Expert Opinion

The nanoscale in pulmonary delivery. Part 1: deposition, fate, toxicology and effects

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This two-part review explores the nanoscale in inhalation delivery. The first part covers the deposition, fate, toxicity and effects of nanoparticles delivered via inhalation. The second part analyses the potential of major inhalation delivery routes. Efficient particle deposition in the lung can be achieved with nanoparticles (50 – 100 nm). However, this particle range has hardly been exploited in a medical setting. Thus, formulation scientists have a rare opportunity to develop new concepts in inhalation delivery. The delivery of nanoparticles raises concern over increased toxicity, but also opens up the possibility for enhanced therapeutic effects and reduced dosage. Toxicity data available so far concerns mainly non-therapeutic molecules, and it remains a moot point as to whether these apply to drug molecules.

Keywords: drug delivery, formulation, inhalation, nano, nanodroplet, nanoparticle, nanoscale, pulmonary delivery

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1. Introduction

The exposure of individuals to airborne nanoparticles is nothing new. '*Human beings have been exposed to airborne nanoparticles throughout their evolutionary stages. Airborne particles are ubiquitous in the air we breathe, and have been an integral component of the earth's atmosphere for as long as generation mechanisms such as fires, volcanic emission, sea spray and dust re-suspension have been in existence*' [1].

The delivery of nanomatter to the lung has attracted interest in the pharmaceutical literature for some time. A small number of articles and reviews on the topic are available, often part of wider reviews on nanoparticles and their use in oral, parenteral or pulmonary drug delivery [2-4]. However, by far the most publications have been on the possible adverse effects of nanomatter in the lungs [1,5]. So far there has not been a full review on the benefits of nanoscale technologies and/or how they could be leveraged for pulmonary delivery.

The adaptation of nanoparticles to pulmonary delivery applications is still in its infancy. If nanoparticles could be delivered to the lungs, their unique properties in avoiding mucociliary clearance [6] and in having a high mass-to-surface ratio may be used for the therapeutic treatment of lung-specific diseases and systemic delivery.

The term nanoscale encompasses a broad scale of size ranges. This calls for a definition of the term 'nano'. Nanotechnology has been described as the manipulation, precision placement, measurement, modelling or manufacture of matter in the sub-100-nm scale [7]. According to the National Institutes of Health Roadmap Initiative, nanomedicine encompasses specific intervention with devices and structures operating at ≤ 100 nm in scale [8]. Alongside this broad definition, the term 'ultrafine particles' is also found. This seems to be

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used for particles < 100 nm [1]. However, the 100-nm limit is constraining, as in effect it would dismiss many recent discoveries. Therefore, in this review, the authors consider all particulates for which at least one dimension is 1 – 1000 nm.

This review first looks at the deposition and fate of nanoparticles in the lungs. This opens the way to discussions of their toxicity and effects in the lungs. The second part of this review deals with specific delivery and formulation routes, such as pressurised metered-dose inhalers, dry powder inhalers and nebulisers.

2. The deposition and fate of nanoparticles in the respiratory tract

Inhalation delivery has traditionally been based on the premise that 'optimum drug delivery to the conducting airways occurs with particles of 2.5 – 6.0 μm. Particles < 2.5 μm are deposited mainly in the alveoli, where they may exert no pharmacodynamic effect and are rapidly absorbed, increasing the risk of systemic adverse events. This size range has been confirmed for mild asthmatics, for whom the particle size of choice should be ~ 2.8 μm [9].

Because of the focus on microparticles, a quasi-disregard for nanoscale inhalation delivery has been widely adopted. As long as a local effect is required for the treatment of respiratory conditions, the nanoscale is not considered a viable option. However, high deposition fractions for nanoparticles with sizes of < 100 nm have been evidenced [10].

2.1 What size range can deposit in the lung?

Particle deposition studies have long identified that nanoparticles can reach the lungs [11]. Two size ranges can lead to maximum deposition: 50 – 100 nm, corresponding to the alveoli, and 1 – 5 μm for the lower airways (as is reviewed later). Most inhalation delivery products only use the upper size range, leaving the lower size range mostly untapped. This is partly due to technological limitations in generating stable aerosols and particles in the range of 50 – 100 nm. However, with the present interest in nanotechnology, this is now the subject of investigations that this review aims to cover.

