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Translocation and cellular entering mechanisms of nanoparticles in the respiratory tract

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Summary

Anthropogenic nano-sized particles (NSP), ie, particles with a diameter of less than 100 nm, are generated with or without purpose as chemically and physically well-defined materials or as a consequence of combustion processes respectively. Inhalation of NSP occurs on a regular basis due to air pollution and is associated with an increase in respiratory and cardiovascular morbidity and mortality. Manufactured NSP may intentionally be inhaled as pharmaceuticals or unintentionally during production at the workplace.

Hence the interactions of NSP with the respiratory tract are currently under intensive investigation. Due to special physicochemical features of NSP, its biological behaviour may differ from that of larger sized particles. Here we review two important themes of current research into the effects of NSP on the lungs: 1) The potential of NSP to cross the blood-air barrier of the lungs, thus gaining access to the circulation and extrapulmonary organs. It is currently accepted that a small fraction of inhaled NSP may translocate to the circulation. The significance of this translocation requires further research. 2) The entering mechanisms of NSP into different cell types. There is evidence that NSP are taken up by cells via well-known pathways of endocytosis but also via different mechanisms not well understood so far. Knowledge of the quantitative relationship between the different entering mechanisms and cellular responses is not yet available but is urgently needed in order to understand the effects of intentionally or unintentionally inhaled NSP on the respiratory tract.

Key words: nanoparticles; respiratory tract; lung; translocation; endocytosis; adhesive interactions

Introduction

Epidemiological studies have shown that exposure to airborne ambient particulate matter is correlated with adverse health effects, particularly increased respiratory and cardiovascular morbidity and mortality (see [1] for review). Further work has highlighted the fact that these correlations depend on particle size, the so-called ultrafine particles (UFP) contributing in significantly stronger fashion to the adverse effects [2]. UFP are particles with a diameter of 100 nm or less in all dimensions [3]. The most important anthropogenic sources of UFP are combustion processes of diesel oil, coal, heavy fuel oil or welding processes [3]. Apart from UFP, engineered nanoparticles (NP), defined as particles at least in one dimension smaller than 100 nm, have recently attracted attention. In contrast to UFP, which usually consist of an elemental carbon core with a shell of attached transition metals and/or organic carbon, NP are physically and chemically well-defined particles specifically designed for a certain use. For example, titanium dioxide NP are used in sun milk, carbon nanotubes are part of carbon fibre bicycles, silver NP have antibacterial properties in socks, etc. Particular interest has been focused on the biomedical applications of NP with respect to imaging and drug delivery [4]. The stability of NP as separate particles is obviously of critical importance for their nano-effects. Although the terms aggregation and agglomeration are frequently used interchangeably, there is a difference in their meaning [5]. Agglomerates of particles develop due to physical bonds (eg, van der Waals or electrostatic forces), whereas aggre-

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Abbreviations:

NP	nanoparticle(s)
NSP	nano-sized particle(s)
UFP	ultrafine particle(s)

gates are held together by chemical bonds. This is important because agglomerates can often be dispersed by ultrasonic treatment or in a certain biological environment, while the forces that hold aggregates together are more stable. The state of agglomeration or aggregation may therefore greatly influence the biological behaviour of particles (including translocation and cellular entering), an aspect which should be borne in mind in studies on NP and UFP.

While human inhalation exposure to UFP occurs on a regular basis, exposure to NP may be restricted to occupational or medicinal settings. Hence the interaction of both UFP and NP with the lungs requires thorough investigation. In the following, the term nano-sized particle (NSP) will be used whenever both UFP and NP are meant.

This review article focuses on the mechanisms by which NSP cross the air-blood barrier, thus entering the systemic circulation, and the mechanisms by which NSP enter cells. For a more detailed review on interactions of NSP with pulmonary structures the reader may refer to [6].

Fate of inhaled and deposited nano-sized particles – hypothetical routes

Since NSP effectively reach the alveolar region of the lungs, the size of the epithelial surface that may simultaneously come into contact with the particles amounts to approximately 140 m^2 [7]. Once NSP have reached the alveoli, they may be retained in the lungs for a long period and may be deposited, ie, come into physical contact with the surface structures. Larger sized particles are quickly displaced from the air-liquid interface to the watery hypophase under the surfactant [8], a process which probably holds true for NSP as well. In the hypophase, the particles may bind to surfactant proteins and glycoproteins or be taken up by macrophages. However, the uptake of NSP by alveolar macrophages seems to be on a far smaller scale than for larger sized particles [9]. The particles thus have a high probability of encountering the alveolar epithelium. Those particles that translocate over the alveolar epithelium may be deposited in the interstitium or translocate over the capillary endothelium and enter the circulation [10, 11]. Since all of the structures mentioned above may either influence the particle behaviour or may be influenced upon contact with NSP, the following paragraphs attempt to provide a mechanistic view of the phenomena of particle translocation and cell entering.

