Chapter 5

In vivo Assessment of Nanomaterials Toxicity

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Additional information is available at the end of the chapter

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Abstract

The concern regarding the safety of nanomaterials for the human body is constantly raising. On one hand, there is an increase in the production of nanomaterials for technological applications, which raises the risk of accidental exposure of the workers during the technological manipulation. On the other hand, nanomaterials can be designed for medical applications and their faith in the human body, along with their interactions with different tissues, becomes of vital importance.

The mechanisms involved in nanomaterials toxicity are discussed, including oxidative stress and inflammation. *In vivo* toxicity evaluation includes different routes of administration or interaction between the nanomaterial and the organism, as well as a short-term or a long-term exposure or evaluation. Also, the characteristics of nanomaterials, including size, shape, impurities, function, surface coating in relation to their toxicity, were discussed. A particular attention has been given to the evaluation of the toxicity of dendrimers, silver nanoparticles, gold nanoparticles and carbon nanotubes.

Keywords: toxicity, oxidative stress, nanoparticles, carbon nanotubes

1. Introduction

Research in the field of nanomaterials and nanotechnology has seen exponential growth in recent years due to multiple applications in different areas such as molecular imaging, drug delivery, engineering technology, and development of materials and medical devices for diagnosis and treatment. Generally, the biological effects of nanomaterials depend on their

electronic, magnetic, optical, and mechanical properties and also on their size, composition, and shape.

However, the impact of these nanomaterials on human and environmental health is still limited. Most studies have investigated only the effects of unintended exposure (inhalation, medical procedures or accidental ingestion) and evaluated especially local effects, at the access site [1, 2]. Nevertheless, due to the development of various biomedical procedures using nanomaterials it is necessary to understand their potential toxic effects at systemic level.

An increasing number of studies published in the last decade have tried to decipher the complexity of interactions between different types of nanomaterials and cells. Nanomaterials usually get into the body either by inhalation and ingestion or transcutaneous [3, 4], most often accidentally in the process of synthesis, through their usage, after wearing gold and silver jewelry or by eating food containing gold. Nevertheless, the behavior of nanoparticles inside the cells is still an enigma, and not all the metabolic and immunological responses induced by these particles are completely understood. In addition, the conclusions obtained have been variable and contradictory according to the type of nanomaterials used, their size, shape, and surface chemistry. Based on the multitude of uses in pharmaceutical and medical applications, a thorough understanding of associated systemic and local toxicity is critically necessary.

2. Nanomaterial design considerations

Generally, the nanomaterials toxicity has been thought to originate in size and surface area, composition, and shape. Size influences the cellular uptake and response to nanomaterials, their distribution and elimination from the body. It has been shown that nanomaterials with small size have an exponential increase in surface area relative to volume, making the surface more reactive in itself and to biological components from surrounding environment. Several *in vitro* studies have shown that the latex nanospheres uptake into the nonphagocytic cells is higher at small sizes (50 - 100 nm) and decrease with the increase of the diameter (over 200 nm) [5]. Usually, nanomaterials with small sizes accumulate mainly in certain tissues (spleen, liver, lung, and kidneys) where they interfere with their biological functions [6, 7].

Chemical composition at the surface influences the chemical interactions of nanomaterials with biomolecules. Most nanomaterials are functionalized on the surface in order to increase blood circulation and to be more biocompatible for use in targeted therapy. Therefore, different functional groups were added to the outside to allow a better interaction with biological components and to aid the passage of nanomaterials in certain cells.

The phytochemical synthesis of nanoparticles using polyphenols from plants or fruits plays an important role in the field of nanomedicine as it offers a safe alternative therapeutic option. David et al. [8] synthesized silver nanoparticles using European black elderberry fruits extract (*Sambucus nigra - SN,* Adoxaceae family). The new materials were tested *in vitro* on spontaneously immortalized, human keratinocytes (HaCaT) exposed to UVB radiation, *in vivo* on acute inflammation model, and in humans on psoriasis lesions. The

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results obtained demonstrated the anti-inflammatory effects of functionalized silver nanoparticles both *in vitro* as well as *in vivo*. Thus, the bionanocomposites reduced cytokine production induced by UVB irradiation and diminished the edema and cytokines levels in the paw tissues, early after the induction of inflammation and presented long-term protective effect. Silver nanoparticles functionalized with natural extracts applied on psoriasis lesions exhibited anti-inflammatory effect [8]. Materials based on gold nanoparticles and natural compounds extracted from native plants of the *Adoxaceae* family (European cranberrybush – *Viburnum opulus L.* and European black elderberry – *Sambucus nigra L.)* possessed anti-inflammatory activity, both in vitro as well as on psoriasis lesions, mainly due to their high content of anthocyanins and other polyphenols [9].

The degradability of the material is also an important factor for acute and long-term toxicity. Thus, the nondegradable nanomaterials can accumulate in cells, where they can cause detrimental effects similar to those of lysosomal storage diseases. By contrast, the biodegradable nanomaterials can undergo changes in cells and may lead to unpredicted toxicity due to unexpected toxic degradation products. They can release toxic products in the biological milieu leading to free radical formation and consequently to cellular damage. Recently, Champion et al. have shown that phagocytosis of nanomaterials depends on the contact with materials and consequently on their shape [10]. Thus, nanorods particles are less internalized in cells compared to spherical materials [11]. Wang et al. suggested that the difference between cellular uptake and cytotoxicity of spherical particles and nanorods depends on chemical compounds used to stabilize the nanomaterials [12]. For example, cetyltrimethylammonium bromide (CTAB), a cationic surfactant used to stabilize gold nanorods, can induce intracellular aggregation and explain nanorods cytotoxicity compared to spherical particles [12, 13]. In order to reduce nanorods toxicity other substances may be used: PEG, citric acid, and transferrin [11].

3. Mechanisms of nanomaterials toxicity

The most important mechanism of *in vitro* and *in vivo* nanotoxicity is related to the induction of oxidative stress by free radical formation, directly into the acidic environment of lysosomes [14]. Free radicals are released from phagocytic cells as a response to foreign material, insufficient amounts of anti-oxidants, presence of transition metals, or environmental factors [15]. The most exposed organs to nanomaterials are the liver and the spleen, especially due to the prevalence of phagocytic cells in their reticulo-endothelial system. In addition, the organs with high blood flow such as kidneys and lungs can be affected.

Hydrogen peroxide generated *in vivo* can react with silver nanoparticles and lead to releasing of Ag^+ ions [16]. In excess, free radicals cause the damage of cellular macromolecules through the oxidation of lipids, proteins, and DNA. Injury of cell membranes results in leakage of cytoplasmic contents and necrosis, whereas rupture of lysosomal membranes can induce apoptosis. In addition, the reactive oxygen species (ROS) can stimulate the inflammation through up-regulation of redox-sensitive transcription factors (nuclear factor kB – NF-kB, activator protein-1 – AP-1), and mitogen-activated protein kinases (MAPK) [15].

Intracellular, nanomaterials may interact with different components, especially with mitochondria and nucleus, considered as main sources of their toxicity. Nanomaterials such as metal nanoparticles, fullerenes, and carbon nanotubes are located in mitochondria and induce apoptosis and ROS formation [15]. The effect on nuclear DNA leads to nuclear damage, cell-cycle arrest, mutagenesis, and apoptosis. The action is due to the diffusion of nanomaterials into the nucleus and formation of ROS, which directly trigger DNA damage and induce chromosomal abnormalities [3]. Silver nanomaterials significantly up-regulate the gene encoding thiore-doxin-interacting protein (Txnip) and determine mitochondria-dependent apoptosis [17]. On the other hand, the oral silver administration did not induce micronucleus formation in rats following 28 days of treatment [18], suggesting lack of short-term toxicity.

It was demonstrated that silver nanoparticles were able to bind to thiol (-SH) groups within proteins and promoted their denaturation [19] including antioxidant enzymes such as superoxide dismutase and glutathione peroxidase. In blood, silver binds to albumin and decreases its ability to transport [20]. Gold nanoparticles can induce genotoxicity and block the transcription due to their ability to bind to DNA [21]. Some nanomaterials can stimulate the up-regulation of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) and xanthine oxidase in macrophages and neutrophils and generate free radicals. After absorption into the systemic circulation, the nanomaterials interact either with blood components and can lead to hemolysis and thrombosis or with the immune system and produce immunotoxicity.

Cho et al. [22] have shown that PEG-coated gold nanoparticles caused a transient inflammation and induced acute liver influx of neutrophils in parallel with increase of adhesion molecule levels (ICAM-1, E-selectin, and VCAM-1), chemokines, and inflammatory cytokines (IL-1β, IL-6, IL-10, IL-12β, and TNF-α) in liver up to 24 hours. Moreover, Lanone et al. suggested that intermediate metabolites of nanomaterials generated in the liver can contribute to hepatotoxicity [6]. Abdelhalim and Jarrar have shown that gold nanoparticles (10, 20, and 50 nm) administered for 3 or 7 days induced alterations in the hepatocytes, portal triads, and sinusoids. The changes were size-dependent and related with time exposure of nanoparticles and were explained by interaction with proteins, hepatic enzymes, and local antioxidant defense system [23].

4. Assessment of nanomaterials toxicity

As current studies show conflicting results on safety and the biocompatibility of nanomaterials, it is recognized that the validation of analytical methods used to determine toxicity is of major importance. A complete evaluation of nanoparticles for therapeutic use includes thorough physicochemical property characterization, biodistribution, and toxicity evaluation, both *in vitro* and *in vivo* [24]. Transmission electron microscopy (TEM) and dynamic light scattering (DLS) are commonly used techniques to measure the size and shape of nanomaterials in biological systems and to evaluate their aggregation into cells [24, 25]. *In vitro* toxicity will follow the cellular viability, apoptosis and oxidative stress markers, and genotoxicity.

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In order to evaluate the acute *in vivo* toxicity of nanomaterials, the Organization for Economic Cooperation and Development (OECD) guidelines recommend oral toxicity test, eye irritation, corrosion and dermal toxicity, and lethal Dose 50 (LD50).

