Expert Opinion

Micro- and nanocarrier-mediated lung targeting

Balak D Kurmi, Jitendra Kayat, Virendra Gajbhiye, Rakesh K Tekade & Narendra K Jain†
Dr Hari Singh Gour University, Department of Pharmaceutical Sciences, Pharmaceutics Research Laboratory, Sagar 470 003, India

Importance of the field: Drug delivery to lungs appears to be an attractive proposition on account of the large surface area of the alveolar region; it provides tremendous opportunities to improve drug therapies both systemically and locally using new drug delivery systems. Administration of drugs directly to the lungs is the most appropriate route in the treatment of asthma and other pulmonary diseases such as tuberculosis, chronic obstructive pulmonary disease and lung cancer.

Areas covered in this review: This review focuses on the utilization of nano- and microcarriers such as microspheres, nanoparticles, liposomes, niosomes and dendrimers for targeted delivery of bioactive molecules to lungs.

What the reader will gain: This review sheds light on the current status of nano- and microcarrier-mediated lung targeting of bioactive compounds.

Take home message: The literature review shows that carriers could supplement sustained drug delivery to the lungs, extended duration of action, reduced therapeutic dose, improved patient compliance, and reduced adverse effects of highly toxic drugs. There is still a need to identify more specific receptors that are present exclusively in the lungs. The identification of such receptors may also facilitate drug targeting to further specific parts of the lungs, such as bronchioles and alveoli.

Keywords: dendrimers, liposomes, lung targeting, microspheres, nanocarriers, nanoparticles

Expert Opin. Drug Deliv. (2010) 7(7):781-794

1. Introduction

The respiratory system (also known as the pulmonary system) is one of the most critical organ systems of the body that supplies the body with oxygen and rids it of carbon dioxide. This process also removes metabolic wastes and maintains the pH balance of the body. The organs involved are the airways, lungs and muscles that mediate the movement of air into and out of the body. Pulmonary drug delivery is becoming more and more important because of the specific physiological environment of the lung as an absorption and treatment organ. Pulmonary drug delivery provides tremendous opportunities to improve drug therapies systemically and locally using advanced drug delivery systems [1]. Gas exchange is the primary function of lungs that plays an important role in metabolic regulations. Lungs maintain systemic blood pressure and remove the metabolic substances from the mixed venous circulation, platelet aggregation, coagulation and anticoagulation [2]. The lung's alveoli are perfused with the pulmonary circulation while bronchial veins bypass the pulmonary circulation and join the pulmonary veins to return oxygenated blood to the heart [3]. Drug delivery to lungs is an attractive horizon owing to the large surface area of the alveolar region, lower thickness of the epithelial barrier, extensive vascularization, relatively low proteolytic activity in the alveolar space as compared with other routes of administration and absence of the first-pass metabolism [4-7]. Ranney [8] demonstrated the importance of targeted drug delivery

informa healthcare

Article highlights.

- Particulate drug carrier systems in aerosol form are becoming an interesting field of research for the delivery of therapeutic compounds to the lungs. The short duration of resultant clinical effects and the requirement for at least three to four daily inhalations of most medications in aerosol form are the main disadvantages of inhaled drug formulation.
- Recent research has suggested that sustained drug delivery to the respiratory tract provides extended duration of action, reduction in therapeutic dose of drugs, improved management of therapy, improved patient compliance, and a reduction in the adverse effects of highly toxic drugs.
- Makino reported higher uptake of polystyrene microspheres in lungs.
- Nanoparticulate carriers can also be used as a potential delivery system for DNA in lung cancer gene therapy.
- Significantly improved nuclear delivery of encapsulated doxorubicin to the target cells can be achieved by targeted liposomes.
- PEGylated dendrimers might be used efficiently for targeting anti-tubercular drug to the lungs.

This box summarizes key points contained in the article.

to the lungs and pointed out that diseases located in this region lend themselves to a drug-targeting approach because of ready access to these organs by both the intravenous and the intratracheal routes.

A suitable route of administration for the drug is the criterion that delivers the most drug to its target. The choice of route depends on the pharmacological, toxicological and physicochemical properties of the drug and vehicle used and on the anatomical changes due to disease. In the treatment of asthma and other pulmonary diseases such as tuberculosis, chronic obstructive pulmonary disease and lung cancer, administration of drugs directly to the lungs might be the most appropriate route [9]. Aerosolized drug administration to the lung has been used for many years for local and selective delivery to treat primarily localized disease states within the bronchi and offers a direct method for the improvement of the kinetic profile in the target tissue by passive targeting. Hence, this route of drug administration can deliver therapeutic agents to the diseased regions while reducing their distribution to other organs [10]. Particulate drug carrier systems in aerosol form are becoming very interesting fields of research for the delivery of therapeutic or diagnostic compounds either locally or systemically. The short duration of resultant clinical effects and the requirement of most medications at least three to four times daily in aerosol form are the main disadvantages of inhaled drug formulations [11]. Often, the rapid absorption of some drugs from the lung epithelium causes undesirable side effect, for example, bronchodilators and corticosteroids [12,13]. This often leads to poor patient compliance with the therapeutic regimen and increases the possibility of associated treatment failure resulting from the risk of self-administration of the drug by the patients [14].

Drug delivery to lungs for the treatment of pulmonary disease with its two to four times a day dosing is far from ideal. The clearance mechanisms involved in the human respiratory tract are highly efficient, which provides little time for drug action after dosing. Hence, daily therapy with prolonged drug action would allow more effective treatment of conditions such as asthma [15]. Recent research has suggested that achieving sustained drug action in the lungs is highly desirable [16,17]. Sustained drug delivery to the respiratory tract provides extended duration of action, a reduction in therapeutic dose of drugs, improved management of therapy, improved patient compliance, and a reduction of the adverse effects of highly toxic drugs [18,19]. The most important factor that is considerable in the field of pulmonary drug targeting is the new scientific information on: i) substances that have the capability of binding with a receptor and can be used as drug carriers that are targetable in nature; ii) endothelial receptors that are involved in the enhancement of drug clearance from the pulmonary region; iii) pulmonary diseases that affect the endothelial and sequestered tissue receptors; iv) techniques and methods that participate in the improvement of the formulation of carriers; v) mass-production improvement methods for the complex biological molecules (biopharmaceuticals) needed for drug targeting; and vi) non-invasive methods for free drug levels and drug clearance rates monitoring within tissues, such as nuclear isotopic and nuclear magnetic resonance methods [8]. Most of the new drug carriers are of nanoscopic size. Drug carriers are substances that deliver the drug in the vicinity of desired site and improve the effectiveness of drugs (**Figure 1**). In this review, the nanocarriers used for drug delivery to lungs are emphasized.

2. Microsphere-mediated lung targeting

Microspheres are spherical particles consisting of natural and synthetic polymer and ideally having particle size < 200 µm. They are used as carriers for targeting of drug to different sites. Inhalation of aerosolized drugs encapsulated in microspheres can also represent an ideal method for drug delivery to the systemic circulation [20]. Macrophages act as reservoirs for viruses and bacteria. In the case of tuberculosis, tubercle bacillus coming from the air stays stable in alveolar macrophages for a long time. Hence, Makino *et al.* [21] prepared polystyrene microspheres and determined the effects of the size and surface properties of the microspheres on phagocytic uptake. The uptake was determined by the amount of superoxide generated from macrophages by the use of chemiluminescence assay, and it was found that the amount of superoxide generated was apparently higher with polystyrene microspheres with a diameter of 1 mm than those with diameters < 1 mm (i.e., 0.2 or 0.5 mm) and with diameter > 1 mm (6 or 10 mm). Surface properties of microsphere also affected the alveolar macrophage uptake.

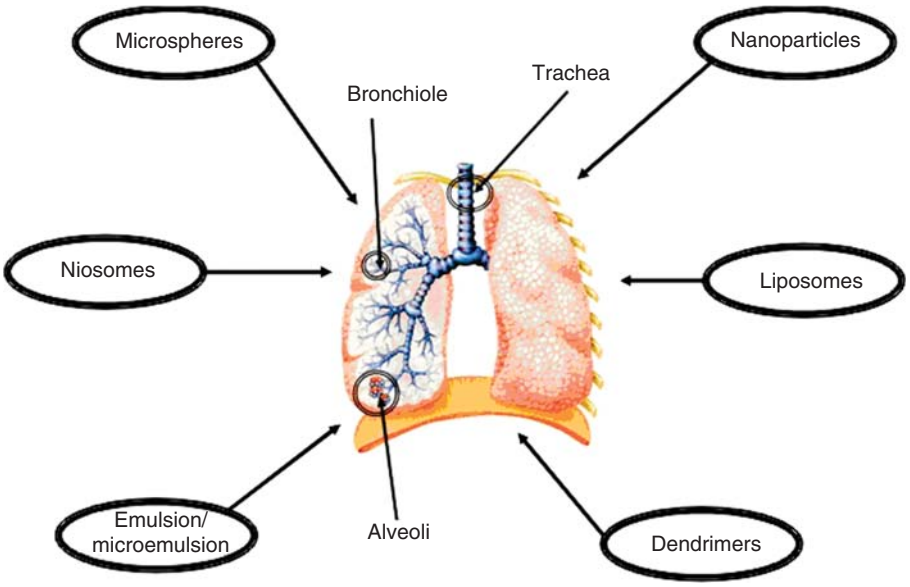

Figure 1. Various carriers for lung targeting.

