Volume 6, Issue 1, 2017, 488 - 493 **ISSN 2284-6808**

Letters in Applied NanoBioScience

www.NanoBioLetters.com

Original Research Article **Open Access Journal**

Received: 10.02.2017 / Revised: 10.03.2017 / Accepted: 11.03.2017 / Published on-line: 30.03.2017

Toxicity of zinc oxide and silver nanoparticles – an overview

Daniela Paula Caraciuc[1, 2, #], Ancuta Telcian[1, 3, #], Liliana Burlibașa[1, 2, #], Magdalena Valentina Lungu [4], Irina Gheorghe [1, 2, *], Luminita Marutescu[1, 2, #], Mariana Carmen Chifiriuc[1, 2]

[1] University of Bucharest, Faculty of Biology, Microbiology Department, 36-46 Mihail Kogălniceanu Street, 050107 Bucharest, Romania

[2] Research Institute of the University of Bucharest – ICUB, Life, Environment and Earth Sciences Division, Splaiul Independentei 91-95, 050095 Bucharest, Romania

[3] SC BIOTEHNOS S.A., 3-5 Gorunului Street, 075100 Otopeni, Ilfov, Romania

[4] National Institute for Research and Development in Electrical Engineering ICPE-CA, Advanced Materials Department, 313 Splaiul Unirii Street, 030138 Bucharest, Romania

#- These authors have equally contributed to this work

*corresponding author e-mail address: ***iryna_84@yahoo.com***

ABSTRACT

The design, manufacture and use of engineered nanoparticles in a wide range of human activities, i.e. medicine, cosmetics, food industry, information and communication, agriculture and water treatment, energy storage, security and cultural heritage will result in their tremendous release into the environment. Apart from their benefits, the toxicity associated with nanomaterials must therefore be addressed. There is an urgent need for the investigation of their impact on health and environment and subsequently, for the development of standardized methods to evaluate their (eco)toxicity. In this minireview we will focus on the mechanisms and features influencing the toxicity of zinc oxide and silver nanoparticles.

Keywords: *Zinc Oxide (ZnO), silver (Ag) Nanoparticles, Nanotechnology, Toxicity.*

1. INTRODUCTION

The developments in nanotechnology have opened new frontiers related to the their applications in a wide range of fields: health, environmental protection, cosmetics, food, electronics, energy, medicines etc. [1]. This wide use of NPs is due to the fact that they are more reactive than conventional size particles, but they could also exhibit greater cytotoxicity [2].

The NPs characterization methods are electronic transmission microscopy (TEM) for determining the size, morphology and aggregation; dynamic dispersion of light (DLS) for analysis of particle size distribution; zeta potential measurement for NPs surface loading, and x - Ray diffraction (XRD) for identification of crystalline particle structure. In some cases, such as silver nanoparticles (Ag NPs), UV spectroscopy can be used to determine the size and size distribution due to size dependent optical activity. Analysis of NPs should be repeated after administration, as various modifications may occur during the application process. NPs are usually applied by mixing them with the cell culture medium. The components dissolved in the medium, especially ions, lead to the agglomeration and precipitation of many NPs, causing significant changes in their physico-chemical properties. Similar effects are expected when NPs come into contact with surfactants or other biological fluids. It has been demonstrated that some NPs tend to form protein crowns in biological systems [3]. Although in recent years progress has been made in using NPs, these still raise problems in terms of genotoxic and carcinogenic effects. Despite the need for standard protocols in assessing the toxicity and bio-performance of each new functional nanomaterial, they are still scarce or currently under development. Nonetheless, nanotoxicology and related adverse effects to the physico-chemical properties of nanomaterials are emerging areas of high importance which have to be continuously revisited as any new material is being developed. [4].

This review summarizes the evaluation of the toxicity, (citotoxicity and genotoxicity) of some inorganic NPs based on ZnO and Ag.

