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## **Nanoparticles used in medical applications for the lung: hopes for nanomedicine and fears for nanotoxicity**

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**Abstract.** Nanotechnology is a promising tool for the development of innovative treatment strategies allowing to overcome obstacles encountered by classical drug delivery. This has led to the development of nanomedicine. Indeed, nano-delivery systems (NDS) may allow the controlled release of therapeutics, protection of drugs against degradation, targeted drug delivery and facilitated transport across barriers. All these advantages of NDS are particularly interesting for treatments of the lung which is a challenging organ in respect to drug delivery. However, for the development of nanomaterials aimed to transport therapeutics, there is also a need to assess the potential health hazards of these new materials, especially as a variety of nanoparticles have been shown to induce toxicity related to their nanometer size leading to the new field of nanotoxicology. We will address both aspects of NDS, specifically in respect to lung treatments: their potential benefits and the possible adverse health effects of these materials.

### **1. Introduction**

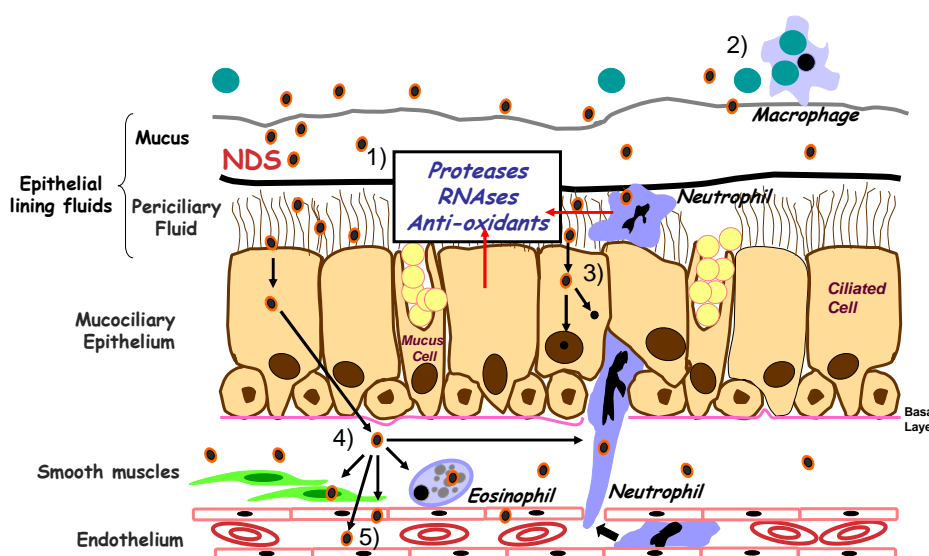
Nanoscience and Nanotechnology are developing very rapidly. The European Commission's strategy for nanotechnology has highlighted the need to invest in knowledge, technology and experience in this field in Europe (COM (2004) 338). An emerging component in this field is nanomedicine which is rapidly developing. However, a comparison of the number of publications on nanomedicine with nanotechnology indicates that nanomedicine still accounts for a little amount of nanotechnology research worldwide [1]. Actually there are two areas in which the impacts of nanomedicine are likely to be most significant: first the diagnostic and medical records sector and second new treatment options [2]. Indeed, various nanoparticles (NPs) are in clinical use or under development for therapeutic purposes with the aim to develop systems for imaging and drug delivery. Nanovectors may in fact allow to overcome several challenges in drug delivery. Embedding of pharmaceuticals into nano-delivery systems (NDS) could protect them from degradation. Furthermore a controlled release of therapeutics would enable persistent drug delivery and treatments at diminished doses. A great advantage of nanomedicine will be targeted drug delivery which allows reducing administration doses and thus lowering side-effects, toxicity and exposure of non-target organs. Certain drugs are difficult or impossible to be efficiently transported through physiological barriers and are administered systemically, which results in possible metabolism and clearance in the liver. Thus higher dosing is necessary to achieve therapeutic effects with possible target and non-target organ toxicity. An

important field of nanomedicine research is consequently the development of new treatment strategies for a controlled, selective and efficient transport of drugs through biological barriers such as the lung. However, the safety of NDS is a principal issue of concern for the development of nanomedicine as nanomaterials will interact with complex biological systems. Several *in vivo* and *in vitro* studies have indeed demonstrated that nanomaterials could induce toxicity in multiple organ systems and NDS may thus have adverse health effects which have been poorly studied thus far.

