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Targeted delivery of nanoparticles for the treatment of lung diseases ☆

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Abstract

Targeted delivery of drug molecules to organs or special sites is one of the most challenging research areas in pharmaceutical sciences. By developing colloidal delivery systems such as liposomes, micelles and nanoparticles a new frontier was opened for improving drug delivery. Nanoparticles with their special characteristics such as small particle size, large surface area and the capability of changing their surface properties have numerous advantages compared with other delivery systems. Targeted nanoparticle delivery to the lungs is an emerging area of interest. This article reviews research performed over the last decades on the application of nanoparticles administered via different routes of administration for treatment or diagnostic purposes. Nanotoxicological aspects of pulmonary delivery are also discussed.

Keywords: Pulmonary delivery; Nanoparticle; Lung cancer; Tuberculosis; Aerosol; Colloidal delivery; Nanotoxicology

Contents

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1. Introduction

Over the last decades, colloidal drug delivery systems and especially nanoparticles have received great attention. Nanoparticles can be administered via different routes of administration such as parenteral, oral, intraocular, transdermal or pulmonary inhalation. Aerosol therapy using particulate drug carrier systems is becoming a popular method to deliver therapeutic or diagnostic compounds either locally or systemically [1] as shown by the development of inhalable insulin [2]. This is due to the large alveolar surface area suitable for drug absorption, the low thickness of the epithelial barrier, extensive vascularization and relatively low proteolytic activity in the alveolar space compared to other routes of administration and the absence of the first-pass metabolism [3–6]. In general, nanoparticle delivery to the lungs is an attractive concept because it can cause retention of the particles in the lungs accompanied with a prolonged drug release if large porous nanoparticle matrices are used [7]. On the other hand studies have shown that nanoparticles uptake by alveolar macrophages can be reduced if the particles are smaller than 260 nm [7–9]. Both effects combined might improve local pulmonary drug therapy. However, the particle size of medically used nanoparticles is too small to be suitable for direct lung delivery. A prerequisite for deep lung delivery is the design of proper carrier systems [1]. Successful delivery of inhaled particles depends mostly on particle size and particle density, and hence, the mass median aerodynamic diameter [10]. The respirable fraction of an inhalable powder is generally the fraction of particles with an aerodynamic diameter ranging between 1 and 5 μm. This size range guarantees a maximum deposition in the deep lung [11]. In this article, we review research performed during the last three decades in the area of nanoparticle delivery with special focus on nanoparticle targeting to the lungs. While direct pulmonary delivery of dry powder formulations containing nanoparticles is rather new, this article will also review nanoparticle delivery to the lungs via different routes of administration.

2. Targeted delivery of nanoparticles to the lungs after intravenous injection

Numerous studies were performed on the body distribution of nanoparticles after iv injection. Different kinds of nanoparticles were used in several studies with different results. Early studies were performed by different researchers investigating the body distribution of nanoparticles. The results showed that nanoparticles mostly accumulate in the organs of the reticuloendothelial system such as liver, spleen and lungs [12–14].

Kreuter et al. [12] studied the body distribution of poly (methyl-2-14C-methacrylate) nanoparticles after intravenous injection into rats and mice. They measured the radioactivity of the nanoparticles in different organs at 0.5, 1, 2, 6, 24 h and 7 days after i.v. injection. The results showed that a maximum accumulation of the nanoparticles in the lungs was after 30 min (21.8% of the administered dose). This amount decreased during the surveillance time of 7 days to 13.2%. After intramuscular administration of nanoparticles, all residual ^{14}C-activity was only found at the injection site. In this study no transportation or distribution of nanoparticles occurred to the lungs during 70 days of observation. Other distribution studies using different nanoparticles showed also that they were taken up by phagocytes of the reticuloendothelial systems and mainly distributed to the liver and in smaller portions to the spleen, bone marrow and lungs [15]. In general, for a passive targeting of nanoparticles to the lungs the particle size should be above 7 μm to be retained in the alveolar capillaries. This may occur through agglomeration of nanoparticles after injection due to a break down of repulsive forces between the particles or by using microparticles larger than 7 μm. In a study performed by Gipps et al. [13], ^{14}C-polyhyxyl cyanoacrylate nanoparticles were intravenously injected into nude mice bearing a human osteosarcoma. The study showed that the maximum amount of radioactivity in the lungs was 2.27% of the dose and occurred after 1 h. This amount decreased during a 14 day observation period to 1.11%. Altogether the highest levels of nanoparticle accumulation were found in the organs of the reticuloendothelial system: liver, spleen, and lungs. The radioactivity in other organs was found to be low at about 2%. Waser and coworkers [14] showed that it is possible to reach relatively high concentrations in the organs of the reticuloendothelial system including the lungs after i.v. injection of radio-loaded nanoparticles. They used three different colloidal formulations including liposomes, ^{14}C-hexylcyanoacrylate nanoparticles and ^{125}I-albumin nanoparticles. Although these researchers showed that a higher drug concentration can be reached in the lungs using nanoparticles, numerous other studies which were performed during the last two decades showed that there was no significant change in the lung accumulation of a drug if it was delivered using nanoparticles compared to free drug. Rolland et al. [16], injected radioactive polymethacrylic nanospheres into mice and analyzed the organ distribution of the nanoparticles. In his study radioactivity in lungs remained insignificant in the first hour.

In another study, Bazile et al. [17] injected ^{14}C-polylactic acid (PLA) nanoparticles (90–250 nm) coated with human serum albumin into male Sprague–Dawley rats. They showed that the dose distribution of these nanoparticles to the lungs was at day 1 and day 7, 0.09% and 0.010% respectively which was rather negligible. Lescure et al. [18] prepared poly (methylidene malonate 2.1.2) nanoparticles and they studied the body distribution using a ^{14}C-labeled after a single dose injection into rats. They showed that less than 2% of the injected dose was detected in the lungs after 1 h and this amount decreased during the next 10 h. Simon et al. [19] studied the body distribution of ^{14}C-labeled amino-modified polystyrene nanoparticles in mice. At 1 min, they showed that for the entire particle size range only 3% of the total particles accumulated in the lungs. This value dropped to less than 1% after 2 min after injection. Page et al. [20] studied the tissue distribution of colistin solution and colistin loaded polyhexylcyanoacrylate nanoparticles in mice after intravenous injection. Their study showed that free colistin was not detected in the lungs of mice during a 24 h period. However, they showed that colistin loaded nanoparticles were detected in low amounts in the lungs after 1 h but after 6, 18 and 24 h they couldn't detect any drug in the lungs. In another study by Leucuta et al. [21], they studied the pharmacokinetics of epirubicin nanoparticles, liposomes and free epirubicin after intravenous injection of the