2. NANOPARTICLES (NPs)

2.1. Zinc oxide Nanoparticles (ZnO NPs). Zinc Oxide (ZnO) represents a remarkable material having a broad spectrum of applications like semiconductor, gas sensor, piezoelectric sensor, electro luminescent material, magnetic material and actuator, ingredients in cosmetics [5]. It has been demonstrated that ZnO is able to inhibit the proliferation of bacteria (bacteriostatic effect) rather than to kill them (bactericidal activity), by the production of reactive oxygen species and disrupting the cell membrane [6]. Jones et al., have observed that the antibacterial activity depends on the size of nanoparticles and by the presence of visible light [7]. ZnO NPs are known to be effective against several types of bacteria and fungi, both under ambient light and ultraviolet (UV) light. The toxicity of ZnO NPs

ZnO NPs can affect the respiratory tract, skin, intestinal tract and the immune system. Several studies suggest that these effects are caused by Zn2 + resulting from the dissolution of NPs outside the cell. Most nanotoxicological studies use in vitro

models that do not provide information about NPs activity in host organisms [2].

2.1.1. Toxicity to cancer cells. In vitro results indicate that the induction of oxidative stress is the most important and probable mechanism responsible for NPs toxicity. ZnO NPs dissolve in the extracellular environment, leading to an increase of Zn2 + concentration in the extracellular compartment, which in turn results in a decreased activity of the intracellular enzymes and transcription factors dependent on Zn2 +. This mechanism has been demonstrated on A549 and BEAS-2B cells. Alternatively, ZnO NPs enter into the cell, being dissolved in the lysosomal compartment. The toxic effects of ZnO NPs include the alteration of the intracellular flow of Ca2 +; the generation of reactive oxygen species, membrane damage and mitochondrial dysfunction (fig. 1) [8].

In BEAS-2B cells, ZnO NPs with 20 nm size have been shown to induce a concentration and time-dependent cytotoxicity, as well as an increased release of LDH (lactic dehydrogenase). Exposure to ZnO NPs also increased the levels of intracellular calcium (Ca2 +) in a concentration- and time dependent manner which was partially attenuated by the antioxidant activity of N-acetylcysteine (NAC). Nifedipine, a calcium channel blocker, has partially mitigated the increase of intracellular Ca2 +, indicating that some of the excess Ca2 + is the result of the influx from outside the cell. Exposure to a sublethal concentration of ZnO NPs increased the expression of four genes for at least 2.5 times that are involved in apoptosis and oxidative stress responses: BNIP PRDX3, PRNP and TXRND1, [9]. The cytotoxicity of ZnO NPs on BEAS-2B cells was attributed to the generation of (ROS), increased oxidative stress, apoptosis, elevated levels of the intracellular Ca2 +, decrease of mitochondrial membrane potential and the production of IL-8. Effects on A549 cells seem to be comparable to the effects of ZnO NPs on BEAS-2B cells. The toxicity of ZnO PNs can be reduced by decreasing their solubility or through doping coverage, resulting in a lower release of Zn2 + [8].

In vitro studies using human leukemia Jurkat cells and human lung carcinoma H1355 cells have demonstrated that ZnO NPs treatment revealed an increase of cytosolic and mitochondrial free Zn2+ concentration. In H1355 cells, ZnO NPs induced depolarization of mitochondrial membrane potential, caspase-3 activation and LDH release [9].

ZnO NPs (8–10 nm) have induced more toxicity to human colon cancer cells (RKO) compared to micrometer sized ZnO (<0.44μm). Both particle types have been found to agglomerate into micrometer-sized particles in cell culture media, and to induce toxicity through apoptotic pathways [10].

The treatment of LoVo cells (human colon carcinoma) with ZnO NPs (11.5 μg/ml) for 24 hours has led to a significant decrease in cell viability, growth of H2O2/OH, lowering O2(-), depolarization of the inner mitochondrial membrane, apoptosis, and IL-8 release. Elevated doses have induced a high cytotoxicity rate after 24 hours of treatment. Experimental data showed that oxidative stress may be a key pathway in the induction of cytotoxicity by ZnO NPs in colon carcinoma cells. In addition, the study of the relationship between the toxicological effects and physico-chemical characteristics of ZnO NPs by analytical electron microscopy suggests that the surface does not play a primary role in their cytotoxicity [11].