In vivo, in vitro and *in silico* studies have confirmed numerous times the possibility to deposit nanoparticles efficiently in the airways.

In **Figure 1**, the deposition fractions of particles with different diameters in selected regions of the respiratory tract (laryngeal, upper and lower bronchial, alveolar) have been plotted as a function of particle size for particles of unit density (i.e., normalised aerodynamic diameter). In this context the term 'deposition' refers to the mean probability of an inspired particle being deposited in the respiratory tract by collection on airway surfaces. Total deposition refers to particle collection in the whole respiratory tract, and regional deposition to particle collection in a region of the respiratory tract [12]. Optimum alveolar deposition can be achieved for two distinct particle sizes: 3 μm and ≤ 50 nm. The latter size distribution offers inhalation delivery opportunities yet untapped by inhalation products, except a few recent mentions in the literature [13].

In a recent study [14], 22 healthy young adults were exposed to an aerosol of non-hygroscopic inert monodispersed metallic nuclei particles coated with sebacate oil. The nominal particle droplet size was 40 – 100 nm. The study showed that the total respiratory tract deposition fraction of ultrafine aerosols increased with a decrease in particle size. Morawska *et al.* [15] studied the total deposition of combustion aerosols *in vivo* (diesel fumes, petrol particles and tobacco smoke, sizes ranging 69 – 183 nm) and confirmed the work carried out by Stahlhofen *et al.* [10]: the total deposition of particles increases with a decrease in size. A theoretical model for the total respiratory tract deposition fraction of ultrafine particles has even been validated for aerodynamic sizes of 40 – 100 nm [14]. Hence, the efficient deposition of nanoparticles in the lungs is possible.

2.2 Deposition mechanisms

A series of reviews and studies are available on the deposition and fate of nanoparticles in the lungs [3,5,11,12]. These are summarised briefly here (see also **Table 1**). Three deposition mechanisms are dominant for uncharged nanoparticles [11]:

- Impaction by the collision of particles with the surface of the airways, due to inertial forces.
- Gravitational sedimentation: an important mechanism for deposition in the smaller bronchi, bronchioles and alveolar spaces, where the airways are small and the air velocity is low. Sedimentation becomes less effective than diffusion for aerodynamic diameters of < 0.5 μm.
- Diffusional deposition: important in small airways and alveoli for particles of < 0.5 μm (i.e., nanoparticles, as identified above).

Heyder *et al.* [12] adopted a similar description of deposition patterns, via the consideration of a thermodynamic domain (i.e., Brownian diffusion) and an aerodynamic domain (sedimentation and impaction, corresponding to gravity and inertial forces). Their work on experimentally determined deposition data showed that particles with aerodynamic diameters of < 0.16 μm deposit only by diffusion in the alveolar region; particles of 0.16 – 2.0 μm deposit by diffusion and sedimentation, mostly in the bronchial region, and particles > 2.0 μm deposit by sedimentation and impaction, mostly in the upper airways.

There is a quasi-unanimous consensus in the scientific community that Brownian diffusion is a significant mechanism for particles less than or equal to ~ 0.5 μm. The particles move by random bombardments of gas molecules and collect against the respiratory walls [3]. For particles smaller then a few nanometres in diameter, the diffusion