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preparations. They measured a higher AUC in the lungs after liposome and nanoparticle administration compared to the free drug. However, the results showed that the accumulation of nanoparticulate systems was not improved and there was no significant difference in the drug's half-life. Chen et al. [22] investigated the body distribution of nanoparticles containing Adriamycin injected into the hepatic artery of hepatoma-bearing rats. They showed that the drug concentration in the lungs of the animals was significantly lower when nanoparticles were used compared to free Adriamycin injection. Teng et al. [23] showed that the incorporation of paclitaxel into gelatin nanoparticles changed the body distribution of the drug in some organs such as liver, spleen, small intestine and kidney but the tissue concentration of the drug in the lungs did not change significantly compared to a conventional iv paclitaxel solution. In contrast to the mentioned works, other scientists showed that using nanoparticulate delivery system for drugs can increase the drug concentration in the lungs [24–26]. Zara et al. [24] studied the pharmacokinetics of doxorubicin loaded solid lipid nanoparticles after intravenous injection and compared the results with a doxorubicin solution. They showed that the drug concentration in the lungs was higher in animals treated with doxorubicin nanoparticles compared to the doxorubicin solution. Santhi et al. [25] studied the body distribution of methotrexate-loaded bovine serum albumin nanospheres in mice after a single i.v. injection. This study showed a 33.14% drug increase using nanospheres compared with a free drug. Löbenberg et al. [26] studied the body distribution of ^{14}C-azidothymidine bound to hexylcyanoacrylate nanoparticles after i.v. injection to rats. They showed that the drug concentration in the lungs was significantly increased compared to the control solution. 480 min after injection the drug concentration in the lungs was 18 times higher than the control solution.

As mentioned earlier, the pioneer work by Kreuter [15] showed that most of the injected nanoparticles will accumulate in the reticuloendothelial system (RES) and do not reach other organs. To overcome the uptake of nanoparticles by the RES, Leu et al. [27] coated the nanoparticles with surfactants and studied the body distribution after an i.v. injection. They showed that surfactant-coated polymethyl [2-^{14}C] methacrylate nanoparticles showed significantly different distribution pattern in rats compared to non-coated and albumin-coated particles. Surfactant-coated nanoparticles did not accumulate in the liver as dramatically as uncoated ones. Although the accumulation of surfactant-coated nanoparticles in the lungs was nearly doubled but still the maximum dose after 24 h was only 7.0%. Nanoparticles coated with rat serum albumin did not show any significant changes in the lung distribution compared to uncoated ones. Coating of the nanoparticles changed the body distribution after i.v. injection, but it did not influence the targeting to the lungs. Gulyaev et al. [28] studied the effect of polysorbate 80 coatings on the body distribution of cyanoacrylate nanoparticles. Although their study was focused on the delivery of nanoparticles to the brain, the body distribution study showed that there was a significant increase of doxorubicin concentration in the liver when nanoparticles were used. At all time points, except for the 4 h time point, (10 min, 1, 2, 4, 6 and 8 h) the doxorubicin concentration in the liver of polysorbate 80 coated nanoparticles was less compared to non-coated nanoparticles but in the lungs this difference was not significant. Araujo et al. [29] studied the influence of surfactant coatings on the body distribution of 2-14C-poly (methyl methacrylate) nanoparticles. They coated the nanoparticles with different concentrations (0.001%–5%) of either polysorbate 80 or poloxamine 908. Their results showed that the lung accumulation of polysorbate 80 coated nanoparticles was decreased compared with uncoated nanoparticles. However, the nanoparticle concentration in the lungs was increased when the nanoparticles were coated with 1% or 5% poloxamine 908. There was no significant change in the lung uptake when other concentrations were used.

As mentioned there are controversial results using different animal models and i.v. injections of nanoparticles to increase the drug concentration in the lungs. However, a multi centre phase II clinical trial showed that Abraxane®, a novel Cremophore free, albumin-bound paclitaxel nanoparticle formulation, showed encouraging results in regard to efficacy and safety in patients with non-small-cell lung cancer (NSCLC) [30]. A significant tumor response and prolonged disease control was documented in forty-three patients. This study shows the potential that nanoparticles may have as carriers of chemotherapeutics to treat tumors and lung cancer in particular. It should be mentioned that, Abraxane® nanoparticles are suspected to dissolve shortly after administration; therefore, it is not known if the nanoparticles simply improve paclitaxel injectability and dissolution or if they contribute to the observed therapeutic efficacy in other ways (e.g. affecting drug biodistribution). More clinical research involving different nanoparticles and drugs is needed to assess if nanoparticles can improve drug therapy especially for cytotoxic drugs; however, preclinical studies suggest that nanoparticles might revolutionize Chemotherapy. The major obstacle, after intravenous injection, is to overcome the massive liver uptake. In conclusion, it seems to be difficult to achieve nanoparticle accumulation in the lungs via intravenous administration. However, in the case of cancer treatment other effects like passive nanoparticle targeting to tumors might still improve lung specific cancer therapy. Table 1 shows a summary of lung targeting using different types of nanoparticles as injections.

3. Antibody conjugated nanoparticles for lung targeting

The development of monoclonal antibodies and utilization of their targeting properties [31] can be used for active targeting. This can be used to improve drug delivery by attaching antibodies to drug molecules or drug delivery systems [32–36]. In an attempt to target lung tumors using antibody modified nanoparticles, Akasaka et al. [37] injected bovine serum albumin (BSA)-conjugated with Lewis lung carcinoma monoclonal antibodies to Lewis lung carcinoma-bearing mice. They showed that nanoparticles made from the BSA-conjugate with monoclonal antibodies were only slightly localized in the carcinoma tissue. Twenty-four hours after injection the amount of the nanoparticles localized in the carcinoma tissue was rather low. This study however, showed that the particle size was more

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Table 1
Summary of intravenous administration of nanoparticles for lung targeting