2.1.2. Toxicity of ZnO NPs on dermal cells. ZnO NPs are present in skin products, such as sunscreen. For this reason, in vitro studies have investigated the exposure effects of skin cells. Meyer et al., have demonstrated the apoptosis induction by ZnO NPs via mitogen-activated protein kinase p38 and cell cycle checkpoint protein p53 pathways in human dermal fibroblasts [12].

2.1.3. Toxicity to human immune system cells. A differential cytotoxic response between human immune cell subsets was observed, with lymphocytes being the most resistant and monocytes being the most susceptible to ZnO NPs - induced toxicity. Significant differences were also observed between previously activated memory lymphocytes and naive lymphocytes, indicating a relationship between cell-cycle potential and NPs susceptibility. Mechanisms of toxicity involve the generation of ROS, displaying the highest levels in monocytes, and the interactions with cellular membranes. An inverse relationship between NPs size and cytotoxicity, as well as NPs size and ROS production was also observed. ZnO NPs induced the production of the proinflammatory cytokines, such as IFN-γ, TNF-α, and IL-12, but at concentrations below those causing appreciable cell death [13, 14].

2.1.4. Toxicity of ZnO NPs on experimental animal models. The toxic effects of in vivo exposure have been revealed in vertebrates (rodents and zebrafish), but also in invertebrates, such as D. melanogaster. Other studies have also shown the potential genotoxic risk of ZnO NPs. Although oxidative stress was suggested as the main mechanism responsible for ZnO NPs toxicity, the direct role of the generated ROS in toxicity remains unclear. Studies have shown that ZnO NPs cause only low rodent subchronic toxicity. In addition, an earlier study using D. melanogaster did not report any toxicity after the ingestion of ZnO nanoparticles. Another study evaluating genotoxicity and oxidative stress induced by ZnO NPs in D. melanogaster showed poor genotoxicity of ZnO NPs [15].

2.1.5. Key Factors and Toxicity Mechanisms of ZnO NPs. The key factors responsible for the toxicity of ZnO NPs are the size, surface characteristics, dissolution, and exposure routes (fig. 2). The size of NPs is directly correlated to several properties, such as surface properties, solubility, chemical reactivity, interactions between nanomaterials and biomolecules that subsequently affect the nanotoxicological behavior of NPs in vivo [16]. Besides size, surface characteristics (e.g., surface charge and rugosity) of NPs are other key factors that determine ZnO NPs toxicity. Hwang and colleagues [17] indicated that metal oxide NPs' cytotoxicity was related to the surface charge.

Another key factor of toxicity is the release of metal ions. Studies have suggested that the cell walls of plant roots and leaves excluded large NPs [18] but absorbed solute metal ions released

from NPs. Brunner et al. [19] found that the six - days toxic effect of soluble metal/metal oxide NPs was higher than that of insoluble ones applied at the same concentration. The solubility of ZnO is related to solution pH and temperature [20].

1.2. Silver nanoparticles (Ag NPs). Ag NPs are particles with size between 1 and 100 nm. Currently, Ag NPs are used as antibacterial / antifungal agents in biotechnology and bioengineering [21]. Recently, Ag NPs have received great attention due to their distinct physico-chemical and biological properties. This interest has derived from the use of Ag NPs in many different products due to their exceptionally small size and potential antibacterial activity [22]. Their multidisciplinary application is generally well known, although their use as antibacterial agents is probably the most studied. There are different synthesis methods for obtaining Ag NPs, for example, laser ablation, gamma irradiation, electronic irradiation, chemical reduction, photochemical methods, microwave processing and synthetic biology methods. With regards to Ag precursors for the synthesis of these nanoparticles, silver nitrate is most often used because it is cheap and affordable [23]. Among metal nanoparticles, Ag NPs have been used the most in antibacterial, antifungal and antiproliferative applications. Ag NPs have been also widely deployed in the field of biomolecular detection, diagnosis, catalysis and microelectronics [24]. The antibacterial activity of silver ions is frequently tested on Gram positive (Staphylococcus aureus) and Gram negative (Escherichia coli) bacteria [25].