For acute oral toxicity test, the mice receive orally colloidal nanomaterials at the limited dose of 5000 mg/kg body weight (LD_{50}). The animals are observed for toxic symptoms for the first 3 hours and after 24 hours the number of survivors is noted. The animals are maintained and observed daily over 14 days for skin symptoms (edema, erythema, ulcers, bloody scabs, discoloration, and scars) and toxic signs (weight loss, water and food consumption, behavior). At 1, 7, and 10 days after exposure, skin biopsies are performed for histopathological evaluations and blood is taken for biochemical (triglyceride, cholesterol, glucose, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT)) and hematological investigations. All animals are sacrificed after 14 days and skin and liver are collected for routine histopathological examination.

For acute eye irritation and corrosion test the animals are treated with 1.50 ppm and 2.5000 ppm colloidal nanoparticles, respectively [26]. Briefly, 0.1 ml of colloidal suspension was placed in the conjunctival sac of one eye/animal and the other eye serves as a control and is treated with the same volume of distilled water. The animals are observed for toxic symptoms at 1, 12, 24, 48, and 72 hours after administration. The eye reactions of conjunctivae respectively cornea and chemosis are graded as recommended the grading system of OECD 405 guidelines. The animals are maintained and observed daily over 14 days for toxic symptoms.

For acute dermal toxicity test the animals are randomly divided in three groups (n=3) as follows: group 1 receives vehicle (distilled water) and groups 2 and 3, receive 50 and 100.000 ppm of colloidal suspension, respectively. All treated groups received the preceding chemicals at 300µl/cm^2. All animal experiments were performed according to the Organization for Economic Cooperation and Development (OECD) 434 guidelines (acute dermal toxicity-fixed dose procedure) [27]. Briefly, colloidal suspensions are applied to a shaved area of skin, an approximately 4 x 3 cm rectangle, then the area is covered with a dressing and non-irritating tape for 24 hours. After 24 hours of exposure, the dressing is removed and the treated area is gently washed with physiological saline and the number of survivors is noted. The animals are maintained and observed daily over 14 days for skin symptoms (edema, erythema, ulcers, bloody scabs, discoloration, and scars) and toxic signs (weight loss, water and food consumption, behavior). At 1, 3, and 7 days after exposure, skin biopsies are performed for histopathological investigations. All animals were sacrificed after a 14 days observation period and skin is collected for routine histopathological examination.

Maneewatttanapinyo et al. observed in an acute toxicity study of colloidal silver nanoparticles that the oral administration of particles at a limited dose of 5.000 mg/kg produced neither mortality nor acute toxic signs during 14 days. All the hematological and biochemical analysis and the histopathological investigations did not show any differences between the groups examined. The instillation of silver nanoparticles at 5.000 ppm determined a transient eye irritation for 24 hours. The skin application of tested substances did not induce toxicity at the macroscopical and microscopical level [28]. Another study showed that gold nanoparticles functionalized with natural compounds extracted from native plants of the Adoxaceae family

(European cranberrybush – *Viburnum opulus L.* and European black elderberry – *Sambucus nigra L.)* had no toxic effects at the dermal toxicity test [9]. All the parameters evaluated showed no significant changes in hematological, biochemical, and histopathological analyzes.

In addition, it is crucial to appreciate the biodistribution of nanomaterials. For this purpose, the materials are conjugated with a label or organic dye that can be tracked in blood and tissue at different time points. The methods have the disadvantage that the dye can be degraded in time and limits its detection in long-term toxicity studies. In addition, it may interfere with the metabolism of nanomaterials and transformation in intermediate compounds.

Moreover, the analysis of biomarkers of inflammation and oxidative stress would enhance mechanistic studies into nanomaterial toxicity. For the evaluation of chronic toxicity and carcinogenic potential of nanomaterials, the OECD guidelines recommend the administration of substances for 12 and 24 months, respectively. After this period, the survival rate, the clinical toxicity signs, animal behavior, tumor incidences, and histopathological findings in the liver, spleen, kidneys, brain, ovary, and testis will be assessed.

5. *In vivo* toxicity of nanomaterials

5.1. Dendrimers

Dendrimers or cascade polymers represent a heterogenic class of compounds consisting of linear or random-coil polymers such as polyethylene glycol, polylactic acid, polyglycolic acid, dextrin, hyaluronic acid, chitosans, polyglutamic acid, etc. [29]. They have different chain length and molecular weight, different chemical structures, and consequently different *in vivo* behavior (biodistribution, pharmacokinetics, stability, and toxicity). Dendrimers offer many advantages including spherical size, low viscosity, narrow polydispersity, and high density, qualities for which they were used as good vehicles for drug or gene delivery. However, their toxicity has not been systematically investigated.

In systemic circulation, positively charged dendrimers and cationic macromolecules can interact with blood components leading to hemolysis [30], which may induce nephrotoxicity and hepatotoxicity [31]. In fact, dendrimer toxicity is dose and generation dependent and is related to the surface charge, the cationic dendrimers being more toxic than anionic compounds. The toxicity of dendrimers is influenced by the nature of the terminal groups, and the addition of inert polyethylene glycol (PEG) or fatty acids can reduce their toxicity and improve biocompatibility [15].

5.2. Silver nanoparticles

Nanoparticles are defined as particles sized between 1 and 100 nanometers (10^{-7}m) [32]. Silver has been widely used in biomedical applications such as preservatives in cosmetics, textiles, water purification systems, coatings in catheters and wound dressings. In the Woodrow Wilson database of nanotechnology-based products, the most common nanomaterial described is silver [33], thus suggesting an increased risk of its exposure [34].

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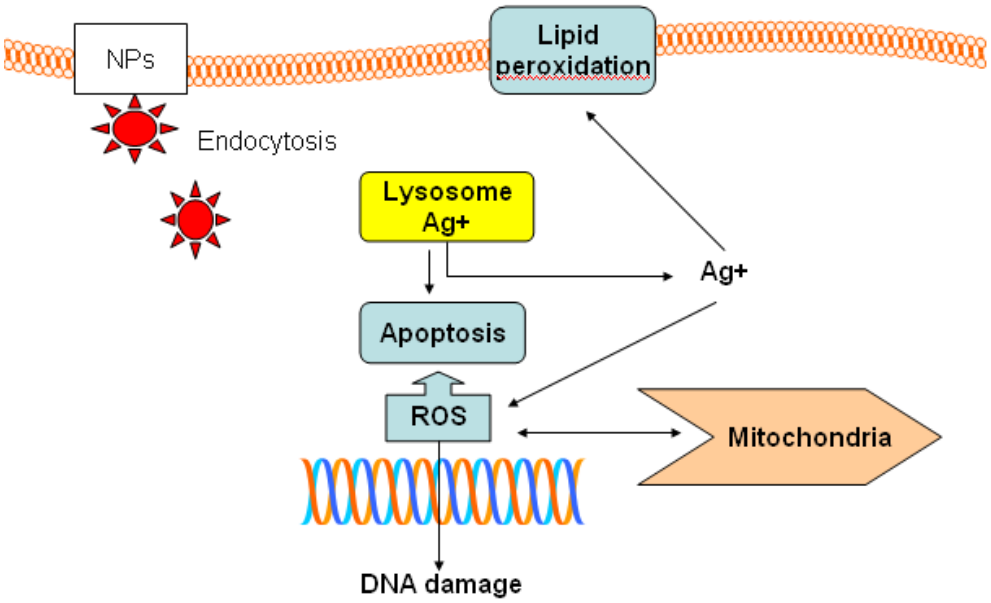
The properties of silver nanoparticles such as size, shape, surface charge and coating, agglomeration, and dissolution rate are particularly important for their biological interactions. Smaller particles have a larger surface area and, therefore, have a greater toxic potential [34]. The rate of particle dissolution depends on its chemical and surface properties as well as on its size and is further affected by the surrounding media [35]. This process leads to surface oxidation and releasing of cytotoxic silver ions.

Experimental data assigned silver nanostructures remarkably antimicrobial [36, 37], antiviral properties [17] and also antitumor effect [38]. The antimicrobian activity led over the years to the development of silver nanoparticles-based products, including textiles, food storage containers, antiseptic sprays, catheters, and bandages. Therefore, it is necessary to evaluate the adverse reactions associated with multiple ways of exposure to silver nanostructures. Recent *in vivo* studies have shown a reproductive toxicity of silver structures on *Caenorhabditis elegans* and thus draw attention to the toxicity of these compounds [17, 39]. In mice treated orally with different sizes of silver nanoparticles 1 mg/kg body weight for 14 days, small particles in brain, lung, liver, kidneys, and testis were found while large particles were not identified in these tissues [40]. In parallel, the transforming growth factor beta (TGF-β) levels were significantly increased in serum in the same groups. In serum of mice treated repeatedly for 28 days, with different doses of silver nanoparticles, increased levels of IL-1, IL-6, IL-4, IL-10, IL-12, and TGF-β in a dose-dependent manner were found, suggesting that frequent administration may cause organ toxicity and inflammation in mice [40].

Several studies have revealed that silver nanoparticles might induce cytotoxicity in phagocytic cells, macrophages, and monocytic cells, a process mediated by the generation of free radicals and induction of apoptosis. These effects are mediated by the release of silver ions, the liver being the major target organ, followed by the spleen, lungs, and kidneys [17]. In addition, silver nanoparticles can alter the mitochondrial membrane and reduce mitochondrial potential, determine glutathione (GSH) depletion, bind to DNA, and thus cause DNA strand breaks and consequently affect DNA replication [41].

Also, silver nanoparticles induced cytotoxicity and apoptosis on HeLa cells [42], determined cytotoxicty on Raw 264.7 cells [43], HepG2 (human hepatoma cells) [44], and human lung cell line [45]. Cytotoxicity and harmful effects on fish cell lines, embryos, and adult fishes were also induced [46]. On microorganisms as *S. typhimurium* and *E. coli* and cultured mammalian cells (CHO-k1) silver nanoparticles exerted cytotoxic effect without to induce genotoxicity [47].

The effects of silver materials on inflammation have not been fully understood and are rather contradictory. Some studies report an anti-inflammatory effect by suppressing cytokine production while others notice the induction of pro-inflammatory cytokines in different biological systems [48]. Several studies attested that exposure to silver induced the release of proinflammatory markers such as TNF-α, macrophage inflammatory proteins (MIPs) and G-CSF from macrophages [48, 49]. The persistence of cytokine production and signaling as response to nanoparticles may lead to inflammatory diseases. Park et al. [40] suggested that silver nanomaterials upon phagocytosis and internalization of silver in lysosomes released silver cytotoxic ions.