Nanoparticle type	Size (nm)	Active ingredient	Lung accumulation	*In vivo* model	Ref
Poly (methyl-2-^{14}C-methylacrylate)	Not provided	^{14}C	21.8% 30 min after inj, 13.2% 7 days after inj	Mice, rats	[12]
^{14}C-polyhyxyl cyanoacrylate	200–300	^{14}C	2.27% 1 h after inj	Mice	[13]
Hexylcyanoacrylate and albumin nanoparticles	750	Diazepam	Higher accumulation compared to control solution	Mice	[14]
Radioactive polymethacrylic	280, 300	^{111}In	No lung accumulation	Mice	[16]
^{14}C-polylactic acid (PLA) nanoparticles	90–250	^{14}C	Negligible after day 1 and 7	Rats	[17]
Poly (methyliden malonate 2.1.2)	250	^{14}C	2% 1 h after injection, negligible after 10 h	Rats	[18]
^{14}C-amino-modified polystyrene	100, 240, 470	^{14}C	Less than 1% 2 min after inj	Mice	[19]
Polyhexylcyanoacrylate	Not provided	Colistin	Negligible after 6, 18 and 24 h	Mice	[20]
Poly (methyl methacrylate)	50	Epirubicin	No improved accumulation compared to control solution	Rats	[21]
Polybutylcyanoacrylate	93.1	Adriamycin	Lower drug concentration compared to free drug solution	Rats	[22]
Gelatin	664	Paclitaxel	No significant change compared to free drug solution	Mice	[23]
Solid lipid nanoparticles	80	Doxorubicin	Higher compared to control solution	Rats	[24]
Bovine serum albumin	712.5	Methotrexate	33.14% increase compared to free drug	Mice	[25]
Hexylcyanoacrylate	230	Azidothymidine	18 times drug concentration increase in lungs compared to control solution	Rats	[26]
Surfactant-coated polymethyl [2-^{14}C] methacrylate	131	^{14}C	7% after 24 h	Rats	[27]
Tween 80-coated *iso*-butylcyanoacrylate	270	Doxorubicin	No significant change	Rats	[28]
Surfactant-coated poly (methyl methacrylate)	130	^{14}C	Poloxamine 908 coated (1%, 5%) nanoparticles showed increased lung accumulation	Rats	[29]
Albumin	130	Paclitaxel	Significant tumor response	NSCLC patients	[30]

important than the affinity of the monoclonal antibodies to the tumor cells. Although the study showed some promising data for targeted delivery of nanoparticles to lung tumors there is still room for improvements.

Endothelial cell targeting is another major research area for antibody oriented nanoparticle delivery [38]. The endothelium represents an important therapeutic target for controlling oxidative stress, thrombosis and inflammation involved in pulmonary diseases. However, rapid blood clearance and lack of affinity to the endothelium compromise targeted drug delivery of antioxidants, enzymes or fibrinolytics. Constitutive endothelial cell adhesion molecules (CAM, such as ICAM-1 and PECAM-1), which are stably expressed and functionally involved in oxidative stress and thrombosis are normally ideal candidates for the targeting of anti-oxidants and fibrinolytics. It has been shown that endothelial cells internalize nanoparticles containing multiple copies of either ICAM-1 or PECAM-1 antibodies conjugates [39,40]. The capacity of endothelial cells to uptake anti-CAM multimeric conjugates depends on the size of the nanoparticles. Conjugates with diameter from 100 to 300 nm can enter endothelial cells, whereas conjugates of larger size (500 nm to 1 μm in diameter) remain attached to the cell surface at 37 °C [39,40]. Therefore, by modulating the size of anti-CAM conjugate carriers, the drugs can be targeted to the surface of endothelial cells or their interior. It seems that utilizing nano carrier conjugated anti-CAM can be a promising delivery concept to endothelial cells; such approaches are specially useful to treat pulmonary diseases.

4. Oral delivery of nanoparticles to target the lungs

In general, the oral route of administration is convenient for drug administration of conventional dosage forms. However, oral delivery of nanoparticles for drug targeting to the lungs has not shown in the past promising results. In a study published by Jani et al. [41], they used negatively charged polystyrene microspheres with covalently linked rhodamine (nominally 100 nm and 1 μm in diameter), and non-ionized polystyrene microspheres with covalently linked fluorescein (nominally 100 nm, 500 nm, 1 μm, 3 μm in diameter). The nanoparticles were administered to female Sprague–Dawley adult rats by gavage. They found that 1 μm diameter microspheres were taken up less efficiently than smaller particles. The liver, tissues of the Payer's patches, villi, lymph nodes and spleen showed evidence of non-ionic microspheres uptake while sections of the heart, kidney and lung tissues showed none. The same group [42] studied the uptake of polystyrene nanoparticles with covalently linked fluorescein (50 nm–3.0 μm) after oral administration and again they couldn't detect any nanoparticles in the lungs. In another study Löbenberg et al. [43] investigated the body distribution of ^{14}C-labeled azidothymidine bound to hexylcyanoacrylate nanoparticles after oral administration. Their study did not show a profound increase of drug concentration in the lungs compared to control oral solution. Also Araujo et al. [44] studied the uptake of polymethyl (2-^{14}C) methacrylate nanoparticles from the gastrointestinal tract after oral administration to rats. They showed that when the nanoparticles were suspended in peanut oil containing oleic acid the uptake of nanoparticles increased by 50%, however, the lung accumulation of the nanoparticles was nearly negligible. These studies show that oral delivery of nanoparticles might not be the proper route of administration to reach the lungs.

5. Delivery of nanoparticles into the lungs for diagnostic purposes

Although there are several studies using nanoparticles for diagnosis of cancers [45–47], so far only one study was published on the pulmonary instillation of nanoparticles for diagnostic

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purposes. In this study, Ketai et al. [48] instilled iodinated nanoparticles intrabronchially to eight dogs. The contrast material used was sterile NC 70146 (1-ethoxycarbonyl) pentyl bis ((3, 5-acetylamino)-2, 4, 6-triiodobenzoate) which was formulated as a nanoparticle stabilized by surfactant. They used spiral computed tomography (CT) 2–3 days later. Their results showed that iodinated nanoparticles instilled into the small airways were transported to the tracheobronchial lymph nodes, where they resulted in a contrast enhancement. Nanoparticles seem to be promising and a powerful tool for imaging purposes of the lungs as shown by Tc-labeled microparticles [49] but further studies need to be performed on the delivery of nanoparticles for diagnostic purposes. Another application for lung imaging is discussed under magnetic nanoparticles in Section 8.

6. Delivery of nanoparticles for the treatment of tuberculosis

Nanoparticulate drug delivery systems have considerable potential for the treatment of tuberculosis. *Mycobacterium tuberculosis* invades and begins its replication within alveolar macrophages before the bacterium spreads out. Therefore, tuberculosis can be seen as a disease involving macrophages which makes nanoparticles an ideal drug carrier. Macrophage targeting was introduced by Löbenberg and Kreuter [50] to deliver anti-viral drugs directly to macrophages which represent an important HIV pool within the body.

