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The impact of nanoparticles on cellular functions

Summary

Nanoparticles can enter cells actively or passively and can trigger various effects. These effects are often coupled with the formation of free radicals, which can be released within the cell or produced on the surface of the particles. Free radicals can induce inflammation, cell death and DNA damage and thus impair human health. The threshold value, i.e. the amount of incorporated nanomaterials that causes an effect, remains unknown. Based on present knowledge, there are no known nanoparticle-specific cellular reactions. However, it is only the knowledge of basic cellular processes that allows us to understand the magnitude of an induced effect,. This, in turn, is a prerequisite for testing drugs and administering medicines. This dossier is therefore designed to provide an overview of selected cell functions. By examining the relevant functional and molecular pathways, we can better understand how nanoparticles potentially cause damage.

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Introduction

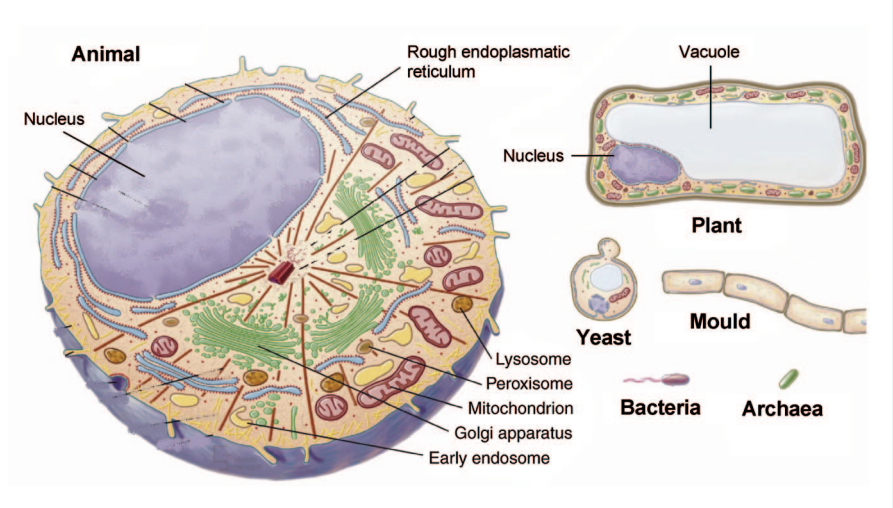
Nanotoxicology deals with the effects of nanomaterials on cells and living organisms. Few toxicological data, however, are available for most nano-objects. There is general agreement that the surface structure of nanoparticles plays a key role in the interaction with cells and therefore requires more consideration. Predictions about potential effects or risks must be based on knowledge about the toxicological behavior of a substance. This must be accompanied by information on cellular mechanisms and feed-back loops in order to be able to evaluate effects or ultimately plan targeted applications. Many research results are gained through standardized techniques and methods anchored in environmental toxicology, for example with soot particles or diesel emission products. This is based on certain parallels to the effects of artificially produced nanoparticles. These techniques have yielded initial successes, but require improvement and further study in the framework of research projects. The following section outlines selected results from basic research on cellular structure, mechanisms of action and the damage potential of nanoparticles.

The cell

Organisms are composed of at least one cell, which makes it the smallest viable unit of life. Unicellular and multicellular organisms exist. A cell is much like a small organism, equipped with all vital functions for growth and reproduction. In principle, there are two types of cells. Cells without a nucleus such as bacteria and the archeobacteria are termed prokaryotes. Plants, fungi and animal cells possess a nucleus and are classified as eukaryotes. Whereas plant cells have a cell wall composed of cellulose, animal cells lack a cell wall but feature cell membrane. Bacteria also have a cell wall, although it is structured differently from that of plants. The cell membrane bears pores that provide direct contact with the environment. This membrane encloses

the cell interior, the cytoplasm. The inside of a eukaryotic cell contains all cellular building blocks such as the nucleus with its DNA (containing the genetic material).

Figure 1:
Cell structure, see text
(after Pollard & Earnshaw, Cell Biology ©)[1]



Other key organelles include the mitochondria (energy suppliers with their own DNA), the endoplasmatic reticulum (transport canals), ribosomes (protein production), the Golgi apparatus (formation of secretory proteins) and the lysosomes (protein degradation). Plant cells possess chloroplasts, in which photosynthesis takes place (the conversion of solar energy into chemical energy), and have a vacuole as a storage site for metabolic wastes (Figure 1). The body contains several hundred different types of cells, whereby shape and size depend on the tasks of the respective cells. They are supplied with oxygen and nutrients by the blood.