Abbreviations: NPs - nanoparticles; ROS – reactive oxygen species; Ag^+ - silver ions

Figure 1. Mechanism of silver nanoparticles toxicity

The increased generation and utilization of silver nanoparticles in clinical practice facilitates the human exposure via inhalation and consequently lung toxicity. Takenaka et al. [50] have studied the biodistribution of different concentrations of inhaled silver particles. The particles were identified in the alveolar macrophages and alveolar walls, in the nasal cavity and regional lymph nodes even 7 days after their administration. Low concentrations of silver ions can be measured in the liver, kidneys, spleen, brain, and heart, suggesting translocation from the lung to other tissues. The authors concluded that nanoparticles were rapidly cleared from the lungs as a consequence of their solubilization, especially less agglomerated forms, those with small size.

Hyun et al. [41] have shown that chronic, repeated exposure to small size silver particles increased the mucins production in nasal respiratory mucosa suggesting the importance of mucus in defense against air pollutants. The respiratory exposure for 90 days to silver particles [52] induced an inflammatory response in alveoli and alteration in lung function, independently of the dose used. Sung et al. suggested that nanoparticles were transferred into the blood from the lung and then to the liver, olfactory bulb, brain, and kidneys. However, the data are contradictory because similar studies have not identified toxicities in tissues after exposure to silver nanostructures. Moreover, they could not specify if the silver was identified in tissues as ions or particles. Another study showed that silver nanomaterials had negligible impact on the nasal cavity and lungs [53]. The different results obtained can be explained by the variability of the synthesis methods, their various sizes, concentrations, and time exposures, the

presence or absence of capping agents, and type of experimental design used. Hence, their risks should be assessed on a case-by-case basis and require more extensive investigations.

Extensive use of silver nanoparticles in textiles and wound dressings allows the particle to come into direct contact with the skin and can alter the structure and function of the skin. Most research on dermal exposure to silver were focused on the study of clinical effects and less on systemic toxicity. Therefore, the real consequences of silver nanostructure penetration in skin need to be assessed. Tian et al. [54] have demonstrated, within an experimental model *in vivo,* a better wound-healing ability of silver nanoparticles compared to silver sulfadiazine, standard burn treatment, and compared the combination between amoxicillin and metronidazole. The effects were superior, both from the cosmetic and functionality point of view. In addition, the results suggested that silver nanoparticles exhibited more potent antibacterial activity than other treatments.

According to some studies, silver nanomaterials can induce apoptosis and reduce the level of matrix metalloproteinase in wounds [55], inhibit angiogenesis [56] and vascular permeability through vascular endothelial growth factor (VEGF) and also interleukin (IL)-1β [38].

In patients with burns, treated with Acticoat wound dressings impregnated with silver nanoparticles, biochemical and hematological changes related to silver absorption were not found and so the authors considered these dressings safe to use in clinical practice [57]. Probably, the permeability of silver nanoparticles within normal skin may be influenced by the quality of the skin barrier. Thus, the nanosilver particles get faster deep into the dermis through hair follicles and sweat ducts if the skin barrier is altered [58].

Rahman et al. [59] studied the effect of nanoparticles on gene expression in mouse brain after intraperitoneal injection. They noticed that high doses of silver nanoparticles exerted neurotoxicity in the caudate nucleus, frontal cortex, and hippocampus by inducing oxidative stress and apoptosis. The mechanism involved was related to silver ions released into the brain or indirectly by neural and humoral mediators.

Silver nanomaterials administered orally are converted into their ionic form due to the low pH of the stomach and induce inflammation evidenced by lymphocyte influx and changes in expression of proinflammatory genes. It is not known whether toxicity is due to the accumulation of particles or it is mediated by humoral or neural mediators [60]. After oral ingestion silver was identified in glial cells and neurons, in brain regions such as the hippocampus and pons. The localization in some regions can be explained by local differences in the permeability of the blood-brain barrier and special turnover in brain cells [61].

In conclusion, the successful translation of silver nanotechnology to the clinic requires the development of safe and eco-friendly synthesis of nanomaterials and understanding the mechanisms of their toxicity and safety control.

5.3. Gold nanoparticles

Gold has long been considered a noble metal, bio-inert, stable with some therapeutic values, being used as anti-inflammatory and anti-rheumatic agent in rheumatoid arthritis treatment.

These qualities were observed particularly in the bulk gold. When the size decreases to nanoscale, the material exhibits different properties due to its surface plasmon resonance excitation characteristics [15, 62, 63]. Besides, the gold nanomaterials can become toxic due to the oxidation of elemental gold or solubilization by cyanidation.

There is a great variety of sizes and shapes of synthesized gold nanoparticles: rods, spheres, tubes, wires, ribbons, plate, cubic, hexagonal, triangular, tetrapods, etc. [64, 65]. Generally, gold nanoparticles are synthesized by the chemical reduction of chloroauric acid (HAuCl4) using various reducing agents [66, 67]: citrate ions, hydroquinone, sodium boron tetrahydride (NaBH4), hydroxyl and sugar pyrolysis radicals, microbial enzymes, plant phytochemicals, or microorganisms such as bacteria and yeast cells [68, 69]. It seems that, on one hand, the biological synthesis of gold nanomaterials is more environmentally friendly than chemical synthesis [70, 71]. On the other hand, the green synthesis of nanomaterials has many disadvantages compared to chemical synthesis: poor mono-dispersity, aggregation, and non-uniform shape. These limits can be overcome through diversification and optimization of the synthesis methods. Thus, there are a lot of natural reducing agents and organic ligands such as peptides, proteins, fungus [72] or polymers (PEG) [73], poly-L- lysine (PLL), poly- D- L-lactic-co- glycolic acid (PLGA), and their co- polymers [74] used to prepare nanomaterials in order to stabilize them.

PEG has gained popularity due to amphiphilic and solubility properties [75]. Polysaccharides, such as glycosaminoglycans (GAGs) began to be incorporated onto nanomaterials. Thus, some researchers synthesized hybrid nanospheres using low molecular weight chitosan as reducing and stabilizing agents [76], chitosan and heparin [77], or hyaluronan [78].

However, the results from literature about gold toxicity are quite contradictory and insufficiently documented. Investigation of toxicity mechanisms is important because in the last years gold nanoparticles were extensively used in various medical applications including drug and protein delivery, gene therapy, *in vivo* delivery and targeting, etc.

Generally, the exposure to gold nanomaterials can occur during synthesis and applications by injection or ingestion [79, 80], inhalation, absorption through skin contact, and release from implants [81]. Another source of exposure is their accumulation in the soil or in organisms such as algae and fish consumed by animals and humans [17].

A very much discussed issue is the relationship between the size of nanoparticles and their toxicity. Small size particles are widely used in medical applications because they have the ability to penetrate into cells and transport various drugs without cell injury. These findings were confirmed by Conner et al.'s studies [82] which found that gold nanoparticles of 18 mm in diameter did not induce damage after penetration into the cell. Moreover, Tsoli et al. [83] demonstrated that very small particles (1 mm diameter) could penetrate into nucleus the without inducing DNA injury. Particles with a large ratio between surface area and volume are not able to be internalized within cells and therefore they cannot be used in biomedical applications. These statements are consistent with the results obtained *in vitro* on four cellular lines (fibroblasts, epithelial cells (HeLa), macrophages (J774A1), and melanoma cells (SK-Mel-28) [21] treated with triphenylphosphine stabilized gold nanoparticles. The cellular

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response is size-dependent, suggesting that the "larger" particles are non-toxic *in vitro*. Goodman et al. demonstrated the ability of gold nanoparticles to inhibit RNA polymerase activity and block DNA transcription [84].

The influence of gold nanomaterials toxicity is related to their shape. Thus, the rod shape has been reported to be more toxic than its spherical counterparts, both on human keratinocyte cells (HaCaT) [11] as well as human breast adenocarcinoma cell line (MCF- 7). The cells treated with nanorods lose the mitochondrial integrity [85], generate free radicals, and stimulate up-regulation of antioxidants, stress response genes, and protein expression [86].

Several reports have noticed that gold nanoparticles caused nephrotoxicity, hemolysis, cytotoxicity and genotoxicity, and size-dependent effects, the smaller size being less toxic than the larger size. Moreover, it has been shown that small particles (10 - 50 nm) dispersed in almost all tissues, mainly in the liver, lungs, spleen, and kidneys, 24 hours post i.v. administration compared to large particles. It has been shown that smaller gold nanoparticles can selectively and irreversibly bind to DNA and cause genotoxicity compared to larger particles [15]. This property can be exploited in cancer therapy because their interaction with DNA tumor cells may lead to cellular death. Thus, Pan et al. have demonstrated that nanoclusters of 0.8, 1.2, and 1.8 nm were four-to six-fold less toxic than nanoclusters of 1.4 nm while larger sizes were not toxic even at high concentrations [21]. Biodistribution of small particles was noticed in almost all tissues 24 hours post i.v. injection compared to larger nanomaterials.

It is possible that gold nanostructures are genotoxic without being cytotoxic and cause alteration of transcription without phenotypic changes [86, 87]. Some reports have revealed that gold nanoparticles with a diameter between 2 and 40 nm are non-toxic on fetal lung fibroblast-like (MRC)-cells while the particles >10 ppm can induce apoptosis and up-regulate the expression of proinflammatory cytokines [88]. Another toxicity mechanism involves oxidative attack on DNA and down-regulation of DNA repair [87]. Inductively coupled plasma mass spectrometry (ICP-MS) studies have shown that lungs, olfactory bulb, spleen, esophagus, tongue, kidney, aorta, heart, septum, and blood are the main tissues that accumulate the gold materials between 30 and 110 nm [89]. The mechanisms of biodistribution are via endocytotic-exocytotic activity and paracellular transport [90]. According to some authors a single nanoparticle has a greater health effect than the agglomerates particles [91]. However this statement was not confirmed in other experiments. The small size facilitates the crossing of the blood-brain barrier and accumulation of gold nanostructures in the brain, placenta, and fetus [92].

