug conjugates as well as limit their clinical translation, since the use of new polymers and excipients will require extensive *in vivo* safety studies [15, 126].

To conclude, polymer drug conjugates have potential as an 'inhalable drug delivery platforms' for the treatment of respiratory diseases and for the non-invasive systemic delivery of therapeutics. However, a general lack of knowledge about ideal physiochemical properties to optimally enhance pulmonary pharmacokinetics, safety, drug release rates and efficacy, together with a general lack of understanding about their pharmacokinetic behaviour and clearance kinetics have thwarted the significant preclinical development and clinical translation of these systems. Focussed efforts are needed to identify the most ideal polymers and polymer formulations (whether as nanoparticles or simple polymer-drug conjugates) that will provide the best inhalable drug delivery platforms in order to accelerate clinical translation of such systems, in a similar way that liposomes have advanced into clinical trials. The ultimate commercial success of inhaled polymer-drug conjugates is therefore dependent on the conduct of more focused research to address these knowledge gaps, rather than mere development of a series of novel and more complex polymer-drug conjugates.

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References:

*** Special interest**

**** Outstanding interest**

[1] Larson N, Ghandehari H. Polymeric Conjugates for Drug Delivery. Chem. Mater. 2012;24:840-53.

[2] Ringsdorf H. Structure and properties of pharmacologically active polymers. J Polym Sci Pol Sym. 1975;51:135-53.

[3*****] Pang X, Yang X, Zhai G. Polymer-drug conjugates: recent progress on administration routes. Expert Opin Drug Deliv. 2014;11:1075-86.

****** *Provides a comprehensive review on the role of polymer-drug conjugates administered by various routes.*

[4] Zelikin AN, Ehrhardt C, Healy AM. Materials and methods for delivery of biological drugs. Nat Chem. 2016;8:997-1007.

[5] Feng Q, Tong R. Anticancer nanoparticulate polymer-drug conjugate. Bioeng Transl Med. 2016;1:277-96.

[6] Delplace V, Couvreur P, Nicolas J. Recent trends in the design of anticancer polymer prodrug nanocarriers. Polym Chem. 2014;5:1529-44.

[7] Chang M, Zhang F, Wei T, Zuo T, Guan Y, Lin G, et al. Smart linkers in polymer–drug conjugates for tumor-targeted delivery. J Drug Target. 2016;24:475-91.

[8] Vicent MJ, Dieudonné L, Carbajo RJ, Pineda-Lucena A. Polymer conjugates as therapeutics: future trends, challenges and opportunities. Expert Opin Drug Deliv. 2008;5:593-614.

[9] Xu H, Ma H, Yang P, Zhang X, Wu X, Yin W, et al. Targeted polymer-drug conjugates: Current progress and future perspective. Colloids Surf B Biointerfaces. 2015;136:729-34.

[10] d'Angelo I, Conte C, Miro A, Quaglia F, Ungaro F. Pulmonary drug delivery: a role for polymeric nanoparticles? Curr Top Med Chem. 2015;15:386-400.

[11] El-Sherbiny IM, El-Baz NM, Yacoub MH. Inhaled nano- and microparticles for drug delivery. Glob Cardiol Sci Pract. 2015;2015:2.

[12] Rytting E, Nguyen J, Wang X, Kissel T. Biodegradable polymeric nanocarriers for pulmonary drug delivery. Expert Opin Drug Deliv. 2008;5:629-39.

[13] Gaspar R, Duncan R. Polymeric carriers: Preclinical safety and the regulatory implications for design and development of polymer therapeutics. Adv Drug Deliv Rev. 2009;61:1220-31.

[14**] Haque S, Whittaker MR, McIntosh MP, Pouton CW, Kaminskas LM. Disposition and safety of inhaled biodegradable nanomedicines: Opportunities and challenges. Nanomed Nanotechnol Biol Med. 2016;12:1703-24.

***Excellent review on challenges and future directions of inhalable nanomedicines*

[15] Loira-Pastoriza C, Todoroff J, Vanbever R. Delivery strategies for sustained drug release in the lungs. Adv Drug Deliv Rev. 2014;75:81-91.