Macrophages/ Phagocytosis – professional particle uptake

Macrophages (feeding cells) play a key role in cellular defense mechanisms. They destroy foreign, pathogenic intruders such as bacteria, parasites and protozoa and help to fight against degenerated cells in the body, especially in the case of metastasing tumors. Precursors of these macrophages are the monocytes, which develop from haemoblasts (monoblasts, then to promonocytes). Monocyte development begins in the bone marrow under the influence of growth factors. Some monocytes remain and others migrate from the bone marrow into the peripheral blood. Certain monocytes remain universally deployable in the body, others – after a period of up to 40 hours – move to various tissues and become tissue macrophages. They can assume different functions depending on tissue type. For example, they can participate in inflammation processes, promote wound healing and fight pathogenic agents. One of the most important functions of macrophages and monocytes is phagocytosis (cellular uptake of particles, bacteria, etc.). The so-called "professional" phagocytes are able to destroy foreign bodies such as bacteria by incorporating them into their cell interior and then submitting them to special, cell-specific defense mechanisms such as free radical formation. The immunological reaction involves specific receptors at the membrane surface of the phagocyting cells.

The surface receptors unspecifically recognize various ligands (bonding sites) such as glycoproteins, oligosaccharides and lectins (sugar-binding proteins) on the cell surface of the bacteria. In contrast, foreign particles must first be mediated by immunoglobulins (Ig) and opsonized (covered with proteins) by components of the complement system (part of the immune system). Such marked particles dock at specific receptors and complement receptors of the monocytes and macrophages. The actual activation and stimulation of the phagocytes is triggered by the receptor-ligand bond (Figure 2). In principle, most cells can phagocytize, although "non- professional" phagocytosis does not involve receptors.

There are various pathways by which nanoparticles can be taken up into cells. New studies show[4] that electrical charge plays an important role in activating certain receptors, especially in the uptake of titanium dioxide-, iron oxide- and quarz-containing nanoparticles. Uncharged particles, such as carbon-containing nanoparticles or diesel emission particles, activate the same receptors as bacteria, viruses or fungi[5]. The particles enclosed in the phagosomes (feeding organelles in macrophages) fuse with the lysosomes, which release enzymes and free radicals in order to digest the pathogen (e.g. bacterium)[6]. Depending on which receptor is activated, the corresponding signal cascades are triggered in the cell, for example to activate the immune system. If the particles cannot be digested, then they can remain within the cells for up to 700 days[7] and thus cause cell damage. This can lead to cell death, which means the particles are retained in the respective organ[8]. The cycle begins anew: the particles are ingested and trigger the continuous formation of free radicals. This process is termed oxidative stress. This, in turn, can lead to chronic inflammatory reactions. Oxidative stress has often been associated with various diseases such as cancer, neurodegenerative illnesses and cardiovascular diseases.

Particle uptake is dependent on particle size and concentration. Human macrophages in the alveoles of the lung measure about 14-21 μm[9]. These cells can effectively incorporate particles when the particles are about the same size as the cells. Phagocytosis becomes less effective when the particles are smaller or larger than the cells themselves. Studies show that 100-200 nm nanoparticles tend not to be phagocytosed. Rather, they often end up in the interstitial space (space between the cells) and therefore reach the epithelium cells and the lymph- and blood vessel system (translocation)[7; 10]. Smaller particles therefore remain in the organism longer because they are translocated into the lymph- and blood vessel system. If the particles are present in higher concentrations, they often form aggregates that can attain sizes exceeding 100 nm. In this size range they can be phagocytosed and therefore are not translocated. It has been shown that high concentrations of silver-, iron- or titanium dioxide-nanoparticles (> 100 nm) were phagocytosed by macrophages and did not enter the organs. Another study demonstrated that a low concentration of 15-nm-sized, inhaled silver nanoparticles in rats already translocate after 30 minutes into the blood, brain and other organs such as the heart and kidneys, whereas the lung remained relatively particle free[10]. This means that smaller nanoparticles in low concentrations have a higher probability of remaining in the body and entering organs than larger particles in higher concentrations. This is because different cellular mechanisms are at work.