Overall, the cellular uptake and toxicity of gold nanoparticles depends not only on the size but also on the surface, functional attachments, and shape [85]. Cationic gold particles were moderately toxic and anionic particles were nontoxic, suggesting the electrostatic binding of the particles to the negatively charged cell membrane and interaction with membrane components as a probable mechanism of toxicity. Kim et al. examined the toxicity of gold nanoparticles (1.3 nm) functionalized with a monolayer of the cationic ligand, N,N,N-trimethylammoniumethanethiol on zebrafish embryos and noticed the occurrence of morphological abnormalities and embryo lethality [93]. The cause of these abnormalities was apoptosis of eye cells and aberrant expression of transcript factors that regulate eye pigmen-

tation development. Other studies showed that 1.4 nm gold nanoparticles capped with triphenylphosphine monosulfonate cause necrosis via the oxidative stress and mitochondrial damage [21], while PEG used to functionalize the 3.7 nm nanoparticles were non toxic on human cervical cancer (HeLa) [92]. The chitosan conjugated gold nanoparticles can transverse the cell membrane of Chinese hamster ovary cells by endocytosis without inducing cell death. Moreover, the authors revealed that >85% of the cells were viable after a long period of exposure [93] suggesting that these nanostructures can be used in cellular imaging and photothermal therapy.

To prevent this problem, researchers have conjugated these nanoparticles with various biomolecules and ligands. Thus, gold nanorods conjugated with PEG or citric acid ligands were less toxic than spherical particles. Surface charges measured by zeta potential determine the properties and functions of nanoparticles. Thus, the gold nanoparticles can promote protein refolding through electrostatic interactions, a mechanism used to refold proteins in a chemical denatured state [94]. In contrast to the silver nanoparticles, silver - gold nanoparticles induced the activation of cells due to the release of ions in the medium [95]. However, more studies are critically needed to establish exactly the potential toxicity of gold nanoparticles to fully evaluate gold nanoparticles for *in vivo* use.

5.4. Carbon nanotubes

5.4.1. The importance of studying carbon nanotube toxicity

An important class of nanomaterials is represented by carbon nanotubes (CNTs). They are cylindrical structures composed of a variable number of layers, each layer being formed of carbon atoms. Their diameter and length are variable, but, according to the number of layers, CNTs can be classified as single-walled (SWCNTs) and multi-walled (MWCNTs). SWCNTs have unique properties, including electrical, mechanical, and thermal particularities, which recommend them for particular applications in the field of electronics, as well as in the biomedical industries. In the meantime, MWCNTs, with a larger diameter, have a great potential to be used as targeted carriers for different substances into cells in medical applications. However, the concern regarding the safety of CNTs for the human body is constantly rising, because of at least two reasons. First of all, there is an increase in the production of CNTs for technological applications, which raises the risk of accidental exposure of the workers during the technological manipulation of the nanomaterial. CNT-based products are already being used in textiles, sport products, microelectronics, and energy storage [98]. On the other hand, for the medical applications, the administration of CNTs is required, and their faith in the human body, along with their interactions with different tissues, become of vital importance, for example, CNTs conjugated to siRNA for cancer therapy or CNTs for the imaging of colorectal cancer that may enter clinical trials in the next years [98].

Also, the environmental risk of CNTs, including even their interactions with some classical contaminants, such as pesticides, must be taken into consideration. There are studies indicating that CNTs could act as pesticide carriers, enhancing pesticide ecotoxicity, and affecting fish behavior, metabolism, and survival [99].

In the meantime, CNTs released into the aquatic medium, in high concentration, exert developmental toxicity, with increased mortality and malformation. The exposure to functionalized SWCNTs in the early stages of the life of rare minnow (*Gobiocypris rarus*) altered the activities of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase and determined reactive oxygen species (ROS) generation and DNA damage [100].

5.4.2. Mechanisms of CNTs' toxicity

Until today, the environmental effects and the toxicity of nanomaterials to organisms are not entirely understood. In order to evaluate these effects, the methods should be cost-effective, rapid toxicity assays should be available, and the tests should be performed with minimal amounts of materials. Drosophila could be such an efficient and cost-effective model organism, which allows tissue-specific nanomaterial assessment through direct microtransfer into target tissues. SWCNTs and MWCNTs were evaluated for their toxicity using this new method of assessment [101].

To date, the studies performed *in vitro* and *in vivo* in order to evaluate the toxicological effects of CNTs have pointed out oxidative stress and inflammation as major mechanisms involved in their toxicity. These two mechanisms influence each other [102]. ROS generation produces lipid peroxidation, protein alteration, and intracellular GSH depletion [103]. Oxidized proteins gain chemically reactive groups and become new agents that can prolong the initial effects of ROS [104]. The next step is NF-kB activation, which in return, will regulate the expression of pro-inflammatory cytokines, such as TNF-α and IL-1β [103]. Also, NF-kB up-regulates the activation of superoxide dismutase [105].

MWCNTs can activate NADPH oxidase, as demonstrated in human macrophages [106] and in the spleen [107]. In the meantime, it was proven that carboxylated MWCNTs can bind to Cu/Zn SOD and the microenvironment of the amino acid residues in the enzyme was slightly changed [108]. MWCNTs inhibit the activity of catalase (CAT). They contribute both to the conformational changes of CAT and to enzyme dysfunction. Also, both in animals and cell cultures, a single-dose exposure to CNTs determines elevated levels of DNA damage [109].

In the past years, a few steps were made in order to understand the deep mechanisms involved in CNT toxicity. For example, for the biological functions, the protein-protein interactions or the protein-protein recognition are essential. It was proven that a graphene sheet can interrupt the hydrophobic interaction between two functional proteins and can disrupt the metabolism of the cell, leading to cell death [110].

For a typical type of CNT, the carbon nanohorns (CNHs), it was proven that they can accumulate in the lysosomes and can induce lysosomal membrane permeabilization. The consequence was the release of lysosomal enzymes, such as cathepsins, with the subsequent mitochondrial dysfunction and generation of ROS in the mitochondria. In return, ROS amplified the mitochondrial dysfunction and led to the activation of caspases followed by cell apoptosis [111].

Unmodified SWCNT aggregates are capable of suppressing the antigen-induced signaling pathways and pro-inflammatory degranulation responses in mast cells. The capacity of SWCNTs to suppress mast cells pro-inflammatory function is explained by remodeling the plasma membrane, with the disaggregation of the cortical actin cytoskeleton and the relocalization of clathrin [112].

In the meantime, embedded SWCNTs nanoparticles in plasma membrane induce cellular calcium outflow alteration [113].

The toxicity of MWCNTs has been evaluated lately at gene level. MWCNTs have microRNA (miRNA) targets, as shown in an *in vivo* Caenorhabditis elegans assay system [114].

CNTs can also determine the perturbation of immune functions in animal models. Different studies had contradictory results, showing that CNTs have, by themselves, either pro-inflammatory or immunosuppressive functions, locally and systematically. When exposed to CNTs, the immortalized airway epithelial cells showed reduced viability, impaired proliferation, and elevated ROS generation and lack of internalization of targets in macrophage cell lines [115].

CNTs can produce anomalies in multicellular behavior, due to ROS generation. In fact this effect is produced by the loss of multicellular chirality, including a decreased migration rate and loss of directional alignment on micropatterned surfaces. The cell chirality is in fact a cellular property important for embriogenic morphogenesis [116].

5.4.3. The impact of different parameters upon CNT cytotoxicity

CNT toxicity is influenced by a variety of parameters, depending both on the physicochemical properties of the material itself and on the environmental variables. The shape, length, diameter, number of layers, surface coating and functionalization, impurities content, the dose, route of administration, and type of dispersion media are just a few of these variables.

CNTs diameter and length influence cell aggregation, ROS generation, and injury extent, the effects depending on the cell type too [117]. For example, long and thick rather than short and thin MWCNTs can induce inflammatory effects [118] as well as those with a larger diameter [119]. When comparing the effects of long and rigid versus shorter and agglomerated MWCNTs at pulmonary level, the long MWCNTs induced a more pronounced pro-fibrotic, inflammatory, and apoptotic response [120].

When comparing the type of functionalization, MWCNTs functionalized with carboxylation and raw MWCNTs can induce oxidative stress and inflammation in macrophages, with the activation of MAPK and NF-kB signaling pathways, up-regulation of IL-1β, IL-6, TNF-α, and iNOs and subsequent cell death. MWCNTs functionalized with polyethylene glycol did not exert the same toxic effects [121].

When testing pristine MWCNTs and MWCNTs functionalized by hydroxylation-oxygenation, amination or carboxylation on human bronchial epithelial cells or on the nematode Caenorhabditis elegans, the results showed that carboxylated MWCNTs were the most cytotoxic and

genotoxic, but with no differences in survival following exposure, proving that surface functionalization can influence the bioactivity of MWCNTs [122].

The exposure of human monocyte-derived macrophages to long rigid MWCNTs elicited a response similar to the exposure to asbestos, in contrast to long-tangled MWCNTs, which induced a weaker protein secretion. The exposure determined lysosomal damage, but only long rigid MWCNTs determined apoptosis [123].

There are results sustaining that the contaminating metals for CNTs are responsible for their toxicity. However, long needle-like MWCNTs without metal impurities are capable of inducing the production of major proinflammatory cytokine IL-1β via the Nod-like receptor pyrin domain containing 3 (NLRP3) flammasome-mediated mechanisms [124].

Also, the dispersion medium can influence the ability of CNTs to bind to proteins and to exert specific biologic effects. For example, SWCNTs and double-walled nanotubes were dispersed in phosphate-buffered saline (PBS) or in bovine serum albumin (BSA). In BSA, CNTs were more stable, the aggregation was reduced, and the levels of IL-6 were increased, while the TNF-α production was decreased, as compared to the same samples, but without BSA [125].

In the meantime, the biotransformation of CNTs in organism is important when evaluating their toxicity. It seems that manganese peroxidase is capable of transforming the SWCNTs, but not oxidized SWCNTs, making the biodegradation of these compounds difficult [126].

5.4.4. Animal models for toxicity evaluation

CNTs from the environment can be a challenge for the respiratory and gastrointestinal mucosa as well as for the dermal system. For medical applications, the systemic administration of CNTs, s.c., i.p., or i.v., is important. Taking these possibilities into consideration, different models for the assessment of CNT toxicity *in vivo* were used. In the meantime, a short-term or a long-term exposure to CNTs can be evaluated.

5.4.4.1. Short-term evaluation

Pharyngeal aspiration of purified SWCNTs in mice determined, in a dose-dependent manner, inflammation, fibrosis, granulomatous pneumonia, and decrease in pulmonary function. The inhalation of nonpurified SWCNTs for 4 days also determined inflammatory response, oxidative stress, collagen deposition, and mutations of k-ras gene locus in the lung of mice [127].