The same approach can be taken for the management of tuberculosis: Nanoparticles with their special characteristics can improve drug bioavailability and reduce the dosing frequency, and may resolve the problem of non-adherence to prescribed therapy and improve patient compliance, which is one of the major obstacles in the control of tuberculosis epidemics [51]. There were several studies using drug-loaded nanoparticles administered orally, subcutaneously or intravenously. Section 9 of this article will discuss the pulmonary delivery of drug-loaded nanoparticles for the treatment of tuberculosis. This section will focus on other routes of administration. Anisimova et al. [52] reported the subcutaneous delivery of a nanoparticle based system using three anti-tuberculosis drugs: isoniazid, rifampin, and streptomycin. They studied *in vitro* the accumulation of these drugs in human monocytes and their anti-microbial activity against *Mycobacterium tuberculosis* residing in human monocyte-derived macrophages. Their results showed that nanoparticle encapsulation increased the intracellular accumulation of all three tested drugs, but only the anti-microbial activity of isoniazid and streptomycin was increased. Also in their study they showed that the activity of encapsulated rifampin against intracellular bacteria was not higher than that of the free drug. In another study Pandey and Khuller [53] administered poly (DL-lactide-co-glycolide) (PLG) nanoparticles encapsulated with three front-line anti-tubercular drugs subcutaneously to mice. They showed that, drug-loaded PLG nanoparticles resulted in undetectable bacterial counts in the lungs and spleen of infected mice. The particle preparation showed a better chemotherapeutic efficacy compared with a daily drug treatment. Later Pandey et al. [54] evaluated the chemotherapeutic potential of oral solid lipid nanoparticles loaded with rifampicin, isoniazid and pyrazinamide against tuberculosis. They showed that after a single oral administration of nanoparticles to mice, the therapeutic concentrations of all three drugs were maintained in the plasma for 8 days and in the organs (such as lungs, liver and spleen) for 10 days, whereas the free drugs were cleared within 1–2 days. Also in *Mycobacterium tuberculosis* infected mice, no tubercle bacilli was detected in the lungs/spleen after 5 oral doses of drug-loaded solid lipid nanoparticles administered every 10 days; whereas 46 daily doses of oral free drugs were necessary to reach the same therapeutic effect. They concluded that nanoparticle based anti-tuberculosis therapy can reduce the dosing frequency and improve patient compliance for a better management of tuberculosis. A similar study was performed by Johnson et al. [55]; they also reported that both treatment using nanoparticle encapsulated and non-encapsulated drugs can significantly reduce the bacterial count and lung histopathology. But since the nanoparticle formulation was administered every 10 days, better patient compliance might be achieved compared to non-encapsulated formulations. In another study Pandey et al. [56] evaluated the efficiency of oral encapsulated ethambutol in combination with PLG nanoparticles loaded with rifampicin, isoniazid and pyrazinamide in a murine tuberculosis model. The study concluded that polymeric nanoparticles using a combination of 4-drugs have a significant potential to shorten the duration of tuberculosis chemotherapy besides reducing the dosing frequency.

These studies showed that oral delivery of anti-tuberculosis drugs incorporated in nanoparticles might be a feasible alternative to conventional oral drug delivery to achieve better patient compliance. This is due to the decreased dosing frequency. However, it is still not known why certain drugs did not have an increased therapeutic effect when delivered to macrophages via nanoparticulate carriers even if their local concentration increased. Also these results are in contrast to the general tendency of oral nanoparticle delivery to the lungs discussed in Section 4. The promising results in the tuberculosis management using nanoparticles might be due to different effects. It is possible that the constant drug plasma levels are more effective than fluctuating drug plasma levels after oral administration of free drugs, or even a small nanoparticle accumulation in the lungs might cause locally an effective increase in drug concentration. Combined with a constant controlled drug release such nearly undetectable drug concentration increases might be the key for an improve drug delivery. More mechanistic studies are needed to gain a better understanding of the improved drug therapy in the treatment of tuberculosis.

7. Nanoparticle based gene delivery to lungs

Gene delivery is an important area of drug delivery since it offers the possibility for direct and in some cases permanent changes of cell and organ functions. Nanoparticles seem to be the right choice for this purpose since they have similar sizes compared to certain viruses which are the natural but pathogenic

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gene delivery systems [57]. Due to safety reasons, natural virus based gene delivery systems have rather a slim chance to be broadly used for gene delivery. However, the engineering of artificial viruses is still not completed [58]. For systemic therapy of lung cancer, Gopalan et al. [59] used DOTAP:cholesterol nanoparticles as an alternate non-immunogenic gene delivery vector. They showed that systemic administration of DNA-nanoparticles might induce multiple signaling molecules both *in vitro* and *in vivo* which are associated with inflammation.

They used small molecule inhibitors against the signaling molecules such as naproxen and showed that these small molecules can suppress nanoparticle-mediated inflammation without affecting transgene expression. Their results might be of clinical significance both in terms of suppressing toxicity, as well as, increasing the therapeutic window. In another study, Kaul et al. [60] investigated the possibility of gelatin nanoparticles as plasmid DNA delivery system on Lewis lung carcinoma (LLC) bearing mice models. They encapsulated reporter plasmid DNA encoding for β-galactosidase (pCMV-β) in gelatin and PEGylated gelatin nanoparticles. They showed that PEGylated gelatin nanoparticles are superior transfection reagents compared to gelatin nanoparticles and lipofectin. Also the *in vivo* expression of β-galactosidase in tumor mass showed that PEGylated gelatin nanoparticles can transfect with 61% efficiency after i.v. administration relative to the intratumoral administration. They attributed the high transfection efficiency of PEGylated gelatin nanoparticles to the biocompatible, biodegradable and long circulating nature of the carrier system. Nanoparticles do not complex DNA molecules and preserve their supercoiled structure which is critical for nuclear uptake and efficient transfection. Fink et al. [61] showed that nanoparticles consisting of a single molecule of DNA condensed with polyethylene glycol-substituted lysine-30-mers efficiently transfected the lung epithelium following intra pulmonary administration into mice. Li et al. [62] developed a ligand targeted and sterically stabilized nanoparticle formulation for the targeted delivery of anti-sense oligodeoxynucleotides and small interference RNA into lung cancer cells. Their results showed that the ligand targeted and sterically stabilized nanoparticles can provide a selective delivery of anti-sense oligodeoxynucleotides and siRNA into lung cancer cells which might be used for cancer therapy. These studies show that nanoparticles have the potential to be used as carrier for safe and effective gene delivery to treat certain lung diseases in the future.

