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Review article

Nanotoxicology – New Research Area in Toxicology

Merve BACANLI, Nurşen BAŞARAN*

Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, 06100, Ankara, TURKEY

Nano sized materials are increasingly used in the fields of industry, science, pharmacy, medicine, electronics, communication and consumer products. On the other hand there is a great concern that these products may have some detrimental effects on human health and environment. Nanotoxicology is a new and important research area in toxicology. This toxicological research area refers to the study of interactions between living organisms and nanomaterials. Studies about nanomaterials shows that some nanomaterials may have cytotoxic and genotoxic effects and may pose health risks. But there is limited knowledge about the toxicity of nanomaterials. The nanotoxicology researchers focused on the relationship between nanomaterial characteristics (size, shape, surface area etc.) and toxic responses (cytotoxicity, genotoxicity, inflammation etc.). This article aims to give a brief summary of what is known today about nanotoxicology.

Key words: Nanomaterial, Nanoparticle, Nanotoxicology.

Nanotoksikoloji – Toksikolojide Yeni Bir Araştırma Alanı

Nano boyutlu materyallerin endüstri, bilim, eczacılık, tıp, elektronik, iletişim gibi alanlar ve tüketici ürünlerinde kullanımı giderek artmaktadır. Bununla birlikte bu ürünlerin insan sağlığına ve çevreye istenmeyen etkileri olabileceğine dair büyük kuşkular bulunmaktadır. Nanotoksikoloji, toksikoloji için yeni ve önemli bir araştırma alanıdır. Bu toksikoloji alanı canlılar ile nanomateryaller arasındaki etkileşmeler hakkında çalışmalar yapmaktadır. Günümüzde nanomateryallerin toksisitesi ile ilgili bilgiler kısıtlıdır. Nanomateryallerle yapılan çalışmalar nanomateryallerin sitotoksik, genotoksik ve sağlığa zararlı etkilerinin olabileceğini göstermiştir. Nanotoksikoloji alanındaki araştırıcılar nanomateryal özellikleri (boyut, şekil, yüzey alanı gibi) ile toksik yanıtlar (sitotoksisite, genotoksisite, enflamasyon gibi) arasındaki ilişki üzerine yoğunlaşmaktadır. Bu makale günümüzde nanotoksikoloji alanında bilinenler hakkında bir özet sunmayı amaçlamaktadır.

Anahtar kelimeler: Nanomateryal, Nanopartikül, Nanotoksikoloji.

***Correspondence:** E-mail: nbasaran@hacettepe.edu.tr, Tel: +90 (312) 305 21 78

INTRODUCTION

Nano sized materials are increasingly used in the fields of industry, science, pharmacy, medicine, electronics, communication and consumer products. The "nano" is derived from the Greek word "nanos" meaning "dwarf" (1). A nanomaterial (NM) defined as a substance with at least one dimension <100 nm in length. There are numerous nano-sized materials in our life. They can take different forms such as tubes, rods, wires or spheres. Depending on their origin, they can be categorized as either engineered or incidental NMs. Engineered nanoparticles (NPs) are particles generated to use the size-related properties inherent in the nanoscale (e.g. conductivity, spectral properties, biodistribution). Incidental NPs, are defined as particles either from unintended anthropogenic sources (e.g. combustion derived) or of natural origin (e.g., particles generated in forest fires). Engineered NMs including NPs and nanofibres are also categorized into four classes which include carbon based materials, metal-based materials

(quantum dots, nanosilver, nanogold…), dendrimers (nanosized polymers), and composites (2).

Nanotoxicology is the study of the toxicity of NMs. It has emerged only recently, years after the beginning of nanotechnology that is considered one of the key technologies of the 21st century, when numerous NMs had already been introduced into some industrial processes and consumer products. Donaldson et al. (3) quated "discipline of nanotoxicology would make an important contribution to the development of sustainable and safe nanotechnology". Growing concerns about the nanotoxicology were derived from prior experiences with air pollution (4) and asbestos (5). Nowadays many NPs, for example carbon nanotubes which are much smaller than asbestos, might have asbestos-like effects on cells (6).

Most of the NM producers demonstate their products as materials having perfect properties (7). Practical use of NMs for many purposes are ranging from applications in medicine to numerous industrial products from electronics to cosmetics. Properties such as small size, large surface area and surface activity, make NMs attractive in too many applications (8). NMs are being used in computer chip technology, automotive catalytic converters, cosmetics (lipsticks, sunscreens, anti-aging creams), dental prosthesis and orthopedic implant wear debris (2, 9). NMs for imaging and drug delivery systems are often intentionally coated with biomolecules such as DNA, proteins and monoclonal antibodies to target specific cells (10). In the future, it is suggested that they can be used in diagnostic aids, drug delivery systems and therapeutic treatments for cancer patients (11, 12). Currently there are over 800 consumer products containing different NMs. It is estimated that the average person consumes 10^{12} NPs per day in a normal diet as a result of food additives. The sales of which were valued at $147 billion in 2007 and are expected to soar over the coming years with a predicted value of $3.1 trillion by 2015 (2).