Twenty-four hours after the intratracheal administration of pristine SWCNTs, acute inflammation was present in the lungs of mice, along with apoptosis through mitochondrial dysfunction and ROS generation [128]. When comparing the effects of MWCNTs on male Sprague-Dawley rats after intratracheal instillation or inhalation administered in different doses, for different periods of time and with different physicochemical characteristics, including functionalization, the results showed that MWCNTs produce inflammation in the lung, but transitory, despite the persistence of CNTs in the lung. The functionalization and the suspension media influenced the pulmonary response after MWCNTs administration [129].

When female mice were exposed to MWCNTs by pharyngeal aspiration technique 4 weeks after the previous exposure to cigarette smoke, it was proven that MWCNTs induced pulmonary toxicity, with a minor role in cigarette smoke exposure [130].

For a follow-up of 6 days after a single dose i.p. administration, transient oxidative stress and inflammation in blood and liver were obtained both for SWCNTs and MWCNTs functionalized with single-stranded DNA [102, 131]. Also, after i.p. administration, MWCNTs translocate in the spleen and determine oxidative stress, inflammation, lymphoid hyperplasia, and increase in the number of cells that undergo apoptosis [132].

5.4.4.2. Long-term evaluation

MWCNTs with different length and iron content were administered by intratracheal instillation to spontaneously hypertensive rats. Seven days and 30 days post exposure, lung inflammation was found, along with increased blood pressure, lesions in abdominal arteries,and accumulation of CNTs in liver, kidneys, and spleen [133].

SWCNTs administered through single intratracheal instillation in rats determined up-regulation of the genes involved in the inflammatory response, with a pattern that is time-dependent, until 180 days, 365 days, and 754 days post-exposure [134].

MWCNTs administered through transtracheal intrapulmonary spraying for 24 weeks translocated into the pleural cavity and induced inflammatory reactions, fibrosis and parietal mesothelial proliferation lesions, the effects being more important for the larger-sized MWCNTs, than for the smaller-sized [135].

Thirteen weeks exposure of rats to MWCNTs by whole-body inhalation, in different doses, determined increased expression of inflammatory cytokines in splenic macrophages, including IL-1β, IL-6, and IL-10. The expression of IL-2 in T lymphocytes was decreased, being a possible cause of reduction in the rats' anti-tumor responses [136]. In the meantime, in a similar experimental model, 0.2 mg MWCNTs/m^3 was established as the lowest dose for the occurrence of lung adverse effects, including inflammation and granulomatous changes [137]. Inhalation exposure of rats to MWCNTs for 28 days determined lung deposits even 90 days post-exposure, especially for short-length MWCNTs, emphasizing their potential to induce genotoxicity [138].

Ma Hock et al. (2009) proved that long-term (13 weeks) exposure of rats to SWCNTs or MWCNTs by inhalation did not produce systemic organ toxicity, for a follow-up period of 90 days post-exposure, even though local pulmonary effects were present. Fibrosis changes were absent [139].

5.4.5. Means to reduce toxicity

A few ways to reduce CNT toxicity are represented by the functionalization, surface coating, and stimulation of the autophagic flux. For example, the amino functionalization can reduce the CNT toxicity to the cells [140], as well as albumin coating for SWCNTs [141]. Also, the

toxicity of carboxylated MWCNTs can be reduced by the stimulation of the autophagic flux, with the extracellular release of the nanomaterial in autophagic microvesicles [142].

6. Perspectives

The extent of use of nanomaterials is currently growing and thus efficient screening methods for toxicity are needed to lower the expenses of testing and to reduce the cost of using. Nanomaterial size, shape, surface chemistry, and degree of aggregation are key factors that influence the toxicity. Generally, the *in vivo* toxicity studies can provide sufficient data in order to understand the absorption, distribution, metabolism, and excretion of nanomaterials. In order to fully evaluate their toxicity, acute and long-term toxicity studies are needed along with the examination of chronic exposure. *In vitro* studies may contribute in addition to explain the mechanisms involved in their toxic effects. However, not all the aspects involved in the use of nanomaterials are fully understood. Therefore, finding the appropriate methods for analysis and carefully designed experiments will contribute to the better understanding of the mechanisms of toxicity, so that nanomaterials could be safely used in biology and medicine.

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References

[1] De Jong WH, Borm PJA: Drug delivery and nanoparticles: applications and hazards. Int. J. Nanomedicine 2008;3:133–149.

[2] Papp T, Schiffmann D, Weiss D, Castranova V, Vallyathan V, Rahman Q: Human health implications of nanomaterial exposure. Nanotoxicology 2008;2:9–27.

[3] AshaRani PV, Mun GLK, Hande MP, Valiyaveetti S: Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano 2009; 3: 279–290.

[4] Stern ST, McNeil SE: Nanotechnology safety concerns revisited. Toxicol Sci 2008; 101:4–21.

[5] Rejman J, Oberle V, Zuhorn IS, Hoekstra D: Size-dependent internalization of particles via the pathways of clarthrin- and caveolae-mediated endocytosis. Biochem. J 2004; 377:159–169.

[6] Lanone S, Boczkowski J: Biomedical applications and potential health risks of nanomaterials: molecular mechanisms. Curr. Mol. Med 2006; 6: 651–663.

[7] Nel A, Xia T, Mädler L, Li N: Toxic potential of materials at the nanolevel. Science 2006; 311:622–627.

[8] David L, Moldovan B, Vulcu A, Olenic L, Perde-Schrepler M, Fischer-Fodor E, Florea A, Crisan M, Chiorean I, Clichici S, Filip A: Green synthesis, characterization and anti-inflammatory activity ofsilver nanoparticles using European black elderberry fruits extract. Colloids Surf B Biointerfaces 2014; 122: 767-77.

[9] Crisan M, David L, Moldovan B, Vulcu A, Dreve S, Perde-Schrepler M, Tatomir C, Filip A, Bolfa P, Achim M, Ciorean I, Kacso I, Berghian Grosan C, Olenic L: New nanomaterials for the improvement of psoriatic lesions. J. Mater. Chem, B 2013; 1: 3152-3158.

[10] Champion JA, Mitragotri S: Shape induced inhibition of phagocytosis of polymer particles. Pharm Res 2009; 26:244–249.

[11] Chithrani BD, Ghazani AA, Chan WCW: Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. Nano Lett 2006; 6:662–668.

[12] Wang S, Lu W, Tovmachenko O, Rai US, Yu H, Ray PC: Challenge in understanding size and shape dependent toxicity of gold nanomaterials in human skin keratinocytes. Chem. Phys. Lett 2008; 463:145–149.

[13] Niidome T, Yamagata M, Okamoto Y, Akiyama Y, Takahashi H, Kawano T, Katayama Y, Niidome Y: PEG-modified gold nanorods with a stealth character in in vivo applications. J. Control. Release 2006; 114:343–347.

[14] Chang YN, Zhang M, Xia L, Zhang J, Xing G: The toxic effects and mechanisms of CuO and ZnO nanoparticles. Materials 2012; 5: 2850–2871.

[15] Aillon KL, Xie Y, El-Gendy N, Berkland CJ, Forrest ML: Effects of nanomaterial physicochemical properties on *in vivo* toxicity. Adv Drug Deliv Rev 2009; 61(6): 457–466. doi:10.1016/j.addr.2009.03.010

[16] Singh RP and Ramarao P: Cellular uptake, intracellular trafficking and cytotoxicity of silver nanoparticles. Toxicol. Lett. 2012; 213: 249–259.

[17] Wei L, Lu J, Xu H, Patel A, Chen ZS, Chen G: Silver nanoparticles: synthesis, properties, and therapeutic applications Drug Discovery Today 2015; http://dx.doi.org/10.1016/.drudis.2014.11.014

[18] Kim YS, Kim JS, Cho HS, Rga DS, Kim JM, Park JD, Choi BS, Lim R, Chang HK, Chung YH, Kwon H, Jeong J, Han BS, Yu J: Twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague–Dawley rats. Inhalation Toxicol 2008; 20: 75–583.

[19] Chen X and Schluesener HJ: Nano-silver: A nanoproduct in medical application. Toxicol Lett 2008; 176: 1-12.

[20] Wadhera A and Fung M: Systemic argyria associated with ingestion of colloidal silver. Dermatol Online J 2005; 11: 12. Available at http://dermatology. cdlib.org.

[21] 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 2007; 3: 1941.

[22] Cho WS, Cho M, Jeong J, Choi M, Cho HY, Kim BSH, Kim HO, Lim YT, Jeong BHC: Acute toxicity and pharmacokinetics of 13 nm-sized PEG-coated gold nanoparticles, *Toxicol and* Appl Pharmacol 2009; 236: 16–24.

[23] Abdelhalim MAK and Jarrar BM: Gold nanoparticles administration induced prominent inflammatory, central vein intima disruption, fatty change and Kupffer cells hyperplasia. Lipids in Health and Disease 2011; 10, 133. doi:10.1186/1476-511X-10-133.

[24] Hall JB, Dobrovolskaia MA, Patri AK, McNeil SE: Characterization of nanoparticles for therapeutics. Nanomedicine 2007; 2:789–803.

[25] Powers KW, Palazuelos M, Moudgil BM, Roberts SM: Characterization of the size, shape, and state of dispersion of nanoparticles for toxicological studies. Nanotoxicology 2007; 1:42–51.

[26] OECD 2002. Test guideline 405. Acute eye irritation and corrosion. In: OECD guidelines for the testing of chemicals Paris. France: Organization for Economic Cooperation and Development. Organization for Economic Cooperation and Development (OECD).

[27] OECD 2004. Test guideline 434. Acute dermal toxicity-fixed dose procedure. *In:* OECD guidelines for the testing of chemicals, Paris, France: Organization for Economic Cooperation and Development (OECD).

[28] Maneewatttanapinyo P, Banlunara W, Thammacharoen C, Ekgasit S, Kaewamatawong T: An evaluation of acute toxicity of colloidal silver nanoparticles. J. Vet. Med. Sci 2011; 73(11): 1417-1423.

[29] Duncan R and Izzo L: Dendrimer biocompatibility and toxicity. Adv. Drug Deliv. Rev 2005; 57:2215–2237.

[30] Malik N, Wiwattanapatapee R, Klopsch R, Lorenz K, Frey H, Weener JW, Meijer EW, Paulus W, Duncan R: Dendrimers: Relationship between structure and biocompatibility in vitro, and preliminary studies on the biodistribution of I-125-labelled polyamidoamine dendrimers in vivo. J. Control. Release 2000; 65:133–148.