Translocation of nano-sized particles across the air-blood barrier

It having been proposed that adverse cardiovascular effects might be related to a translocation of NSP to the systemic circulation, several studies have addressed the question whether and to what extent NSP cross the air-blood barrier.

Animal models of NSP inhalation and instillation have provided evidence that a small fraction of the particles applied translocate to the circulation and can reach extrapulmonary organs via the bloodstream. These findings were observed for titanium dioxide NP in the rat [10, 11] as well as for diesel exhaust particles in the hamster [12]. In the human lung one study reported a rapid and significant translocation of inhaled carbonaceous NP to the systemic circulation and extrapulmonary organs [13]. In contrast, other subsequent studies failed to confirm this finding and detected only a low degree of translocation for iridium [14] or carbon NSP [15–17]. The studies of Nemmar et al. [13] and Mills et al. [16] had a very similar design and Mills et al. [16] have argued convincingly that the strong translocation of ^{99m}Tc labeled particles observed by Nemmar et al. [13] was mainly related to the translocation of soluble ^{99m}Tc-pertechnetate or pertechnegas which was cleaved from the carbonaceous particles. Based on these studies, it is reasonable to conclude that a small fraction of NSP translocates across the air-blood barrier to the circulation, but the significance of this translocation for human health requires further study.

A very specific property that has been assigned to inhaled NSP refers to translocation from the nasal epithelium to the brain. Elder et al. [18] showed that ultrafine manganese oxide particles translocate to the olfactory bulb and other regions of the central nervous system, although the question whether entire particles were translocated or solubilised manganese was transported to the central nervous system [19] warrants further research. However, additional support for translocation of ultrafine particles via olfactory axons comes from a study by Oberdörster et al. [20], who showed that inhaled radioactive carbon particles were significantly enhanced in the olfactory bulb after exposure, in contrast to other brain regions that showed only inconsistent increases in carbon particles.

Cellular entering mechanisms

There is little evidence for paracellular transport of NSP in the healthy lung, but this route may be enhanced when additional air- or blood-borne substances, such as ozone, hydrogen peroxide or histamine, increase the permeability of epithelial or endothelial barriers [21]. Most particles crossing the alveolar epithelium will however use a transcellular route for which endocytosis has been observed as a key mode of cellular particle uptake in several studies: after inhalation exposure, single and agglomerated gold NSP were observed in membrane-bound vesicles in AM and alveolar type I cells of rat lungs [22]. In a macrophage cell line, gold NP were found in pinocytotic vesicles and lysosomes [23]. Similarly, titanium dioxide NP were localised inside vesicular structures, including multivesicular and lamellar bodies, in an immortalised alveolar epithelial cell line [24]. Further support for an active energy-dependent transport process comes from the observation that low temperature and metabolic inhibitors inhibit the uptake of magnetic silica-coated NP [25]. Receptor-mediated endocytosis of particles via scavenger receptors was shown to be involved in macrophages [26, 27].

Apart from endocytosis, however, other routes of cellular entry are likely. Very few titanium dioxide NP were observed inside red blood cells after inhalation exposure in rats [10], suggesting passive movement of NP across the plasma membrane. This mechanism was confirmed by the presence of polystyrene NP as well as titanium dioxide NP in red blood cell culture [28]. Ambient UFP were observed in mitochondria of macrophage and epithelial cell lines [29] indicating direct access to the enzymes of the respiratory chain. C60 fullerenes were found inside the nucleus and the free cytoplasm of human monocyte-derived macrophages [30], and 1.4 nm gold particles were bound to the nuclear DNA of eleven different normal or cancer cell lines [31]. The latter finding raises the possibility that some particles entering the nucleus may directly interact with nuclear DNA. The particle entering

Figure 1

Summary of possible entering mechanisms of nano-sized particles. Particles may actively be taken up by cells via phagocytosis (A), macropinocytosis (B), clathrin-mediated endocytosis (C), clathrin- and caveolae-independent endocytosis (D) or by caveolae-mediated endocytosis (E). In most cases of active uptake, particles will be transported via vesicular structures to form phagolysosomes or endosomes (A–D) but they may also be transported to the endoplasmic reticulum, cytosol or through the cell as part of transcytotic processes (E). Apart from these mechanisms, a passive movement through the plasma membrane with subsequent access to all subcellular compartments, including nucleus and mitochondria, has been proposed (F). The significance of particular intracellular localizations and entering mechanisms for specific cellular responses awaits further study. Figure modified after [38].