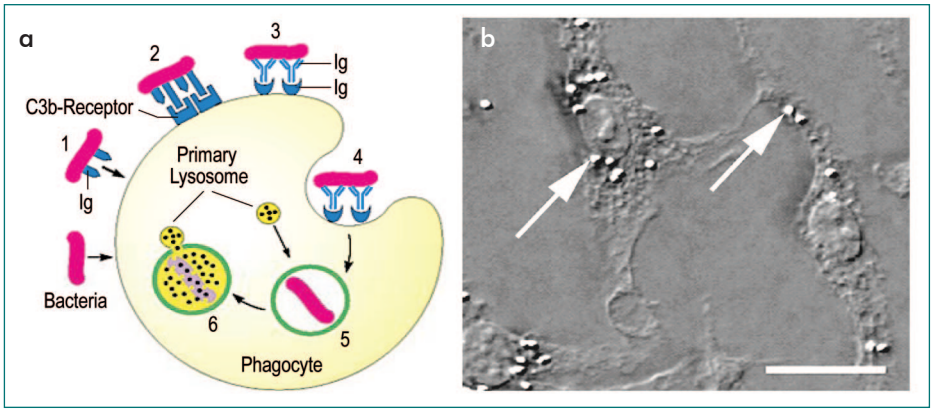


Figure 2a: The phagocytosis process based on ingestion of a bacterium, 1: Opsonization of the bacteria cell with Ig, 2: Further opsonization of the bacterium and bonding to specific receptors, 3: Bonding of an Ig marked bacterium to the specific receptor, 4 and 5 Ingestion of the bacterium in the cell, 6: Fusion of lysosomes with the phagosome and digestion of the bacterium by lysosomal enzymes from the lysosomes (after Klein, modified[2]).
Figure 2b: Phagocyting macrophages, the arrows point to the ingested microparticles (after Simkó et al.)[3]

Cellular uptake – the non-professional particle uptake

Nanoparticles, much like viruses, can enter non-phagocyting cells and react with subcellular structures. What then happens in the cell that retains the particles, and whether the particles trigger or catalyze chemical reactions, depends on the chemical composition and size of the particles[11]. As noted above, uptake takes place without specific membrane receptors. Rather, the process is passive or involves an adhesive interaction based on physical forces (van der Waals forces, electrostatic charges, steric interactions and/or surface charges)[12; 13]. In this case, vesicle (phagosome) formation does not necessarily occur. The particles float freely in the cell, and the cell organelles can be exposed to the particles. For example, incorporated C_{60}-molecules become distributed everywhere, including inside the nucleus[14]. This type of uptake poses a severe threat to the cells and its organelles because it can involve direct contact and interaction with the cytoplasm and its proteins. Experimentally, nanoparticles have been detected outside on the cell membrane, in the cytoplasm[15; 16], in the mitochondra[11; 17], in lipid vesicles[16; 18], on the nuclear membrane[15] and even within the nucleus[11; 16]. Depending on a particle's localization, different cellular effects can be triggered. If the particles damage the DNA then immediate cell death can occur. Where the particles are intracellular localized also depends on their size. Larger particles (2.5-10 μm) have been recorded in the cytoplasm (in vacuoles), smaller particles (>100 nm) in the mitochondria17. C_{60}-molecules measure about 0.7 nm and enter the cells via different mechanisms, for example through the canals (ion canals) or pores in the cell membrane[14]. The various forms of cellular particle uptake are a topic of current research efforts.

Cellular effects

When certain materials or forces act externally on cells, the resulting physiological processes depend either on the causative agent or on the cell type. Cell activity is regulated by an extremely dynamic process and is cell type specific. This means that cell activity depends on the cell's morphological and functional differentiation. This activity can also be regulator specific, i.e. different stimuli affect the cells in different ways and therefore trigger specific biological feed-back loops. Changes or disturbances to this very fine-tuned, directed process can lead to dysfunctions as well as to malignant degenerations.

The regulated and dynamic interaction between proteins and protein cascades is essential for the specific and efficient course of most cellular reactions. If we understand the activation mechanisms of primary (trigger) processes through extracellular signals, then we may be able to selectively apply specific agents to trigger a desired cell reaction. Here, gene activations and protein production (protein expression) and potential changes in proteins (translational modifications, e.g. phosphorylation) play the decisive role. The assumption is that certain stimuli activate certain intracellular proteins in a regulatory manner, which themselves set off one or more cellular signal cascades. Activating a signal cascade can trigger specific cell activation processes, which in turn lead to cell type dependent biological effects. Positive effects, but also pathophysiological conditions, may be the result. The formation of free radicals within a cell also activates specific signal cascades.

The exact mechanism by which cellular particle uptake can trigger pro-inflammatory effects remains unknown. Nonetheless, studies point to the formation of free radicals; this is associated with a change in the intracellular calcium concentration. Moreover, transcription factors (specific proteins for gene activation) are activated and cytokines produced[19]. The activation of these complex mechanisms shows that particle uptake triggers cellular effects.