[16] Driscoll KE, Carter JM, Howard BW, Hassenbein DG, Pepelko W, Baggs RB, et al. Pulmonary inflammatory, chemokine, and mutagenic responses in rats after subchronic inhalation of carbon black. Toxicol Appl Pharmacol. 1996;136:372-80.

[17] Brown DM, Wilson MR, MacNee W, Stone V, Donaldson K. Size-dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. Toxicol Appl Pharmacol. 2001;175:191-9.

[18] Kajiwara T, Ogami A, Yamato H, Oyabu T, Morimoto Y, Tanaka I. Effect of particle size of intratracheally instilled crystalline silica on pulmonary inflammation. J Occup Health. 2007;49:88-94.

[19] Inoue K-i, Takano H, Yanagisawa R, Hirano S, Sakurai M, Shimada A, et al. Effects of airway exposure to nanoparticles on lung inflammation induced by bacterial endotoxin in mice. Environ Health Perspect. 2006;114:1325-30.

[20] Cho W-S, Duffin R, Howie S, Scotton CJ, Wallace W, MacNee W, et al. Progressive severe lung injury by zinc oxide nanoparticles; the role of Zn2+ dissolution inside lysosomes. Part Fibre Toxicol. 2011;8:27.

[21] Harris JM, Chess RB. Effect of pegylation on pharmaceuticals. Nat Rev Drug Discov. 2003;2:214-21.

[22] Dai L, Wang L, Deng L, Liu J, Lei J, Li D, et al. Novel Multiarm Polyethylene glycol-Dihydroartemisinin Conjugates Enhancing Therapeutic Efficacy in Non-Small-Cell Lung Cancer. Sci Rep. 2014;4:5871.

[23] Veronese FM, Mero A. The impact of PEGylation on biological therapies. BioDrugs. 2008;22:315-29.

[24*****] Veronese FM, Pasut G. PEGylation, successful approach to drug delivery. Drug Discov Today. 2005;10:1451-8.

****** *Highlights the need for PEGylation of drugs/polymers for improving in vivo behaviour*

[25] Schuster BS, Suk JS, Woodworth GF, Hanes J. Nanoparticle diffusion in respiratory mucus from humans without lung disease. Biomaterials. 2013;34:3439-46.

[26] Katsumi H, Takashima M, Sano J, Nishiyama K, Kitamura N, Sakane T, et al. Development of polyethylene glycol-conjugated alendronate, a novel nitrogen-containing bisphosphonate derivative: evaluation of absorption, safety, and effects after intrapulmonary administration in rats. J Pharm Sci. 2011;100:3783-92.

[27] Bayard FJ, Thielemans W, Pritchard DI, Paine SW, Young SS, Backman P, et al. Polyethylene glycol-drug ester conjugates for prolonged retention of small inhaled drugs in the lung. J Control Release. 2013;171:234-40.

[28] McLeod VM, Chan LJ, Ryan GM, Porter CJH, Kaminskas LM. Optimal PEGylation can Improve the Exposure of Interferon in the Lungs Following Pulmonary Administration. J Pharm Sci. 2015;104:1421-30.

[29] Gursahani H, Riggs-Sauthier J, Pfeiffer J, Lechuga-Ballesteros D, Fishburn CS. Absorption of polyethylene glycol (PEG) polymers: the effect of PEG size on permeability. J Pharm Sci. 2009;98:2847-56.

[30] Luo T, Loira-Pastoriza C, Patil HP, Ucakar B, Muccioli GG, Bosquillon C, et al. PEGylation of paclitaxel largely improves its safety and anti-tumor efficacy following pulmonary delivery in a mouse model of lung carcinoma. J Control Release. 2016;239:62-71.

[31] Khor SY, Hu J, McLeod VM, Quinn JF, Porter CJ, Whittaker MR, et al. The Pharmacokinetics and Biodistribution of a 64 kDa PolyPEG Star Polymer After Subcutaneous and Pulmonary Administration to Rats. J Pharm Sci. 2016;105:293-300.