ROUTES of EXPOSURE

It has been demonstrated that the NPs enter the body mainly via dermal, inhalation, and oral routes (13-15). For ultrafine particles, the main entry road is respiratory system (16). Intravenous and oral administrations have a more rapid systemic effect compared to other routes and once in systemic circulation, most substances are subject to first-pass metabolism within the liver where they may accumulate or distribute via vasculature to end organs including brain (17). Liver is the site for first-pass metabolism, and it is particularly vulnerable to NM toxicity. The hepatotoxic potential of silica NPs could cause mononuclear inflammatory cell infiltrates at the portal area with concomitant hepatocyte necrosis (17).

Skin exposure to NMs can occur during the intentional application of topical creams and other drug treatments or accidental exposure (18, 19). There are controversial data about the dermal absorption of NMs although stratum corneum, the outer layer of epidermis, is a good barrier for chemical exposure. Oberdorster et al. (19) showed the penetration of a variety of NPs in the dermis and translocation to the systemic vasculate via lymphatic system and regional lymph. In some studies, the cytotoxicity of NMs applied to the skin was demonstrated. Cultured keratinocytes were exposed to extracts of several types of silver containing dressings. Of these, extracts of nanocrystalline silver coated dressings were most cytotoxic (20).

Because of its large surface area, localization/accumulation of drugs within the pulmonary tissue, lung is an attractive target for drug delivery due to the non-invasive nature of inhalation therapy (21, 22). Inhaled NPs can be deposited in all regions of respiratory tract. Being different than micron sized particles that are largely trapped and cleared by upper airway mucociliary escalator system, particles less than 2.5 μm can get down to the alveoli. The deposition of inhaled ultrafine particles (aerodynamic-diameter < 100 nm) mainly takes place in the alveolar region (18). After absorption from the respiratory tract, NMs can enter blood and lymph to reach cells in the bone marrow, lymph nodes, spleen and heart (18, 23). In respiratory tract, alveolar macrophages engulf and process particles that are not cleared by mucociliary action and coughing. Upon phagocytosis macrophages are activated to

release substantial amounts of oxygen radicals, proteolytic enzymes, proinflammatory mediators, etc. these mediators may lead to both acute and chronic lung inflammation. Ultrafine NPs are suggested to have more toxic properties than larger particles with the same chemical identity due to their larger surface area. Ultrafine silver particles were taken up by alveolar macrophages and aggregated silver particles persisted there for up to 7 days. Aggregated silver NPs and some other NMs have been shown to be cytotoxic to alveolar macrophage cells as well as epithelial lung cells (24). Another report by Warheit et al. (25) investigated acute lung toxicity and observed that intratracheally instilled single-wall carbon nanotubes produced granulomas in rats at very high doses. Citrate-capped gold NPs (13 nm in diameter) were found to be toxic to a human carcinoma lung cell line (26).

NMs can reach the gastrointestinal tract after mucociliary clearance from the respiratory tract through the nasal region, or can be ingested directly in food, water, cosmetics, drugs, and drug delivery devices (18, 19). Numerous kinds of NMs can pass through the gastrointestinal tract and are rapidly eliminated in feces and urine. However some NMs can accumulate in the liver during first-pass metabolism (19). Chung et al. (27) recently reported the occurrence of systemic argyria after ingestion of colloidal nanosilver proves its translocation from the intestinal tract. Nanocopper was reported to cause damage to liver, kidney and spleen. Injections and implants are other possible routes of exposure, primarily limited to engineered materials. Thus, nanoscale particles can end up in different parts of the body depending on size and other characteristics as well as routes of entry (1).

EFFECTS of PHYSICOCHEMICAL PROPERTIES of NANOMATERIALS on TOXICITY

Although NMs have the same material at the macro and nano scale, they might have some different toxicological effects because of their unique properties (7). The unusual physicochemical properties of NMs are attributable to their small size, surface area, shape, chemical composition (purity, crystallinity, electrophilic properties etc.), surface structure (morphology), solubility and aggregation. Physicochemical characteristics of the NMs are very important with respect to their biologic effects (1).

NMs can cross biological barriers, gaining entry to the body because of their small size. Size governs their kinetics including absorption, distribution, metabolism and excretion. Once inside the body, the NPs are small enough, they may readily enter to the cells and may easily interact with biomolecules which have the potential to destabilise normal cellular functioning (2). When particle size decreases, the surface area will increase. The smaller the particle is, the larger the surface area it has. Larger surface area enhances the catalytic activity of material and thus has been reported to increase its reactivity because surface atoms have a tendency to have unsatisfied high energy bonds. On the other hand because of their small size, electrons are not free to move as in the bulk material. Because of this movement restriction, particles react differently with light. At the nanoscale, the majority of atoms are split between the inside and the surface of the object whereas at the macro scale, the atoms are inside the object. The melting point is also lower for smaller particles (2).

The other important factor on toxicity of NMs is their shape and morphology. Numerous studies showed that shape of NM can highly influence their rate of uptake. Spherical NPs show higher uptake than nanoroods, while internalisation of these cylindrical shaped materials is strongly influenced by their dimensions (28, 29).