Makino *et al.* prepared polystyrene microspheres of 1 mm diameter with different surface groups such as primary amine, sulfate, hydroxyl, or carboxyl groups. Their results showed that the alveolar macrophages trapped most effectively polystyrene microspheres with primary amine groups, whereas carboxyl groups bearing microspheres were taken up by alveolar macrophages to a slightly lesser extent, and other microspheres were taken up in smaller amounts. They concluded that microspheres having soft surfaces are easily accessible to alveolar macrophages, and effectively trapped by macrophages. Alveolar macrophage uptake undoubtedly increases drug delivery to lungs.

Cisplatin (*cis*-diaminedichloroplatium [CDDP]) is one of the most potent anticancer agents that cause several side effects, including renal disturbances, nausea, vomiting and auditory toxicity. Huo *et al.* [22] prepared cisplatin-loaded poly(lactic-*co*-glycolic) acid (PLGA) microspheres and studied their pharmacokinetics and tissue distribution in rabbit. Their results suggested a significantly higher concentration of CDDP in lung (212 µg/g, 15 min) than those of other tissues and blood. Compared with CDDP intravenous injection, the drug concentration of cisplatin in lung after intravenous injection of CDDP-PLGA-MS was enhanced from 1.37 to 212 µg/g (15 min). Carboplatin (CPt) is a potent antitumor drug used for many types of cancer treatment, including pulmonary small-cell carcinoma, carcinoma of ovary and testis, and epithelium carcinoma of the head and neck. Lu *et al.* [23] prepared carboplatin-loaded gelatin microspheres having arithmetic mean diameter 13.20 µm with 98% of the microspheres ranging from 5.0 to 28.6 µm and found that the targeting efficacy (*te*) of lung increased by a factor of 9.4 compared with spleen and 90.5 compared with liver. Compared with the original drug CPt, the targeting ratio of lung $[(te)_{NS}/(te)_{S}]$ increased by a factor of 11.8, compared with kidney, 36.5, compared with liver, and the ratio of peak concentration in lung (*Ce*) increased by a factor of 4.1 compared with CPt and suggested that microencapsulation improved the antitumor effect of CPt (S-180 lung neoplasm models) (**Figure 2**).

Recently, Yang *et al.* [24] developed porous PLGA microspheres by using ammonium bicarbonate (ABC) as an effervescent porogen agent, to avoid macrophage uptake, and to enhance the drug encapsulation efficiency. They found that microparticles prepared with 7.5% w/w (ABC/PLGA) had a mass median aerodynamic diameter (MMAD) of 4.0 ± 1.2 µm and fine particle fraction (FPF) of 32.0 ± 9.1%, deposited at the Andersen Cascade Impactor (ACI) stage. The highly porous large particles avoided phagocytosis by macrophages, while non-porous small particles were quickly taken up by the macrophages. Enrofloxacin has a broad spectrum of antibacterial activity against Gram-negative bacteria, Gram-positive bacteria, and mycoplasma species in animals [25-27]. Traditional enrofloxacin formulation exerts adverse effects, such as drug residues, resistant bacteria and allergic hypersensitivity reactions [28-30]. Tang *et al.* [31] prepared enrofloxacin microspheres by emulsifying with gelatin and liquid paraffin, and studied their pharmacokinetics and lung targeting characteristic in dogs after intravenous administration and found that the half-life of the distribution phase was reduced by 77.78%, the half-life of the elimination phase was lengthened from 5.15 to 33.86 h, and the rate of body clearance in the lung was shortened by 55.72% as compared with marketed Enrofloxacin injection (Baytril, Bayer Healthcare, USA). The targeting ratio to the lung ($Te_{(microsphere)}/Te_{(Baytril)}$ increased by a factor of 1.77 compared with liver and 3.51 compared with spleen. Collectively, the Enrofloxacin microsphere showed

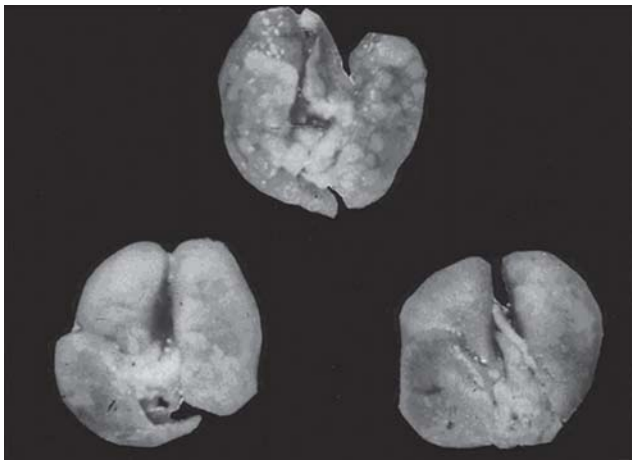

Figure 2. Antitumor effect of different microsphere formulations in mice bearing pulmonary carcinoma. Upper: no treatment; lower left: CPt-MS (carboplatin gelatin microsphere, 8 mg CPt/kg); lower right: CPt (carboplatin, 16 mg CPt/kg).
Adapted with permission from [23].

potent lung-targeting characteristics in dogs. Microspheres have also been used for targeting drugs for tuberculosis. *Mycobacterium tuberculosis,* a causative agent in the lung, is phagocytosed by alveolar macrophages, where it mainly resides. Long-term oral administration of high doses of multiple drugs for treating tuberculosis leads to serious side effects, such as liver damage. Delivering drugs to sites deep within the lungs where *M. tuberculosis* resides is difficult [32-36]. ESAT-6 (early secreted antigenic target, 6 kDa) from *M. tuberculosis* is an important T-cell antigen for cell-mediated immunity in the early phase of tuberculosis infection. As the lung is the organ in which infection is initiated, immune responses in the lung play a significant role in restricting the initial infection with *M. tuberculosis.* Carpenter *et al.* [37] assessed whether efficient cell-meditated immune responses in the lung and draining mediastinal lymph nodes could be stimulated by pulmonary administration of ESAT-6 encapsulated in poly(lactide) (PLA) microspheres. Intranasal instillation of microencapsulated ESAT-6 induced higher numbers of ESAT-6-specific IFN-γ and IL-4-secreting cells in the lung and lymphnode. Similarly, ESAT-6-specific recall responses were strongest following intranasal immunization of mice with microsphere-encapsulated antigen. Fluorescence-activated cell sorting (FACS) demonstrated a higher proportion of T cells expressing CD44 in the mediastinal lymph nodes (MLN) from mice immunized intranasally with microencapsulated ESAT-6. These data support the notion that the immune system is compartmentalized and responses are often strongest in compartments proximal to the site of vaccine application.

Tomoda and Makino [38] prepared inhalable and monodisperse poly(lactide-*co*-glycolide) (PLGA) microspheres targeting tubercle bacilli residing in alveolar macrophages and studied the effects of pulmonary surfactant on the Rifampicin (RFP) release rate from RFP-loaded poly(lactide-*co*-glycolide) microspheres. They found that release rate of RFP from RFP/PLGA microspheres was accelerated by adsorption of pulmonary surfactant on the particle surface and the results suggested that when RFP/PLGA microspheres are administrated by inhalation, the RFP release rates from the particles, which are not taken up by alveolar macrophages and remain in the alveoli, will be small. On the other hand, the RFP release rates and release amounts will be high after RFP/PLGA microspheres are taken up by alveolar macrophages existing in phagosomes, but become relatively small after RFP/PLGA microspheres move into phagosome–lysosomes by the fusion of phagosomes with lysosomes. Changes in surface properties of PLGA microspheres also affect the uptake efficiency by alveolar macrophages. The absolute values of the electrophoretic mobility of PLGA microspheres increase by the adsorption of pulmonary surfactants on the surfaces of PLGA microspheres. The surface of PLGA microspheres became harder and the electric charge density increased with the adsorption of pulmonary surfactant on the surfaces of PLGA microspheres. After intravenous administration, microspheres 7 μm or more in diameter are rapidly accumulated in the lungs by mechanical filtration, whereas microspheres with a diameter of 5 μm or less are mainly taken up by cells of the reticuloendothelial system predominantly in the liver [39]. Clarithromycin acts like erythromycin and has a similar spectrum of antibacterial activity and is used for respiratory tract infections, including atypical pneumonias and soft tissue infections [40]. Ozkan *et al.* [41] prepared clarithromycin-loaded microspheres from human serum albumin having diameter of 7 – 15 μm and injected into the tail vein of mice for size distribution and *in vivo* evaluation. Their results showed that microsphere accumulation in the lung began at 10 min and increased gradually until 6 h, followed by decline. The microspheres were also found after 24 h. No microsphere accumulation was observed in the liver at any time. Hyaluronan is a microsphere-forming material because of its mucoadhesiveness secreted endogenously in the lung, and is biodegradable in the alveolar macrophages [42-45], protects against injury in several respiratory diseases [46] and prevents pleural thickening in tuberculosis patients [47]. Hwang *et al.* [48] prepared ofloxacin containing hyaluronan microspheres with a mean diameter of 2 – 5 μm and delivered to lung alveolar macrophages for the treatment of tuberculosis. The area under the ofloxacin concentration curve from time zero to infinity (AUC) was estimated for plasma and lungs in rats following intratracheal, intravenous and oral administration of hyaluronan microspheres (HMO), ofloxacin microspheres (MO), and an aqueous solution of ofloxacin (OS), at an equivalent ofloxacin dose of 8 mg/kg rat. The AUC ratio between the lung and plasma for intratracheal-administered HMO was 10.9-, 9.3- and 1.8-fold greater than intravenous OS, oral OS and intratracheal MO, respectively. The *in vitro* uptake of

ofloxacin from HMO by air-surface cultured alveolar macrophages (RAW264.7) was 2.1- and 1.7-fold higher than ofloxacin uptake from OS and MO. The results showed that pulmonary administration of ofloxacin by means of HMO would improve the treatment efficacy of ofloxacin against tuberculosis compared with other forms of ofloxacin (OS and MO) and with other routes of administration (intravenous and by mouth).