8. Magnetic nanoparticles used for lung targeting

Using magnetic nanoparticles, either for diagnostic or treatment purposes, was the centre of interest during the last two decades, however there were only two articles published on the specific delivery of nanoparticles to the lungs. In one of the first studies, Mykhaylyk et al. [63] evaluated the pharmacokinetics of doxorubicin magnetic conjugate (DOX-M) nanoparticles in a mouse model. They investigated the efficiency of a non-uniform magnetic field on the clearance of the magnetic DOX-M. In this work they injected DOX-M suspensions into the eye sinus vein of adult male mice, and applied a magnetic field centered over the left lung. They showed that a non-uniform magnetic field was a potent factor in modifying the DOX-M conjugate pharmacokinetics. The magnetic field application resulted in considerable enrichment of DOX-M in the lungs, and a depletion in the liver of the magnetic carrier compared to a reference without a magnetic field. They showed that the application of a magnetic field can significantly increase the bioavailability of DOX-M in the lungs. Although their work in mice showed some promising results, the outcome of the application of magnetic fields in humans for increasing the localization of a drug in the lungs containing magnetic nanoparticles has not yet been proven. Contrary to the results from the previous study, Wu et al. [64] showed that an external magnetic field applied to rats after intravenous injection of dextran coated Fe_3O_4 did not change the accumulation of the nanoparticles in the lungs. Generally, the use of magnetic nano-carriers has merit for diagnostic or treatment purposes. The delivery of magnetic nanoparticles to the lungs might be worth more detailed research to be used as effective drug delivery system or as a safe diagnostic tool.

9. Pulmonary delivery of nanoparticles

9.1. Delivery of nanoparticles using dry powder carriers

Pulmonary delivery of nanoparticles via different dry powder formulations is gaining more attention in recent years. As mentioned before, the large alveolar surface area, the low thickness of the epithelial barrier and an extensive vascularization make the pulmonary route an ideal route for administration of active ingredients [65]. Since nanoparticles are in a size range which is not suitable for deep lung delivery, the major challenge for pulmonary delivery of nanoparticles is to find a proper carrier system [66]. Several researchers have prepared carrier systems for nanoparticles to improve the delivery of nanoparticles to the alveolar area. Kawashima et al. [67] used ultrafine hydrophilic particles, hydroxypropylmethyl cellulose phthalate (HPMCP) nanospheres, to improve the aerosolization properties of a dry powder inhalation of a hydrophobic drug, pranlukast hydrate. In their study they prepared a drug containing HPMCP nanospheres and then mixed the surface modified drug powder dispersion with lactose and then spray- or freeze-dried the suspension to obtain a dry powder. This powder was mixed with larger lactose particles for better dispersion when used in an inhaler. They performed an *in vitro* inhalation test using a twin impinger and showed that the inhalation properties of the surface modified powder dramatically improved. They showed that the emission of the powder increased two fold and the dry powder delivery to the deep lung might increase 3 fold compared to the original unmodified powder. They related this improvement to the increased surface roughness and hydrophilicity of the surface-modified particles, which resulted in an increased dispersibility in air. In another study the same group [68] incorporated insulin into PLGA nanospheres and administered them using a sieve type ultrasonic nebulizer into the trachea of guinea pigs. They showed that the insulin loaded nanospheres were able to reduce the blood glucose significantly

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Table 2
Summary of pulmonary delivery of nanoparticles using dry powder carriers

Nanoparticle type	Nanoparticle size (nm)	Carrier particle	Carrier particle size (μm)	Active ingredient	Method for the preparation of carrier	Ref
Hydroxypropylmethyl cellulose phthalate (HPMCP)	51.6	Lactose	0.6–9.3	Pranlukast	Spray drying and freeze drying	[67]
Carboxylate-modified polystyrene (PS) and Nyacol 9950 colloidal silica	PS: 25, 170, 1000 Nyacol: 100	Large porous particles (LPP), nanoparticles attached to each other	4±0.2	–	Spray drying	[8]
Gelatin and *iso*-butyl cyanoacrylate	173, 242	Lactose	2.50–2.60	–	Spray drying	[66]
Chitosan	388, 419	Lactose	2–3	Insulin	Spray drying	[65]
Iso-butyl cyanoacrylate	244	Effervescent carrier powder	2.17	Ciprofloxacin	Spray drying	[1]

and the hypoglycemic effect was prolonged over 48 h compared to a nebulized aqueous solution of insulin as a reference. They linked their results to the sustained release of insulin from the nanospheres deposited widely throughout the lung. In another attempt, Tsapis et al. [8] introduced large porous carriers of nanoparticles for pulmonary drug delivery. They used a spray drying technique to produce large porous particles (LPP) which have extremely thin walled structures. They added two different surfactants, 1, 2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dimyristoyl-*sn*-glycero-3-phophoethanolamine (DMPE), and lactose to the nanoparticles suspension and spray dried the mixture. The particles were made from nanoparticles which attached to each other using lactose and the mentioned surfactants when spray dried. They showed that the spray dried powder has proper characteristics for pulmonary delivery and can be re-dissolved in a mixture of ethanol/water.

The concept of administering nanoparticles incorporated into a carrier matrix for lung delivery was first introduced by Sham et al. [66]. For the preparation of the carrier powder containing nanoparticles (gelatin or *iso*-butyl cyanoacrylate), they dissolved lactose as a carrier powder in a nanoparticle suspension and spray dried it. They showed that the particle size of the nanoparticles before and after spray drying might change significantly due to the heat involved in the spray drying process. This study proved the possibility of delivering and releasing nanoparticles in the lungs and allows specific applications such as treatment of lung cancer, cystic fibrosis or asthma.

Grenha et al. [65] incorporated insulin loaded chitosan nanoparticles in microspheres using aerosol excipients like lactose and mannitol by means of a spray drying technique for pulmonary delivery as a dry powder. They showed that the microencapsulation process does not affect the insulin release from nanoparticles. They propose this method for systemic delivery of macromolecules through the pulmonary route of administration to promote peptide absorption.

The active release mechanism of nanoparticles from inhaled carrier particles was first introduced by Ely et al. [1]. They used a spray drying technique for preparing effervescent carrier particles containing cirprofloxacin nanoparticles thereby adding an active release mechanism to the pulmonary route of administration. They showed that effervescent carrier particles can be synthesized with an adequate particle size for deep lung deposition. Their results also showed that the effervescent carrier particles released 56±8% ciprofloxacin into solution compared with 32±3% when lactose carrier particles were used. The mean nanoparticle size did not significantly change upon release when the nanoparticles were incorporated into an effervescent formulation. The active release can also overcome the agglomeration of nanoparticles when the carrier matrix dissolves.