An inherent property of many NMs is their hydrophobicity and thus a propensity to agglomerate particularly under physiological conditions. With regard to human exposure, it is therefore likely that under more circumstances NMs will be able in the form of aggregates rather than individual units (2).

Surface charge will govern the formation of agglomerates according to the factors like pH or ionic strength of the aqueous environment they are in (30). This physicochemical property plays an important role in cellular

uptake of NMs. The plasma membrane is negatively charged, as is the intracellular environment, thus anionic NMs may be endocytosed at a lower rate than those are cationic (31). But this is not a rule and it does not affect the uptake of negatively charged NMs (32). However, positively charged NMs appear to be associated with greater cytotoxic responses when compared to negatively charged NMs. But it is unclear as to whether the cell death is the result of surface charge or if it is because of the increased uptake associated with positively charged NMs (33). Additionaly, DNA is negatively charged, so positively charged NMs may be easily react with DNA molecule (2).

All these unique properties make NPs very interesting for a number of industrial and medical applications. But these properties raise also important safety concerns.

TOXICITY of NANOMATERIALS

Since NPs can differ from the bulk materials because of their unique properties such as size, surface area, physico-chemical structure, shape and charge, their toxicity can be quiet different. On the other hand, there has been limited data about the toxicity of man-made NPs. There is serious lack of information about the toxicity of NPs. Toxicity of NPs may closely be related to their size as shown by studies of ultrafine particles in the respiratory tract (34). The small size of NPs is one of the key factors which may make them harmful to human health (35).

NPs are able to pass biological barriers and to penetrate into the cell. They can even penetrate into the nucleus and cause harmful interactions with biological systems (19). Protein misfolding and protein fibrillation induced by NPs were reported to cause some problems in the brain (36). In some cases NMs are even shown to transfer across the placental barrier (37). Gold NPs were shown to cross the materno-foetal barrier (38) and fullerenes were found to have a fatal effect on mouse embryos (39). However 10-30 nm sized polyethylene glycol coated (PEGylated) gold NPs cannot cross the perfused human plasenta and were not detected in foetal circulation (38).

The proposed toxicological mechanisms of NMs include oxidative stress, cytotoxicity, genotoxicity and inflammatory responses (40).

Oxidative stress and reactive oxygen species

NMs can induce oxidative stress, which refers to a redox imbalance within cells usually as a result of increased intracellular reactive oxygen species (ROS) and decreased antioxidants (2). In general, small and transient increases in ROS can be tolerated by most cell types, whereas higher levels which persist over a longer time period, are more likely to result in cell damage (37). ROS are highly reactive molecules that can interact with cellular macromolecules such as DNA, proteins and lipids (2).

Composition of NMs and their high surface area are associated with the generation of ROS by NMs. Consequently, the smaller NP, the higher oxidative stress they induce (41, 42). NMs have been described to possibly generate ROS by different mechanisms: direct generation of ROS as a result of exposure to an acidic environment such as the lysosomes (43), interaction of the NMs with cellular organelles such as mitochondria (44), interaction of NMs with redox active proteins such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and interaction of NMs with cell surface receptors and activation of intracellular signaling pathways (37). Oxidative stress induced by NPs is reported to enhance inflammation though upregulation of redox-sensitive transcription factors (45).

There are too many studies demonstrating the induction of ROS by NMs. Park et al. (46) showed that perinuclear distrubition of titanium dioxide (TiO_2) NPs correlated with the induction of ROS in the same region. For quantum dots (QDs), the induction of ROS has also been reported (47). Interactions of silver NPs (AgNPs) with human fibro sarcoma (HT-1080) and human skin/carcinoma (A431) cells have showed some signs of oxidative stress such as decreased reduced glutathione (GSH) and superoxide dismutase (SOD) levels (48). When human lung fibroblast cells interacted with gold NPs (AuNPs), cells reported to generate significantly more lipid

hydroperoxides (49). It was also shown that the amount of alkylated proteins by malondialdehyde (MDA), a product of cellular lipid peroxidation was significantly more in the AuNPs treated samples (43).

Cytotoxicity

The membrane stability can be affected by NPs either directly like physical damage or indirectly like oxidation which can cause cell death. Interactions of NPs with membranes are associated with surface properties of NPs (50). The higher surface area over volume ratio of NMs augments the surface available for interaction with cellular components (51). NP induced cytotoxicity has been reported by several groups. But the data about the cytotoxicity of NPs are conflicting. There is a lack of consensus in the published data on NP cytotoxicity due to variable methods, materials and cell lines.

The identification of cytotoxicity of NPs toward mammalian germ line stem cells has aroused great concern over the biosafety of NMs. The results showed that AgNPs were the most toxic with manifestations like drastic reduction of mitochondrial function, increased membrane leakage, necrosis and induction of apoptosis (52). Also cytotoxic activity of AgNPs were evaluated by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and results showed a dose dependent decrease in cell viability compared to control cells (53).