[32] Khor SY, Hu J, McLeod VM, Quinn JF, Williamson M, Porter CJ, et al. Molecular weight (hydrodynamic volume) dictates the systemic pharmacokinetics and tumour disposition of PolyPEG star polymers. Nanomed Nanotechnol Biol Med. 2015;11:2099-108.

[33] Pasut G, Veronese FM. Polymer–drug conjugation, recent achievements and general strategies. Prog Polym Sci. 2007;32:933-61.

[34] Syrett JA, Haddleton DM, Whittaker MR, Davis TP, Boyer C. Functional, star polymeric molecular carriers, built from biodegradable microgel/nanogel cores. Chem Commun (Camb). 2011;47:1449-51.

[35] Cameron DJA, Shaver MP. Aliphatic polyester polymer stars: synthesis, properties and applications in biomedicine and nanotechnology. Chem Soc Rev. 2011;40:1761-76.

[36] Kaminskas LM, McLeod VM, Jones SA, Boyd BJ, Porter CJ. Dendrimers for Biomedical Applications. HANDBOOK OF NANOBIOMEDICAL RESEARCH: Fundamentals, Applications and Recent Developments: Volume 1 Materials for Nanomedicine: World Scientific; 2014. p. 279-328.

[37] Kalomiraki M, Thermos K, Chaniotakis NA. Dendrimers as tunable vectors of drug delivery systems and biomedical and ocular applications. Int J Nanomedicine. 2016;11:1-12.

[38**] Kaminskas LM, McLeod VM, Porter CJH, Boyd BJ. Association of Chemotherapeutic Drugs with Dendrimer Nanocarriers: An Assessment of the Merits of Covalent Conjugation Compared to Noncovalent Encapsulation. Mol Pharm. 2012;9:355-73.

** *Describes the benefits of covalent conjugation to polymer-based delivery systems compared to physical encapsulation*

[39] Kaminskas LM, McLeod VM, Ryan GM, Kelly BD, Haynes JM, Williamson M, et al. Pulmonary administration of a doxorubicin-conjugated dendrimer enhances drug exposure to lung metastases and improves cancer therapy. J Control Release. 2014;183:18-26.

[40*] Ryan GM, Bischof RJ, Enkhbaatar P, McLeod VM, Chan LJ, Jones SA, et al. A Comparison of the Pharmacokinetics and Pulmonary Lymphatic Exposure of a Generation 4 PEGylated Dendrimer Following Intravenous and Aerosol Administration to Rats and Sheep. Pharm Res. 2016;33:510-25.

* *Detailed study comparing the pulmonary pharmacokinetics and lymphatic exposure of an inhaled dendrimer in rats and sheep*

[41**] Ryan GM, Kaminskas LM, Kelly BD, Owen DJ, McIntosh MP, Porter CJ. Pulmonary administration of PEGylated polylysine dendrimers: absorption from the lung versus retention within the lung is highly size-dependent. Mol Pharm. 2013;10:2986-95.

** *Comprehensive description of the impact of molecular weight on the pulmonary pharmacokinetics of PEGylated dendrimers in rats.*

[42] Landers JJ, Cao Z, Lee I, Piehler LT, Myc PP, Myc A, et al. Prevention of Influenza Pneumonitis by Sialic Acid–Conjugated Dendritic Polymers. J Infect Dis. 2002;186:1222-30.

[43] Reuter JD, Myc A, Hayes MM, Gan Z, Roy R, Qin D, et al. Inhibition of Viral Adhesion and Infection by Sialic-Acid-Conjugated Dendritic Polymers. Bioconjug Chem. 1999;10:271-8.

[44] Inapagolla R, Guru BR, Kurtoglu YE, Gao X, Lieh-Lai M, Bassett DJ, et al. In vivo efficacy of dendrimer-methylprednisolone conjugate formulation for the treatment of lung inflammation. Int J Pharm. 2010;399:140-7.

[45] Morris CJ, Smith MW, Griffiths PC, McKeown NB, Gumbleton M. Enhanced pulmonary absorption of a macromolecule through coupling to a sequence-specific phage display-derived peptide. J Control Release. 2011;151:83-94.