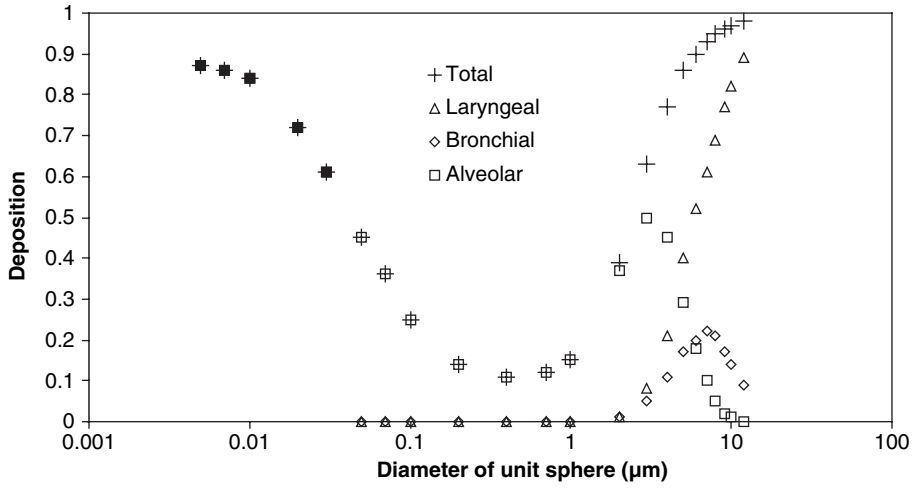

Figure 1. The deposition of unit density spheres in the respiratory tract for steady breathing at a mean flow rate of 750 cm^3/s, breathing cycle 4 s, tidal volume 1500 cm^3.
Data from [12].

Table 1. The principal mechanism of particle deposition in the lungs.

Size	Dominant mechanism	Heyder *et al.* [12]
> 0.5 µm	Impaction	> 2.0 µm
> 0.5 µm	Sedimentation	0.16 – 2.0 µm
< 0.5 µm	Diffusion	< 0.16 µm

Data from [12].

coefficient is very high, leading to a very high probability of deposition. The deposition of ultrafine particles is governed primarily by the diffusion process, and the diffusion coefficient of particles is the sole parameter for determining the deposition of ultrafine particles [14]. *In vitro* data on human lung casts performed with particle sizes of 1 – 40 nm confirmed that for the entire ultrafine particle size range, diffusional deposition is the dominant mechanism [16].

It must be remembered that in deposition studies, aerosol droplets and particles are highly dynamic systems [3]. Particle size does not remain constant once it reaches the respiratory tract. Volatile aerosols become smaller with evaporation, hygroscopic aerosols grow bigger with moisture from the respiratory tract, and particulate aerosols can agglomerate. Therefore, knowledge of the primary particle size will not be enough to predict deposition, and the formulation of nanoparticles for pulmonary delivery will require knowledge of the dynamics of aerosol behaviour for efficient targeting.

2.3 Parameters influencing particle deposition

A range of parameters influence particle deposition. Increasing air velocity increases impaction deposition, but decreases sedimentation and diffusion deposition. Breathing patterns: tidal volumes, respiratory time and flow rates all influence deposition. Total respiratory tract deposition increases with mean respiratory time and tidal volume (maximum inspiration volume). Total deposition has been shown to be dependent on mean residence time (T_m) and tidal volume (V_t), according to the following equation [14]:

$$TDF = (DT_m)^{0.5} V_t^{0.49} \quad (1)$$

D is the diffusion coefficient of the particles in air. The respiratory period and T_m of particles in the respiratory tract and the V_t are the two most important breathing parameters affecting the deposition of ultrafine particles. Deposition increases to almost the same extent with an increase in T_m or V_t [14]. Age does not substantially influence the deposition patterns of ultrafine particles (i.e., < 100 nm), except maybe for very small particles (1 – 2 nm) and for very young subjects (3 years of age, *in vitro* cast data) [16]. It is unclear how gender effects nanoparticle deposition, although Kim and Jaques [14] did notice differences in the total deposition fractions between healthy male and female adults at the same breathing pattern. The total deposition was higher for women than for men for particle sizes < 40 nm.

Hofmann *et al*. studied the influence of airway structure on particle deposition in an *in vitro* modelling work of human and rat lungs [17]. The parameters studied were airway generation, airway diameters and cumulative path lengths. Of the three parameters, airway diameter was the most appropriate morphometric parameter to classify local particle deposition in both human and rat models for particle sizes ranging from 1 nm to 10 µm. This of course is

variable between patients: there are inter-individual differences in the human population that affect the deposition and clearance, due to factors such as age, existing respiratory conditions, the state of the mucous layer and exposure to other respiratory hazards (e.g., cigarette smoke) [18].

2.4 The fate of deposited particles

Once deposited on the surface of the airways, the fate of nanoparticles depends on their solubility and their landing site (see **Table 2**). The efficiency of inhaled nanoparticles depends on their deposition, as well as their subsequent fate in the lungs.

Inhaled nanoparticles can be dissolved in lung fluid, act locally or can be absorbed systemically, or can be translocated out of the respiratory tract when insoluble [5]. If the deposited matter is fairly soluble in body fluids, it will enter the blood circulation. For matter that is relatively insoluble, clearance is governed mainly by the mechanical removal of particles by phagocytosis by alveolar macrophages and mucociliary transport.