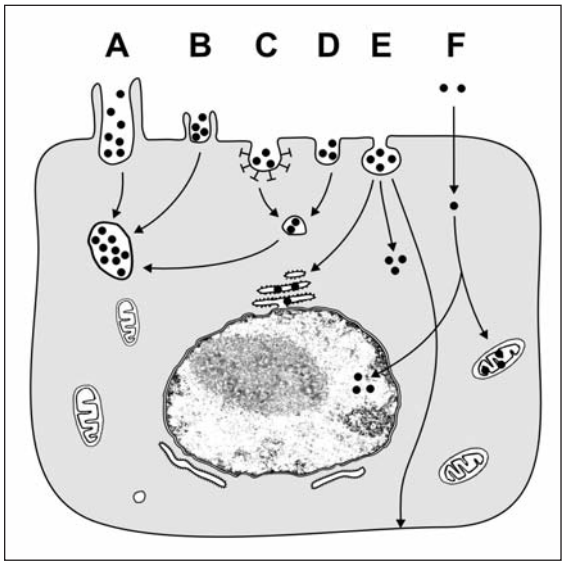
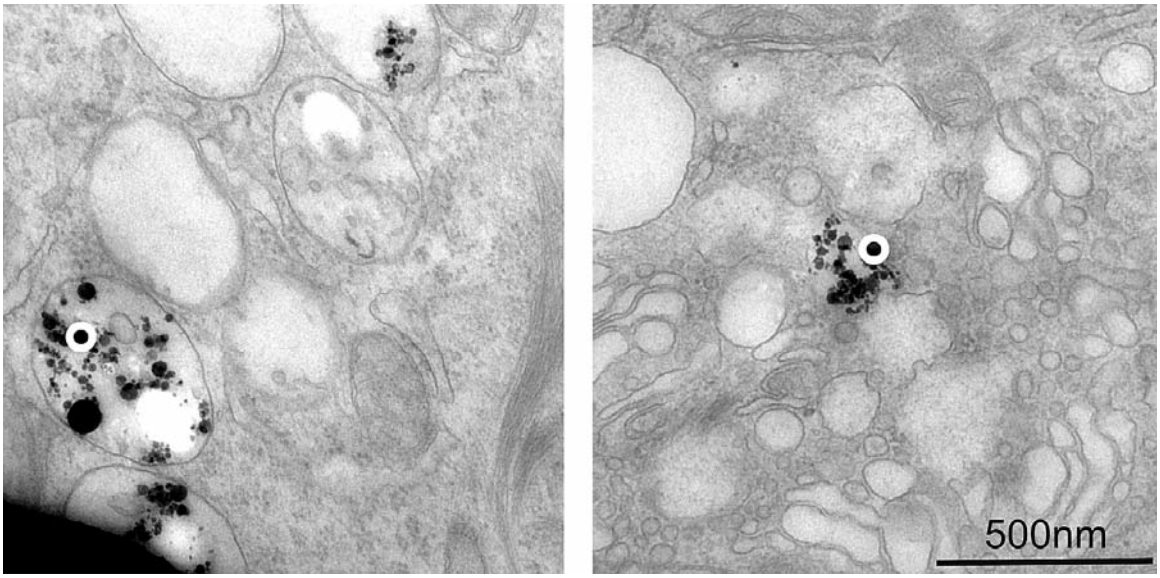


Figure 2

Titanium dioxide nanoparticles inside an epithelial cell (A549 cell line). The left electron micrograph shows an agglomerate of differently sized titanium dioxide nanoparticles inside a vesiclular structure. In contrast, the titanium dioxide NP shown in the right micrograph are free within the cytoplasm. The white circle indicates the region from which an elemental analysis was performed by energy filtered transmission electron microscopy. Reproduced from [35].