Key factors such as size and shape affect the antibacterial activity of Ag NPs. The smaller the size of antimicrobial particles, the higher the surface-to-volume ratio. A large surface offers many opportunities for interaction with bacteria. Thus, the small particle size is effective as an antibacterial agent. Pal and colleagues reported that triangular silver nanoparticles are more active than spherical and rod-shaped silver nanoparticles against Escherichia coli, suggesting that the shape of Ag NPs is a key factor in determining antimicrobial activity [25].

1.2.1.Toxicity of silver nanoparticles

1.2.1.1. Nanotoxicological in vitro studies. Ag NPs are the most common nanomaterials found in consumer products (including cosmetics, textiles, food cans and sprays), appliances (refrigerators, washing machines) and medical applications (wound dressings, medical devices, imaging methods). However, the extensive use of Ag NPs may result in contamination of the environment and human exposure by inhalation with a toxic potential.

With regards to silver, there have been numerous in vitro studies, and the majority of the experiments have shown that cytotoxic and genotoxic effects of Ag NPs are dependent on size and dose and the type of coverage and cell. Analysis of in vitro immuno-compatibility of THP1 cells-XBlue showed that Ag NPs in concentrations of 5 mg/ml or less do not cause the activation of monocytes. However, concentrations of 50 pg/ml or greater significantly altered the activation of monocytes [25].

Ag NPs are used increasingly more often in wound dressings, catheters and various household products due to their antimicrobial activity. The toxicity of Ag NPs was studied using normal human lung fibroblasts (IMR-90) and human glioblastoma cells (U251). The toxicity was evaluated by following changes in cell morphology, cellular viability, metabolic activity and oxidative stress. Ag NPs induced the decrease of the ATP cellular content causing damages to mitochondria and an increase in the production of ROS in a dose-dependent manner. DNA damage measured by gel electrophoresis with a single cell (SCGE) micronuclei test and blocked cytokinesis (CBMN) was also dose-dependent and more prominent in the cancer cells. Treatment with Ag NPs has resulted in stopping the cell cycle in G2/M phase. TEM analysis indicated the presence of Ag NPs inside the nucleus, mitochondria, demonstrating their involvement in mitochondrial toxicity and direct damage to DNA [26].

It has been reported that Natural Killer cells have been activated after exposure to a quantity of 100 μg/ml of Ag NPs. This effect has been observed only in HepG2 cells (cell line of human liver carcinoma), but not in A549 (lung cancer). Activation of immune cells, including monocytes, is dependent on NF-kB. Studies have shown that Ag NPs may provide an activation signal for NF-κB and induce the production of inflammatory mediators, such as TNF-α, IL-8, IL-2 and IL-6 [27]. It has also been observed that Ag NPs can induce endothelial dysfunction and injury of human umbilical vein through the activation of NF-kB, which is associated with oxidative stress induced by Ag NPs [28].

The role of oxidative stress in the toxicity of Ag NPs has been also demonstrated on human hepatocarcinoma cells.

Toxicity induced by silver ions was studied in parallel by using AgNO as a source of silver ions. By using cation exchange treatment, it was confirmed that the Ag NPs solution contained a negligible amount of free silver ions [29]. The results indicate that cells treated with Ag have a limited exposure to Ag ions (+), despite the Ag (+) release in cell cultures. Ag NPs induced intracellular oxidative stress. The cytotoxicity of Ag NPs was comparable with that of Ag ions (+) and suggests that the cytotoxicity is primarily the result of oxidative stress and is independent of Ag (+) toxicity [30]. Ag NPs (15, 30 and 55 nm) were investigated for their potential role in the initiation of oxidative stress in macrophages. The results showed that exposure of cells to high Ag NPs doses has led to abnormal changes in terms of morphology and absorption characteristics after 24 hours. Assessments were made using mitochondrial toxicity viability and cell membrane permeability together with ROS. After 24 hours of exposure, the viability significantly decreased with increasing the Ag NPs dose [31].