Several phases of removal are usually accepted: i) a rapid phase to remove particles from extrathoracic airways; ii) a fast phase to remove particles from ciliated thoracic airways; iii) a slow phase to remove matter deposited in the non-ciliated thoracic airspaces. The most prevalent mechanism for solid particle clearance in the alveolar region is mediated by alveolar macrophages, through phagocytosis of deposited particles. This is followed by a gradual movement of the macrophages with internalised particles towards the mucociliary escalator. The retention half-time of solid particles in the alveolar region based on this clearance mechanism is ~ 70 days in rats and up to 700 days in humans [5]. Within 6 – 12 h after deposition in the alveoli, virtually all of the particles are phagocytosed, but it would appear that there are significant particle size-dependent effects on the effectiveness of this process [19]. The optimal particle size for phagocytosis by alveolar macrophages has been estimated to be 1 – 3 µm, and smaller particles result in a rate of phagocytosis that is progressively slower [20]. As a result of their small size, defence against nanoparticles would be less efficient, as their recognition by macrophages is suggested to be impaired or less effective. However, in cell culture, alveolar macrophages are able to phagocytose nanoparticles, and are consequently stimulated [21,22].

Those molecules and particles that are not removed by phagocytosis, such as nanoparticles in the deep lungs where there are no macrophages, readily gain access to epithelial and interstitial sites, blood circulation and even the lymphatic nodes. Once nanoparticles have translocated to the blood circulation, they can be distributed throughout the body. Different mechanisms have been proposed for uptake into systemic circulation and biological tissues. One involves transcytosis across the epithelium of the respiratory tract into the interstitium and access to the blood circulation directly or via the lymphatics. The other is a not generally recognised mechanism and appears to involve nanoparticle uptake by sensory nerve endings embedded in the airway epithelium [5]. The interchange of fine particles across the alveolar epithelium is more prominent in large species (dogs, primates) than rodents [23], suggesting that a high rate of translocation is likely to occur in humans. In addition to the conventional clearance processes, experimental animal models suggest that nanoparticles can translocate to extrapulmonary organs [24], suggesting a potential systemic health hazard [25]. Evidence in humans for the translocation of inhaled nanoparticles into blood circulation is ambiguous, with one study showing rapid appearance in the blood [24], and significant accumulation in the liver [26], and another study, using the same labelled particles, reports no such accumulation [27]. Kreyling *et al.* [25] for example, do not mention any translocation of iridium particles in rat lungs after 1 week. Particle uptake is chemistry dependent and it is possible to find evidence for the absence of nanoparticle translocation, as in the *in vivo* inhalation study of Mills *et al.* [28] on the uptake of ^{99m}Tc-labelled carbon nanoparticles. Ten healthy volunteers inhaled the aerosolised particles, and although radioactivity was very rapidly found in blood samples, no nanoparticles were detected, indicating no translocation.

Lymphatic uptake is particularly important for nanoparticles. This has been evidenced in a radiolabelling study of ^{99m}Tc solid lipid nanoparticles. The particles were delivered by nebulisation, and had an average size of 200 nm. The results showed significant uptake of the radiolabelled nanoparticles into the lymphatics after inhalation [29]. Furthermore, lymphatic drainage has also been held responsible for the alveolar clearance of deposited nanoparticles up to a certain particle diameter (i.e., 500 nm) [30]. Again, the phagocytic activity of bronchoalveolar macrophages seems to be the vector for the uptake of foreign particles in the lymphs [29,31]. The delivery of drug nanoparticles to the lymph nodes is particularly desirable in lung cancer treatment.

3. Toxicity and effects of nanoparticles

Small is beautiful, but '*smaller is not always better: nanotechnology yields nanotoxicity*' [32]. This statement seems to have become the creed of any critics against nanoparticles. As is shown in the following subsections, the data available so far on inhalation toxicology at the nanoscale does not primarily concern therapeutic molecules, and, therefore, is not directly applicable to inhalation therapies.