Additional studies have shown that free radical formation can be triggered by nanoparticles such as fullerenes, carbon nanotubes, quantum dots, or exhaust emission particles[7]. The resulting overproduction or the chronic production of "reactive oxygen species" (ROS) can damage DNA, proteins and lipids, which then influence cellular processes[19]. This so-called oxidative stress reaction can signal cell damage, but also occurs in cell respiration (during metabolic processes) and in the activation of inflammatory reactions[20].

Nanoparticles trigger ROS formation in different ways. For example, ROS can develop directly on the surface of particles. Moreover, metallic particles function as catalysts and thus trigger ROS formation[20]. Nanoparticles also create mechanical damage, e.g. in the mitochondria, and thus create oxidative stress, which can lead to cell death[11; 17; 21]. As noted above, nanoparticles are also actively incorporated by phagocytosis, initiating ROS formation[22; 23]. The surface is the decisive factor. Smaller particles have a larger surface area to mass ratio and thus induce more ROS than larger particles[21; 24; 25]. This can lead to inflammatory reactions and activate the immune system, much like in bacterial infections[8; 23].

To a certain degree, cellular antioxidants can neutralize the free radicals and thus maintain a balanced "redox homeostasis". When more ROS is formed than neutralized, the system can shift, and certain biomolecules such as DNA or proteins can be oxidized and/or split. Such changes can cause mutations in the DNA, but also epigenetic damage[12; 22]. Studies show that nanoparticles composed of different materials (diesel exhaust particles, carbon black, metallic particles) can exert a gene toxic effect on humans[12].

Nanoparticles can also influence fundamental cell functions and cell physiological processes such as cell proliferation, cell metabolism and even cell death. Many illnesses develop due to uncontrolled cell reproduction (e.g. cancer), but also due to premature cell death, for example in neurodegenerative diseases. It has been shown[26] that carbon nanotubes can alter cell proliferation, programmed cell death (apoptosis) and certain cell parameters. Interestingly, the changes are not only concentration dependent; they also depend on the purity of the nanomaterials used as well as on cell type. This means that nanoparticle toxicity depends on numerous factors such as cell type, particle concentration, as well as particle features such as shape, material, surface, particle size and purity.

Conclusions

It is well-known that the active or passive uptake of nanoparticles can cause cellular effects. The biological relevance of these effects can be assessed only in a few cases. Unfortunately, no data is available on dose dependency or threshold values. Therefore, to investigate synthetic nanoparticles with nanotoxicological approaches, new techniques and devices have to be developed in order to evaluate the possible adverse health effects of nanoparticles. Currently, it appears that such results and effects underlie known mechanisms. This means that no specific or exclusively nanoparticle-caused cellular effects are to be expected. This calls for a better understanding of fundamental cellular processes, both to avoid potential risks and to use the opportunities that nanotechnologies offer in medical applications.

Notes and References

[1] Pollard, T. D., Earnshaw, W. C., 2007, *Cell Biology;* in Reihe, Bd. XX, Springer, 2. Aufl.: Original American edition published by Saunders.

[2] Klein, J., 1991, *Klein, J., Immunologie,* in Reihe: Immunologie, hg. v. Schmidt, R. E., Weinheim, New York, Basel: VCH Verlagsgesellschaft mbH.

[3] Simkó, M., Droste, S., Kriehuber, R. and Weiss, D. G., 2001, Stimulation of phagocytosis and free radical production in murine macrophages by 50 Hz electromagnetic fields, *Eur J Cell Biol 80(8),* 562-6

[4] Kobzik, L., 1995, Lung macrophage uptake of unopsonized environmental particulates. Role of scavenger-type receptors, *J Immunol 155(1),* 367-76.

[5] Inoue, K., Takano, H., Yanagisawa, R., Hirano, S., Ichinose, T., Shimada, A. and Yoshikawa, T., 2006, The role of toll-like receptor 4 in airway inflammation induced by diesel exhaust particles, *Arch Toxicol 80(5),* 275-9.

[6] Park, J. B., 2003, Phagocytosis induces superoxide formation and apoptosis in macrophages, *Exp Mol Med 35(5),* 325-35.

[7] Oberdorster, G., Oberdorster, E. and Oberdorster, J., 2005, Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles, *Environ Health Perspect 113(7),* 823-39.

[8] Brown, D. M., Donaldson, K. and Stone, V., 2004, Effects of PM10 in human peripheral blood monocytes and J774 macrophages, *Respir Res 5,* 29.

[9] Oberdorster, G., 2002, Toxicokinetics and effects of fibrous and nonfibrous particles, *Inhal Toxicol 14(1),* 29-56.

[10] Takenaka, S., Karg, E., Roth, C., Schulz, H., Ziesenis, A., Heinzmann, U., Schramel, P. and Heyder, J., 2001, Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats, *Environ Health Perspect 109 Suppl 4,* 547-51.