Based on the aforementioned researches performed in this area, it seems that pulmonary delivery of nanoparticles as dry powders for both local and systemic effects is a promising and feasible route of administration for the treatment of lung diseases and potentially for the systemic delivery of nano-sized drug delivery systems. However, the nebulization parameters of the nanoparticle delivery matrix must be optimized to prevent particle aggregation to reach an optimized drug delivery into the deep lungs. Table 2 shows a summary of pulmonary delivery of nanoparticles using dry powder carriers.

9.2. Delivery of nanoparticle suspensions using nebulization

Another method for the delivery of nanoparticles was spraying or nebulization of a nanoparticle suspension using a nebulizer. In a study Dailey et al. [69] introduced a novel surfactant free biodegradable nanoparticle system for aerosol therapy. They formulated nanoparticle suspensions from a branched polyester, diethylaminopropyl amine-poly (vinyl alcohol)-grafted-poly (lactide-co-glycolide) (DEAPA-PVAL-g-PLGA), as well as with increasing amounts of carboxy methyl cellulose. They showed that this new polymer has high encapsulation efficiency for drug molecules by utilizing electrostatic interactions. They claimed that using these nanoparticles "alveolar deposition can be easily achieved by either jet or ultrasonic nebulization of the nanoparticle suspensions". They also showed that not only polymer hydrophilicity was necessary to maintain stability during nebulization, but also the formation of well-defined nanoparticles is an important factor. In their study they showed that formulations containing free DEAPE-PVAL-g-PLGA tend to aggregate and, therefore, only anionic formulations will be suitable for nebulization. Also they showed that a critical amount of carboxy methyl cellulose is needed to prevent particles from agglomeration. In another study, Videira et al. [70] evaluated the role of lymphatic drainage in the uptake of inhaled solid lipid nanoparticles (SLN). They studied the biodistribution of SLNs (200 nm) following the aerosolisation of a ^{99m}Tc-SLN suspension in a group of rats. Their study showed an important and significant uptake of the radio-labeled SLN into the lymphatic system after inhalation, and a high rate of distribution in

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periaortic, axillary and inguinal lymph nodes. Nanoparticle accumulation in the regional lymph nodes suggests that the translocation mechanism of SLN may involve phagocytosis by macrophages followed by migration to the lymphatic system [70]. This study showed that inhalation can be an effective route to deliver radio-labeled SLN to the lungs, representing an alternative to the intraperitoneal route for targeting colloidal carriers to the lymphatics. This technology may provide the possibility of using radio-labeled SLN as a lymphoscintigraphic agent. Additionally, it can allow the delivery of cytotoxic drugs to lung cancers in different stages. As mentioned in Section 6, Pandey et al. [71,72] studied different routes of administration for the treatment of tuberculosis. In one study which was not previously discussed, they compared different routes of administration with aerosol delivery of anti-tubercular drugs (rifampin, isoniazide and pyrazinamide) using loaded to PLG nanoparticles in guinea pigs using. They separated the animals into different groups and treated them with either a free drug orally or intravenously administered, nebulized drug-loaded PLG nanoparticles and nebulized empty PLG nanoparticles at the same dose. They sprayed the nanoparticle suspensions using a saline solution and a compressor powered-nebulizing system with an exposure time of 3-4 min/animal. The results showed that T_{max} and AUC were much higher for the nebulized PLG nanoparticles compared to oral or intravenous administration. In their study, they showed that five doses every ten days of nebulized drug-loaded nanoparticles had the same effect as 46 daily oral doses. No colony-forming unit (cfu) count was detected in previously infected guinea pigs. Untreated animals and animals nebulized with drug-free nanoparticles showed comparable bacterial load in their lungs. They repeated the study but instead of PLG nanoparticles they used solid lipid nanoparticles [72]. They compared the chemotherapeutic potential of nebulized solid lipid nanoparticles incorporating three major anti-tuberculosis drugs, rifampicin, isoniazid and pyrazinamide against experimental tuberculosis with the same drugs administered orally. They showed that after a single nebulization to guinea pigs, drugs levels were maintained in plasma for 5 days and in the organs (lungs, liver and spleen) for 7 days, whereas the free drugs were cleared by 1–2 days. Also their study showed that mean residence time and bioavailability improved using nebulized nanoparticles. They showed that no tubercle bacilli could be detected in the lungs/spleen of infected guinea pigs after 7 doses of treatment every 7 days whereas 46 daily doses of orally administered drugs were required to obtain the same therapeutic benefit. Therefore nebulization of nanoparticles containing anti-tubercular drugs improves the bioavailability and reduces the dosing frequency for better management of pulmonary tuberculosis. Taking the results of the oral nanoparticle administration into account (discussed in Section 6) both results confirm that an improved drug delivery of the anti-tuberculosis drugs can be linked to the use of a nanoparticle based delivery system. In conclusion, nanoparticles are a very promising drug delivery system via different routes of administration in the treatment of tuberculosis.

In another study, Yamamoto et al. [73] prepared surface modified PLGA nanospheres with chitosan to improve the pulmonary delivery of calcitonin by mucoadhesion after deposition in the lungs. They administered the nanoparticles into the trachea of guinea pigs using a nebulizer. They showed that chitosan modified PLGA nanospheres, loaded with elcatonin, reduced blood calcium levels by 80% of the initial calcium concentration. The treatment also prolonged the pharmacological action up to 24 h, which when compared to unmodified nanospheres was significantly longer. They attributed these results to the retention of nanospheres adhered to the bronchial mucus and lung tissue combined with a sustained drug release at the adherence site. Also they showed that chitosan, either alone or on the surface of the surface modified nanospheres, enhanced the drug absorption possibly by opening the intercellular tight junctions.

In another study, McConville et al. [74] formulated itraconazole nanoparticles using either evaporative precipitation into an aqueous solution (EPAS) or spray freezing into liquid (SFL) technologies. They demonstrated that nanoparticles of itraconazole, a poorly water-soluble drug, can be dispersed into aqueous liquid and nebulized effectively to the lungs using a murine model which resulted in high drug concentrations in the lungs. They showed that local delivery of itraconazole nanoparticles is a promising alternative to oral or intravenous administration, thus potentially decreasing the incidence of side effects associated with a high drug serum concentration.

These studies show that the delivery of nanoparticle suspensions using nebulization is a possible route of administration. However, the used nanoparticle suspension must be physically and chemically stable to reach clinical relevance in the near future. Table 3 shows a summary of pulmonary delivery of nanoparticle suspensions using nebulization.