Terbutaline sulfate (TBS) is a β-adrenergic receptor agonist used as a bronchodilator for the treatment of bronchial asthma, chronic bronchitis and emphysema [49]. Sahin *et al.* [50] prepared terbutaline-loaded bovine serum albumin microspheres with mean particle size in the range 22 – 30 μm. Drug release showed a biphasic pattern characterized by an initial fast release, followed by a slower release decreased by increasing the concentration of glutaraldehyde, a crosslinking agent. In the absence of trypsin, the time required for complete degradation of microspheres was increased from 144 to 264 h when the glutaraldehyde concentration increased from 0.1 to 0.7 ml. Biodistribution studies after intravenous administration of ^{99m}TC-labeled microspheres into the tail vein of Swiss albino male mice indicated that the degree of uptake by the lungs was higher than that of the other organs and suggested that terbutaline sulphate-loaded microspheres can be used for passive lung targeting.

3. Nanoparticle-mediated lung targeting

Lung cancer causes a high number of deaths every year worldwide in both males and females. Present research in targeted therapeutics for lung cancer includes inhalable and injectable systems. There are many types of nanoparticle system now being explored for drug delivery to lungs, especially cancer therapeutics [51]. The material properties of each nanoparticle system have been developed to enhance delivery to the tumor. For example, hydrophilic surfaces can be used to provide the nanoparticles with stealth properties for longer circulation times and positively charged surfaces can enhance endocytosis. The types of nanoparticle used at present in research for cancer therapeutic applications include polymeric nanoparticles [52], micelles [53], protein nanoparticles [54], ceramic nanoparticles [55], viral nanoparticles [56] and metallic nanoparticles [57]. Functionalization of nanoparticles provides a stealth surface to prevent opsonization, which is the adherence of serum proteins to the nanoparticle surface [58,59]. Short circulation times decrease the efficiency of the delivery of the nanoparticle to the tumor site. Incorporation of a hydrophilic polymer, such as poly(ethylene glycol) (PEG), to the surface of the nanoparticle allows for a reduction in opsonization, which reduces removal by the reticuloendothelial system [60,61].

Interaction of leukocyte function-associated antigen-1 (LFA-1) and intercellular cell adhesion molecule-1 (ICAM-1) can play a critical role in tumor metastasis and progression [62,63]. Cyclo-(1,12)-PenITDGEATDSGC (cLABL), a cyclic peptide, specifically inhibited homotypic and heterotypic T-cell adhesion to epithelial and endothelial cell monolayers by disrupting the LFA-1/ICAM-1. Recently Chittasupho *et al.* [64] conjugated cLABL interaction to poly (DL-lactic-*co*-glycolic acid) nanoparticles (cLABL-NP) targeting ICAM-1 on A549 lung epithelial cells. Their internalization studies have demonstrated that nanoparticles can be targeted to these epithelial cells by means of ICAM-1 and can be internalized into the cells rapidly, as confirmed by fluorescence microscopy. The results indicated that PLGA nanoparticles targeted by cLABL localized within A549 cells to provide sustained release. Uptake of cLABL-NP to A549 cells was found to be significantly more rapid compared with untargeted nanoparticles with 2.3-fold higher fluorescence intensity compared with the control nanoparticles after 5 min of incubation.

Tsenga *et al.* [65] developed gelatin nanoparticles biotinylated for lung cancer targeting, and compared the activity of GP (gelatin nanoparticles), GP–Av (GP modified with NeutrAvidinFITC) and GP–Av–bEGF (GP–Av conjugated with biotinylated EGF). When the biopolymer carrier is engrafted with particular ligands such as epithelial growth factor (EGF) it can be used for more specific recognition and interaction with cancer cells because of the overexpression of EGF receptor (EGFR) on human tumors, especially on non-small-cell lung cancer. They studied the accumulation of nanoparticles into different cell lines such as A549 (adenocarcinoma cells), HFL1 (normal lung fibroblast) and H520 (lung sequencema cell). The results revealed that A549 cells showed a high expression of EGFR, whereas HFL1 cells showed a moderate expression and H520 cells showed nearly no expression of EGFR. After incubation of GP–Av–bEGF with cells for 3 h, the highest binding capacity was observed in A549 cells (81%), whereas it was less distinctive in HFL1 cells (55%) and in H520 cells (40.8%).

Brzoska *et al.* [66] prepared gelatin nanoparticles as well as nanoparticles based on human serum albumin (HAS). These were administered in primary airway epithelium cells and the cell line 16HBE14o- in a concentration- and temperature-dependent manner and it was found that nanoparticle incorporation is an active, endocytosis-like process, and not diffusion through the cellular membrane. The cytotoxicity in primary pulmonary epithelium cells and 16HBE14o- cells showed only little or no cytotoxicity. This aspect makes them highly suitable as drug carriers or for gene therapy on the human airway epithelium. On the other hand, polyalkylcyanoacrylate nanoparticles are highly toxic for airway epithelium cells. The toxicity is dependent on alkyl side chain length, with short side chains being more cytotoxic than longer ones [67].

Nguyen *et al.* [68] developed a highly efficient nanocomposite aerosol for pulmonary gene delivery, consisting of a biodegradable polymer core, poly[vinyl-3-(diethylamino)propylcarbamate-*co*-vinyl acetate-*co*-vinyl alcohol]-*g*-poly(DL-lactide-*co*-glycolide), (P(68)-10). They studied the transfection efficiency to lung cells by coating different concentrations of

carboxy methyl cellulose (CMC) and poloxamer. They prepared plain nanoparticles without coating materials (P(68)-10), nanocomposites with 0.0075% CMC (P(68)-10 CMC 1), nanocomposites with 0.00075% CMC (P(68)-10 CMC 2) and nanocomposites with 0.0075% CMC P(68)-10 poloxamer. The MTT cytotoxic assay demonstrated that the P(68)-10 CMC 2 formulation was not toxic because 80% of the cells remained intact. Lack of cell death was a result of the low transfection efficiency and negative zeta potential of the particles. Negatively charged particles showed less interaction with the negatively charged cell membrane, whereas all other nanocomposite formulations with positive surface charges displayed higher toxicity profiles.

Zou *et al.* [69] prepared PLGA nanoparticles by using bioadhesive agent and stabilizer Carbopol (CP) 940 to establish bioadhesive PLGA nanoparticles and Pluronic F68- and Pluronic F127-stabilized PLGA nanoparticles, and found both 1% F68-NP and 1% F127-NP showed relatively lower transfection efficiency than Lipofectamine 2000 (control) over 48 h. In the case of 0.02% CP-NP, the transfection efficiency in 48 h was comparable to that of Lipofectamine 2000, but transfection efficiency was still lower in 24 h owing to its sustained release property. They demonstrated that CP-stabilized PLGA nanoparticles performed better in A549 cell transfection and they could be used as a potential delivery system for DNA in lung cancer gene therapy. Kawashima *et al.* [70] prepared PLGA nanoparticles with insulin for guinea-pig lungs and demonstrated a significant reduction in blood glucose level, with a prolonged effect over 48 h as compared with insulin solution. Zhang *et al.* [71] also studied insulin-loaded nanoparticles but with a different polymer, poly(butyl cyanoacrylate). Their results suggested that the duration of a hypoglycemic effect extends over 20 h (glucose level < 80% of the original levels).

Yamamoto *et al.* [72] produced chitosan-modified PLGA nanoparticles and encapsulated the peptide elcatonin and found that they reduced blood calcium levels to 80% of initial calcium concentrations. These results suggested that chitosan modified PLGA nanoparticles provide prolonged effects up to 24 h after pulmonary administration in guinea pigs as compared to unmodified nanoparticles.