3.1 Toxicity concerns versus experimental data

A genuine concern exists with the safety of nanoparticles in their interaction with the environment and its inhabitants. This fear is partly fuelled by a range of toxicology reports that have suggested that nanoparticles pose a unique risk to everything from bacteria to mammals [33]. These fears have

Table 2. The fate of deposited particles in the lungs.

Type of particle	Uptake or clearance*
Soluble particles	→ Dissolution → blood circulation
Insoluble particles	→ Local action
	or → Translocation, transcytosis, systemic or sensory nerves uptake → Lymphatic uptake (~ 500 nm)
	→ Clearance → macrophages → mucociliary

*Arrows indicate a sequential order of events.

been relayed by citizen awareness groups, which have called for a moratorium on releasing new products containing nanoparticles and on lab-based research using nanomaterials until health officials come up with standards for dealing with nanomaterials [101]. There is some evidence to suggest that some nanoparticles are more toxic than their larger chemical forms and as such they should be treated as new chemicals, but a lack of extensive toxicology data on engineered nanoparticles does not allow for an adequate risk assessment. This does not necessarily need to be read negatively, but is a reminder that nanoparticles have new and unique biological properties, and that the potential risks associated with nanoparticles are not the same as those of the bulk material of the same chemistry, for better or for worse.

Pulmonary delivery is not exempt from these concerns. The biokinetics of nanoparticles are different from larger particles. When inhaled, they are efficiently deposited in all regions of the respiratory tract; they evade specific defence mechanisms, and they can translocate out of the respiratory tract via different pathways and mechanisms. Inhaled nanoparticle effects are no longer confined to the lung, as particles are suggested to translocate to the blood, and lung inflammation invokes systemic responses [34]. The mechanism of action of nanoparticles in the lung remains to be fully understood. It has been suggested that poorly soluble substances retained in the lungs can cause oxidative stress, either directly from free radicals on their surfaces, or indirectly by triggering the influx of defensive cells into the lungs, leading to inflammation, fibrosis or cancer [1].

3.2 The toxicity of inhaled non-therapeutic molecules

Although the health and safety issues of inhaled nanoparticles have been known for over a decade [35,36], the bulk of toxicity data on inhaled material concerns non-therapeutic molecules. There is a certain amount of data on the toxicity of nanoparticles [15,37,38], yet this data is mainly based on a limited selection of nanoparticles to which a large majority of people are exposed (diesel soot, TiO_2, air pollutants), but do not constitute the basis of inhalation therapies.

Toxicology studies of spherical and fibrous nanoparticles have established that natural (e.g., asbestos) and man-made (e.g., biopersistent vitreous) fibres are associated with an increased risk of pulmonary fibrosis and cancer, after prolonged exposures [39]. An underlying assumption of these studies is that a lot of the effects observed must be driven by the presence of nanoparticles [38]. However, the principal mechanism for these health outcomes is not well understood.

Inhalation toxicology databases on nanomaterials are rather sparse, and discussions on the potential effects of their widespread use in commercial products are just beginning to emerge [102]. The present knowledge in the field has recently been reviewed [40], and there has been significant recognition of the need for some form of regulation from the European Union Commission [103]. Even though there are presently no studies of exposure and response to engineered nanomaterials (i.e., therapeutic particles) in humans, their potential health hazards can be extrapolated from studies on exposure to nanoparticles in the workplace [41]. One of the most studied occupational groups is coal miners, who have experienced high morbidity and mortality from pulmonary diseases [42].

3.3 Nanoparticles and systemic effects

Although it has been shown that nanoparticles can enter the human body through several routes, for example ingestion, dermal and injection [43-45], one of the most critical route is pulmonary uptake. Their pathogenic effects depend primarily on achieving a sufficient lung burden, defined by the rates of deposition and clearance [46]. In recent studies of the biological and health effects of nanoparticles of < 100 nm versus fine particulate matter (≥ 2 μm), it has been shown that nanoparticles are more toxic than fine particles [47]. Interestingly, epidemiological studies on the health effects of nanoparticles have provided evidence that these may well induce adverse health effects independent from those of larger fine particles and other airborne toxic matter [48]. Several epidemiologic studies have found correlations between ambient nanoparticles and adverse respiratory or cardiovascular effects, resulting in morbidity and mortality in susceptible parts of the population [49,50], but others have not detected such correlations [51]. There is a reasonable amount of evidence that inhaled particles may be associated with cardiovascular deaths and hospital admissions [52], and that they may also impact on alveolar inflammation, blood coagulation pathways and cardiovascular function [48,52-54].