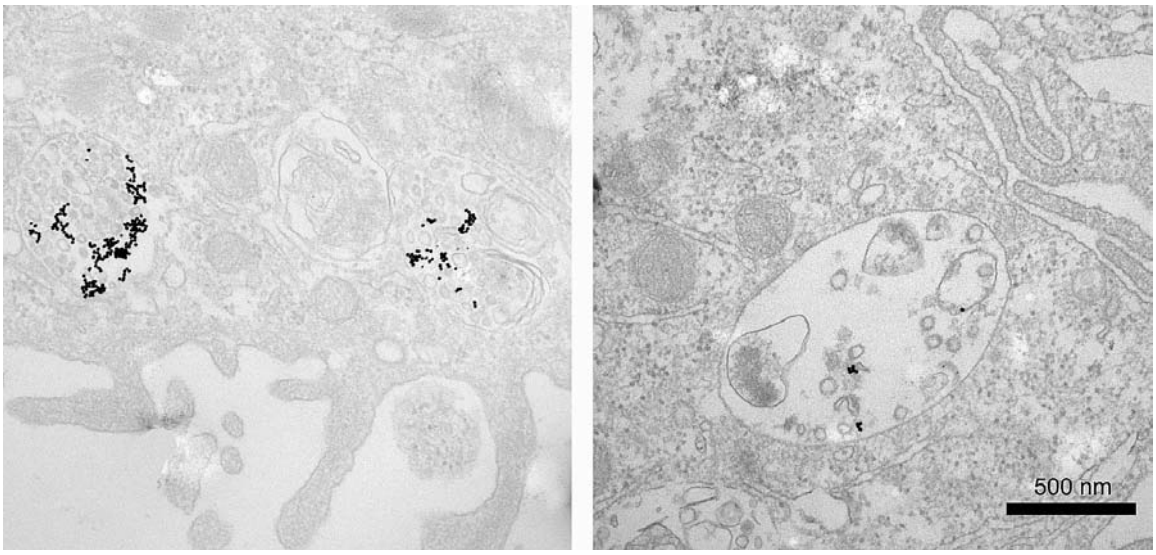


Figure 3

Colloidal gold nanoparticles inside an epithelial cell (A549 cell line). Both electron micrographs show colloidal gold nanoparticles with an individual particle diameter of approximately 15–20 nm. In the left figure variously sized agglomerates of NP are observed within vesicular structures. The right figure shows one single gold NP and two agglomerates in the nanometer range consisting of three to five individual particles.

processes different from endocytosis have been related to adhesive interactions due to electrostatic forces, Van der Waals or steric interactions [32]. Little is known, however, about the quantitative relationship between active endocytotic routes and passive routes of entry. We have recently established a quantitative approach to guarantee an unbiased way of investigating this relationship in tissues and cells [33]. In addition, the significance of a specific intracellular localization needs to be analyzed in quantitative terms to distinguish between sporadic and scientifically relevant particle distributions. The different mechanisms of cellular entering and intracellular trafficking are summarized in figure 1. Some examples of intracellular NP localization visualized by transmission electron microscopy are shown in figures 2 and 3.

Composition and surface characteristics of the particles obviously influence the entering mechanism and the intracellular trafficking of particles [34–36]. In particular, protein adsorption may determine the mechanism of NSP trafficking as evidenced for the translocation of poly(ethylene glycol-co-hexadecyl)cyanoacrylate NP into rat brain endothelial cells [37]. Future studies therefore need to define the experimental setting of particle exposure both from the particle and from the biological side in great detail.

Open questions

1. Although it is accepted that a small fraction of inhaled particles translocates across the air-blood barrier, there is currently no rationale for relating these particles to the adverse health effects associated with particle exposure. Therefore, future studies are warranted addressing the following questions: Do particles need to translocate to the circulation to induce adverse cardiovascular health effects? What are the cumulative effects of particle translocation? What is the fate of the translocated particles?

2. Similarly, there is evidence that most nanosized particles entering cells are taken up by endocytosis but other routes of cellular entering also exist. Systematic studies are needed investigating the mechanisms of cellular particle entering and the effects of endogenous and exogenous factors on these mechanisms. Quantitative studies have to be performed to relate the cellular reactions such as the generation of reactive oxygen species and inflammatory proteins to a specific intracellular localization.