Vaughn *et al.* [73] prepared itraconazole (ITZ) nanoparticles with Polysorbate 80 and Poloxamer 407, and delivered them to mice by the pulmonary route and found significantly high lung tissue concentrations as compared with oral gavage of solutions of either drug or nanoparticles. Pandey *et al.* [74] delivered PLGA nanoparticles by means of inhalation to the lungs of guinea-pigs. They found that therapeutic drug concentrations were maintained in the plasma for 6 – 8 days and in the lungs for 9 – 11 days. The same group also prepared poly(DL-lactide-*co*-glycolide) (PLG) nanoparticles encapsulating three antitubercular drugs, that is, rifampicin (RIF), isoniazid (INH) and pyrazinamide (PZA). Their studies showed that an enhanced sustained release of the drugs (without altering the drug dosage) is possible with PLG-NP. Following oral administration of drug-loaded PLG-NP, the drugs were present at or above MIC in the plasma for 4 days in the case of RIF, and 9 days in the case of INH and PZA. Oral administration of free drugs daily or drug-loaded PLG-NP every 10 days to *M. tuberculosis* H37Rv-infected mice for 46 days resulted in complete bacterial clearance from the organs [75]. Sharma *et al.* [76] prepared PLGA nanoparticles and functionalized them with wheat germ agglutinin (WGA), a lectin having bioadhesive properties that could potentially interact with lectin receptors on the alveolar epithelium of lungs. They found that antibiotic-containing nanoparticles elevated drug levels slightly longer than non-functionalized particles, 6 – 14 days in the plasma and 15 days in the lungs.

Apart from simple nanoparticles, magnetic nanoparticles have also emerged as interesting carrier systems during the last two decades for diagnostic and/or treatment purposes. Mykhaylyk *et al.* [77] formulated doxorubicin magnetic conjugate nanoparticles (DOX-M) and evaluated the pharmacokinetics in a mouse model. They injected DOX-M suspensions into the eye sinus vein of adult male mice and applied a magnetic field centered over the left lung, and found that the magnetic field can significantly increase the bioavailability of DOX-M in the lungs, whereas Wu *et al.* [78] showed that after intravenous injection of dextran-coated Fe_3O_4 in rats using an applied external magnetic field did not change the accumulation of nanoparticles in the lungs. Monoclonal antibodies can also improve the drug delivery by attaching them to a carrier system [79-83]. Akasaka *et al.* [84] prepared nanoparticles made from bovine serum albumin (BSA) and conjugated them with monoclonal antibodies (Lewis lung carcinoma monoclonal). They injected them into Lewis lung carcinoma-bearing mice and found only slight localization of nanoparticles in the carcinoma tissue, and even after 24 h the number of nanoparticles was low. This study, however, showed that the particle size was more important than the affinity of the monoclonal antibodies to the tumor cells.

4. Liposome-mediated lung targeting

Liposomes are potential drug and/or gene carrier owing to their high loading capacity for a variety of compounds, allowing chemical modification for a wide range of applications, minimizing the systemic toxicity of the incorporated drugs and improving their stability [85-88]. Wijagkanalan *et al.* [89] evaluated the targeting efficiency to alveolar macrophages by intratracheal administration of mannosylated liposomes (Man-liposomes). Mannose receptors, a 175 kDa transmembrane protein of the C-type lectin family, are expressed exclusively on the surface of alveolar macrophages that can recognize mannose terminal molecules with high affinity [90]. Wijagkanalan *et al.* [89] found concentration-dependent *in vitro* uptake by alveolar macrophages of Man-liposomes. The internalization of Man-liposomes with

7.5% (Man-7.5-liposomes) and 5.0% (Man-5.0-liposomes) Man-C4-Chol was considerably higher than that of Man-liposomes with 2.5% of Man-C4-Chol (Man-2.5-liposomes) and bare-liposomes. Internalization of Man-liposomes was significantly inhibited by an excess of mannan, suggesting mannose receptor-mediated endocytosis. Moreira *et al.* [91] examined the ability of a growth factor antagonist, ([D-Arg6, D-Trp7;9-NmePhe8]-substance P(6 – 11), named antagonist G), for selectively targeting polyethylene glycol-grafted liposomes (i.e., sterically stabilized liposomes) to a human classical small-cell lung cancer (SCLC) cell line, H69. Their results showed that radiolabeled antagonist G-targeted sterically stabilized liposomes (SLG) bound to H69 cells with higher avidity than free antagonist G and were internalized (reaching a maximum of 13,000 SLG/cell), mainly through a receptor-mediated process that was confirmed by confocal microscopy. It was shown that SLG significantly improved the nuclear delivery of encapsulated doxorubicin to the target cells, increasing the cytotoxic activity of the drug over non-targeted liposomes. ^{125}I tyraminylinulin-containing SLG were long circulating, with a half-life of 13 h in mice and also improved therapy of SCLC by sterically stabilized liposomes.

Zhang *et al.* [92] studied the biodistribution of 9-nitrocamptothecin (9-NC)-loaded liposomes following pulmonary delivery and found that the mean residence time (MRT) of 9-NC liposomes in the lungs was 3.4 times that of 9-NC solution. The total $AUC_{0\text{-}t}$ of all tissues in mice of the liposomes was 2.2-fold higher than that of the solution, indicating that the liposomes had sustained-release characteristics. After intravenous and intratracheal administration the targeting efficiency value (*Te*) was calculated from the equation: $Te = AUC_{0\text{-}\infty}$ (target tissue)/$\sum_{i=0}^{n}$ = ($AUC_{0\text{-}\infty}$) non-target tissue. The targeting efficiency of 9-NC liposomes to the lung was 0.14 and 2.02, respectively, which showed that intratracheal instillation can deliver the drug mainly to the lung and decrease the accumulation of the drug in other tissues at different concentrations, and the lung damage by liposomes was less severe than that by the 9-NC solution. Mylonakis *et al.* [93] examined the efficacy and safety of a liposomal cisplatin (Lipoplatin)-gemcitabine versus a cisplatin-gemcitabine combination and found that it caused lower toxicity, mainly nephrotoxicity, as well as higher efficacy than cisplatin, when combined with gemcitabine in advanced non-small-cell lung cancer (NSCLC), and there were no associated deaths, or life-threatening adverse events. Trammer *et al.* [94] prepared aerolized cyclosporine A (CsA) and compared the kinetic behavior of a propylene glycol solution of cyclosporine (CsA-PG) with a liposomal formulation (L-CsA). Their results showed that permeability across the human bronchial cell line Calu-3 (cell culture model) revealed low permeability for CsA. The apparent permeability for CsA-PG being twice as high as for L-CsA, the degree and rate of drug transfer into human blood were more pronounced for CsA-PG than for L-CsA, with the AUC of CsA-PG being about 1.6 times higher than that of the L-CsA formulation.

Folate receptors have also been used for targeting cancer and are frequently overexpressed on epithelial cancer cells and have been exploited to deliver specifically liposome-encapsulated drug molecules *in vitro*. Lee and Low [95] prepared folate-conjugated liposomes with the help of PEG (folate-PEG) and were loaded with doxorubicin (DOX). They found that the uptake of folate-PEG-liposomal DOX by KB (a human nasopharyngeal epidermal carcinoma) cells was 45-fold higher than that of non-targeted liposomal DOX and 1.6 times higher than that of free DOX, whereas the cytotoxicity was 86 and 2.7 times higher, respectively.

Waldrep *et al.* [96] developed concentrated, high-dose liposomal formulations initially using immunosuppressive (CsA) and anti-inflammatory drugs (budesonide [Bud]) for targeted pulmonary delivery with maximal aerosol output and particle size ranges within the optimal range of 1 – 13 µm. Their results indicated that with increasing drug-liposome concentrations there was reduced nebulized mass output. Transferrin receptor (TfR) is one of the successful targeting molecules [97], with a density of 10,000 – 100,000 molecules per cell commonly found on tumor cells [98]. Takada *et al.* [99] prepared liposomes labeled with [C^{14}] coenzyme Q_{10} in the lipid bilayer coated with various polysaccharide derivatives, that is, palmitoyl conjugates of pullulan, pullulan phosphate, amylopectin, amylopectin phosphate and amylopectin sulfate. After intravenous injection of the liposomes into guinea-pigs, the lung uptake of *O*-palmitoyl amylopectin- and *O*-palmitoyl amylopectin phosphate-coated liposomes was 5 and 3 times higher, respectively, at 30 min than that of the conventional liposomes.

Therapeutic activity of stealth liposomes loaded with doxorubicin (DXR) was examined by Moreira *et al.* [100] in Balb/c nude mice xenografts inoculated subcutaneously with the human small-cell lung cancer cell line (H69). Mice were treated with non-targeted liposomes (SL) or liposomes targeted with antagonist G coupled to the liposome surface (SLG). SLG showed 30 – 44-fold higher binding to H69 cells harvested from H69 xenografts as compared with SL. At 48 and 72 h post-injection, tumor accumulation of [^{125}I] tyraminylinulin-containing liposomes was shown to be dependent on liposome size but independent of the presence of the targeting ligand. Maximum tumor uptake of either SLG or SL ranged from 2 to 4% of injected dose per gram of tissue. Asaia *et al.* [101] investigated liposomes composed of 5′-*O*-dipalmitoylphosphatidyl (DPP) derivative of 2′-C-cyano-2′deoxy-1-β-D-arabino-pentofuranosylcytosine (CNDAC) DPP-CNDAC and cholesterol (DPP-CNDAC/CH liposomes). CNDAC is a new antitumor agent involving a DNA-self-strand-breaking mechanism after incorporation of the agent into the DNA strand [102,103]. CNDAC phosphorylated by deoxycytidine kinase inhibits DNA polymerase for DNA elongation and breaks the DNA strand during enzymatic DNA-chain elongation [104]. These DPP-CNDAC/CH liposomes were markedly accumulated in mice lung bearing B16BL6 melanoma and reduced the lung colonization in a

dose-dependent manner, and activity was significantly superior to conventional liposomal formulation or soluble CNDAC [101]. Chono *et al.* [105] prepared mannosylated ciprofloxacin (CPFX, a first-generation fluoroquinolone antimicrobial drug)-liposomes with particle size ~ 1000 nm and found that the targeting efficiency of ciprofloxacin to rat alveolar macrophages following pulmonary administration of mannosylated CPFX-liposomes was significantly greater than that of ciprofloxacin incorporated into unmodified liposomes.