Due to their small size, AuNPs have been found to easily enter cells (10). Tkachenko et al., looked at the nuclear targeting ability of AuNPs alone, and then at AuNPs with a full-length peptide containing both the receptor-mediated endocytosis and nuclear localization signal segments from an adenovirus in HepG2 cells. The type of surface coating played an important role in the cytotoxicity of AuNPs. Viability of HepG2 cells after 12 h in the presence of NP-peptide complexes was only slightly compromised (<5%) as compared to that of a control batch. (54).

Cai et al. (55) showed that TiO_2 NPs have some cytotoxic effects on HeLa in the presence of ultraviolet (UV) light and they suggested that this was associated with the photo-excited TiO_2 promoting oxidative stress. 7 nm sized cerium oxide NPs have caused cytotoxicity with absorption on cell membrane (56). Nano sized gold NPs were shown to have size dependent cytotoxicity (57).

Several *in vitro* studies have demonstrated the cytotoxicity of single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) in guinea pig alveolar macrophages (58). In a study exposing human embryo kidney cells to SWNTs for one to five days, Cui et al. found dose- and time-dependent decreases in cell-adhesion ability, cell proliferation, and increases in induction of apoptosis (59). Monteiro-Riviere et al. (60) reported that keratinocytes incubated with higher concentrations of MWNTs for longer exposure times, the percentage of cells with MWNTs inside increased from 59% at 24 hours to 84% after 48 hours. In addition, a dose- and time-dependent decrease in cell viability was observed.

Genotoxicity

Due to their small size and large surface area, NMs may have unpredictable genotoxic effects and the most important genotoxic effect is DNA damage induction which can cause mutagenesis, and carcinogenesis. NMs are small enough, so they may pass through cellular membranes and they may interact with DNA directly. When they promote oxidative stress and inflammatory responses, they may also interact with DNA indirectly (2).

DNA damage induced by NPs, single-strand DNA breaks, double-strand breaks, DNA deletions and genomic instability in the form of increase in 8-hydroxy-2-deoxyguanosine (8-OHdG) levels are formed (61). Long term exposure of cells to NPs caused genome instability, altered cell cycle kinetics and induced protein expression of p53, which have a critical role in responding to various stresses that cause damages in DNA and in DNA repair related proteins (7, 62).

Colognato et al. (63) showed that cobalt NPs were capable of inducing genotoxicity in human peripheral blood leukocytes. They demonstrated a dose dependent increase in the frequency of micronucleated lymphocytes. Silica NPs can induce ROS production, DNA strand breaks and oxidized bases (64). Zinc

oxide NPs (ZnO NPs) are widely used in cosmetics and sunscreens and in different cell lines, genotoxic effects of ZnO NPs were observed (65, 66).

AuNPs, AgNPs and TiO_2 NPs are important for ROS production and genotoxicity (7). But there are too many discordant studies about them. For example Li et al. (67) studied about the genoxicity of 5 nm AgNPs with Ames and micronucleus assay. In Ames test, AgNPs did not induce mutations in five different *S. typhimurium* strains. However, in micronucleus test, AgNPs displayed concentration-dependent genotoxicity in human lymphoblast TK6 cell line.

AuNPs were reported to be capable of inducing DNA damage indirectly through an oxidative stress response (2). But results in two genotoxic tests, comet assay and micronucleus assay, showed that AuNPs in different size were not genotoxic and showed no systemic and local adverse effects (68).

TiO_2 NPs are also mutagenic, capable of inducing point mutations and DNA damage (69). TiO_2 NPs caused increased micronuclei frequency in micronucleus assay. In comet assay, TiO_2 NPs had a significant olive tail moment which indicated unrepaired DNA strand breaks (70).

In vitro experiments have shown that C60 fullerenes to be generally noncytotoxic with no mutagenic effects in Chinese Hamster Ovary and mice lung epithelial cells, respectively (71, 72). Another study has found that C60 treatment also increases formamidopyrimidine-DNA glycosylase sensitive sites (69). However *in vivo* studies in mice demonstrated that treatment with C60 fullerenes has caused DNA damage in liver and lung and increase in the levels of DNA adducts like 8-OHdG. Also oral administration of SWCNTs in mice is found to be associated with increase in 8-OHdG levels in liver and lung (73).

Cobalt and its alloy are commonly used in hip joint replacements and other orthopedic joint replacements. Genotoxic effects were observed in some studies with these NPs (74). Analysis of peripheral blood leukocytes of patients with cobalt alloy joint replacements showed positive DNA damage in comet assay (63). Cobalt and Cobalt-Chromium NPs induced a dose-dependent increase in micronucleus frequency as well as chromosomal loss, gains, deletions and polyploidy (75).

Inflammatory responses

Inflammation is an important physiological process in response to tissue injury and is mediated by inflammatory cells that secrete a large variety of soluble factors, including cytokines, migration inhibition factors, reactive nitrogen species and ROS. These factors are important defences against infection and tissue injury (76, 77). Oxidative stress induced by NPs is reported to enhance inflammation through upregulation of redox-sensitive transcription factors such as nuclear factor kappa B (NFκB), activating protein 1 (AP-1), extracellular signal regulated kinases (ERK) c-Jun, N-terminal kinases, JNK, and p38 mitogen-activated protein kinases pathways (34, 45, 78). The increase of TNF-α levels can cause damage of cell membrane and apoptosis (7). Additionally, chronic inflammation has been strongly associated with carcinogenesis (79).