[46] Bai S, Ahsan F. Synthesis and Evaluation of Pegylated Dendrimeric Nanocarrier for Pulmonary Delivery of Low Molecular Weight Heparin. Pharm Res. 2009;26:539-48.

[47] Bai S, Thomas C, Ahsan F. Dendrimers as a carrier for pulmonary delivery of enoxaparin, a low-molecular weight heparin. J Pharm Sci. 2007;96:2090-106.

[48] Dong Z, Hamid KA, Gao Y, Lin Y, Katsumi H, Sakane T, et al. Polyamidoamine dendrimers can improve the pulmonary absorption of insulin and calcitonin in rats. J Pharm Sci. 2011;100:1866-78.

[49] Bharatwaj B, Mohammad AK, Dimovski R, Cassio FL, Bazito RC, Conti D, et al. Dendrimer Nanocarriers for Transport Modulation Across Models of the Pulmonary Epithelium. Mol Pharm. 2015;12:826-38.

[50] Zhong Q, Bielski ER, Rodrigues LS, Brown MR, Reineke JJ, da Rocha SRP. Conjugation to Poly(amidoamine) Dendrimers and Pulmonary Delivery Reduce Cardiac Accumulation and Enhance Antitumor Activity of Doxorubicin in Lung Metastasis. Mol Pharm. 2016;13:2363-75.

[51*****] Zhong Q, Merkel OM, Reineke JJ, da Rocha SR. Effect of the Route of Administration and PEGylation of Poly(amidoamine) Dendrimers on Their Systemic and Lung Cellular Biodistribution. Mol Pharm. 2016;13:1866-78.

***** *Pharmacokinetic evaluation of PEGylated PAMAM dendrimers via various routes of administration*

[52] Kaminskas LM, Boyd BJ, Karellas P, Krippner GY, Lessene R, Kelly B, et al. The Impact of Molecular Weight and PEG Chain Length on the Systemic Pharmacokinetics of PEGylated Poly l-Lysine Dendrimers. Mol Pharm. 2008;5:449-63.

[53*****] Xu C, Wang P, Zhang J, Tian H, Park K, Chen X. Pulmonary Codelivery of Doxorubicin and siRNA by pH-Sensitive Nanoparticles for Therapy of Metastatic Lung Cancer. Small. 2015;11:4321-33.

***** *Demonstrates the importance of pulmonary delivery of PEI-doxorubicin conjugates and SiRNA complex in the treatment of lung cancers*

[54] Xu C, Tian H, Wang P, Wang Y, Chen X. The suppression of metastatic lung cancer by pulmonary administration of polymer nanoparticles for co-delivery of doxorubicin and Survivin siRNA. Biomater Sci. 2016;4:1646-54.

[55] Kleemann E, Neu M, Jekel N, Fink L, Schmehl T, Gessler T, et al. Nano-carriers for DNA delivery to the lung based upon a TAT-derived peptide covalently coupled to PEG–PEI. J Control Release. 2005;109:299-316.

[56] Choudhury CK, Roy S. Structural and dynamical properties of polyethylenimine in explicit water at different protonation states: a molecular dynamics study. Soft Matter. 2013;9:2269-81.

[57] Griesenbach U, Geddes DM, Alton EWFW. Gene therapy for cystic fibrosis: an example for lung gene therapy. Gene Ther. 2004;11:S43-S50.

[58] Mintzer MA, Simanek EE. Nonviral vectors for gene delivery. Chem. Rev. 2009;109:259-302.

[59] Dong D-W, Tong S-W, Qi X-R. Comparative studies of polyethylenimine–doxorubicin conjugates with pH-sensitive and pH-insensitive linkers. J Biomed Mater Res A. 2013;101A:1336-44.

[60] Zamora-Avila DE, Zapata-Benavides P, Franco-Molina MA, Saavedra-Alonso S, Trejo-Avila LM, Resendez-Perez D, et al. WT1 gene silencing by aerosol delivery of PEI-RNAi complexes inhibits B16-F10 lung metastases growth. Cancer Gene Ther. 2009;16:892-9.