Zaru *et al.* [106] investigated applicability of RIF (an antitubercular drug) containing chitosan (CHT)-coated liposomes as a carrier for delivery of drugs to the lungs by nebulization. They found that mucoadhesive properties of CHT-coated liposomes were substantially better (compared with non-coated ones) whereas the toxicity of liposomal RIF towards A549 epithelial cells was lower compared with free drug. Schijelers *et al.* [107] studied the circulation kinetics of PEG-coated liposomes by the incorporation of different amounts of phosphatidylserine (PS) and variation of lipid dose in a rat model of an acute unilateral *Klebsiella pneumoniae*. Their results suggested that the degree of liposome localization at the target site is positively linearly related to the area under the blood concentration–time curve of the liposome formulations, irrespective of PEG coating. Abraham and Walubo [108] studied the disposition of gentamicin to the normal rat brain, lung, kidney and liver after intraperitoneal injection of gentamicin encapsulated in positive, negative and neutral liposomes and found that the average concentrations of gentamicin in the liver and the brain were highest with positive liposomes, whereas gentamicin concentrations in the kidneys and lungs were not influenced by surface charge of the liposomes.

5. Dendrimer-mediated lung targeting

Dendrimer is a Greek word consisting of 'dendron' meaning tree and 'meros' means part; structurally it is hyperbranched, monodisperse, three-dimensional macromolecules resembling the architecture of a tree. Dendrimers are one of the newest carrier systems used for delivery of drug(s) with better prospects [109-112]. Typically, a dendrimer consists of three main structural components: a multifunctional central core, branched units and surface groups [113]. The partical size of dendrimers, ranging from 1 to 100 nm, makes them less susceptible to uptake by the reticuloendothelial system [114]. Papagiannaros *et al.* [115] studied the cytotoxic activity of doxorubicin–PAMAM dendrimer attached to liposome against human lung cell lines. The liposome composed of hexadecylphosphocholine (antitumor ether lipid), egg yolk phosphatidylcholine, stearylamine (HePC:EPC:SA, 10:10:0.1) formulation 1 and EPC:SA (10:0.1) formulation 2 and their results demonstrated that the doxorubicin–PAMAM complex retained high growth-inhibiting activity in NCI-H460 and DMS114 lung cell, which was decreased when the complex attached to EPC:SA liposomes, whereas incorporation of HePC increased the activity. Perumal *et al.* [116] studied the influence of surface functionality on their uptake in A549 lung epithelial cells by using flow cytometry and fluorescence microscopy (**Figure 3**). Their results showed that anionic dendrimers are taken up mainly by caveolae-mediated endocytosis in A549 lung epithelial cells, whereas cationic and neutral dendrimers are taken up by a non-clathrin, non-caveolae-mediated mechanism.

Kukowska-Latallo *et al.* [117] studied the effect of Exosurf (synthetic lung surfactant) on dendrimer-mediated transfection in eukaryotic cells and found that Exosurf significantly enhanced the dendrimer luciferase plasmid transfection rate of human T-cell leukemia. The Jurkat cell line improved significantly from 10 to 90% of cells at 24 h, while the non-ionic surfactant tyloxapol enhanced the dendrimer-mediated gene transfer owing to increased cell membrane porosity and DNA uptake. Kumar *et al.* [118] synthesized rifampicin (anti-tuberculosis drug)-loaded mannosylated dendrimers and studied macrophage uptake. They found that rifampicin-loaded mannosylated dendrimers enhance alveolar macrophage uptake. Kumar [119] assessed the lung targeting efficiency of rifampicin-loaded PEGylated poly (propylene imine) (PPI) dendrimers after intravenous administration and found higher accumulation of drug in the lung in the case of PEGylated dendrimers as compared with plain PPI loaded with rifampicin and free rifampicin. Kannan *et al.* [120] prepared the methylprednisolone (MP)–polyamidoamine dendrimer (PAMAM G4-OH) complex for treatment of asthma and found that the residence times for the dendrimers were significantly higher than for free drug after intranasal delivery, > 35% of the dendrimer was recovered from the lung in 24 h after intranasal delivery. Elfinger *et al.* [121] also characterized lactoferrin receptor on bronchial epithelial cells of the lung for targeting purposes. They carried out gene delivery to lungs by using lactoferrin-conjugated polyethylenimine branched polymer.

6. Niosome-mediated lung targeting

Niosomes are non-ionic surfactant-based unilamellar and multilamellar vesicles used as a carrier system for drug delivery. Zhang and Lu [122] prepared the lung-targeted carboplatin-loaded niosome (CBP-NS) with mean diameter 3.72 μm to improve the treatment efficacy and reduce side effects. Their results showed that the antitumor effects were significantly increased by injection of CBP-NS as compared with CBP in the treatment of mice with lung carcinoma. Gude *et al.* [123] studied the effects of niosomal-encapsulated cisplatin and theophylline on activated macrophages in a murine B16F10 melanoma model. In an *ex vivo* study they found that cisplatin-encapsulated niosomes have significant antimetastatic activity on secondary growth of tumors in lung and reduced toxicity compared with free cisplatin. However, theophylline failed to show an antimetastatic effect alone or in combination with cisplatin or with activated

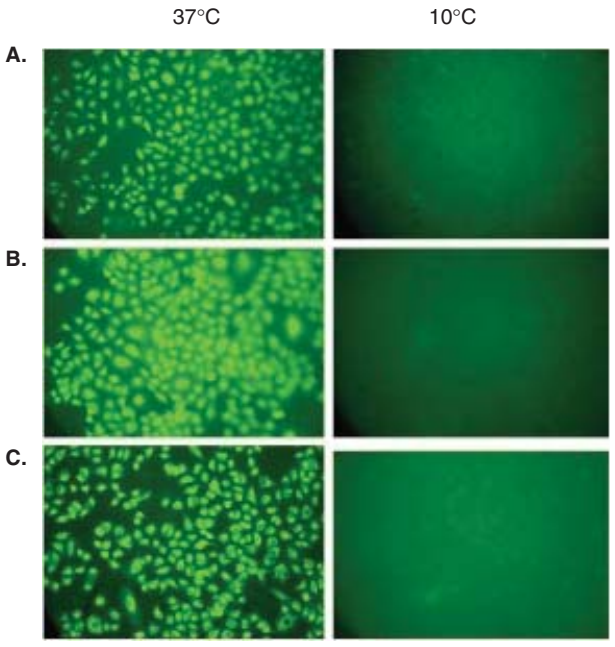

Figure 3. Fluorescence microscopic images (40) of A549 lung epithelial cells after incubation with the FITC-labeled dendrimers for 1 h at 37 and 10°C for (A) amine dendrimer, (B) carboxyl dendrimer and (C) hydroxyl dendrimer. The cell uptake is arrested for all the three dendrimers at low temperature, suggesting that they are endocytosed by an active process.
Adapted with permission from [116].

macrophages. Jain and Vyas [124] prepared rifampicin-loaded niosomes of Span-85 and cholesterol in various molar fractions with 8 – 15 μm diameters. After oral administration in rats they found that 65% of the drug could be localized in the lungs by controlling the niosome size.

7. Conclusion

Nano- and microcarrier systems play an important role in the targeting of drug(s), proteins and peptides. Carriers provide sustained drug delivery to the lungs, extend duration of action, reduce the therapeutic dose, improve patient compliance, and reduce the adverse effects of highly toxic drugs. Recently, bioactive molecules such as antibodies attached to the surface of the carriers have provided a better platform for drug delivery to the lungs. However, studies that clearly demonstrate the receptors that are highly expressed on lungs, as compared with other vital organs, are scarce. There is also a need to identify the receptors that are present exclusively on lungs. The identification of such receptors may further facilitate drug targeting not only to lungs but also to specific parts of lungs, such as the bronchioles and alveoli. Further research efforts are needed to ensure the safety of long-term *in vivo* applications. There is an urgent requirement for cautiously designed toxicology and toxicokinetic studies for each nanocarrier type; the protocols would have to be customized to address the likely clinical use. Any nanocarrier that attains the 'generally regarded as safe' (GRAS) status will enjoy overwhelming therapeutic acceptance in terms of safety and efficacy as well as unlimited market potential.