Table 3
Summary of pulmonary delivery of nanoparticle suspensions using nebulization

Nanoparticle type	Nanoparticle size (nm)	Nebulization device	Active ingredient	*In vivo* models	Ref
DEAPA-PVAL-g-PLGA [a]	76.2–213.6	Pari® LC Star and Optineb®	–	–	[69]
Solid lipid nanoparticles (SLN)	200	Ultrasonic nebulizer	^{99m}Tc	Rat	[70]
PLG [b]	186–290	Compressor-nebulizer system	Rifampin, isoniazide, pyrazinamide	Guinea pig	[71]
Solid lipid nanoparticles (SLN)	Not provided	Compressor-nebulizer system	Rifampin, isoniazide, pyrazinamide	Guinea pig	[72]
Surface modified PLGA [c] with chitosan	650	Ultrasonic nebulizer	Calcitonin	Guinea pig	[73]
Itraconazole nanocrystals	300–800	Aeroneb Pro micropump nebulizer	Itraconazole	Mice	[74]

[a] diethylaminopropyl amine-poly (vinyl alcohol)-grafted-poly (lactide-co-glycolide).
[b] poly (DL-lactide-co-glycolide) (50:50).
[c] poly (lactide-co-glycolide).

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10. Toxicity of inhaled nanoparticles

10.1. Toxicity of inhaled ultrafine particles

It has been shown that nanoparticles can translocate from the respiratory tract, via different pathways to other organs/tissues and induce direct adverse responses in remote organs [75]. In particular, such responses may be initiated through the interaction of nanoparticles with sub-cellular structures following endocytosis by different target cells. Therefore, special attention must be given to such effects, which could have serious consequences in a compromised organism or a compromised organ [75]. Most of the toxicological data is based on our knowledge from nanoparticles inhaled during daily life such as carbon black, diesel particulates, silica and titanium oxide nanoparticles [6], which are considered ultrafine particles (<100 nm in diameter). It has been shown that the toxicity of nanoparticles increases with decreasing particle size. Ultrafine carbon black particles are known to produce greater pulmonary toxicity in rats when compared to large-sized carbon black particle [76–78]. Single wall carbon nanotubes also show some degree of toxicity after inhalation [74,75]. Warheit et al. [79] studied the toxicity of single wall carbon nanotubes (SWCNT). In their study 15% of the rats who were exposed to high-dose (5 mg/kg) SWCNT showed mortality 24 h post instillation. They related this mortality to the mechanical blockage of the upper airways by the instillate and not the inherent pulmonary toxicity of the instilled SWCNT particulate. They showed that pulmonary exposure to SWCNT in rats produces a non-dose dependent series of multifocal granulomas, which were evidence of a foreign tissue body reaction and were non-uniform in distribution and not progressive beyond 1 month post exposure. Shvedova et al. [80] showed that aspiration of SWCNTs elicited an unusual inflammatory response in the lungs of exposed mice. This inflammatory reaction is probably triggered by damage to pulmonary epithelial type I cells which include a strong neutrophilic pneumonia followed by recruitment and activation of macrophages [80]. This early response can switch from the acute phase to fibrogenic events resulting in a significant pulmonary deposition of collagen and elastin. This phase is accompanied by a change in the production and release of proinflammatory (tumor necrosis factor-α, interleukin-1 β) to anti-inflammatory profibrogenic cytokines (transforming growth factor-β, interleukin-10). These inflammatory and fibrogenic responses are accompanied by a detrimental decline in pulmonary function and enhanced susceptibility to infection [75]. In another study, Barlow et al. [81] showed that exposure of type II cells to carbon black nanoparticles resulted in a significant release of macrophage chemoattractant. Xia et al. [82] compared the cellular effects of ambient ultrafine particles with manufactured titanium dioxide (TiO_2), carbon black, fullerol, and polystyrene nanoparticles on a phagocytic cell line (RAW 264.7) that is representative of a lung target for nanoparticles.

They showed that, among the particles tested, ambient ultrafine particles and cationic polystyrene nanospheres were capable of inducing cellular reactive oxygen species (ROS) production, GSH depletion and toxic oxidative stress. This toxicity involves mitochondrial injury through increased calcium uptake and structural organellar damage. However, TiO_2 and fullerol did not induce toxic oxidative stress. Also they showed that, increased TNF-α production could be seen with ultrafine particles induced by oxidant injury, but cationic polystyrene nanospheres induced mitochondrial damage and cell death without inflammation.

Wiebert et al. [83] studied the pulmonary retention of 35 nm ^{99m}Tc labeled carbonaceous particles on healthy and asthmatic volunteers who inhaled the test particles. Their study showed that there was no evidence of a quantitatively important translocation of deposited particles to the systemic circulation from healthy lungs. Although, only a small fraction of 35-nm combustion particles could find access from peripheral lungs to systemic circulation and extrapulmonary organs, it is possible that responses in the lungs lead to the onset of cardiovascular disease. However, they mentioned that the fraction of nanoparticles which possibly translocated from the lungs into the circulation (less than 1%) may not be sufficient to cause harmful effects.

Lin et al. [84] evaluated *in vitro* the cytotoxicity and oxidative stress caused by cerium oxide (CeO_2) nanoparticles in a human lung cancer cell. They showed that free radicals generated by exposure to CeO_2 nanoparticles produced significant oxidative stress in the cells, as reflected by reduced glutathione and α-tocopherol levels; these toxic effects of CeO_2 nanoparticles are dose and time dependent. Elevated oxidative stress increases the production of malondialdehyde and lactate dehydrogenase, which are indicators of lipid peroxidation and cell membrane damage, respectively.

Several studies have been undertaken to evaluate the effects of various ultrafine nanoparticles on alveolar macrophage functions. Brown et al. [80] and Warheit et al. [85] showed both in humans and rats that inhalation of low toxicity ultrafines results in impaired pulmonary clearance mechanisms. Also their work demonstrated that, rats exposed to ultrafine titanium dioxide particles show evidence of pigment-laden macrophages and macrophage aggregates in the alveolar spaces up to 6 months postexposure [86]. Pre-exposure of alveolar macrophages to ultrafine particles also significantly reduced subsequent macrophage phagocytotic abilities and the effect was shown to vary dependent upon the particle properties [87]. In another study, Möller et al. [88] investigated the intracellular effects of ultrafines on alveolar macrophages using flow cytometry and cytomagnetometry. They showed that ultrafine particles can impair phagosome transport and increase cytoskeletal stiffness at high concentrations which leads to a reduced phagocytotic capability, inhibited cell proliferation, and decreased cell viability. Inoue et al. [89] evaluated the effects of nanoparticles on lung inflammation related to bacterial endotoxin lipopolysaccharides in mice. They administered two sizes of carbon black nanoparticles and evaluated parameters of lung inflammation and coagulation. Their results showed that nanoparticles can aggravate lung inflammation related to bacterial endotoxin, which is more prominent with smaller particles. Also they showed that, nanoparticles can promote coagulatory disturbance accompanied by lung inflammation.