[61] De Paula D, Bentley MVLB, Mahato RI. Hydrophobization and bioconjugation for enhanced siRNA delivery and targeting. RNA. 2007;13:431-56.

[62] Merkel OM, Beyerle A, Librizzi D, Pfestroff A, Behr TM, Sproat B, et al. Nonviral siRNA delivery to the lung: investigation of PEG-PEI polyplexes and their in vivo performance. Mol Pharm. 2009;6:1246-60.

[63] Nguyen J, Xie X, Neu M, Dumitrascu R, Reul R, Sitterberg J, et al. Effects of cell-penetrating peptides and pegylation on transfection efficiency of polyethylenimine in mouse lungs. J Gene Med. 2008;10:1236-46.

[64] Zhao J, Feng S-S. Nanocarriers for delivery of siRNA and co-delivery of siRNA and other therapeutic agents. Nanomed Nanotechnol Biol Med. 2015;10:2199-228.

[65] Wang Y, Gao S, Ye W-H, Yoon HS, Yang Y-Y. Co-delivery of drugs and DNA from cationic core-shell nanoparticles self-assembled from a biodegradable copolymer. Nat Mater. 2006;5:791-6.

[66] Marasini N, Giddam AK, Khalil ZG, Hussein WM, Capon RJ, Batzloff MR, et al. Double adjuvanting strategy for peptide-based vaccines: trimethyl chitosan nanoparticles for lipopeptide delivery. Nanomedicine (Lond). 2016;11:3223-35.

[67] Marasini N, Skwarczynski M, Toth I. Intranasal delivery of nanoparticle-based vaccines. Ther Deliv. 2017;8:151-67.

[68] Croisier F, Jérôme C. Chitosan-based biomaterials for tissue engineering. Eur Polym J. 2013;49:780-92.

[69] Muhsin MDA, George G, Beagley K, Ferro V, Wang H, Islam N. Effects of Chemical Conjugation of l-Leucine to Chitosan on Dispersibility and Controlled Release of Drug from a Nanoparticulate Dry Powder Inhaler Formulation. Mol Pharm. 2016;13:1455-66.

[70] Lee C, Choi JS, Kim I, Oh KT, Lee ES, Park E-S, et al. Long-acting inhalable chitosan-coated poly(lactic-co-glycolic acid) nanoparticles containing hydrophobically modified exendin-4 for treating type 2 diabetes. Int J Nanomedicine. 2013;8:2975-83.

[71] Garg T, Rath G, Goyal AK. Inhalable chitosan nanoparticles as antitubercular drug carriers for an effective treatment of tuberculosis. Artif Cells Nanomed Biotechnol. 2016;44:997-1001.

[72] Yang M, Yamamoto H, Kurashima H, Takeuchi H, Yokoyama T, Tsujimoto H, et al. Design and evaluation of inhalable chitosan-modified poly (DL-lactic-co-glycolic acid) nanocomposite particles. Eur J Pharm Sci. 2012;47:235-43.

[73] Ghaz-Jahanian MA, Abbaspour-Aghdam F, Anarjan N, Berenjian A, Jafarizadeh-Malmiri H. Application of Chitosan-Based Nanocarriers in Tumor-Targeted Drug Delivery. Mol Biotechnol. 2015;57:201-18.

[74] Suarato G, Li W, Meng Y. Role of pH-responsiveness in the design of chitosan-based cancer nanotherapeutics: A review. Biointerphases. 2016;11:04b201.

[75] Cho YI, Park S, Jeong SY, Yoo HS. In vivo and in vitro anti-cancer activity of thermo-sensitive and photo-crosslinkable doxorubicin hydrogels composed of chitosan-doxorubicin conjugates. Eur J Pharm Biopharm. 2009;73:59-65.

[76] Marasini N, Skwarczynski M, Toth I. Oral delivery of nanoparticle-based vaccines. Expert Rev Vaccines. 2014;13:1361-76.

[77] Muhsin MDA, George G, Beagley K, Ferro V, Armitage C, Islam N. Synthesis and Toxicological Evaluation of a Chitosan-l-Leucine Conjugate for Pulmonary Drug Delivery Applications. Biomacromolecules. 2014;15:3596-607.