## **2. Promising benefits of nanovectors for improved drug delivery to the lungs**

Several recent reviews have highlighted the promising role of nanomedicine for the treatment of respiratory diseases [3-5]. Indeed, the treatment of the airways could be limited by the anatomical and physiological barriers of the respiratory tract, which may be bypassed by the use of NDS. In the conducting airways, the mucociliary clearance allows the transport of foreign substances such as bacteria and particles to the pharynx, for their elimination by expectoration or ingestion. In the distal lung, the alveolar clearance is achieved by alveolar macrophages that are present in the lumen of alveoli and which are professional phagocytes. An important challenge of nanomedicine will be to circumvent these elimination roads. Furthermore, the use of NDS will allow to protect protein drugs from proteases present in the mucus and surfactant, especially in inflamed lungs. Another promising feature of NDS, beside their potential to cross the lung lining fluids, is their capacity to be taken up by respiratory epithelial cells [6] as modern treatment strategies aim to introduce expression vectors, genes, antibodies or siRNA into cells to induce or inhibit local production of proteins involved in pulmonary diseases [7-10]. As the airway epithelium is highly resistant to transfection, due to the extracellular lining fluids [11], the use of NDS is a promising tool to achieve penetration. Attempts using NDS to increase transfection efficiencies have recently been conducted for pulmonary treatments with promising results. In this regard, it is also interesting to note that endocytosis of NPs by epithelial cells has been shown to be size dependent and smaller NPs are more efficiently taken up [6,12]. The epithelium of the conducting airways also presents tight junctions, preventing paracellular crossing of foreign substances, but NPs have been shown to translocate through the epithelium in *in vitro* and animal studies, with smaller NPs being more efficient [13,14] thus allowing to foresee treatment strategies of the submucosa. Indeed, pathophysiological modifications, such as fibrosis and infiltration of inflammatory cells, are important features of several respiratory diseases. However, body distribution studies of inhaled NPs show low levels of translocation [15]. It is important to note that translocation studies have only been performed after a sole exposure to NPs and a more significant translocation is thus likely to occur in case of chronic exposure and coating of NPs may also allow to increase their translocation. Interestingly, long-term studies over 6 months have shown initial interstitial uptake and a return of NPs to the alveolar lumen, where they were cleared by macrophages [16]. In pathological conditions, the administration may even be more difficult. For instance, the epithelium in chronic bronchitis is characterized by mucus hypersecretion and squamous cell differentiation at late stages. Due to the hypersecretion, the delivery of drugs targeting the alveolar region may also be more difficult in COPD or asthma. Pathological situations could also increase the translocation, as shown in animal models of inflammation [17,18] which may be due to increased paracellular translocation. The differences in route of administration (inhalation versus instillation) may explain the divergence in the extent of translocation observed between studies. Instillations are generally performed at high doses as a bolus whereas inhalation studies are usually carried out at much lower doses. *In vitro* studies have furthermore highlighted the role of NP physicochemical characteristics in their ability to cross the epithelial barrier [19]. However, interpretation of *in vitro* studies has to be done with great caution since they are generally carried out at high doses. In addition, to direct translocation through the epithelial barrier, an indirect transfer via macrophages is plausible as migration of NP loaded-alveolar macrophages has been observed towards the blood circulation and extrapulmonary organs [20]. Due to the high surface area and small air-blood distance of the alveolar epithelium, the lower respiratory tract may be treated by intravenous administration of pulmonary drugs. This treatment route is already studied for antitubercular drugs and NDS have been shown to

increase their pulmonary delivery [21]. The translocation through the alveolar epithelium of drugs, by the use of NDS, is also considered for systemic treatment strategies for non pulmonary diseases. Insulin is already administered by inhalation (Exubera®) and NDS have been shown to increase plasma levels [22,23]. Indeed the alveolar clearance by macrophages may be bypassed by NPs, as it has been shown that these are less efficiently taken up by macrophages than micrometer sized particles [13,24], whereas epithelial and interstitial uptake is increased for smaller particles [13]. Furthermore this administration route allows circumventing first-pass metabolism and side effects seen in systemic oral or intravenous delivery. Indeed the lung presents lower levels of drug efflux transporters and metabolizing enzymes than the gastrointestinal tract.