1.2.1.2. In vivo studies regarding the cytotoxicity Ag NPs. A quantitative in vivo study was performed on zebra fish and a low transport and Ag NPs toxicity was demonstrated. Oxidative stress and apoptosis were evaluated in zebra fish liver, and the hepatotoxic behavior of Ag NPs was concluded [32]. Tran et al. [33] demonstrated a minimal pulmonary inflammation or cytotoxicity in mice after 10 days of exposure to Ag NPs.

1.3. Assessement of NPs genotoxicity. Micronuclei (MN) are formed during mitosis when delayed chromosomes are left outside the metaphysical plaque due to mitotic defects (aneuploid effect), or when chromosomal fragments lacking centromeres are not attached to the microtubules of the division spindle (clastogenic effect). In these cases, the genetic material could not be incorporated into the nucleus of daughter cells [34]. These chromosomal fragments surrounded by the nuclear membrane are known as MN, and they are morphologically similar to the normal nucleus, but are much smaller in size [35].

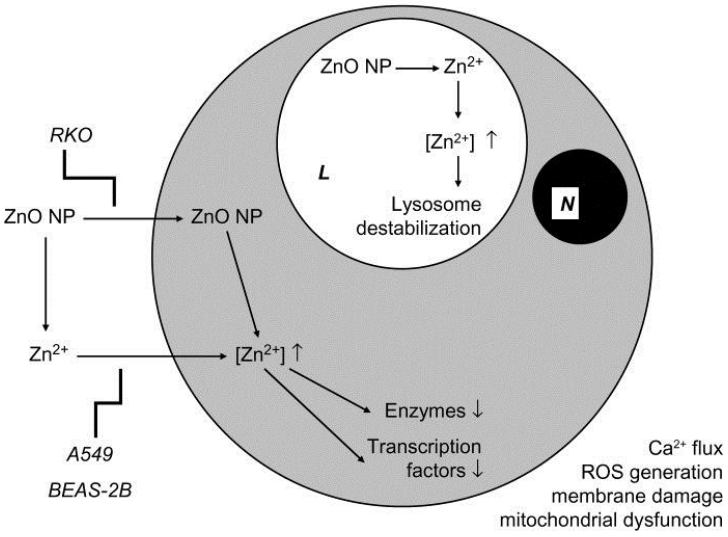

Figure 1. The in vitro effects of zinc oxide nanoparticles [8].

Genotoxicity tests are used to detect genetic lesions through various mechanisms in vitro and in vivo systems [36]. Until now, most international authorities recommend a test scheme consisting of in vitro and in vivo methods to detect genotoxicity / mutagenicity induced by different substances [36]. A variety of potentially genotoxic agents can induce MN formation, which will lead to cell death, genomic instability, or ultimately to the development of cancer cells, which is also the reason why micronuclei have become the most widely used biomarker for determining fault chromosomal [37].

There is a need to validate animal models for NM toxicity studies. Difficulties consist in developing an appropriate approach for interpreting studies and making decisions on the parameters to be considered when examining toxicity in vivo models. The tests used for genotoxicity assessment are similar to those used in in vitro studies [38]. It is worth noting that there is no information on the genotoxicity of Ag-ZnO NPs in literature. Studies have been conducted separately on ZnO NPs and Ag NPs.