[78] Berezin AS, Skorik YA. Chitosan-isoniazid conjugates: Synthesis, evaluation of tuberculostatic activity, biodegradability and toxicity. Carbohydr Polym. 2015;127:309-15.

[79] Yeh TH, Hsu LW, Tseng MT, Lee PL, Sonjae K, Ho YC, et al. Mechanism and consequence of chitosan-mediated reversible epithelial tight junction opening. Biomaterials. 2011;32:6164-73.

[80] Trapani A, Di Gioia S, Ditaranto N, Cioffi N, Goycoolea FM, Carbone A, et al. Systemic heparin delivery by the pulmonary route using chitosan and glycol chitosan nanoparticles. Int J Pharm. 2013;447:115-23.

[81*****] Choi M, Cho M, Han BS, Hong J, Jeong J, Park S, et al. Chitosan nanoparticles show rapid extrapulmonary tissue distribution and excretion with mild pulmonary inflammation to mice. Toxicol Lett. 2010;199:144-52.

****** *Demonstrates the impact of chitosan nanoparticles on the lungs and pharmacokinetic behaviour*

[82] Woods A, Patel A, Spina D, Riffo-Vasquez Y, Babin-Morgan A, de Rosales RT, et al. In vivo biocompatibility, clearance, and biodistribution of albumin vehicles for pulmonary drug delivery. J Control Release. 2015;210:1-9.

[83] Yamamoto H, Kuno Y, Sugimoto S, Takeuchi H, Kawashima Y. Surface-modified PLGA nanosphere with chitosan improved pulmonary delivery of calcitonin by mucoadhesion and opening of the intercellular tight junctions. J Control Release. 2005;102:373-81.

[84] Makhlof A, Werle M, Tozuka Y, Takeuchi H. Nanoparticles of glycol chitosan and its thiolated derivative significantly improved the pulmonary delivery of calcitonin. Int J Pharm. 2010;397:92-5.

[85] Tripodo G, Trapani A, Torre ML, Giammona G, Trapani G, Mandracchia D. Hyaluronic acid and its derivatives in drug delivery and imaging: Recent advances and challenges. Eur J Pharm Biopharm. 2015;97, Part B:400-16.

[86] Mero A, Campisi M. Hyaluronic Acid Bioconjugates for the Delivery of Bioactive Molecules. Polymers. 2014;6:346.

[87] Rouse JJ, Whateley TL, Thomas M, Eccleston GM. Controlled drug delivery to the lung: Influence of hyaluronic acid solution conformation on its adsorption to hydrophobic drug particles. Int J Pharm. 2007;330:175-82.

[88] Lee-Sayer SSM, Dong Y, Arif AA, Olsson M, Brown KL, Johnson P. The Where, When, How, and Why of Hyaluronan Binding by Immune Cells. Front Immunol. 2015;6:150.

[89] Jiao Y, Pang X, Zhai G. Advances in Hyaluronic Acid-Based Drug Delivery Systems. Curr Drug Targets. 2016;17:720-30.

[90] Surendrakumar K, Martyn GP, Hodgers EC, Jansen M, Blair JA. Sustained release of insulin from sodium hyaluronate based dry powder formulations after pulmonary delivery to beagle dogs. J Control Release. 2003;91:385-94.

[91] Costabile G, d'Angelo I, d'Emmanuele di Villa Bianca R, Mitidieri E, Pompili B, Del Porto P, et al. Development of inhalable hyaluronan/mannitol composite dry powders for flucytosine repositioning in local therapy of lung infections. J Control Release. 2016;238:80-91.

[92] Martinelli F, Balducci AG, Kumar A, Sonvico F, Forbes B, Bettini R, et al. Engineered sodium hyaluronate respirable dry powders for pulmonary drug delivery. Int J Pharm. 2017;517:286-95.

[93] Kuehl C, Zhang T, Kaminskas LM, Porter CJ, Davies NM, Forrest L, et al. Hyaluronic Acid Molecular Weight Determines Lung Clearance and Biodistribution after Instillation. Mol Pharm. 2016;13:1904-14.