Their effect on the immune system is more uncertain. It has been argued that they could both defend against microorganisms or, in contrast, intensify allergic immune responses to common allergens [55]. Once nanoparticles have translocated to the systemic circulation, they can be

distributed throughout the body. The liver is the major distribution site, followed by the spleen. Distribution to the heart, kidneys, and immune-modulating organs has been reported. It is important to note that here is still little evidence to support the hypothesis that nanoparticles drive adverse effects, and that it is unclear how ultrafine particles may cause adverse reactions [4].

3.4 Parameters affecting particle toxicity

The adverse effects of nanoparticles depends on their chemical composition, their bioavailability and their toxic effects on mucosal and neuronal cells, as well as other tissue sites they enter from the general circulation (see **Box 1** for a summary of parameters influencing particle toxicology). The debate is ongoing about the chemical and physical properties that influence the toxicity of inhalable nanoparticles [56-58]. Investigation into air-pollution particles has demonstrated that nanoparticles, in contrast with larger particles, have a range of characteristics that promote their toxicity: surface area/size and the chemistry of their surface activity [59,60]. Collectively, these studies indicate how particle size, surface chemistry and possibly charge govern translocation across epithelial and endothelial cell layers. For a full and exhaustive review of these studies, the reader is referred to Mehta *et al.* [61] or Heckel *et al.* [62].

3.4.1 *Particle size*

Particle size is often said to be a dominant factor in inflammatory response, as shown in inhalation studies evaluating the biological effects of ambient particulate matter *in vitro* and *in vivo* [63]. Similar results have been obtained for a variety of different metals [64-68]. Thus, it has been suggested that the biokinetic properties of nanoparticles may be different from those of larger particles, and that they can translocate out of the respiratory tract via different pathways and mechanisms. The lungs may be both a target and a route of exposure for systemic toxicity. Once the particles have reached pulmonary interstitial sites, uptake into blood circulation, in addition to lymphatic pathways, can occur; again, this pathway is dependent on particle size, favouring nanoparticles [69]. Particle surface area has been shown to be closely associated with lung responses, including inflammation and tissue damage in rat lungs [20,70-72]. More recently, surface area has been shown to better predict lung tumours in rats exposed to poorly soluble low toxicity nanoparticles [73,74].

However, it is possible to find evidence where size reduction does not increase toxic effects, but, on the contrary, it would seem to reduce them. Warheit *et al.* [75] showed that quartz particles (1 – 3 μm) produced dose-dependent inflammatory responses in rat lungs *in vivo* after installation that would not be observed with nanoscale TiO_2 particles. The results described provide the first example of nanoscale particle types that are not more cytotoxic or inflammogenic to the lung, compared with larger sized particles of similar composition. These findings would contradict the hypothesis that surface area or size is a major factor associated with the pulmonary toxicity of nanoscale particle types.

Equally, a reduced size does not necessarily lead to enhanced effects. Weda *et al.* showed for example that a reduction in size did not necessarily equate an improvement in treatment efficacy [76], at least in the upper range of the nanoscale. Their work studied the influence of lactose carrier size (three carrier sizes were investigated: 250 – 315 μm, 90 – 106 μm and 3.4 μm) on the efficacy of salbutamol sulphate delivered by a dry powder inhaler in the cumulative dose range of 50 – 400 μg. No improvement in FEV_1 (forced expiratory volume in 1 s) was detected over the size range studied. They concluded that rather large differences in aerodynamic particle size distribution of polydisperse aerosols do not necessarily result in differences in efficacy. Similarly, they conducted a further study to find out if side effects were enhanced if the particle size of the aerosol was reduced. In this study, the same lactose–salbutamol sulphate dry powder inhaler formulation was used [77]. They found some evidence of increased side effects; however, these were modest. Serum potassium levels were found to increase for doses 800 μg and higher, and heart rate variations were only detectable at large cumulative doses (i.e., 1600 μg). These conclusions are valid for micronsized aerosols, and for varying the size of the carrier, not the active agent; conclusions at the nanoscale may differ.