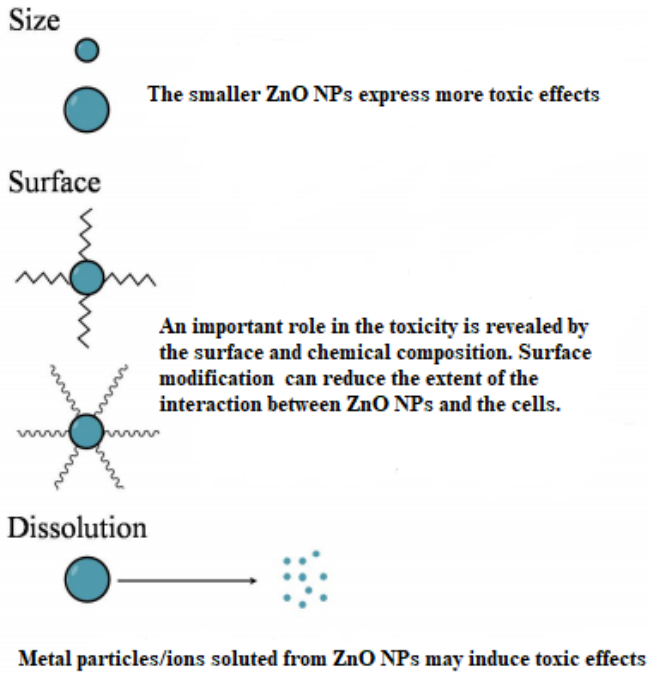

Figure 2. Schematic representation of the factors responsible for ZnO NPs toxicity.

4. CONCLUSIONS

The ZnO and Ag NPs are the most attractive nanomaterials used for different applications in different fields, being found in different products (including cosmetics, drugs, textiles, food cans and sprays), appliances (refrigerators, washing machines) and studied for various medical applications (wound dressings, medical devices, imaging diagnosis methods). Apart from their benefits, the toxicity associated with nanomaterials must therefore be addressed. In this review we have discussed the main factors and mechanisms of the toxicity of ZnO NPs and Ag NPs to both humans and the environment. The NPs generally exhibit greater toxicity than microparticles with the same composition; the cytotoxicity and genotoxicity of ZnO NPs and Ag NPs is dependent on the size, shape, surface charge and rugosity, solubility, while the main molecular mechanisms involved, yet not entirely elucidated, are the release of reactive oxygen species, depletion of Zn dependent enzymatic activity, activation of apoptosis, induction of DNA damages and of cellular membrane permeability etc. The results reported by different authors using different in vitro and in vivo experimental approaches demonstrate the urgent need for standardized methods for the investigation of the NPs impact on health and environment.

5. REFERENCES

[1]. I. Popa, I.M. Nicola, V. Ceara, C.Boboc, C.A. Danec. Obtinerea si utilizarea nanoparticulelor de argint, CPE, Bucureti, România; IMT, Bucuresti, România, **2015**.

[2]. M.C. Chifiriuc, A.C. Ratiu, M. Popa M, A. Al. Ecovoiu. Drosophotoxicology: An Emerging Research Area for Assessing Nanoparticles Interaction with Living Organisms, *Int. J. Mol. Sci*. 2016, 17, 36; **2016**.

[3]. A.J. Omlor, J. Nguyen, R. Bals, Q.T. Din. Nanotechnology in respiratory medicine, *Respiratory Research*, 16:64, **2015**.

[4]. J. Wolfram, M. Zhu, Y. Yang, J. Shen, E. Gentile, D. Paolino et al. Safety of nanoparticles in medicine, *Current Drug Targets*, 16, 1671-1681, **2015**.

[5]. M. Hasanpoor, M. Aliofkhazraei, H. Delavari. In-situ study of mass and current density for electrophoretic deposition of zinc oxide nanoparticles, *Ceramics International, Elsevier*, 42, 6, 6906-6913, **2016**.

[6]. K.L.Phan, S.F. Taylor, R.C. Welsh,S.H. Ho, J.C. Britton, I. Liberzon. Neural correlates of individual ratings of emotional salience: a trial-related fMRI study. *Neuroimage*, 21,768–780, **2004**.

[7]. N. Jones, B. Ray, K.T. Ranjit, A.C. Manna. Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms, *FEMS Microbiol Lett*., 279(1), 71-6. **2008**.

[8]. R.J. Vandebriel, W.H. De Jong. A review of mammalian toxicity of ZnO nanoparticles, *Nanotechnology, Science and Applications*, 5, 61–71, **2012**.

[9]. Y.Y. Kao, Y.C. Chen, T.J. Cheng, Y.M. Chiung, P.S. Liu. Zinc Oxide Nanoparticles Interfere With Zinc Ion Homeostasis to Cause Cytotoxicity. *Toxicological Sciences*, 125, 2, 462–472, **2012**.