[94] Mattheolabakis G, Milane L, Singh A, Amiji MM. Hyaluronic acid targeting of CD44 for cancer therapy: from receptor biology to nanomedicine. J Drug Target. 2015;23:605-18.

[95] Song E, Han W, Li C, Cheng D, Li L, Liu L, et al. Hyaluronic Acid-Decorated Graphene Oxide Nanohybrids as Nanocarriers for Targeted and pH-Responsive Anticancer Drug Delivery. ACS Appl Mater Interfaces. 2014;6:11882-90.

[96] Katoh S, Zheng Z, Oritani K, Shimozato T, Kincade PW. Glycosylation of CD44 negatively regulates its recognition of hyaluronan. J Exp Med. 1995;182:419-29.

[97] Cai S, Alhowyan AAB, Yang Q, Forrest WCM, Shnayder Y, Forrest ML. Cellular Uptake and Internalization of Hyaluronan-based Doxorubicin and Cisplatin Conjugates. J Drug Target. 2014;22:648-57.

[98**] Ishiguro S, Cai S, Uppalapati D, Turner K, Zhang T, Forrest WC, et al. Intratracheal Administration of Hyaluronan-Cisplatin Conjugate Nanoparticles Significantly Attenuates Lung Cancer Growth in Mice. Pharm Res. 2016;33:2517-29.

** *Reports on the potential of hylaronan-cisplatin conjugates as a promising inhalable platform for the improved treatment of lung cancer*

[99] Xie Y, Aillon KL, Cai S, Christian JM, Davies NM, Berkland CJ, et al. Pulmonary delivery of cisplatin-hyaluronan conjugates via endotracheal instillation for the treatment of lung cancer. Int J Pharm. 2010;392:156-63.

[100] Cai S, Xie Y, Bagby TR, Cohen MS, Forrest ML. Intralymphatic chemotherapy using a hyaluronan-cisplatin conjugate. J Surg Res. 2008;147:247-52.

[101] Misra S, Hascall VC, Markwald RR, Ghatak S. Interactions between Hyaluronan and Its Receptors (CD44, RHAMM) Regulate the Activities of Inflammation and Cancer. Front Immunol. 2015;6:201.

[102] Murata M, Yonamine T, Tanaka S, Tahara K, Tozuka Y, Takeuchi H. Surface modification of liposomes using polymer-wheat germ agglutinin conjugates to improve the absorption of peptide drugs by pulmonary administration. J Pharm Sci. 2013;102:1281-9.

[103] Dailey LA, Jekel N, Fink L, Gessler T, Schmehl T, Wittmar M, et al. Investigation of the proinflammatory potential of biodegradable nanoparticle drug delivery systems in the lung. Toxicol Appl Pharmacol. 2006;215:100-8.

[104] Ungaro F, d'Angelo I, Miro A, La Rotonda MI, Quaglia F. Engineered PLGA nano- and micro-carriers for pulmonary delivery: challenges and promises. J Pharm Pharmacol. 2012;64:1217-35.

[105] Karra N, Nassar T, Ripin AN, Schwob O, Borlak J, Benita S. Antibody conjugated PLGA nanoparticles for targeted delivery of paclitaxel palmitate: efficacy and biofate in a lung cancer mouse model. Small. 2013;9:4221-36.

[106] Oh JE, Nam YS, Lee KH, Park TG. Conjugation of drug to poly(D,L-lactic-co-glycolic acid) for controlled release from biodegradable microspheres. J Control Release. 1999;57:269-80.

[107] Zhang X, Malhotra S, Molina M, Haag R. Micro- and nanogels with labile crosslinks - from synthesis to biomedical applications. Chem Soc Rev. 2015;44:1948-73.

[108] Mo Y, Lim L-Y. Preparation and in vitro anticancer activity of wheat germ agglutinin (WGA)-conjugated PLGA nanoparticles loaded with paclitaxel and isopropyl myristate. J Control Release. 2005;107:30-42.