3.4.2 *Particle chemistry*

Particle chemistry is known to alter inflammatory potency [21], and tumour rates in rat lungs [78]. Only few data are available that show the influence of particle physicochemical surface properties on pulmonary responses, and most of this work has been done with cytotoxic quartz particles [38,79]. A recent study has shown how the surface properties of nanoparticles can be modified via oxygen radical generation and how this surface modification could reduce cytotoxicity by several orders of magnitude [80]. Additional concerns have been raised over the role of particle morphology. Calvert and Coles [81,82] mention the potential health risk of material such as carbon nanotubes, with specific attention to single-walled carbon nanotubes. Although carbon nanotubes are known to trigger adverse reactions [39], their surface modifications with glycans seem to reduce their toxicity [83]. For a complete overview of the toxicology of carbon nanomaterial the authors suggests a very recent comprehensive article by Hurt *et al.* [84]. So far, only a very limited number of *in vitro* and *in vivo* toxicity studies have been carried out on material with this kind of morphology [85]. In studies involving silica and NiO particles, assuming the same size range, crystalline nanoparticles have been shown to be more toxic than amorphous nanoparticles [86], although for TiO_2 particles the opposite seems true, as mentioned earlier. Furthermore, in other

Box 1. General considerations on the toxicity of nanoparticles for inhalation.

Parameters that influence toxicity:
Chemical composition
Bioavailability
Morphology
Solubility
Size/surface area

It is not to be assumed that size increase necessarily increases therapeutic effects or side effects.

studies, inflammation and cytotoxicity have been also associated with particle-surface free radical activity [87].

On the contrary, Geiser *et al.* [88] studied the influence of particulate surface chemistry and its interactions with the lung surface lining layer. They found that, regardless of the nature of the surface, due to the surface tension of the lung fluid falling temporarily to relatively low levels [89], nanoparticles are submerged in the lining layer after their depositions in the small airways and alveoli, and their effects proportionally reduced.

3.4.3 *Soluble versus insoluble particles*

Particle insolubility seems to exacerbate negative effects. Poorly soluble substances that are retained in the lungs can cause oxidative stress and consequently lead to inflammation, fibrosis and cancer. Nanoparticles have been shown to be more potent in causing these effects than the same dose of larger particles [90,91]. This is true for insoluble compounds such as TiO_2, carbon black or silica particles [37,38]. In all these cases, there is some evidence of enhanced toxicity as the particle size is reduced, although this must be moderated on a case by case basis by the chemical nature of the particles [92].

3.4.4 *The case of therapeutic molecules*

Therapeutic molecules are not the same as insoluble organic molecules, and benefits have been associated with nanoscale particles, from diagnostic agents to therapeutic molecules [93]. A recent study on the influence of salbutamol sulphate aerosol particle size in the sub- and supra-micrometer range on pharmacodynamic (FEV_1) effect was recently presented by Crampton *et al.* [13]. They found that on a mass-for-mass basis, sub-micrometer particles (size fractions: 0.4 – 5.0 µm) were found to be more efficacious than their supra-micrometer counterparts, which was attributed to a greater penetration to distal regions of lung and/or more uniform deposition throughout the lung due to diffusion. Another study aimed at comparing nebulised nanosuspensions of budesonide versus micronised formulations (Pulmicort®; AstraZeneca) showed that a mean particle size reduction from 4.0 to 0.5 µm increased both the delivered and respirable doses by 53 – 88%, and enhanced the bioavailability and increased the residence time (reduced clearance) [8]. Aerosol cascade impactor studies of beclomethasone NanoCrystals® (Elan) have also shown significantly higher respirable fractions and lower unwanted systemic uptake via throat deposition for nanosuspensions compared with micronized formulations [94]. In a Phase I clinical trial, nebulised nanocrystal (75 – 300 nm) of budesonide in suspension doubled the C_{max}, and almost halved the T_{max} of larger, 4.4-µm Pulmicort Respules® (AstraZeneca) [95]. Similarly, itraconazole nanostructured nebulised formulations proved to be more efficient in treating invasive pulmonary aspergillosis in murine models compared with oral formulations [96]. Finally, doxorubicin-loaded nanoparticles showed higher cytoxicity in lung cancer cells compared with free doxorubicin, thus confirming the potential of nanoparticles in treating lung cancer via the inhalation route [97].