8. Expert opinion

The pulmonary region of human beings is a critical organ having different parts such as bronchi, bronchioles and alveoli. These parts may be involved in various types of disease, for example, obstructive lung diseases, respiratory tract infections, respiratory tumors, pleural cavity diseases, pulmonary vascular diseases, and so on. Owing to the specific physiological environment and large surface area of the alveolar region, lower thickness of the epithelial barrier, extensive vascularization, relatively low proteolytic activity in the alveolar space as compared with other routes of administration and absence of the first-pass metabolism (i.e., gut), drug delivery to lungs become more desirable for treating lung-associated dysfunctions and diseases. Particle-based micro- and nanotechnology plays an important role in providing a new and proficient drug delivery system that can overcome the problems that arise with conventional treatment of lung diseases. Targeted therapy of drug to lungs provides maximum deposition of suitable therapeutic agents at the lung surface, minimizes potential drug side effects and reduces healthcare cost with increased efficacy. This review has focused mainly on targeted delivery of drugs to the lungs through various micro- and nanocarriers such as nanoparticles, liposomes, dendrimers, niosomes, microspheres, and so on. The ligand-based carrier system is a recently attractive strategy for the localization of therapeutic agent to lung owing to its specificity to a particular disease (monoclonal antibody, folate receptor overexpression on cancer cells) and organ-expressed receptor (lactoferrin receptor on bronchial epithelial surface). There is still a need to identify and specify the receptors that are present exclusively on lungs. The identification of such receptors may further facilitate drug targeting to lungs, including specific parts of lungs such as bronchioles and alveoli. However, the carrier systems discussed above are at a preliminary stage of use in lung targeting. These carrier systems need to be explored in human subjects to establish their potential use in lung targeting of bioactive compounds.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Azarmi S, Roa WH, Lobenberg R. Targeted delivery of nanoparticles for the treatment of lung diseases. Adv Drug Deliv Rev 2008;60:863-75
2. Banov CH. Anatomy and physiology of the lower and upper airway. J Allergy Clin Immunol 1989;84:1044-6
3. Wolfson MR, Shaffer TH. Respiratory physiology: structure, function, and integrative responses to intervention with special emphasis on the ventilatory pump. Cardiopulm Phys Ther 2004;4:40
4. Patton JS, Platz RM. Pulmonary delivery of peptides and proteins for systemic action. Adv Drug Deliv Rev 1992;8:179-96
5. Clark A. Formulation of proteins and peptides for inhalation. Drug Deliv Systems Sci 2002;2:73-7
6. Courrier HM, Butz N, Vandamme TF. Pulmonary drug delivery systems: recent developments and prospects. Crit Rev Ther Drug Carr Syst 2002;19:425-98

• **This review demonstrated the advantages of pulmonary drug delivery and desirable factors important for pulmonary delivery.**

7. Gill S, Lobenberg R, Ku T, et al. Nanoparticles: characteristics, mechanisms of action and toxicity in pulmonary drug delivery-a review. J Biomed Nanotechnol 2007;3:107-19
8. Ranney DF. Drug targeting to the lungs. Biochem Pharmacol 1986;35:1063-9

• **This review demonstrated new scientific information and considerable factors in the field of pulmonary drug targeting.**

9. Raeburn D, Underwood SL, Villamil ME. Techniques for drug delivery to the airways, and the assessment of lung function in animal models. J Pharmacol Toxicol Methods 1992;27:143-59
10. Thompson DC. Pharmacology of therapeutic aerosols. Marcel Dekker, New York; 1992
11. Byron PR. Prediction of drug residence times in regions of the human respiratory tract following aerosol inhalation. J Pharm Sci 1986;75:433-8
12. Burton A, Schanker LS. Absorption of corticosteroids from the rat lung. Steroids 1974;23:617-24
13. Brown RA, Schanker LS. Absorption of aerosolized drugs from the rat lung. Drug Metab Dispos 1983;11:355-60
14. Smolensky MH, D'Alonzo GE, Kunkel G, et al. Day-night patterns in bronchial patency and dyspnoea: basis for one daily and unequally divided twice daily dosing schedules. Chronobiol Int 1987;4:303-7
15. Barnes PJ. Nocturnal asthma: mechanisms and treatment. Br Med J 1984;12:1397-8
16. Surendrakumar K, Martyn GP, Hodgers ECM, et al. Sustained release of insulin from sodium hyaluronate based dry powder formulations after pulmonary delivery to beagle dogs. J Control Release 2003;91:385-94
17. Cook RO, Pannu RK, Kellaway IW. Novel sustained release microspheres for pulmonary drug delivery. J Control Rel 2005;104:79-90
18. Zeng XM, Martin GP, Marriott C. The controlled delivery of drugs to the lung. Int J Pharm 1995;124:149-64

• **This review demonstrated the importance of controlled drug delivery to lungs.**

19. Hardy JG, Chadwick TS. Sustained release drug delivery to the lungs. Clin Pharmacokinet 2000;39:1-4
20. Ventura CA, Tommasini S, Crupi E, et al. Chitosan microspheres for intrapulmonary administration of moxifloxacin: interaction with biomembrane models and in vitro permeation studies. Eur J Pharm Biopharm 2008;68:235-44
21. Makino K, Yamamoto N, Higuchi K, et al. Phagocytic uptake of polystyrene microspheres by alveolar macrophages: effects of the size and surface properties of the microspheres. Colloids Surf Biointerfaces 2003;27:33-9

• **The paper provides information about the effects of size and surface properties of microspheres on alveolar macrophages' phagocytic uptake.**

22. Huo D, Deng S, Li L, et al. Studies on the poly(lactic-co-glycolic) acid microspheres of cisplatin for lung-targeting. Int J Pharm 2005;289:63-7
23. Lu B, Zhang JQ, Yang H. Lung-targeting microspheres of carboplatin. Int J Pharm 2003;265:1-11
24. Yang Y, Bajaj N, Xu P, et al. Development of highly porous large PLGA microparticles for pulmonary drug delivery. Biomaterials 2009;30:1947-53
25. Schroder J. Enrofloxacin: a new antimicrobial agent. J S Af Vet Assoc 1989;60:122-4
26. Prescott JF, Yielding KM. In vitro susceptibility of selected veterinary bacterial pathogens to ciprofloxacin, enrofloxacin and norfloxacin. Can J Vet Res 1990;54:195-7
27. Hannan PCT, Windsor GD, Jong A, et al. Comparative susceptibilities of various animal-pathogenic mycoplasmas to fluoroquinolones. Antimicrob Agents Chemother 1997;41:2037-40
28. Bebear CM, Renaudin J, Charron A, et al. Mutations in the gyrA, parC, and parE genes associated with fluoroquinolone resistance in clinical isolates of Mycoplasma hominis. Antimicrobiol Agents Chemother 1999;43:954-6
29. Wu CC, Shryock TR, Lin TL, et al. Antimicrobial susceptibility of Mycoplasma hyorhinis. Vet Microbiol 2000;76:25-30
30. Gautier-Bouchardon AV, Reinhardt AK, Kobisch M, et al. In vitro development of resistance to enrofloxacin, erythromycin, tylosin, tiamulin and oxytetracycline in Mycoplasma gallisepticum, Mycoplasma iowae and Mycoplasma synoviae. Vet Microbiol 2002;88:47-58
31. Tang S, Zhou Y, Li R, et al. Pharmacokinetics and lung-targeting characterization of a newly formulated enrofloxacin preparation. J Vet Pharmacol Ther 2007;30:443-50
32. Suarez S, O'Hara P, Kazantseva M, et al. Respirable microspheres containing rifampicin for the treatment of tuberculosis: screening in an infections disease model. Pharm Res 2001;18:1315-19
33. Makino K, Nakajima T, Shikamura M, et al. Efficient intracellular delivery of

rifampicin to alveolar macrophages using rifampicin-loaded PLGA microspheres: effect of molecular weight and composition of PLGA on release of rifampicin. Colloids Surf Biointerfaces 2004;36:35-42

34. Sharma R, Saxena D, Dwivedi AK, et al. Inhalable microparticles containing drug combinations to target alveolar microphages for treatment of pulmonary tuberculosis. Pharm Res 2001;18:1405-10

35. Schwartz YS, Dushikin MI, Vavilin VA, et al. Novel conjugate of moxifloxacin and carboxymethylated glucan with enhanced activity against Mycobacterium tuberculosis. Antimicrob Agents Chemother 2006;50:1982-8

36. Muttil P, Kaur J, Kumar K, et al. Inhalable microparticles containing large payload of anti-tuberculosis drugs. Eur J Pharm Sci 2007;32:140-50

37. Carpenter ZK, Williamson ED, Jim E, et al. Mucosal delivery of microparticle encapsulated ESAT-6 induces robust cell-mediated responses in the lung milieu. J Control Rel 2005;104:67-77

38. Tomoda K, Makino K. Effects of lung surfactants on rifampicin release rate from monodisperse rifampicin-loaded PLGA microspheres. Colloids Surf B Biointerfaces 2007;55:115-24

39. Illum L, Davis SS, Wilson CG, et al. Blood clearance and organ deposition of intravenously administered colloidal particles. The effects of particle size, nature and shape. Int J Pharm 1982;12:135-46