NPs are described to be more toxic than larger particles with the same chemical entidy, causing inflammation or allergic response. It was suggested that NPs, because of their small size, could act like haptens to modify protein structures (80).

TiO_2 NPs and ultrafine carbon black NPs have been associated with inflammatory potency in the lungs of the rats following intratracheal instillation (2, 81). Also some studies reported that exposure to TiO_2 NPs resulted in pulmonary inflammation, pulmonary edema, macrophages accumulation and pneumonocyte apoptosis (7). Silica NPs induced inflammatory and oxidative stress responses both *in vitro* and *in vivo* (82, 83). In a study using rat alveolar macrophages (NR8333) exposed to AgNPs, demonstrated significant levels of TNF-α, IL-1β comparing to the control group (53). In another study, Park et al. (84) showed that the phagocytosis of AgNPs stimulated inflammatory signaling through the ROS generation in macrophages followed by the induced secretion of TNF-α.

CONCLUSION

NMs, depending on the size, shape, elemental materials and the surface functional groups were observed to have a range of detrimental effects on cells. However the toxicological data about NPs has been collected mainly from occupational and environmental research with natural NMs. Nano sized particles are known to be generated in certain place conditions. There is a still serious lack of information about the toxicity of NPs. Exposure to NPs is inevitable since NPs become more widely used but there is still doubts and much more to be understood regarding their safety. Possible interactions between NPs and living organisms and the results of long-term NP exposure are not yet fully understood.

When making a toxicological assessment with NMs, knowledge what material has been tested, uptake and distribution of NM in the body and the effects of NMs must be considered. A critical point to determine the toxicity of NM is to know the characterization of NP. Also determination of real exposure concentrations from *in vivo* and epidemiologic studies are necessary. So more research is required to understand the mechanisms and pathways in the body and the toxicity of NMs. As the development of nanotechnological applications continue to grow, the demand for safety and risk assessment studies will increase in the future.

REFERENCES

1. Arora S, Rajwade JM, Paknikar KM, Nanotoxicology and in vitro studies: The need of the hour, Toxicol Appl Pharm 258(2), 151-165, 2012.
2. Singh N, Manshian B, Jenkins GJS, Griffiths SM, Williams PM, Maffeis TGG, Wright CJ, Doak SH, NanoGenotoxicology:The DNA damaging potential of engineered nanomaterials, Biomaterials 30(23-24), 3891-3914, 2009.
3. Donaldson K, Stone V, Tran CL, Kreyling W, Borm PJA, Nanotoxicology, Occup Environ Med 61(9), 727-728, 2004.
4. Dockery DW, Pope CA, Xu X, Spengler JD, Ware JH, Fay ME, Ferris Jr BG, Speizer FE, An association between air pollution and mortality in six US cities, N Engl J Med 329(24), 1753-1759, 1993.
5. Kane AB, Hurt RH, Nanotoxicology: the asbestos analogy revisited, Nat Nanotech 3(7), 378-379, 2008.
6. Elsaesser A, Howard CV, Toxicology of nanoparticles, Adv Drug Delivery Rev 64(2), 129-137, 2012.
7. Aydın A, Sipahi H, Charehsaz M, Nanoparticles toxicity and their routes of exposures, Rec Adv in Novel Drug Car Syst Chapter 18, 483-500, 2012.
8. Landsiedel R, Kapp MD, Schulz M, Wiench K, Oesch F, Genotoxicity investigations on nanomaterials: methods, preparation and characterization of test material, potential artifacts and limitations—Many questions, some answers, Mutat Res/Rev in Mutat Res 681(2), 241-258, 2009.
9. Brown C, Fisher J, Ingham E, Biological effects of clinically relevant wear particles from metal-on-metal hip prostheses, Proc Inst Mech Eng H J Eng Med 220(2), 355-369, 2006.
10. Lewinski N, Colvin V, Drezek R, Cytotoxicity of nanoparticles, Small 4(1), 26-49, 2008.
11. Ferrari M, Cancer nanotechnology: opportunities and challenges. Nat Rev Cancer 5(3), 161-171, 2005.
12. Sahoo S, Parveen S, Panda J, The present and future of nanotechnology in human health care, Nanomed-Nanotechnol 3(1), 20-31, 2007.
13. Stern ST, McNeil SE, Nanotechnology safety concerns revisited, Toxicol Sci 101(1), 4-21, 2008.
14. Fischer HC, Chan WCW, Nanotoxicity: the growing need for in vivo study, Curr Opin Biotechnol 18(6), 565-571, 2007.
15. Ryman-Rasmussen JP, Riviere JE, Monteiro-Riviere NA, Variables influencing interactions of untargeted quantum dot nanoparticles with skin cells and identification of biochemical modulators, Nano Letters 7(5), 1344-1348, 2007.
16. Donaldson K, Brown D, Clouter A, Duffin R, MacNee W, Renwick L, Tran L, Stone V, The pulmonary toxicology of ultrafine particles, J Aerosol Med 15(2), 213-220, 2002.
17. Yıldırımer L, Thanh NTK, Loizidou M, Seifalian AM, Toxicology and clinical potential of nanoparticles, Nano Today 6(6), 585-607, 2011.
18. Hagens WI, Oomen AG, de Jong WH, Cassee FR, Sips AJAM, What do we (need to) know about the kinetic properties of nanoparticles in the body? Regul Toxicol Pharm 49(3), 217-229, 2007.