Sayes et al. [90] evaluated several different particles and variables which strongly impact the ability of *in vitro* screening

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studies to accurately reflect *in vivo* pulmonary toxicity of several particle types in rats. The results of their *in vivo* and *in vitro* cytotoxicity and inflammatory cell measurements demonstrated little correlation. They concluded that *in vitro* cellular systems need to be further developed, standardized and validated (relative to *in vivo* effects) in order to provide useful screening data on the relative toxicity of inhaled particle types. Takenaka et al. [91] investigated the role of alveolar macrophages in the fate of ultrafine particles (<100 nm) in the lung. They exposed rats to ultrafine gold particles and then examined lavaged cells and lung tissue for up to 7 days. They found gold nanoparticles on day 0 and day 7 in the lungs. Also they showed that 29% and 6% of the retained gold particles were lavageable on days 0 and 7 respectively. A low amount of gold was found in the blood. Also they reported the presence of gold in the cytoplasm of alveolar macrophages. Their results indicated that inhaled ultrafine gold particles in alveolar macrophages and type I epithelial cell are processed by endocytotic pathways. The uptake of the gold particles by alveolar macrophages is limited and systemic particle translocation takes place only to a very low degree.

As shown many nanotoxicology studies were performed in the area of environmental health sciences. It is now up to pharmaceutical sciences to translate this knowledge and address serious safety concerns regarding the concept of nanoparticle delivery to the lungs. Some of these aspects are discussed in Section 10.2.

10.2. Toxicity of polymeric nanoparticles used in drug delivery

Today there is plenty of information available about the toxicity of inhaled environmentally occurring dust nanoparticles, mostly ultrafine particles. While the absorbed dose of such dust particles is generally low (mostly less than 1%) [75], drug delivery strategies must yield high deposition rates to be therapeutic and economically feasible. For deep lung delivery there are different aspects which have to be considered; one is the acute toxicity of the drug delivery system on the epithelia; and secondly the interaction of nanoparticles with the alveolar environment. The first aspect was reviewed by Forbes and Ehrhardt [92] and the second by Gill et al. [6]. In pharmaceutical sciences generally natural or synthetic polymeric nanoparticles are used for drug delivery. Their known biocompatibility from other routes of administration e.g. intravenous is encouraging but the acute toxicity of these delivery systems must be established for the pulmonary epithelia too. To assess such effects, cell culture models seem to be the best way to proceed [92]. Forbes and Ehrhardt suggest different cell culture models which might be used to assess pulmonary drug delivery systems. They report that A549 (alveolar) and BEAS-2B (airways) cell lines have been used to study the nanotoxicological aspects of inhaled environmental pollutants [92]. These two cell lines which do not form functional tight junctions are considered suitable for toxicology studies of inhaled nanoparticles. Standard toxicity assays such as cellular metabolic activity, membrane integrity and the release of proinflammatory and inflammatory mediators can be performed with these cells after nanoparticles uptake. This adoption of screening methodology from environmental sciences will extend the safety information about the toxicological aspects of inhaled polymeric nanoparticles used for drug delivery.

However, there is currently no standard cell culture model available to mimic the epithelium permeability in the alveolar region except for pneumocyte monolayers in primary culture [93]. However, such cell models are available for the bronchial epithelium. Here 16HBE14o- and Calu-3 cells have shown to be suitable models.

16HBE14o-cells were used by Brzoska et al. [94]. They investigated the suitability of nanoparticles synthesized from porcine gelatin, human serum albumin and polyalkylcyanoacrylate as drug and gene carriers for the pulmonary administration.

They investigated *in vitro* the effect of these particles on primary airway epithelium cells and 16HBE14o-cells. They showed that the nanoparticles incorporated into bronchial epithelial cells have little or no cytotoxicity and cause no inflammation. In another study, Dailey et al. [95] studied the potential of biodegradable polymeric nanoparticles of PLGA and a novel PLGA derivative, diethylaminopropylamine polyvinyl alcohol-grafted-poly (lactic-co-glycolic acid), to provoke inflammatory reactions in mice lungs after intratracheal instillation. As control, they used two sizes of polystyrene nanospheres (75 and 220 nm) in their study. They instilled nanoparticles and then evaluated the inflammatory parameters such as lactate dehydrogenase (LDH) release, protein concentration, macrophage inflammatory protein-2 mRNA induction and polymorphonucleocyte recruitment in the bronchial alveolar lavaged fluid. Their results suggest that biodegradable polymeric nanoparticles designed for pulmonary delivery may not induce the same inflammatory response as non-biodegradable polystyrene particles of comparable size.

The second safety aspect of deep lung deposition is the interaction of nanoparticles with the alveolar environment. The alveolar space is covered with a thin surfactant film [6].

This film has important physiological functions e.g. to accelerate gas exchange and to lower the surface tension in the alveolar space. Compromising these functions by inhalable nanoparticles might cause life threatening consequences. Therefore, the compatibility of a delivery system with the alveolar environment must be considered.

Stuart et al. [96] investigated the interactions between gelatin nanoparticles and artificial lung surfactants using biophysical *in vitro* methods. They used dipalmitoylphosphatidylcholine (DPPC), the main lipid component of the lung surfactant film, as a model system and measured the surface pressure of the DPPC monolayer in the presence of gelatin nanoparticles using a Langmuir trough. Their results showed that interactions between the nanoparticles and the lung surfactant film did not destabilize the monolayer. This demonstrates that pulmonary nanoparticle delivery is a possible and safe route of administration. However, dose and deposition margins have still to be defined.

In summary, the results of the different studies show that although there are some concerns regarding the safety of ultrafine inhalable nanoparticles, the inhalation of biocompatible polymeric nanoparticles used for drug or gene delivery might expose little or no toxicity if the particle size is not smaller than 100 nm.

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The application of inhalable nanoparticles will hopefully find its way into clinical studies soon.

11. Conclusion

Pulmonary drug delivery is becoming more and more important. This is due to the specific physiological environment of the lung as an absorption and treatment organ. The development of inhalable insulin can be seen as a milestone in pulmonary drug delivery.

It demonstrates that dry powder delivery systems allow the absorption of large molecules into the systemic circulation. The clinical application of drug delivery systems like nanoparticles is still in its preclinical phase. Pulmonary drug delivery offers tremendous opportunities to improve drug therapies systemically and locally using advanced drug delivery systems like nanoparticles. However, nanotoxicological aspects of inhaled drug delivery systems have to be considered and *in vitro* methods be established to ensure safety.

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