**Figure 1.** Nanovectors for improved drug delivery to the lungs.

Nanodelivery systems (NDS) present several possibilities to improve treatments of the upper airways:

- 1) NDS could protect the drugs from degradation by enzymes present in the epithelial lining fluids.
- 2) The nanometre size of NDS may enable to circumvent the clearance by macrophages.
- 3) Facilitated crossing of the lining fluids and endocytosis of NDS will improve transfection efficiencies. Particularly important target cells are epithelial cells, neutrophils (eg in COPD) and eosinophils (eg in asthma).
- 4) Transcytosis of NDS will allow the treatment of the submucosa.
- 5) Crossing of the endothelium will allow systemic treatment by NDS as well as intravenous administration of pulmonary drugs. The crossing of the air/blood barrier is however even more prominent at the alveolar epithelium.

## 2.1. Benefits of NDS for innovative treatment strategies of the lung: monoclonal antibody therapies for COPD

Chronic obstructive pulmonary disease (COPD) is currently the fourth leading cause of death in the world, and if current trends continue, it is likely to rank as the third most important cause of death by the year 2030 (WHO, World Health Statistic 2008). COPD has a well recognised inflammatory component that is central to the development and progression of the illness and contributes also to many of the extra-pulmonary features. As cytokines and chemokines play a critical role in the orchestration of COPD, the use of monoclonal antibodies are valuable treatment options for more specific therapies [8,9]. Special emphasis has been put on the inhibition of neutrophil infiltration, which plays an important role in the pathogenesis of COPD. A multitude of antibodies targeting

cytokines, chemokines, growth factors and their receptors are considered for the treatment of COPD [8,9,25,26] and some have already been studied in clinical trials with COPD patients. The anti IL-8 antibodies Abgenix [27] improved dyspnea, but not lung function and this limited clinical benefit has been attributed to the suboptimal dosing in the airways, due to intravenous administration. Thus, the use of NDS may allow administration by inhalation, increasing local concentrations and avoiding possible side effects. Moreover, several clinical trials, with the anti TNF $\alpha$  antibody infliximab have been conducted but have shown no effects in COPD patients so far [28-30]. A clinical trial using the EGFR inhibitor BIBW 2948 in COPD patients has been conducted but was poorly tolerated. Therefore a higher local dosage is needed to allow a better inhibition of this receptor [31] and NDS should enable a better dosage at the site of action and a prolonged release of drugs. The use of monoclonal antibodies could have higher selectivity and reduced side effects compared to the use of inhibitors [9]. Thus, delivery of monoclonal antibodies such as Cetuximab (already in clinical trials for cancer therapy) could be a valuable option for the treatment of COPD.

### 3. Toxicity of nanomaterials

While a lot of attention has been paid to the development of new engineered nanomaterials and to new applications of nanotechnologies, comparatively less research has been performed to assess the potential hazard of these new materials. Concerns about the potential impacts of NPs on health effects have been expressed since NPs of same chemical composition possess different physicochemical properties than particles of larger size [6,13]. Indeed, when the particle size decreases, the proportion of atoms on the surface is increased to manifolds. Thus, NPs could have a greater reactivity and increased catalytic potential. This increase in the surface of engineered particles provides thus benefits compared to larger particles but could also result in much higher biological activities than bigger particles [34]. Answers to these questions are essential to identify the hazards related to nanomaterials and to accept or to compel knowingly the expected explosion of nanotechnology in general, and nanomedicine in particular. NP toxicity studies are mostly conducted with materials that are not designed for medical use (CB, CNT, metal oxides...) and which are more relevant for environmental and occupational exposures. However, in regard to development of NDS for clinical applications, the present knowledge of NP toxicity from studies on occupationally relevant NPs could be of interest. However, most of the toxicity studies have been carried out at very high doses which may not represent real-world conditions. Nevertheless these studies have allowed to identify several potential health hazards of NPs. Especially these studies have put forward the need of nanomaterial specific toxicity testing strategies and the role of physico-chemical characteristics (surface area, surface reactivity, charge as well as size and shape) in the toxicity of NPs (for review see [32]).