[10]. P.J. Moos, K. Chung, D. Woessner, M. Honeggar, N.S. Cutler, J.M. Veranth. ZnO particulate matter requires cell contact for toxicity in human colon cancer cells. *Chem Res Toxicol* 23, 733–739, **2010**.

[11]. B. De Berardis, G. Civitelli, M. Condello, P. Lista, R. Pozzi, G. Arancia, S. Meschini. Exposure to ZnO nanoparticles induces oxidative stress and cytotoxicity in human colon carcinoma cells. *Toxicology and Applied Pharmacology*, 246, 116–127, **2010**.

[12]. K. Meyer, P. Rajanahalli, M. Ahamed, J. J. Rowe, Y. Hong. ZnO nanoparticles induce apoptosis in human dermal fibroblasts via p53 and p38 pathways. *Toxicology in Vitro*, 25, 1721–1726, **2011**.

[13]. C.C. Huang, R.S. Aronstam , D.R. Chen , Y.W. Huang. Oxidative stress, calcium homeostasis, and altered gene expression in human lung epithelial cells exposed to ZnO nanoparticles. *Toxicol In Vitro*. 24(1), 45-55, **2010**.

[14]. C. Hanley, A. Thurber, C. Hanna, A. Punnoose, J. Zhang, D. G. Wingett. The Influences of Cell Type and ZnO Nanoparticle Size on Immune Cell Cytotoxicity and Cytokine Induction. *Nanoscale Res Lett*, 4(12), 1409–1420, **2009**.

[15]. C.T. Ng, L.Q. Yong, M.P. Hande, C.N. Ong, L.E. Yu, B.H. Bay, G.H. Baeg. Zinc oxide nanoparticles exhibit cytotoxicity and genotoxicity through oxidative stress responses in human lung fibroblasts and *Drosophila melanogaster*, *International Journal of Nanomedicine*, 12, 1621–1637, **2017**.

[16]. Y. Zhao, H. Meng, Z. Chen, Z. Feng, Z. Chai. Dependence of nanotoxicity on nanoscale characteristics and strategies for reducing and eliminating nanotoxicity. *In Nanotoxicology, American Scientific Publisher*, 265–280, **2007**.

[17]. X. Hu, S. Cook, P. Wang, H.M. Hwang. In vitro evaluation of cytotoxicity of engineered metal oxide nanoparticles. *Sci. Total Environ*, 407, 3070–3072, **2009**.

[18]. T.E. Proseus, J.S. Boyer. Turgor Pressure Moves polysaccharides into growing cell walls of Chara corallina. *Ann. Bot*. 95, 967–979, **2005**.

[19]. T.J. Brunner, P. Wick, P. Manser, P. Spohn, R.N. Grass, L.K. Limbach, A. Bruinink, W.J. Stark. In vitro cytotoxicity of oxide nanoparticles: Comparison to asbestos, silica, and the effect of particle solubility. *Environ. Sci. Technol*. 40, 4374–4381, **2006**.

[20]. Y.N. Chang, M. Zhang, L. Xia, J. Zhang, G. Xing. The Toxic Effects and Mechanisms of CuO and ZnO Nanoparticles *Materials*, 5, 2850-2871, **2012**.

[21]. J.B. Chappel, G.D. Greville. Effect of silver ions on mitochondrial adenosinetriphosphates. *Nature (London)*, 174, 930–931, **2004**.

[22]. N. Duran, C.P. Silveira, M. Durán, D.S.T. Martinez. Silver nanoparticle protein corona and toxicity: a mini review, *J Nanobiotechnol*, 13, 55, **2015**.

[23]. M. Balaz, L. Balazova, N. Daneu, E. Dutkova, M. Balazova, Z. Bujnakova, Y. Shpotyuk. Plant-Mediated Synthesis of Silver Nanoparticles and Their Stabilization by Wet Stirred Media Milling. *Nanoscale Research Letters*, 12, 83, **2017**.

[24]. Y.K. Mohanta, S.K. Panda, A.K. Bastia, T.K. Mohanta. Biosynthesis of Silver Nanoparticles from Protium serratum and Investigation of their Potential Impacts on Food Safety and Control, *Frontiers in Microbiology*, doi: 10.3389/fmicb.2017.00626. **2017**.