19. Oberdörster G, Oberdörster E, Oberdörster J, Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles, Environ Health Perspect 113(7), 823, 2005.
20. Paddle-Ledinek JE, Nasa Z, Cleland HJ, Effect of different wound dressings on cell viability and proliferation, Plast Reconstr Surg 117(7S), 110S-118S, 2006.
21. Patton JS, Byron PR, Inhaling medicines: delivering drugs to the body through the lungs, Nat Rev Drug Discov 6(1), 67-74, 2007.
22. Yang W, Peters JI, Williams Iii RO, Inhaled nanoparticles—A current review, Int J Pharmaceut 356(1–2), 239-247, 2008.
23. Oberdörster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, Carter J, Karn B, Kreyling W, Lai D, Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy, Particl Fibre Toxicol 2(1), 8, 2005.
24. Soto K, Garza K, Murr L, Cytotoxic effects of aggregated nanomaterials, Acta Biomater 3(3), 351-358, 2007.
25. Warheit DB, Laurence BR, Reed KL, Roach DH, Reynolds GAM, Webb TR, Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats, Toxicol Sci 77(1), 117-125, 2004.
26. Patra, HK, Banerjee S, Chaudhuri U, Lahiri P, Dasgupta AK, Cell selective response to gold nanoparticles, Nanomed-Nanotechnol 3(2), 111-119, 2007.
27. Chung IS, Lee M-Y, Shin DH, Jung, HR, Three systemic argyria cases after ingestion of colloidal silver solution, Int J Dermatol 49(10), 1175-1177, 2010.
28. Chithrani BD, Chan WC, Elucidating the mechanism of cellular uptake and removal of protein-coated gold nanoparticles of different sizes and shapes, Nano Letters 7(6), 1542-1550, 2007.
29. Gratton SE, Ropp PA, Pohlhaus PD, Luft JC, Madden VJ, Napier ME, DeSimone JM, The effect of particle design on cellular internalization pathways, P Natl Acad Sci 105(33), 11613-11618, 2008.
30. Jiang J, Oberdörster G, Biswas P, Characterization of size, surface charge, and agglomeration state of nanoparticle dispersions for toxicological studies, J Nanopart Res 11(1), 77-89, 2009.
31. Harush-Frenkel O, Debotton N, Benita S, Altschuler Y, Targeting of nanoparticles to the clathrin-mediated endocytic pathway, Biochem Bioph Res Co 353(1), 26-32, 2007.
32. Lockman PR, Koziara JM, Mumper RJ, Allen DD, Nanoparticle surface charges alter blood-brain barrier integrity and permeability, J Drug Target 12(9-10), 635-641, 2004.
33. Nan A, Bai X, Son SJ, Lee SB, Ghandehari H, Cellular uptake and cytotoxicity of silica nanotubes, Nano Lett 8(8), 2150-2154, 2008.
34. Curtis J, Greenberg M, Kester J, Phillips S, Krieger G. Nanotechnology and nanotoxicology, Toxicol Rev 25(4), 245-260, 2006.
35. El-Ansary A, Al-Daihan S, On the toxicity of therapeutically used nanoparticles: an overview, J Toxicol 2009, doi:10.1155/2009/754810.
36. Oberdorster G, Elder A, Rinderknecht A, Nanoparticles and the brain: cause for concern? J Nanosci Nanotechno 9(8), 4996-5007, 2009.
37. Soenen SJ, Rivera-Gil P, Montenegro J-M, Parak WJ, De Smedt SC, Braeckmans K, Cellular toxicity of inorganic nanoparticles: Common aspects and guidelines for improved nanotoxicity evaluation, Nano Today 6(5), 446-465, 2011.
38. Myllynen PK, Loughran MJ, Howard CV, Sormunen R, Walsh AA, Vähäkangas KH, Kinetics of gold nanoparticles in the human placenta, Reprod Toxicol 26(2), 130-137, 2008.
39. Tsuchiya T, Oguri I, Yamakoshi YN, Miyata N, Novel harmful effects of (60) fullerene on mouse embryos in vitro and in vivo, FEBS Lett 393(1), 139-145, 1996.
40. Nel A, Xia T, Madler L, Li N, Toxic potential of materials at the nanolevel, Science 311(5761), 622-627, 2006.
41. Brown DM, Wilson MR, MacNee W, Stone V, Donaldson K, Size-dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines, Toxicol Appl Pharm 175(3), 191-199, 2001.
42. Knaapen AM, Borm PJA, Albrecht C, Schins RPF, Inhaled particles and lung cancer. Part A: Mechanisms, Int J Cancer 109(6), 799-809, 2004.
43. Stroh A, Zimmer C, Gutzeit C, Jakstadt M, Marschinke F, Jung T, Pilgrimm H, Grune T, Iron oxide particles for molecular magnetic resonance imaging cause transient oxidative stress in rat macrophages, Free Radical Bio Med 36(8), 976-984, 2004.
44. Jain TK, Reddy MK, Morales MA, Leslie-Pelecky DL, Labhasetwar V, Biodistribution, clearance, and biocompatibility of iron oxide magnetic nanoparticles in rats, Mol Pharm 5(2), 316-327, 2008.
45. Kabanov AV, Polymer genomics: an insight into pharmacology and toxicology of