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References

1 Künzli N, Tager IB. Air pollution: from lung to heart. Swiss Med Wkly. 2005;135 (47-48):697–702.
2 Peters A, Wichmann HE, Tuch T, Heinrich J, Heyder J. Respiratory effects are associated with the number of ultrafine particles. Am J Respir Crit Care Med. 1997;155 (4):1376–83.
3 Donaldson K, Tran L, Jimenez LA, Duffin R, Newby DE, Mills N, et al. Combustion-derived nanoparticles: A review of their toxicology following inhalation exposure. Part Fibre Toxicol. 2005;2:10.
4 Murthy SK. Nanoparticles in modern medicine: State of the art and future challenges. Int J Nanomed. 2007;2(2):129–41.
5 Nichols G, Byard S, Bloxham MJ, Botterill J, Dawson NJ, Dennis A, et al. A review of the terms agglomerate and aggregate with a recommendation for nomenclature used in powder and particle characterization. J Pharm Sci. 2002;91(10):2103–9.
6 Mühlfeld C, Rothen-Rutishauser B, Vanhecke D, Blank F, Ochs M, Gehr P. Interaction of nanoparticles with pulmonary structures and cellular responses. Am J Physiol Lung Cell Mol Physiol. 2008;294(5):L817–29.
7 Gehr P, Bachofen M, Weibel ER. The normal human lung: Ultrastructure and morphometric estimation of diffusion capacity. Respir Physiol. 1978;32(2):121–40.
8 Schürch S, Gehr P, Im Hof V, Geiser M, Green F. Surfactant displaces particles toward the epithelium in airways and alveoli. Respir Physiol. 1990;80(1):17–32.
9 Geiser M, Casaulta M, Kupferschmid B, Schulz H, Semmler-Behnke M, Kreyling W. The role of macrophages in the clearance of inhaled ultrafine titanium dioxide particles. Am J Respir Cell Mol Biol. 2008;38(3):371–6.
10 Geiser M, Rothen-Rutishauser B, Kapp N, Schürch S, Kreyling W, Schulz H, et al. Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. Environ Health Perspect. 2005;113(11):1555–60.
11 Mühlfeld C, Geiser M, Kapp N, Gehr P, Rothen-Rutishauser B. Re-evaluation of pulmonary titanium dioxide nanoparticle distribution using the "relative deposition index": Evidence for clearance through microvasculature. Part Fibre Toxicol. 2007; 4:7.
12 Nemmar A, Hoylaerts MF, Hoet PHM, Nemery B. Possible mechanisms of the cardiovascular effects of inhaled particles: systemic translocation and prothrombotic effects. Toxicol Lett. 2004;149(1-3):243–53.
13 Nemmar A, Hoet PHM, Vanquickenborne B, Dinsdale D, Thomeer M, Hoylaerts MF, et al. Passage of inhaled particles into the blood circulation in humans. Circulation. 2002;105 (4):411–4.
14 Kreyling W, Semmler M, Erbe F, Mayer P, Takenaka S, Schulz H, et al. Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. J Toxicol Environ Health A. 2002;65:1513–30.
15 Wiebert P, Sanchez-Crespo A, Seitz J, Falk R, Philipson K, Kreyling WG, et al. Negligible clearance of ultrafine particles retained in healthy and affected human lungs. Eur Respir J. 2006; 28 (2): 286-290.
16 Mills NL, Amin N, Robinson SD, Anand A, Davies J, Patel D, et al. Do inhaled carbon nanoparticles translocate directly into the circulation in humans? Am J Respir Crit Care Med. 2006; 173(4):426–31.
17 Möller W, Felten K, Sommerer K, Scheuch G, Meyer G, Meyer P, et al. Deposition, retention and translocation of ultrafine particles from the central airways and lung periphery. Am J Respir Crit Care Med. 2008;177(4):426–32.
18 Elder A, Gelein R, Silva V, Feikert T, Opanashuk L, Carter J, et al. Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. Environ Health Perspect. 2006;114(8):1172–8.
19 Ghio AJ, Bennett WD. Metal particles are inappropriate for testing a postulate of extrapulmonary transport. Environ Health Perspect. 2007;115(2):A70.
20 Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W, et al. Translocation of inhaled ultrafine particles to the brain. Inhal Toxicol. 2004;16(6-7):437–45.