[31] Neerman MF, Zhang W, Parrish AR, Simanek EE: In vitro and in vivo evaluation of a melamine dendrimer as a vehicle for drug delivery. Int. J. Pharm 2004; 281:129–132.

[32] Granqvist CG, Buhrman RA, Wyns J, Sievers AJ: Far-infrared absorption in ultrafine Al particles. Phys Rev Lett 1976, 37(10):625–629.

[33] http://www.nanotechproject.org/ inventories/consumer/analysis and draft/

[34] Johnston HJ, Hutchison G, Christensen FM, Peters S, Hankin S, Stone V: A review of the in vivo and in vitro toxicity of silver and gold particulates: particle attributes and biological mechanisms responsible for the observed toxicity. Crit. Rev. Toxicol 2010; 40: 328–346.

[35] Misra SK, Dybowska A, Berhanu D, Luoma SN, Valsami-Jones E: The complexity of nanoparticle dissolution and its importance in nanotoxicological studies. Sci. Total Environ 2012; 38: 225–232.

[36] Shahverdi AR, Fakhimi A, Shahverdi HR, Minaian S: Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against Staphylococcus aureus and Escherichia coli. Nanomedicine 2009; 3: 168-171.

[37] Kim JS, Kuk E, Yu KN, Kim, JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, et al.: Antimicrobial effects of silver nanoparticles. Nanomedicine 2007; 3: 95–101.

[38] Rutberg FG, Dubina MV, Kolikov VA Moiseenko FV, Ignat'eva EV, Volkov NM, Snetov VN, Stogov Y: Effect of silver oxide nanoparticles on tumor growth in vivo. Dokl. Biochem. Biophys 2008; 421: 191-193.

[39] Roh JY, Sim SJ, Yi J, Park K, Chung KH, Ryu DY, Choi J: Ecotoxicity of silver nanoparticles on the soil nematode Caenorhabditis elegans using functional ecotoxicogenomics. Environ Sci Technol 2009; 15; 43(10):3933-40.

[40] Park EJ, Bae E, Yi J, Kim Y, Choi K, Lee SH, Yoon J, Lee BC, Park K: Repeated dose toxicity and inflammatory responses in mice by oral administration of silver nanoparticles. Environ. Toxicol. And Pharmacol 2010; 10: 162-168.

[41] Guo D, Zhu L, Huang Z, et al.: Anti-leukemia activity of PVP-coated silver nanoparticles via generation of reactive oxygen species and release of silver ions. Biomaterials 2013; 34: 7884–7894.

[42] Miura N and Shinohara Y: Cytotoxic effect and apoptosis induction by silver nanoparticles in HeLa cells. Biochem. Biophys. Res. Common 2009; 390 (3): 733-737.

[43] Park EJ, Yi J, Kim Y, Choi K, Park K: Silver nanoparticles induce cytotoxicity by a Trojan horse mechanism. Toxicol. In Vitro 2010; 24 (3): 872-878.

[44] Kawata K, Osawa M, Okabe S: In vitro toxicity of silver nanoparticles at noncytotoxic doses to HepG2 human hepatoma cells. Environ Sci Technol 2009; 43: 6046–6051.

[45] Foldbjerg R, Dang DA, Autrup H: Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549. Archives Toxicol 2010; 85:743–750.

[46] Wise JPSr, Goodale BC, Wise SS, Craig GA, Pongan AF, et al.: Silver nanospheres are cytotoxic and genotoxic to fish cells. Aquatic Toxicol 2010; 97: 34–41.

[47] Kim JS, Song KS, Sung JH, Ryu HR, Choi BG, Cho HS, Lee JK, Yu IJ: Genotoxicity, acute oral and dermal toxicity, eye, and dermal irritation and corrosion and skin sensitization evaluation of silver nanoparticles. Nanotoxicology Early online, 2010; 1-8.

[48] Park MVDZ, Neigh AM, Jolanda PV, Fonteyne DL, Verharen HW, Briede JJ, Loveren HV, Jong WH: The effect of particle size on the cytotoxicity, inflammation, developmental toxicity and genotoxicity of silver nanoparticles. Biomaterials 2011; 32: 9810-9817.

[49] Nishanth RP, Jyotsna RG, Schlager JJ, Hussain SM, Reddanna R: Inflammatory responses of RAW 264.7 macrophages upon exposure to nanoparticles: role of ROS-NF-kB signaling pathway. Nanotoxicology 2011; 5(4):502-16.

[50] Takenaka S, Karg E, Roth C, Schulz H, Ziesenis A, Heinzmann U, Schramel P, Heyder J: Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. Environ Health Perspect 2001; 109:547–551.

[51] Hyun JS, Lee BS, Ryu HY, Sung JH, Chung KH, Yu IJ: Effects of repeated silver nanoparticles exposure on the histological structure and mucins of nasal respiratory mucosa in rats. Toxicol Lett 2008; 182:24–28.

[52] Sung JH, Ji JH, Park JD, Yoon JU, Kim DS, Jeon KS, Song MY, Jeong J, Han BS, Han JH, Chung YH, Chang HK, Lee JH, Cho MH, Kelman BJ, Yu IJ: Subchronic inhalation toxicity of silver nanoparticles. Toxicol Sci 2009; 108:452–461.

[53] Lee HY, Choi YJ, Jung EJ, Yin HQ, Kwon JT, Kim JE, et al.: Genomics-based screening of differentially expressed genes in the brains of mice exposed to silver nanoparticles via inhalation. J. Nanopart. Res 2009; 12: 1567–1578.

[54] Tian J, Wong KK, Ho CM, Lok CN, Yu WY, Che CM, Chiu JF, Tam PK: Topical delivery of silver nanoparticles promotes wound healing. Chem Med Chem 2007; 2:129–136.

[55] Warriner R, Burrell B: Infection and the chronic wound: a focus on silver, Adv. Skin. Wound Care 2005; 18(1): 2-12.

[56] Kalishwaralal K, Barathmanikanth S, Pandian SRK, Deepak V, Gurunathan S: Silver nano - a trove for retinal therapies. J. Control Release 2010; 145(2): 76-90.

[57] Vlachou E, Chipp E, Shale E, Wilson YT, Papini R, Moiemen NS: The safety of nanocrystalline silver dressings on burns: A study of systemic silver absorption. Burns 2007; 33:979–985.

[58] Hoet PHM, Bruske-Hohlfeld I, Salata OV: Nanoparticles—Known and unknown health effects. J Nanobiotechnol 2004; 2:12.

[59] Rahman MF, Wang J, Patterson TA, Saini UT, Robinson BL, Newport GD, Murdock R. C, Schlager JJ, Hussain SM, Ali SF: Expression of genes related to oxidative stress in the mouse brain after exposure to silver-25 nanoparticles. Toxicol Lett 2009; 187:15–21.

[60] Cha K, Hong HW, Choi YG, Lee MJ, Park JH, Chae HK, Ryu G, Myung H: Comparison of acute responses of mice livers to short-term exposure to nano-sized or micro-sized silver particles. Biotechnol Lett 2008; 30:1893–1899.

[61] Rungby J and Danscher G: Localization of exogenous silver in brain and spinal cord of silver exposed rats. Acta Europathol 1983; 60: 92–98.

[62] Marsich E, Travan A, Donati I, Luca AD, Benincasa M, Crosera M, Paoletti: Biological response of hydrogels embedding gold nanoparticles. Colloids and SurfaceB: Biointerfaces 2011; 83: 331-339.

[63] Jenkins JT, Halaney DL, Sokolov KV, Ma LL, Shipley HJ, Mahajan S, Louden CL, Asmis R, Milner TE, Johnsons KP, Feldman MD: Excretion and toxicity of gold–iron nanoparticles. Materials Science and Engineering C 2013; 33(1): 550–556.

[64] Wu HY, Liu M, and Huang MH: Direct synthesis of branched gold nanocrystals and their transformation into spherical nanoparticles. J. Phys. Chem. B 2006; 110: 19291-19294.

[65] Ghosh P, Han G, De M, Kim CK, Rotello VM: Gold nanoparticles in delivery applications. Advanced Drug Delivery Reviews 2008; 60: 1307–1315.

[66] Krause RWM, Mamba BB, Malefetse TJ, Bambo FM, Malefetse TJ: Cyclodectrins: Chemistry and Physics. ISBN: Chapter 9 cyclodextri polymers: Synthesis and application in water treatment. Editor. JIe Hu: Transworld Research Network, Kerala. India. 2010; 185-209.

[67] Lu X, Tuan HY, Korgelc BA, and Xia Y: Facile synthesis of gold Nanoparticles with narrow size distribution by using AuCl or AuBr as the precursor. Chemistry 2008; 14(5): 1584–1591.

[68] Prathna TC, Mathew L, Chandrasekaran N, Raichur AM, Mukherjee A: Biomimetic Synthesis of Nanoparticles: Science, Technology & Applicability.School of Bio Sciences & Technology, VIT University, Department of Materials Eng., Indian Institute of Science. India.www.intechopen.com, 2013.

[69] Chauhan A, Zubair S, Tufail S, Sherwani A, Sajid M, Raman SC, Azam A, Owais M: Fungusmediated biological synthesis of gold nanoparticles: potential in detection of liver cancer. Int J Nanomedicine 2011; 6: 2305 – 2319.

[70] Srivastava SK, Yamada R, i Ogino C, Kondo A: Biogenic synthesis and characterization of gold nanoparticles by Escherichia coli K12 and its heterogeneous catalysis in degradation of 4-nitrophenol. Nanoscale Research Letters 2013; 8:70.

[71] Narayanan KB, Sakthivel N: Biological synthesis of metal nanoparticles by microbes. Adv Colloid Interface Sci.2010; 156:1–13.

[72] Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, Ramani R, Parischa R, Ajayakumar PV, Alam M, Sastry M, Kumar R: Bioreduction of AuCl4- ions by the fungus *Verticillum* species and surface trapping of gold nqanoparticles formed. Angew. Chem. Int. Ed 2001; 40:3585–3588.

[73] Pillay J, Ozoemena KI, Tshikhudo RT, Moutloali RM: Monolayer-Protected Clusters of Gold Nanoparticles: Impacts of Stabilizing Ligands on the Heterogeneous Electron Transfer Dynamics and Voltammetric Detection. Langmuir 2010; 26(11): 9061–9068.