40. Laurence DR, Bennett PN, Brown MJ. Clinical pharmacology. Churchill Livingstone, New York; 1997

41. Ozkan Y, Dıkmen N, Isimer A, et al. Clarithromycin targeting to lung: characterization, size distribution and in vivo evaluation of the human serum albumin microspheres. Farmaco 2000;55:303-7

42. Shi R, Zhang J, Li C, et al. Emergency of ofloxacin resistance in Mycobacterium tuberculosis clinical isolates from China: gyrA mutation analysis by denaturing HPLC and DNA sequencing. J Clin Micribiol 2006;44:4566-8

43. Brown MB, Jones SA. Hyluronic acid: a unique topical vehicle for the localized delivery of drugs to the skin. J Eur Acad Dermatol Venereol 2005;19:308-18

44. Pritchrad K, Lansley AB, Martin GP, et al. Evaluation of the bioadhesive properties of hyaluronan derivatives: detachment weight and mucociliary transport rate studies. Int J Pharm 1996;129:137-45

45. Vachon E, Martin R, Plumb J, et al. CD44 is a phagocytic receptor. Blood 2006;107:4149-58

46. Cantor JO, Turino GM. Can exogenously administered hyaluronan improve respiratory function in patients with pulmonary emphysema. Chest 2004;125:288-92

47. Zhou A, Guo L, Tang L. Effect of an intrathoracic injection of sodium hyaluronic acid on the prevention of pleural thickening in excess fluid of tuberculous thoracic cavity. Clin Exp Pharmacol Physiol 2003;30:203-5

48. Hwang SM, Kim DD, Chung SJ, et al. Delivery of ofloxacin to the lung and alveolar macrophages via hyaluronan microspheres for the treatment of tuberculosis. J Control Release 2008;129:100-6

49. Ahuja S, Ashman J. Analytical profiles of drug substances. Academic Press, New York; 1990

50. Sahin S, Selek H, Ponchel G, et al. Preparation, characterization and in vivo distribution of terbutaline sulfate loaded albumin microspheres. J Control Release 2002;82:345-58

51. Haley B, Frenkel E. Nanoparticles for drug delivery in cancer treatment. Urol Oncol 2008;26:57-64

52. Betancourt T, Brown B, Peppas LB. Doxorubicin-loaded PLGA nanoparticles by nanoprecipitation: preparation, characterization and in vitro evaluation. Nanomed 2007;2:219-32

53. Sutton D, Nasongkla N, Blanco E, et al. Functionalized micellar systems for cancer targeted drug delivery. Pharm Res 2007;24:1029-46

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

55. Montet X, Abou KM, Reynolds F, et al. Nanoparticle imaging of integrins on tumor cells. Neoplasia 2006;8:214-22

56. Flenniken ML, Liepold LO, Crowley BE, et al. Selective attachment and release of a chemotherapeutic agent from the interior of a protein cage architecture. Chem Commun 2005;4:447-9

57. Lowery AR, Gobin AM, Day ES, et al. Immunonanoshells for targetedphotothermal ablation of tumor cells. Int J Nanomed 2006;1:149-54

58. Bhadra D, Bhadra S, Jain S, et al. A PEGylated dendritic nanoparticulate carrier of fluorouracil. Int J Pharm 2003;257:111-24

59. Fawaz F, Bonini F, Guyot M, et al. Influence of poly(DL-lactide) nanocapsules on the biliary clearance and enterohepatic circulation of indomethacin in the rabbit. Pharm Res 1993;10:750-6

60. Gref R, Luck M, Quellec P, et al. 'Stealth' corona-core nanoparticles surface modified by polyethylene glycol (PEG): influences of the corona (PEG chain length and surface density) and of the core composition on phagocytic uptake and plasma protein adsorption. Colloids Surf Biointerfaces 2000;18:301-13

61. Otsuka H, Nagasaki Y, Kataoka K. PEGylated nanoparticles for biological and pharmaceutical applications. Adv Drug Deliv Rev 2003;55:403-19

62. Skelding AK, Barry DR, Shafren DR. Systemic targeting of metastasic human breast tumor xenografts by Coxsackievirus A21. Breast Cancer Res Treat 2009;113:21-30

63. Koyama S, Eihara T, Fukao K. Expression of intercellular adhesion molecule-1 (ICAM-1) during the development of invasion and/or metastasis of gastric carcinoma. J Cancer Res Clin Oncol 1992;118:609-14

64. Chittasupho C, Xie SX, Baoum A, et al. ICAM-1 targeting of doxorubicin-loaded PLGA nanoparticles to lung epithelial cells. Eur J Pharm Sci 2009;37:141-50

65. Tsenga CL, Wanga TW, Dongb GC, et al. Development of gelatin nanoparticles with biotinylated EGF conjugation for lung cancer targeting. Biomaterials 2007;28:3996-4005

66. Brzoska M, Langer K, Coester C, et al. Incorporation of biodegradable nanoparticles into human airway epithelium cells-in vitro study of the suitability as a vehicle for drug or gene delivery in pulmonary diseases.

Biochem Biophys Res Commun 2004;318:562-70

- **The paper describes the active incorporation of nanoparticles in a concentration- and temperature-dependent manner into human airway epithelium cells.**

67. Lherm C, Muller RH, Pusieux F, et al. Alkylcyanoacrylate drug carriers: II. cytotoxicity of cyanoacrylate nanoparticles with different alkyl chain length. Int J Pharm 1992;84:13-22
68. Nguyen J, Reul R, Betz T, et al. Nanocomposites of lung surfactant and biodegradable cationic nanoparticles improve transfection efficiency to lung cells. J Control Release 2009;140:47-54
69. Zou W, Liu C, Chen Z, et al. Studies on bioadhesive PLGA nanoparticles: a promising gene delivery system for efficient gene therapy to lung cancer. Int J Pharm 2009;370:187-95
70. Kawashima Y, Yamamoto H, Takeuchi H, et al. Pulmonary delivery of insulin with nebulized DL-lactide/ glycolide copolymer (PLGA) nanospheres to prolong hypoglycemic effect. J Control Release 1999;62:279-87

- **The paper describes insulin delivery through the pulmonary route.**

71. Zhang Q, Shen Z, Nagai T. Prolonged hypoglycemic effect of insulin-loaded polybutylcyanoacrylate nanoparticles after pulmonary administration to normal rats. Int J Pharm 2001;218:75-80
72. Yamamoto H, Kuno Y, Sugimoto S, et al. 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
73. Vaughn JM, McConville JT, Burgess D, et al. Single dose and multiple dose studies of itraconazole Nanoparticles. Eur J Pharm Biopharm 2006;63:95-102
74. Pandey R, Sharma A, Zahoor A, et al. Poly (DL-lactide-co-glycolide) nanoparticlebased inhalable sustained drug delivery system for experimental tuberculosis. J Antimicrob Chemother 2003;52:981-6
75. Pandey R, Zahoor A, Sharma S, et al. Nanoparticle encapsulated antitubercular drugs as a potential oral drug delivery system against murine tuberculosis. Tuberculosis 2003;83:373-8
76. Sharma A, Sharma S, Khuller GK. Lectin-functionalized poly (lactide-co-glycolide) nanoparticles as oral/aerosolized antitubercular drug carriers for treatment of tuberculosis. J Antimicrob Chemother 2004;54:761-6
77. Mykhaylyk O, Dudchenko N, Dudchenko A. Doxorubicin magnetic conjugate targeting upon intravenous injection into mice: high gradient magnetic field inhibits the clearance of nanoparticles from the blood. J Magn Magn Mater 2005;293:473-82

- **The paper provides information on magnetic nanoparticle-mediated lung targeting.**

78. Wu T, Hua MY, Chen JP, et al. Effects of external magnetic field on biodistribution of nanoparticles: a histological study. J Magn Magn Mater 2007;311:372-5
79. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975;256:495-7
80. Funaro A, Horenstein AL, Santoro P, et al. Monoclonal antibodies and therapy of human cancers. Biotechnol Adv 2000;18:385-401
81. Trail PA, Bianchi AB. Monoclonal antibody drug conjugates in the treatment of cancer. Curr Opin Immunol 1999;11:584-8
82. Trail PA, King HD, Dubowchik GM. Monoclonal antibody drug immunoconjugates. Cancer Immunol Immunother 2003;52:328-37
83. Lambert JM. Drug-conjugated monoclonal antibodies for treatment of cancer. Curr Opin Pharmacol 2005;5:543-9
84. Akasaka Y, Ueda H, Takayama K, et al. Preparation and evaluation of bovine serum albumin nanospheres coated with monoclonal antibodies. Drug Design Deliv 1988;3:85-97

- **The paper provides information on monoclonal antibody-conjugated nanoparticles of bovine serum albumin for tumor accumulation.**

85. Hattori Y, Kawakami S, Nakamura K, et al. Efficient gene transfer into macrophages and dendritic cells by in vivo gene delivery with mannosylated lipoplex via the intraperitoneal route. J Pharmacol Exp Ther 2006;318:828-34
86. Kawakami S, Sato A, Nishikawa M, et al. Mannose receptor-mediated gene transfer into macrophages using novel mannosylated cationic liposomes. Gene Ther 2000;7:292-9
87. Kawakami S, Wong J, Sato A, et al. Biodistribution characteristics of mannosylated, fucosylated, and galactosylated liposomes in mice. Biochim et Biophys Acta 2000;1524:258-65
88. Opanasopit P, Sakai M, Nishikawa M, et al. Inhibition of liver metastasis by targeting of immunomodulators usingmannosylated liposome carriers. J Control Release 2002;80:283-94
89. Wijagkanalan W, Kawakami S, Takenaga M, et al. Efficient targeting to alveolar macrophages by intratracheal administration of mannosylated liposomes in rats. J Control Release 2008;125:121-30