[25]. G. Nam, S. Rangasamy, B. Purushothaman, J.M. Song. The Application of Bactericidal Silver Nanoparticles in Wound Treatment, *Nanomater Nanotechnol*, 5, 23, **2015**.

[26]. P.V. AshaRani, M. Prakash, S. Valiyaveettil. Anti-proliferative activity of silver nanoparticles. *BMC Cell Biology*, 10, 65, **2009**.

[27]. T.M. Stępkowski , K. Brzóska , M. Kruszewski. Silver nanoparticles induced changes in the expression of NF-κB related genes are cell type specific and related to the basal activity of NF-κB. *Toxicol In Vitro.* 28(4), 473-8. **2014**.

[28]. J. Shi, X. Sun, Y. Lin, X. Zou, Z. Li, Y. Liao, M. Du, H. Zhang. Endothelial cell injury and dysfunction induced by silver nanoparticles through oxidative stress via IKK/NF-κB pathways. *Biomaterials*. S0142-9612(14)00491-8. **2014**.

[29]. S.W. Kim , K.S. Kim, K. Lamsal , Y.J. Kim , S.B. Kim , et al. An in vitro study of the antifungal effect of silver nanoparticles on oak wilt pathogen Raffaelea sp. *J Microbiol Biotechnol*. 19(8), 760-4, **2009**.

[30]. S.C.Yen, M.D. Mashitah. Optimization of process variables for the synthesis of silver nanoparticles by *Pycnoporus sanguineus* using statistical experimental design. *Journal of the Korean Society for Applied Biological Chemistry*, 56, 1, 11–20, **2013**.

[31]. A. Manna, T. Imae, K. Aoi, M. Okada, T. Yogo. Synthesis of Dendrimer-Passivated Noble Metal Nanoparticles in a Polar Medium: Comparison of Size between Silver and Gold Particles. *Chem. Mater*., 13 (5), 1674–1681, **2001**.

[32]. C. Chakraborty, A.R. Sharma, G. Sharma, S.S. Lee. Zebrafish: A complete animal model to enumerate the nanoparticle toxicity, *J Nanobiotechnol*, 14:65, **2016**.
[33]. Q.H. Tran, V.Q. Nguyen, A.T. Le. Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives, *Adv. Nat. Sci. Nanosci. Nanotechnol*. 4 033001 (20). **2013**.
[34]. O. Torres-Bugarín, N.M. Romero, M.L.R. Ibarra, A. Flores-García et al. Genotoxic Effect in Autoimmune Diseases Evaluated by the Micronucleus Test Assay: Our Experience and Literature Review, *BioMed Research International*, http://dx.doi.org/10.1155/2015/194031. **2015**.
[35]. S.H. Kang, J.Y. Kwon, K.K. Lee, Y.R. Seo. Recent Advances in In Vivo Genotoxicity Testing: Prediction of Carcinogenic Potential Using Comet and Micronucleus Assay in Animal Models, *Journal of Cancer Prevention*,18, 4, **2013**.
[36]. Rim K.T., Kim S.J. *A Review on Mutagenicity Testing for Hazard Classification of Chemicals at Work: Focusing on in vivo Micronucleus Test for Allyl Chloride*, Safety and Health Research Institute. Published by Elsevier Ltd. http://dx.doi.org/10.1016/j.shaw.2015.05.005, **2015**.
[37]. L. Luzhna, P. Kathiria, O. Kovalchuk. Micronuclei in genotoxicity assessment: from genetics to epigenetics and beyond, *Frontiers in Genetics*, 4, 131, **2013**.
[38]. C.T. Ng, J.J. Li, B.H. Bay, L.Y.L.Yung. Current Studies into the Genotoxic Effects of Nanomaterials, *Journal of Nucleic Acids*, doi:10.4061/2010/947859, **2010**.

6. ACKNOWLEDGEMENTS

The financial support of the research project PN II PCCA 215/2014 – Innovative nanostructured materials and coatings with antimicrobial activity for medical applications is gratefully acknowledged.