4. Conclusion

Efficient particle deposition in the lungs happens for two particle size ranges: 50 – 100 nm and 1 – 5 µm. Of the two, the larger has been fully exploited in inhalation drug delivery, and the lower one is mostly unused. It is possible to deliver and formulate nanoparticles to the lungs, and for these particles to have a local or systemic effect. Indeed *in vitro* and *in vivo* deposition studies have confirmed this potential. A range of parameters preside over efficient deposition, which is mostly diffusion driven. These parameters include airways structure, breathing patterns and the diffusion characteristics of the nanoparticles, as well as the characteristics of the aerosol itself (e.g., ballistic properties).

Data from environmental epidemiological studies, and the established knowledge of occupational diseases associated with exposure to respirable dust, lend credibility to the adverse health effects associated with exposure to nanoparticles. Yet, more research is required to better understand the underlying toxic mechanisms, and the relevant doses of exposure that may lead to the gradual shift from health to disease.

Toxicity data is limited to inorganic insoluble nanomaterials (such as TiO_2, carbon soot, construction material dust or carbon nanotubes), and may not be relevant to therapeutically active soluble molecules at low dosages. As a matter of fact, there is a body of evidence that the nanoscale could lead to enhanced therapeutic effects, drawn from a limited pool of respiratory medicines.

5. Expert opinion

The nanoscale is a largely untapped opportunity in inhalation delivery. Deposition studies have long identified the advantages of delivering nanoparticles to the lung to achieve enhanced therapeutic effects; yet, so far, few products or studies have fully leveraged its benefits. *In vitro* and *in vivo* studies are all eloquent in this matter, nanoparticles

can be deposited, and as matter of fact fine particles in some aerosol products may already play a role in enhancing product performance. As yet, this opportunity has not been consciously fully exploited, and, thus, offers formulation scientists a rare opportunity to develop new concepts in inhalation delivery. It is of course difficult to formulate nanoparticles, or generate stable nanodroplets, as is reviewed Part 2 of this review. This technology difficulty needs to be resolved.

The lack of toxicity data on therapeutically active nanoparticles is a hindrance to developing nanoscale inhalation systems: the pharmaceutical industry is not exploiting nanoscale opportunities, and consequently no research is performed on the toxicity of inhaled nanoparticles, which in turn results in the present lack of meaningful toxicity data. Only when this cycle is broken, will confidence be restored and research progress. Too many publications base their warnings against nanoscale inhalation formulations on epidemiological studies that do not deal with therapeutic molecules, but inherently toxic material, such as diesel soot, or TiO_2. Although these studies provide a salutary caution against naive expectations from nanoscale technologies, their results are not entirely translatable to therapeutic drugs. One of the problems of toxicity studies with nanomedicines for inhalation is that most of them are aimed at denouncing the toxic dimension of the nanoscale, and therefore the premise of the research pre-empts its conclusions. Rare are the studies that aim to reveal the positive aspects of the nanoscale – the few results available from studies with therapeutic medicines actually very encouraging. One could even contemplate a future where nanoparticles provide equivalent efficacy to existing therapeutic molecules, but at lower dosages and with reduced side effects. This of course will be chemistry dependent, in the sense that the chemistry of the active molecule will dictate what is achievable. It is reasonable therefore to warn against a too conservative approach with inhalation nanotechnology, as it remains a moot point whether the known hazards and risks will be found with therapeutic nanoparticles.

The search for better medicines, through the advent of better nanotechnologies, will no doubt help to provide information on the real toxicity of nanoparticles delivered via inhalation. One of the limiting factors for further studies in the field is a lack of nano-formulations, and as these are developed over the next few years, the lack of data on nanotoxicology from inhalation therapies will improve.

Already there are signs at conferences that research is progressing in the field, driven by a need for pharmaceutical firms to find intellectual protection for next-generation products. These new formulations often do come from the close circuit of drug delivery. As nanoscience progresses in other areas than pharmaceutical science (in particular in particle manufacturing and device design), the corresponding discoveries are making their way into drug delivery (e.g., bubble jet technology to produce nanodroplets), and the nanoscale gradually becomes a reality in inhalation delivery.

Declaration of interest

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

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