21 Meiring JJ, Borm PJA, Bagate K, Semmler M, Seitz J, Takenaka S, et al. The influence of hydrogen peroxide and histamine on lung permeability and translocation of iridium nanoparticles in the isolated perfused rat lung. Part Fibre Toxicol. 2005;2:3.
22 Takenaka S, Karg E, Kreyling WG, Lentner B, Möller W, Behnke-Semmler M, et al. Distribution pattern of inhaled ultrafine gold particles in the rat lung. Inhal Toxicol. 2006;18 (10):733–40.
23 Shukla R, Bansal V, Chaudhary M, Basu A, Bhonde RR, Sastry M. Biocompatibility of gold nanoparticles and their endocytotic fate inside the cellular compartment: A microscopic overview. Langmuir. 2005;21(23):10644–54.
24 Singh S, Shi T, Duffin R, Albrecht C, van Verlo D, Höhr D, et al. Endocytosis, oxidative stress and IL-8 expression in human lung epithelial cells upon treatment with fine and ultrafine TiO2: Role of the specific surface area and of surface methylation of the particles. Toxicol Appl Pharm. 2007;222(2):141–51.
25 Kim JS, Yoon TJ, Yu KN, Noh MS, Woo M, Kim BG, et al. Cellular uptake of magnetic nanoparticle is mediated through energy-dependent endocytosis in A549 cells. J Vet Sci. 2006; 7(4):321–6.
26 Arredouani MS, Palecanda A, Koziel H, Huang YC, Imrich A, Sulahian TH, et al. MARCO is the major binding receptor for unopsonized particles and bacteria on human alveolar macrophages. J Immunol. 2005;175(9):6058–64.
27 Kanno S, Furuyama A, Hirano S. A murine scavenger receptor MARCO recognizes polystyrene nanoparticles. Toxicol Sci. 2007;97(2):398–406.
28 Rothen-Rutishauser BM, Schürch S, Haenni B, Kapp N, Gehr P. Interaction of fine particles and nanoparticles with red blood cells visualized with advanced microscopic techniques. Environ Sci Technol. 2006;40(14):4353–9.
29 Li N, Sioutas C, Cho A, Schmitz D, Misra C, Sempf J, et al. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. Environ Health Perspect. 2003;111(4):455–60.
30 Porter AE, Muller K, Skepper J, Midgley P, Welland M. Uptake of C60 by human monocyte macrophages, its localization and implications for toxicity: studied by high resolution electron microscopy and electron tomography. Acta Biomater. 2006;2(4):409–19.
31 Tsoli M, Kuhn H, Brandau W, Esche H, Schmid G. Cellular uptake and toxicity of Au55 clusters. Small. 2005;1(8-9):841–4.
32 Rimai DS, Quesnel DJ, Busnaia AA. The adhesion of dry particles in the nanometer to micrometer-size range. Colloids Surf A: Physicochem Eng Aspects. 2000;165:3–10.
33 Mühlfeld C, Mayhew TM, Gehr P, Rothen-Rutishauser B. A novel quantitative method for analysing the distributions of nanoparticles between different tissue and intracellular compartments. J Aerosol Med. 2007;20(4):395–407.
34 Patil S, Sandberg A, Heckert E, Self W, Seal S. Protein adsorption and cellular uptake of cerium oxide nanoparticles as a function of zeta potential. Biomaterials. 2007;28(31):4600–7.
35 Rothen-Rutishauser, Mühlfeld C, Blank F, Musso C, Gehr P. Translocation of particles and inflammatory responses after exposure to fine particles and nanoparticles in an epithelial airway model. Part Fibre Toxicol. 2007;4:9.
36 Yacobi NR, Phuleria HC, Demaio L, Liang CH, Peng CA, Sioutas C, et al. Nanoparticle effects on rat alveolar epithelial cell monolayer barrier properties. Toxicol in vitro. 2007;21(8): 1373–81.
37 Kim HR, Andrieux K, Gil S, Taverna M, Chacun H, Desmaele D, et al. Translocation of poly (ethylene glycol-co-hexadecyl) cyanoacrylate nanoparticles into rat brain endothelial cells: role of apolipoproteins in receptor-mediated endocytosis. Biomacromolecules. 2007;8(3):793–9.
38 Unfried K, Albrecht C, Klotz LO, von Mikecz A, Grether-Beck S, Schins RPF. Cellular responses to nanoparticles: Target structures and mechanisms. Nanotoxicology. 2007;1:1–20.

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