[74] Zhang XD, Wu HY, Wu D, Wang YY, Chang JH, Zhai ZB, Meng AM, Liu PX, Zhang LA, Fan FY: Toxicologic effects of gold nanoparticles in vivo by different administration routes. Int. J. of Nanomed 2010; 5: 771–781.

[75] Pissuwan D, Niidome T, Cortie MB : The forthcoming applications of gold nanoparticles in drug and gene delivery systems. Journal of Controlled Release 2011; 149(1): 65-71.

[76] Guo, R. Zhang, L. Zhu, Z. Jiang X: Direct facile approach to the fabrication of chitosan-gold hybrid nanospheres. Langmuir 2008; 24(7): 3459-3464.

[77] Huang H and Yang X: Synthesis of polysaccharide-stabilized gold and silver nanoparticles: A green method. Carbohydr Res 2004; 339: 2627-2631.

[78] Kemp M, Kumar A, Mousa S, Park TJ, Ajayan P, Kubotera N, Mousa SA, Linhardt R.J: Synthesis of gold and silver nanoparticles stabilized with glycosaminoglycans having distinctive biological activities. Biomacromolecules 2009; 10 (3): 589-595.

[79] Lewinski N, Colvin V, Drezek R: Cytotoxicity of nanoparticles. Small 2008; 4(1): 26-49.

[80] Yah CS, Iyuke SE, Simate GS: Nanoparticles toxicity and their routes of exposures. PJPS 2012; 25(2): 477-491.

[81] Byrne HJ, Lynch I, De Jong WH, Kreyling WG, Loft S, Park MVDZ, Riediker M, Warheit D: Protocols for assessment of biological hazards of engineered nanomaterials. The European Network on the Health and Environmental Impact of Nanomaterials 2010;1-30.

[82] Connor EE, Mwamuka J, Gole A, Murphy CJ, Wyatt MD: Gold nanoparticlesv are taken up by human cells but do not cause acute cytotoxicity. Small 2005;1: 325–327.

[83] Tsoli M, Kuhn H, Brandau W, Esche H, Schmid G: Cellular Uptake and Toxicity of Au55 Clusters. Small 2005; 1(8-9): 841–844.

[84] Goodman CM, Chari NS, Han G, Hong R, Ghosh P, Rotello, VM: DNA binding by functionalized gold anoparticles: mechanism and structural requirements. Chem Biol Drug Des 2006; 67: 297–304.

[85] Qiu Y, Liu Y, Wang L, Xu L, Ba R, Ji Y, Wu X, Zhao Y, Chen C: Surface chemistry and aspect ratio mediated cellular uptake of Au nanorods. Biomaterials 2010; 31(30): 7606–7619.

[86] Li JJ, Hartono D, Ong CN, Bay BH, Yung LYL: Autophagy and oxidative stress associated with gold nanoparticles. Biomaterials 2010; 31: 5996-6003.

[87] Coradeghini R, Gioria S, García CP, Nativo P, Franchini F, Gilliland D, Ponti J, Rossi F: Size dependent toxicity and cell interaction mechanisms of gold nanoparticles on mouse fibroblasts. Toxicology Letters 2013; 217(3): 205–216.

[88] Yen HJ, Hsu SH, Tsai CL: Cytotoxicity and immunological response of gold and silver nanoparticles of different sizes. Small 2009; 5(13):1553-61.

[89] Yu LE, Yung LYL, Ong CN, Tan YL, Balasubramaniam KS Hartono D, Shu G, Wenk MR, Ong WY: Translocation and effects of gold nanoparticles after inhalation exposure in rats. Nanotoxicology 2007; 1(3), 235 – 242.

[90] Sadauskas E, Jacobsen NR, Danscher G, Stoltenberg M, Vogel U, Larsen A, Kreyling W, Wallin H: Biodistribution of gold nanoparticles in mouse lung following intratracheal instillation. Chemistry Central Journal 2009; 3:16.

[91] Gosens I, Post JA, de la Fonteyne LJJ, Jansen EHJM, Geus JW, Cassee FR, De Jong WH: Impact of agglomeration state of nano- and submicron sized gold particles on pulmonary inflammation. Particle and Fibre Toxicology 2010; 7:37.

[92] Lasagna-Reeves C, Gonzalez-Romero D, Barria MA, Olmedo I, Clos A, Ramanujam VMS, Urayama A, Vergara L, Kogan MJ, Soto C: Bioaccumulation and toxicity of gold nanoparticles after repeated administration in mice. Biochem Biophys Res Commun 2010; 393:649–655.

[93] Kim KT, Zaikova T, Hutchison JE, Tanguay RL: Gold nanoparticles disrupt zebrafish eye development and pigmentation. Toxicol Sci 2013, 133(2):275–288.

[94] Goodman CM, Mccusker CD, Yilmaz T, Rotello VM: Toxicity of gold Nanoparticles functionalized with cationic and anionic side chains. Bioconjugate Chem 2004; 15: 897-900.

[95] 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: 10644.

[96] Hirn S, Behnke MS, Schleh C, Wenk A, Lipka J, Schäffler M, Takenaka S, Möller W, Schmidn G, Simon U, Kreyling WG: Particle size-dependent and surface charge-dependent biodistribution of gold nanoparticles after intravenous administration. Eur J of Pharm Biopharm 2011; 77(3):407-16.

[97] Mahl D, Diendorf J, Ristig S, Greulich C, Li ZA, Farle M, Kuller M, Epple M: Silver, gold and alloyed silver-gold nanoparticles: characterization and comparative cell-biologic action. J. Nanopart. Res 2012; 14, 1153. DOI 10.1007/s1151-012-1153-5.

[98] Amenta V, Aschberger K.: Carbon nanotubes: potential medical applications and safety concerns, Wiley Interdiscip Rev Nanomed Nanobiotechnol, 2014; 27. doi: 10.1002/wnan.1317. [Epub ahead of print]

[99] Campos-Garcia J, Martinez DS, Alves OL, Leonardo AF, Barbieri E:. Ecotoxicological effects of carbofuran and oxidised multiwalled carbon nanotubes on the freshwater fish Nile tilapia: Nanotubes enhance pesticide ecotoxicity. Ecotoxicol Environ Saf. 2015 Jan; 111: 131-137. doi: 10.1016/j.ecoenv.2014.10.005.

[100] Zhu B, Liu GL, Ling F, Song LS, Wang GX.: Development toxicity of functionalized single-walled carbon nanotubes on rare minnow embryos and larvae. Nanotoxicology, 2014 Sep 11: 1-12 [Epub ahead of print]

[101] Vega-Alvarez S, Herrera A, Rinaldi C, Carrero-Martinez FA.: Tissue-specific direct microtransfer of nanomaterials into Drosophila embryos as a versatile in vivo test bed for nanomaterial toxicity assessment. Int J Nanomedicine. 2014; 9: 2031-2041. doi: 10.2147/IJN.S56459. eCollection 2014.

[102] Clichici S, Biris AR, Tabaran F, Filip A.: Transient oxidative stress and inflammation after intraperitoneal administration of multiwalled carbon nanotubes functionalized with single strand DNA in rats. Toxicol Appl Pharmacol. 2012. 259(3): 281-292. doi: 10.1016/j.taap.2012.01.004.

[103] Rahman I, MacNee W.: Oxidative stress and regulation of glutathione in lung inflammation. Eur. Respir. J. 2000; 16: 534–554.

[104] Berlett BS, Stadtman ER.: Protein oxidation in aging, disease and oxidative stress. J. Biol. Chem. 1997; 272 (33): 20313–20316.

[105] Tasaka S, Amaya F, Hashimoto S, Ishizaka A.: Roles of oxidants and redox signaling in the pathogenesis of acute respiratory distress syndrome. Antioxid. Redox Signal. 2008; 10(4): 739–753.

[106] Ye S, Wang Y, Jiao F, Zhang H, Lin C, Wu Y, Zhang Q.: The role of NADPH oxidase in multi-walled carbon nanotubes-induced oxidative stress and cytotoxicity in human macrophages. J. Nanosci. Nanotechnol. 2011; 11(5): 3773–3781.

[107] Mitchell LA, Gao J, Wal RV, Gigliotti A, Burchiel SW, McDonald JD.: Pulmonary and systemic immune response to inhaled multiwalled carbon nanotubes. Toxicol. Sci. 2007; 100 (1): 203–214.

[108] Guan J, Liu G, Cai K, Gao C, Liu R.: Probing the interactions between carboxylated multi-walled carbon nanotubes and copper-zinc superoxide dismutase at a molecular level. Luminiscence. 2014 Oct 29. doi: 10.1002/bio.2807. [Epub ahead of print]

[109] Møller P, Hemmingsen JG, Jensen DM, Danielsen PH, Karottki DG, Jantzen K, Roursgaard M, Cao Y, Kermanizadeh A, Klingberg H, Christophersen DV, Hersoug LG, Loft S.: Applications of the comet assay in particle toxicology: air pollution and engineered nanomaterials exposure. Mutagenesis. 2015; 30(1): 67-83.

[110] Luan B, Huynh T, Zhao L, Zhou R.: Potential toxicity of graphene to cell functions via disrupting protein-protein interactions, ACS Nano, 2014 Dec 17[Epub ahead of print]

[111] Yang M, Zhang M, Tahara Y, Chechetka S, Miyako E, Iijima S, Yudasaka M.: Lysosomal membrane permeabilization: carbon nanohorn-induced reactive oxygen species generation and toxicity by this neglected mechanism. Toxicol Appl Pharmacol. 2014; 280(1): 117-126. doi: 10.1016/j.taap.2014.07.022.

[112] Umemoto EY, Speck M, Shimoda LM, Kahue K, Sung C, Stokes AJ, Turner H.: Single-walled carbon nanotube exposure induces membrane rearrangement and suppression of receptor-mediated signalling pathways in model mast cells. Toxicol Lett. 2014; 229(1): 198-209. doi: 10.1016/j.toxlet.2014.06.009.

[113] Wang J, Liu R, Su Y, Li W.: Embedded carbon nanotubes nanoparticles in plasma membrane induce cellular calcium outflow imbalancing. J Nanosci Nanotechnol. 2014; 14(6): 4058-4065.