[109] Dozono H, Yanazume S, Nakamura H, Etrych T, Chytil P, Ulbrich K, et al. HPMA Copolymer-Conjugated Pirarubicin in Multimodal Treatment of a Patient with Stage IV Prostate Cancer and Extensive Lung and Bone Metastases. Target Oncol. 2016;11:101-6.

[110] Singh J, Desai S, Yadav S, Narasimhan B, Kaur H. Polymer drug conjugates: recent advancements in various diseases. Curr Pharm Des. 2016;22:2821-43.

[111] Duncan R. The dawning era of polymer therapeutics. Nat Rev Drug Discov. 2003;2:347-60.

[112] Shamay Y, Golan M, Tyomkin D, David A. Assessing the therapeutic efficacy of VEGFR-1-targeted polymer drug conjugates in mouse tumor models. J Control Release. 2016;229:192-9.

[113] Lomkova EA, Chytil P, Janouskova O, Mueller T, Lucas H, Filippov SK, et al. Biodegradable Micellar HPMA-Based Polymer-Drug Conjugates with Betulinic Acid for Passive Tumor Targeting. Biomacromolecules. 2016;17:3493-507.

[114] Camacho KM, Menegatti S, Mitragotri S. Low-molecular-weight polymer–drug conjugates for synergistic anticancer activity of camptothecin and doxorubicin combinations. Nanomedicine (Lond). 2016;11:1139-51.

[115] Forbes B, O'Lone R, Allen PP, Cahn A, Clarke C, Collinge M, et al. Challenges for inhaled drug discovery and development: Induced alveolar macrophage responses. Adv Drug Deliv Rev. 2014;71:15-33.

[116] Jones RM, Neef N. Interpretation and prediction of inhaled drug particle accumulation in the lung and its associated toxicity. Xenobiotica. 2012;42:86-93.

[117] Li C, Liu H, Sun Y, Wang H, Guo F, Rao S, et al. PAMAM nanoparticles promote acute lung injury by inducing autophagic cell death through the Akt-TSC2-mTOR signaling pathway. J Mol Cell Biol. 2009;1:37-45.

[118] Grenha A, Grainger CI, Dailey LA, Seijo B, Martin GP, Remuñán-López C, et al. Chitosan nanoparticles are compatible with respiratory epithelial cells in vitro. Eur J Pharm Sci. 2007;31:73-84.

[119] Beyerle A, Irmler M, Beckers J, Kissel T, Stoeger T. Toxicity pathway focused gene expression profiling of PEI-based polymers for pulmonary applications. Mol Pharm. 2010;7:727-37.

[120] Mura S, Hillaireau H, Nicolas J, Le Droumaguet B, Gueutin C, Zanna S, et al. Influence of surface charge on the potential toxicity of PLGA nanoparticles towards Calu-3 cells. Int J Nanomedicine. 2011;6:2591.

[121*****] Grabowski N, Hillaireau H, Vergnaud J, Santiago LA, Kerdine-Romer S, Pallardy M, et al. Toxicity of surface-modified PLGA nanoparticles toward lung alveolar epithelial cells. Int J Pharm. 2013;454:686-94.

* *Reports on possible toxicities of FDA-approved PLGA nanoparticles on lung alveolar epithelial cells*.

[122] Cantor JO. Potential therapeutic applications of hyaluronan in the lung Int J Chron Obstruct Pulmon Dis. 2007;2:283-8.

[123] Mascarenhas MM, Day RM, Ochoa CD, Choi W-I, Yu L, Ouyang B, et al. Low molecular weight hyaluronan from stretched lung enhances interleukin-8 expression. Am J Respir Cell Mol Biol. 2004;30:51-60.

[124] Noble PW, Jiang D. Matrix regulation of lung injury, inflammation, and repair: the role of innate immunity. Proc Am Thorac Soc. 2006;3:401-4.

[125] Makadia HK, Siegel SJ. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers (Basel). 2011;3:1377-97.

[126] Healy AM, Amaro MI, Paluch KJ, Tajber L. Dry powders for oral inhalation free of lactose carrier particles. Adv Drug Deliv Rev. 2014;75:32-52.