nanomedicines, Adv Drug Deliver Rev 58(15), 1597-1621, 2006.
46. Park E-J, Yi J, Chung K-H, Ryu D-Y, Choi J, Park K, Oxidative stress and apoptosis induced by titanium dioxide nanoparticles in cultured BEAS-2B cells, Toxicol Lett 180(3), 222-229, 2008.
47. Hardman R, A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors, Environ Health Persp 114(2), 165, 2006.
48. Arora S, Jain J, Rajwade JM, Paknikar KM, Cellular responses induced by silver nanoparticles:In vitro studies, Toxicol Lett 179(2), 93-100, 2008.
49. Li JJ, Hartono D, Ong C-N, Bay, B-H, Yung L-YL, Autophagy and oxidative stress associated with gold nanoparticles, Biomaterials 31(23), 5996-6003, 2010.
50. Vasir JK, Labhasetwar V, Quantification of the force of nanoparticle-cell membrane interactions and its influence on intracellular trafficking of nanoparticles, Biomaterials 29(31), 4244-4252, 2008.
51. Pisanic Ii TR, Blackwell JD, Shubayev VI, Fiñones RR, Jin S, Nanotoxicity of iron oxide nanoparticle internalization in growing neurons, Biomaterials 28(16), 2572-2581, 2007.
52. Braydich-Stolle L, Hussain S, Schlager JJ, Hofmann M-C, In vitro cytotoxicity of nanoparticles in mammalian germline stem cells, Toxicol Sci 88(2), 412-419, 2005.
53. Carlson C, Hussain SM, Schrand AM, K. Braydich-Stolle L, Hess KL, Jones RL, Schlager JJ, Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species, J Phys Chem B 112(43), 13608-13619, 2008.
54. Tkachenko AG, Xie H, Coleman D, Glomm W, Ryan J, Anderson MF, Franzen S, Feldheim DL, Multifunctional gold nanoparticle-peptide complexes for nuclear targeting, J Am Chem Soc 125(16), 4700-4701, 2003.
55. Cai R, Kubota Y, Shuin T, Sakai H, Hashimoto K, Fujishima Akira, Induction of cytotoxicity by photoexcited TiO_2 particles, Cancer Res 52(8), 2346-2348, 1992.
56. Thill A, Zeyons O, Spalla O, Chauvat Franck, Rose J, Auffan M, Flank AM, Cytotoxicity of CeO_2 nanoparticles for Escherichia coli. Physico-chemical insight of the cytotoxicity mechanism, Environ Sci Tech 40(19), 6151-6156, 2006.
57. Pan Y, Neuss S, Leifert A, Fischler M, Wen F, Simon U, Schmid G, Brandau W, Jahnen-Dechent W, Size-Dependent Cytotoxicity of Gold Nanoparticles, Small 3(11), 1941-1949, 2007.
58. Jia G, Wang H, Yan L, Wang X, Pei R, Yan T, Zhao Y, Guo X, Cytotoxicity of carbon nanomaterials: single-wall nanotube, multi-wall nanotube, and fullerene, Environ Sci Tech 39(5), 1378-1383, 2005.
59. Cui D, Tian F, Ozkan CS, Wang M, Gao H, Effect of single wall carbon nanotubes on human HEK293 cells, Toxicol Lett 155(1), 73-85, 2005.
60. Monteiro-Riviere NA, Inman AO, Challenges for assessing carbon nanomaterial toxicity to the skin, Carbon 44(6), 1070-1078, 2006.
61. Song M-F, Li Y-S, Kasai H, Kawai K, Metal nanoparticle-induced micronuclei and oxidative DNA damage in mice, J Clin Biochem Nutr 50(3), 211, 2012.
62. Mroz R, Schins RPF, Li H, Jimenez LA, Drost EM, Holownia A, MacNee W, Donaldson K, Nanoparticle-driven DNA damage mimics irradiation-related carcinogenesis pathways, Eur Respir J 31(2), 241-251, 2008.
63. Colognato R, Bonelli A, Ponti J, Farina M, Bergamaschi E, Sabbioni E, Migliore L, Comparative genotoxicity of cobalt nanoparticles and ions on human peripheral leukocytes in vitro, Mutagenesis 23(5), 377-382, 2008.
64. Valko M, Rhodes CJ, Moncol J, Izakovic MM, Mazur M, Free radicals, metals and antioxidants in oxidative stress-induced cancer, Chem Biol Interact 160(1), 1-40, 2006.
65. Sharma V, Anderson D, Dhawan A, Zinc oxide nanoparticles induce oxidative stress and genotoxicity in human liver cells (HepG2), J Biomed Nanotech 7(1), 98-99, 2011.
66. Sharma V, Singh SK, Anderson D, Tobin DJ, Dhawan A, Zinc oxide nanoparticle induced genotoxicity in primary human epidermal keratinocytes, J Nanosci Nanotechno 11(5), 3782-3788, 2011.
67. Li Y, Chen DH, Yan J, Chen Y, Mittelstaedt RA, Zhang Y, Biris AS, Heflich RH, Chen T, Genotoxicity of silver nanoparticles evaluated using the Ames test and micronucleus assay, Mutat Res-Gen Tox En 745(1), 4-10, 2012.
68. Schulz M, Ma-Hock L, Brill S, Strauss V, Treumann S, Gröters S, van Ravenzwaay B, Landsiedel R, Investigation on the genotoxicity of different sizes of gold nanoparticles administered to the lungs of rats, Mutat Res-Gen Tox En 745(1), 51-57, 2012.
69. Xu A, Chai Y, Nohmi T, Hei TK, Genotoxic responses to titanium dioxide nanoparticles and fullerene in gpt delta transgenic MEF cells, Part Fibre Toxicol 6(3), 2009.