[114] Zhao Y, Wu Q, Li Y, Nouara A, Jia R, Wang D.: In vivo translocation and toxicity of multi-walled carbon nanotubes are regulated by microRNAs. Nanoscale 2014; 6(8): 4275-4284. doi: 10.1039/c3nr06784j

[115] Walling BE, Lau GW.: Perturbation of pulmonary immune functions by carbon nanotubes and susceptibility to microbial infection. J Microbiol. 2014; 52(3): 227-234.

[116] Singh AV, Mehta KK, Worley K, Dordick JS, Kane RS, Wan LQ.: Carbon nanotube-induced loss of multicellular chirality on micropatterned substrate is mediated by oxidative stress. ACS Nano 2014; 8(3): 2196-2205.

[117] Haniu H, Saito N, Matsuda Y, Tsukahara T, Usui Y, Maruyama K, Takanashi S, Aoki K, Kobayashi S, Nomura H, Tanaka M, Okamoto M, Kato H.: Biological responses according to the shape and size of carbon nanotubes in BEAS-2B and MESO-1 cells. Int J Nanomedicine 2014; 9: 1979-1990, doi: 10.2147/IJN.S58661. eCollection 2014.

[118] Yamashita K, Yoshioka Y, Higashisaka K, Morishita Y, Yoshida T, Fujimura M, Kayamuro H, Nabeshi H, Yamashita T, Nagano K, Abe Y, Kamada H, Kawai Y, Majumi T,

Yoshikawa T, Itoh N, Tsunoda S, Tsutsumi Y.: Carbon nanotubes elicit DNA damage and inflammatory response relative to their size and shape. Inflammation 2010; 33(4): 276–280.

[119] Wang X, Jia G, Wang H, Nie H, Yan L, Deng XY, Wang S.: Diameter effects on cytotoxicity of multi-walled carbon nanotubes. J. Nanosci. Nanotechnol. 2009; 9(5): 3025–3033.

[120] van Berlo D, Wilhelmi V, Boots AW, Hullmann M, Kuhlbusch TA, Bast A, Schins RP, Albrecht C.: Apoptotic, inflammatory, and fibrogenic effects of two different types of multi-walled carbon nanotubes in mouse lung. Arch Toxicol, 2014 Sep; 88(9): 1725-1737. doi: 10.1007/s00204-014-1220-z.

[121] Zhang T, Tang M, Kong L, Li H, Zhang T, Xue Y, Pu Y.: Surface modification of multiwall carbon nanotubes determines the pro-inflammatory outcome in macrophage. J Hazard Mater, 2015 Mar 2; 284:73-82. doi: 10.1016/j.jhazmat.2014.11.013.

[122] Chatterjee N, Yang J, Kim HM, Jo E, Kim PJ, Choi K, Choi J.: Potential toxicity of differential functionalized multiwalled carbon nanotubes (MWCNT) in human cell line (BEAS2B) and Caenorhabditis elegans. J Environ health A. 2014; 77(22-24): 1399-1408. doi: 10.1080/15287394.2014.951756

[123] Palomaki J, Sund J, Vippola M, Kinaret P, Greco D, Savolainen K, Puustinen A, Alenius H.: A secretomics analysis reveals major differences in the macrophage responses towards different types of carbon nanotubes. Nanotoxicology 2014 Oct 17:1-10. [Epub ahead of print]

[124] Cui H, Wu W, Okuhira K, Miyazawa K, Hattori T, Sai K, Naito M,Suzuki K, Nishimura T, Sakamoto Y, Ogata A, Maeno T, Inomata A, Nakae D, Hirose A, Nishimaki-Mogami T.: High-temperature calcined fullerene nanowhiskers as well as long needle-like multi-wall carbon nanotubes have abilities to induce NLRP3-mediated IL-1β secretion. Biochem Biophys Res Commun. 2014 Sep 26; 452(3): 593-599. doi: 10.1016/j.bbrc.2014.08.118.

[125] Liu WT, Bien MY, Chuang KJ, Chamg TY, Jones T, BeruBe K, Laley G, Tsai DH, Chuang HC, Cheng TJ.: Taiwan Cardio Pulmonary Research (T-CPR) Group. Physicochemical and biological characterization of single-walled and double-walled carbon nanotubes in biological media. J Hazard Mater. 2014 Sep 15; 280: 216-225. doi: 10.1016/j.jhazmat.2014.07.069.

[126] Zhang C, Chen W, Alvarez PJ.: Manganese peroxidase degrades pristine but not surface-oxidized (carboxylated) single-walled carbon nanotubes. Environ Sci Technol. 2014 Jul 15; 48(14):7918-7923. doi: 10.1021/es5011175.

[127] Shvedova AA, Kisin E, Murray AR, Johnson VJ, Gorelik O, Arepalli S, Hubbs AF, Mercer RR, Keohavong P, Sussman N, Jin J, Yin J, Stone S, Chen BT, Deye G, Maynard A, Castranova V, Baron PA, Kagan VE.: Inhalation vs. aspiration of single-walled carbon nanotubes in C57BL/6 mice: inflammation, fibrosis, oxidative stress, and

mutagenesis. Am J Physiol Lung Cell Mol Physiol. 2008 Oct; 295(4): L552–L565. doi: 10.1152/ajplung.90287.2008.

[128] Park EJ, Zahari NE, Kang MS, Lee Sj, Lee K, Lee BS, Yoon C, Cho MH, Kim Y, Kim JH.:Toxic response of HIPCO single-walled carbon nanotubes in mice and RAW264.7 macrophage cells. Toxizol Lee. 2014 Aug 17; 229(1): 167-177 doi: 10.1016/j.toxlet. 2014.06.015.

[129] Silva RM, Doudrick K, Franzi LM, TeeSy C, Anderson DS, Wu Z, Mitra S, Vu V, Dutrow G, Evans JE, Westerhoff P, Van Winkle LS, Raabe OG, Pinkerton KE.: Instillation versus inhalation of multiwalled carbon nanotubes: exposure-related health effects, clearance, and the role of particle characteristics. ACS Nano 2014 Sep 23; 8(9): 8911-8931. doi: 10.1021/nn503887r

[130] Han SG.: Pulmonary response of mice to a sequential exposure of side-stream cigarette smoke and multi-walled carbon nanotubes. Inhal Toxicol 2014 May; 26(6): 327-332. doi: 10.3109/08958378.2014.890683.

[131] Clichici S, Mocan T, Filip A, Biris A, Simon S, Daicoviciu D, Decea N, Parvu A, Moldovan R, Muresan A.: Blood oxidative stress generation after intraperitoneal administration of functionalized single-walled carbon nanotunes in rats. Acta Physiol Hung. 2011 Jun; 98(2): 231-241. doi: 10.1556/APhysiol.98.2011.2.15.

[132] Clichici S, Biris AR, Catoi C, Filip A, Tabaran F.: Short-term splenic impact of single-strand DNA functionalized multi-walled carbon nanotubes intraperitoneally injected in rats. J Appl Toxicol. 2014 Apr; 34(4): 332-344. doi: 10.1002/jat.2883.

[133] Chen R, Zhang L, Ge C, Tseng M, Bai R, Qu Y, Beer C, Autrup H, Chen C.: Subchronic toxicity and cardiovascular responses in spontaneously hypertensive rats after exposure to multiwall carbon nanotubes by Iintratracheal instillation. Chem Res Toxicol 2015 Jan 12 [Epub ahead of print]

[134] Fujita K, Fukuda M, Fukui H, Horie M, Endoh S, Uchida K, Shichiri M, Morimoto Y, Ogami A, Iwahashi H.: Intratracheal instillation of single-wall carbon nanotubes in the rat lung induces time-dependent changes in gene expression. Nanotoxicology 2014 Jun 9: 1-12. [Epub ahead of print]

[135] Xu J, Alexander DB, Futakuchi M, Numano T, Fukamachi K, Suzui M, Omori T, Kanno J, Hirose A, Tsuda H: Size- and shape-dependent pleural translocation, deposition, fibrogenesis, and mesothelial proliferation by multiwalled carbon nanotubes. Cancer Sci. 2014 Jul; 105(7): 763-769. doi: 10.1111/cas.12437.

[136] Kido T, Tsunoda M, Kasai T, Sasaki T, Umeda Y, Senoh H, Yanagisawa H, Asakura M, Aizawa Y, Fukushima S.: The increases in relative mRNA expressions of inflammatory cytokines and chemokines in splenic macrophages from rats exposed to multi-walled carbon nanotubes by whole-body inhalation for 13 weeks. Inhal Toxicol. 2014 Oct; 26(12): 750-758. doi: 10.3109/08958378.2014.953275.

[137] Kasai T, Umeda Y, Ohnishi M, Kondo H, Takeuchi T, Aiso S, Nishizawa T, Matsumoto M, Fukushima S.: Thirteen-week study of toxicity of fiber-like multi-walled carbon nanotubes with whole-body inhalation exposure in rats. Nanotoxicology. 2014; 17: 1-10.

[138] Kim JS, Sung JH, Choi BG, Ryu HY, Song KS, Shin JH, Lee JS, Hwang JH, Lee JH, Lee GH, Jeon K, Ahn KH, Yu IJ:.In vivo genotoxicity evaluation of lung cells from Fischer 344 rats following 28 days of inhalation exposure to MWCNTs, plus 28 days and 90 days post-exposure. Inhal Toxicol. 2014 Mar; 26(4): 222-234. doi: 10.3109/08958378.2013.878006.

[139] Warheit DB.: Long-term inhalation toxicity studies with multiwalled carbon nanotubes: closing the gaps or initiating the debate?Toxicol Sci. 2009; 112(2): 273-275. doi: 10.1093/toxsci/kpf237

[140] Chen W, Xiong Q, Ren Q, Guo Y, Li G.: Can amino-functionalized carbon nanotubes carry functional nerve growth factor? Neural Regen Res., 2014; 9(3): 285-292. doi: 10.4103/1673-5374.128225.

[141] Liu Y, Ren L, Yan D, Zhong W.: Mechanistic Study on the Reduction of SWCNT-induced Cytotoxicity by Albumin Coating. Part Part Syst Charact, 2014; 31(12): 1244-1251.

[142] Orecna M, De Paoli SH, Janouskova O, Tegegn TZ, Filipova M, Bonevich JE, Holada K, Simak J.: Toxicity of carboxylated carbon nanotubes in endothelial cells is attenuated by stimulation of the autophagic flux with the release of nanomaterial in autophagic vesicles. Nanomedicine 2014 Jul; 10(5): 939-948. doi: 10.1016/j.nano. 2014.02.001.