[11] Xia, T., Kovochich, M., Brant, J., Hotze, M., Sempf, J., Oberley, T., Sioutas, C., Yeh, J. I., Wiesner, M. R. and Nel, A. E., 2006, Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm, *Nano Lett 6(8),* 1794-807.

[12] Peters, A., Veronesi, B., Calderon-Garciduenas, L., Gehr, P., Chen, L. C., Geiser, M., Reed, W., Rothen-Rutishauser, B., Schurch, S. and Schulz, H., 2006, Translocation and potential neurological effects of fine and ultrafine particles a critical update, *Part Fibre Toxicol 3,* 13.

[13] Geiser, M., Rothen-Rutishauser, B., Kapp, N., Schurch, S., Kreyling, W., Schulz, H., Semmler, M., Im Hof, V., Heyder, J. and Gehr, P., 2005, Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells, *Environ Health Perspect 113(11),* 1555-60.

[14] Porter, A. E., Muller, K., Skepper, J., Midgley, P. and Welland, M., 2006, Uptake of C60 by human monocyte macrophages, its localization and implications for toxicity: studied by high resolution electron microscopy and electron tomography, *Acta Biomater 2(4),* 409-19.

[15] Stefani, D., Wardman, D. and Lambert, T., 2005, The implosion of the Calgary General Hospital: ambient air quality issues, *J Air Waste Manag Assoc 55(1),* 52-9.

[16] Garcia-Garcia, E., Andrieux, K., Gil, S., Kim, H. R., Le Doan, T., Desmaele, D., d'Angelo, J., Taran, F., Georgin, D. and Couvreur, P., 2005, A methodology to study intracellular distribution of nanoparticles in brain endothelial cells, *Int J Pharm 298(2),* 310-4.

[17] Li, N., Sioutas, C., Cho, A., Schmitz, D., Misra, C., Sempf, J., Wang, M., Oberley, T., Froines, J. and Nel, A., 2003, Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage, *Environ Health Perspect 111(4),* 455-60.

[18] Penn, A., Murphy, G., Barker, S., Henk, W. and Penn, L., 2005, Combustion-derived ultrafine particles transport organic toxicants to target respiratory cells, *Environ Health Perspect 113(8),* 956-63.

[19] Brown, D. M., Donaldson, K., Borm, P. J., Schins, R. P., Dehnhardt, M., Gilmour, P., Jimenez, L. A. and Stone, V., 2004, Calcium and ROS-mediated activation of transcription factors and TNF-alpha cytokine gene expression in macrophages exposed to ultrafine particles, *Am J Physiol Lung Cell Mol Physiol 286(2),* L344-53.

[20] Risom, L., Lundby, C., Thomsen, J. J., Mikkelsen, L., Loft, S., Friis, G. and Moller, P., 2007, Acute hypoxia and reoxygenation-induced DNA oxidation in human mononuclear blood cells, *Mutat Res 625(1-2),* 125-33.

[21] Sioutas, C., Delfino, R. J. and Singh, M., 2005, Exposure assessment for atmospheric ultrafine particles (UFPs) and implications in epidemiologic research, *Environ Health Perspect 113(8),* 947-55.

[22] Risom, L., Moller, P. and Loft, S., 2005, Oxidative stress-induced DNA damage by particulate air pollution, *Mutat Res 592(1-2),* 119-37.

[23] Long, H., Shi, T., Borm, P. J., Maatta, J., Husgafvel-Pursiainen, K., Savolainen, K. and Krombach, F., 2004, ROS-mediated TNF-alpha and MIP-2 gene expression in alveolar macrophages exposed to pine dust, *Part Fibre Toxicol 1(1),* 3.

[24] Stone, V., Tuinman, M., Vamvakopoulos, J. E., Shaw, J., Brown, D., Petterson, S., Faux, S. P., Borm, P., MacNee, W., Michaelangeli, F. and Donaldson, K., 2000, Increased calcium influx in a monocytic cell line on exposure to ultrafine carbon black, *Eur Respir J 15(2),* 297-303.

[25] Wilson, M. R., Lightbody, J. H., Donaldson, K., Sales, J. and Stone, V., 2002, Interactions between ultrafine particles and transition metals in vivo and in vitro, *Toxicol Appl Pharmacol 184(3),* 172-9.

[26] Kaiser, J. P., Wick, P., Manser, P., Spohn, P. and Bruinink, A., 2008, Single walled carbon nanotubes (SWCNT) affect cell physiology and cell architecture, *J Mater Sci Mater Med 19(4),* 1523-7.

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