#### 3.1. Pulmonary toxicity of nanomaterial

Respiratory effects of NPs are mainly inflammation, oxidative stress and functional disturbances. A variety of *in vivo* studies demonstrates inflammatory effects of NPs as transitory responses and assess whether the degree of the inflammatory response is related to the exposure dose of NPs. In fact, inhalation [33-35] or instillation exposures [36-38] of various types of nanomaterials have shown to induce inflammation in lungs. These studies revealed local invasion of leukocytes, increased numbers of inflammatory cells in broncho-alveolar lavage fluid (BALF), release of LDH and increased cytokine production. In addition, typical granulomatous reactions are observed after pulmonary exposures to CNTs [39-41]. Furthermore functional disturbances could be observed after NP exposure including airway hyper-reactivity (AHR) to non-specific stimuli, tissue injury leading to disturbance in respiratory functions [42-45] and effects on existing pulmonary inflammation [46]. These disturbances are generally studied in pathologic animal models, like asthma etc. Translocation could be increased by lung injury and thus the presence of cadmium NPs in the liver, after inhalation of high doses, has been attributed to their pulmonary toxicity [47]. In the context of pulmonary toxicity, it is important to note that computational models predict increased deposition of inhaled NPs in diseased or constricted

airways [48] and obstructive lung diseases have indeed been shown to increase pulmonary retention [49].

The carcinogenic potential of low toxicity low solubility dusts (LTSD), like CB and TiO<sub>2</sub>, has been established in experimental animals [50] but these were generally performed at high concentrations and in sensitive species. There are very few epidemiological studies on human exposures and thus lung cancer risks could not directly be associated with engineered nanomaterials. The mechanisms leading to NP carcinogenicity are unknown and may involve primary genotoxic insult or secondary genotoxic response due to particle induced inflammation [51].

### 3.2. Cellular mechanisms

The cellular mechanisms, responsible for these effects, have been postulated to involve an oxidative stress induction by nanomaterials. Nel *et al* and Xia *et al* proposed a hierarchical oxidative stress model to explain the biological effect of NPs, stating that “minor levels of oxidative stress induce protective effects that may yield to more damaging effects at higher levels of oxidative stress” [52,53]. The induction of a cascade of cellular events, due to this oxidative stress, leads to the induction of redox sensitive pathways in the cells. Modifications of cell cycle, proliferation, pro-inflammatory response and cell death by apoptosis or necrosis could be the consequences. Many recent reviews on the mechanisms of action of NPs sustain this oxidative stress paradigm [52-54] while few have questioned the central role of oxidative stress in these responses to NP [55,56]. Several studies pointed out the roles of size and surface reactivity in the ability to induce an oxidative stress [33,53]. The adverse effects on the target tissues could either be due to abiotic or biotic reactive oxygen species (ROS) induced by NP or to ROS produced indirectly from the inflammatory process induced by NP exposures. The activation of redox sensitive transcription factors, such as NF-kappaB, AP1 or Nrf2, by this oxidative stress could lead to the transcription of a number of pro-inflammatory genes and the increased release of numerous pro-inflammatory factors. However, adaptive responses may diminish these effects which make the extrapolation on health effects in humans very difficult.

A modulation of intracellular calcium by carbon black NPs has also been demonstrated [57]. This transient increase of intracellular calcium by NPs has been shown to trigger impaired phagosome transport and cytoskeletal dysfunctions. The underlying mechanism may involve direct effects of NPs on ion channels which control the cellular calcium homeostasis. Higher NP concentrations have also been shown to induce apoptosis in a variety of cells [58-62]. Differential signalling events are responsible for these apoptotic effects, involving death receptor, mitochondria or lysosomes.

## 4. Conclusions

Nanomedicine presents a great opportunity for the radical improvement of current therapies and development of new treatment options for diseases previously thought difficult or impossible to treat. In particular the treatment of lung disorders could be achieved by the use of specially designed NDS. In this paper we reviewed the multiple advantages of NDS to improve lung treatments, particularly the special feature of NPs to be taken up by cells and to translocate through the epithelial barrier. However, the need exists of a balance between therapeutic efficacy and safety of the NDS with the aim to increase the benefit to risk ratio. Studies of pulmonary toxicity of environmental and occupational NPs allow to foresee possible adverse health effects of NDS. There is now a great need to perform studies with chronic exposures at low doses which are more realistic to be encountered in real live conditions. The understanding of the mechanisms behind these NP specific effects is essential for the development of safe NDS allowing the rapid expansion of new applications of nanomedicine for innovative treatment strategies.

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