70. Kang SJ, Kim BM, Lee YJ, Chung HW, Titanium dioxide nanoparticles trigger p53-mediated damage response in peripheral blood lymphocytes, Environ Mol Mutagen 49(5), 399-405, 2008.
71. Mrđanović J, Solajić S, Bogdanović V, Stankov K, Bogdanović G, Djordjevic A, Effects of fullerenol $C_{60}(OH)_{24}$ on the frequency of micronuclei and chromosome aberrations in CHO-K1 cells, Mutat Res-Gen Tox En 680(1), 25-30, 2009.
72. Shinohara N, Matsumoto K, Endoh S, Maru J, Nakanishi J, In vitro and in vivo genotoxicity tests on fullerene C_{60} nanoparticles, Toxicol Lett 191(2), 289-296, 2009.
73. Folkmann JK, Risom L, Jacobsen NR, Wallin H, Loft S, Moller P, Oxidatively damaged DNA in rats exposed by oral gavage to C60 fullerenes and single-walled carbon nanotubes, Environ Health Persp 117(5), 703, 2009.
74. Ng CT, Li JJ, Bay B-H, Yung L-YL, Current studies into the genotoxic effects of nanomaterials, J Nucleic Acids 2010, doi:10.4061/2010/947859.
75. Tsaousi A, Jones E, Case CP, The in vitro genotoxicity of orthopaedic ceramic (Al_2O_3) and metal (CoCr alloy) particles, Mutat Res-Gen Tox En 697(1), 1-9, 2010.
76. Jaiswal M, LaRusso NF, Burgart LJ, Gores GJ, Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism, Cancer Res 60(1), 184-190, 2000.
77. Valinluck V, Sowers LC, Inflammation-mediated cytosine damage: a mechanistic link between inflammation and the epigenetic alterations in human cancers, Cancer Res 67(12), 5583-5586, 2007.
78. Vega-Villa KR, Takemoto JK, Yáñez JA, Remsberg CM, Forrest ML, Davies NM, Clinical toxicities of nanocarrier systems, Adv Drug Deliver Rev 60(8), 929-938, 2008.
79. Federico A, Morgillo F, Tuccillo C, Ciardiello F, Loguercio C, Chronic inflammation and oxidative stress in human carcinogenesis, Int J Cancer 121(11), 2381-2386, 2007.
80. Klimuk SK, Semple SC, Nahirney PN, Mullen MC, Bennett CF, Scherrer P, Hope MJ, Enhanced anti-inflammatory activity of a liposomal intercellular adhesion molecule-1 antisense oligodeoxynucleotide in an acute model of contact hypersensitivity, J Pharmacol Exp Ther 292(2), 480-488, 2000.
81. Li XY, Gilmour PS, Donaldson K, MacNee W, Free radical activity and pro-inflammatory effects of particulate air pollution (PM10) in vivo and in vitro, Thorax 51(12), 1216-1222, 1996.
82. Kaewamatawong T, Shimada A, Okajima M, Inoue H, Morita T, Inoue K, Takano H, Acute and subacute pulmonary toxicity of low dose of ultrafine colloidal silica particles in mice after intratracheal instillation, Toxicol Pathol 34(7), 958-965, 2006.
83. Lin W, Huang Y-W, Zhou X-D, Ma Y, In vitro toxicity of silica nanoparticles in human lung cancer cells, Toxicol Appl Pharm 217(3), 252-259, 2006.
84. Park E-J, Yi J, Kim Y, Choi K, Park K, Silver nanoparticles induce cytotoxicity by a Trojan-horse type mechanism, Toxicol In Vitro 24(3), 872-878, 2010.

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