- **The paper demonstrated mannose receptor targeting by liposomes.**

90. Largent BL, Walton KM, Hoppe CA, et al. Carbohydrate- specific adhesion of alveolar macrophages to mannose-derivatized surfaces. J Biol Chem 1984;259:1764-9
91. Moreira JN, Hansen CB, Gaspar R, et al. A growth factor antagonist as a targeting agent for sterically stabilized liposomes in human small cell lung cancer. Biochim Biophys Acta 2001;1514:303-17
92. Zhang LJ, Xing B, Wu J, et al. Biodistribution in mice and severity of damage in rat lungs following pulmonary delivery of 9-nitrocamptothecin liposomes. Pulm Pharmacol Ther 2008;21:239-46
93. Mylonakis N, Athanasiou A, Ziras N, et al. Phase II study of liposomal cisplatin (LipoplatinTM) plus gemcitabine versus cisplatin plus gemcitabine as first line treatment in inoperable (stage IIIB/IV) non-small cell lung cancer. Lung Cancer 2010;68(2):240-7
94. Trammer B, Amann A, Ukomadu EH, et al. Comparative permeability and diffusion kinetics of cyclosporine A liposomes and propylene glycol solution from human lung tissue into human blood ex-vivo. Eur J Pharmacol Biopharm 2008;70:758-64

95. Lee RJ, Low PS. Folate-mediated tumor cell targeting of liposome-entrapped doxorubicin in vitro. Biochim Biophys Acta 1995;1233:134-44

96. Waldrep JC, Arppe J, Jansa KA, et al. High dose cyclosporin A and budesonide-liposome aerosols. Int J Pharm 1997;152:27-36

97. Iinuma H, Maruyama K, Okinaga K, et al. Intracellular targeting therapy of cisplatin-encapsulated transferrin-polyethylene glycol liposome on peritoneal dissemination of gastric cancer. Int J Cancer 2002;99:130-7

98. Singh M. Transferrin as a targeting ligand for liposomes and anticancer drugs. Curr Pharm Des 1999;5:443-51

99. Takada M, Yuzuriha T, Katayama K, et al. Increased lung uptake of liposomes coated with polysaccharides. Biochim Biophys Acta 1984;802:237-44

100. Moreira JN, Gaspar R, Allen TM. Targeting stealth liposomes in a murine model of human small cell lung cancer. Biochim Biophys Acta 2001;1515:167-76

101. Asaia T, Shutob S, Matsudab A, et al. Targeting and anti-tumor efficacy of liposomal 5′-O-dipalmitoylphosphatidyl 2′-C-cyano-2′-deoxy-1-beta-D-arabino-pentofuranosylcytosine in mice lung bearing B16BL6 melanoma. Cancer Lett 2001;162:49-56

102. Matsuda A, Nakajima Y, Azuma A, et al. Synthesis of a new potent antitumor nucleoside 2′-C-cyano-2′ deoxy-1-beta-D-arabino-pentofuranosylcytosine. Nucleic Acids Symp 1990;22:51-2

103. Matsuda A, Nakajima Y, Azuma A, et al. Nucleosides and nucleotides 100. 2′-C-cyano-2′-deoxy-1-beta-D-arabino-pentofuranosylcytosine (CNDAC): design of a potential mechanism-based DNA-strand-breaking antineoplastic nucleoside. J Med Chem 1991;34:2917-19

104. Hayakawa Y, Kawai R, Otsuki K, et al. Evidence supporting the activity of 2′-C-cyano-2′-deoxy-1-beta-D-arabino-pentofuranosylcytosine as a terminator in enzymatic DNA-chain elongation. Bioorg Med Chem Lett 1998;8:2559-62

105. Chono S, Tanino T, Seki T, et al. Efficient drug targeting to rat alveolar macrophages by pulmonary administration of ciprofloxacin incorporated into mannosylated liposomes for treatment of respiratory intracellular parasitic infections. J Control Release 2008;127:50-8

106. Zaru M, Manca ML, Fadda AM, et al. Chitosan-coated liposomes for delivery to lungs by nebulisation. Colloids Surf Biointerfaces 2009;71:88-95

- **The paper provides information on aerosolized pulmonary delivery of liposomes.**

107. Schijelers RM, Bakker-Woudenberg IAJM, Storm G. Localization of sterically stabilized liposomes in experimental rat Klebsiella pneumoniae pneumonia: dependence on circulation kinetics and presence of poly(ethylene)glycol coating. Biochim Biophys Acta 2000;1468:253-61

108. Abraham AM, Walubo A. The effect of surface charge on the disposition of liposome-encapsulated gentamicin to the rat liver, brain, lungs and kidneys after intraperitoneal administration. Int J Antimicrobiol Agents 2005;25:392-7

109. Gajbhiye V, Kumar PV, Tekade RK, et al. Dendrimers as therapeutic agents: a systematic review. J Pharm Pharmacol 2009;61:989-1003

- **First review covering all therapeutic aspects of different types of dendrimer.**

110. Gajbhiye V, Kumar PV, Tekade RK, et al. Pharmaceutical and biomedical potential of PEGylated dendrimers. 2007;13:415-29

111. Gajbhiye V, Kumar PV, Sharma A, et al. PEGylated PPI dendritic architectures for sustained delivery of anti-inflammatory agent. Curr Nanosci 2008;4:267-77

112. Gajbhiye V, Kumar PV, Sharma A, et al. Dendrimer mediated transdermal & oral delivery of bioactives. Ind J Pharm Sci 2008;70:431-9

- **A review covering transdermal and oral drug delivery aspects of different types of dendrimer.**

113. Gajbhiye V, Kumar PV, Tekade RK, et al. PEGylated PPI dendritic architectures for sustained delivery of H2 receptor antagonist. Eur J Medi Chem 2009;44:1155-6

114. El-Sayed M, Rhodes CA, Ginski M, et al. Transport mechanism (s) of poly (amidoamine) dendrimers across Caco-2 cell monolayers. Int J Pharm 2003;262:151-7

- **The paper suggested information on size of dendrimer for avoiding reticuloendothelial system uptake.**

115. Papagiannaros A, Dimas K, Papaioannou GH, et al. Doxorubicin–PAMAM dendrimer complex attached to liposomes: cytotoxic studies against human cancer cell lines. Int J Pharm 2005;302:29-38

116. Perumal OP, Inapagolla R, Kannan S, et al. The effect of surface functionality on cellular trafficking of dendrimers. Biomaterials 2008;29:3469-76

- **The paper provides information on the influence of surface functionality of dendrimers desirable for uptake by lung epithelial cells.**

117. Kukowska-Latallo JF, Chen C, Eichman J, et al. Enhancement of dendrimer-mediated transfection using synthetic lung surfactant exosurf neonatal in vitro. Biochem Biophys Res Commun 1999;264:253-61

118. Kumar PV, Asthana A, Dutta T, et al. Intracellular macrophage uptake of rifampicin loaded mannosylated dendrimers. J Drug Target 2006;14:546-56

119. Kumar PV. PhD Thesis entitled 'Dendritic architecture for the delivery of some anti-tubercular bioactives', Dr. Harisingh Gour University, Sagar 470003 [India], 2007

120. Kannan RM, Inagopalla R, Kannan S, et al. Aiche annual meeting. Dendrimer-based nanodevices for asthma drug delivery: synthesis, in vitro and in vivo studies. Cincinnati:15015 poster session. Drug Gene Deliv 2005

121. Elfinger M, Maucksch C, Rudolph C. Characterization of lactoferrin as a targeting ligand for nonviral gene delivery to airway epithelial cells. Biomaterials 2007;28:3448-55

- **The paper suggested a ligand for lung targeting.**

122. Zhang JQ, Lu B. Studies on lung targeted niosomes of carboplatin. Acta Pharm Sin 2001;36:303-6

123. Gude RP, Jadhav MG, Rao SGA, et al. Effects of niosomal cisplatin and combination of the same with theophylline and with activated macrophages in murine B16F10 melanoma model.

Cancer Biother Radiopharm 2002;17:183-92

124. Jain CP, Vyas SP, Preparation and characterization of niosomes containing rifampicin for lung targeting. J Microencapsul 1995;12:401-7

Affiliation

Balak D Kurmi[1], Jitendra Kayat[1], Virendra Gajbhiye[1], Rakesh K Tekade[1] & Narendra K Jain[†1,2]
[†]Author for correspondence
[1]Dr Hari Singh Gour University, Department of Pharmaceutical Sciences, Pharmaceutics Research Laboratory, Sagar 470 003, India
[2]Professor, Dr Hari Singh Gour University, Department of Pharmaceutical Sciences, Pharmaceutics Research Laboratory, Sagar 470 003, India
E-mail: jnarendr@yahoo.co.in