Silver and Gold Nanoparticles: a Toxicological Aspect

Dang Minh Hieu

Hanoi University of Science and Technology – No. 1, Dai Co Viet Str., Hai Ba Trung, Ha Noi, Viet Nam

Received: September 06, 2016; accepted: June 9, 2017

Abstract

Silver and gold nanoparticles have been found in vast number of applications, especially in medicine. With increasing and intensively uses of these nanoparticles, there is a growing concern, recently, on their environmental impacts when they are released into environments. In this study, the size- and shape-dependent cytotoxicity of silver and gold nanoparticles have been examined. Silver nanoparticles inhibited the growth of the mold Aspergillus niger, and the one-dimension (Np1) and two-dimension (Np2) nanoparticles indicated more effective than the round ones (Np0). On the other hand, gold nanoparticles of the three types: nanostars (AuNS), polyethylene glycol coated nanostars (PEG-NS) and TAT peptide tagged nanostars (TAT-NS), placed impact on the BT549 human breast cancer cells with reduction in the cell viability. The PEG-NS showed more remarkable impact on the cells in compare to the others.

Keywords: Silver nanoparticles, Gold nanoparticles, Toxicity.

1. Introduction

Nanoparticles (NPs) and related technology have gained great development over the last two decades. Metal NPs now can be found easily commercial available and in various applications especially in medicine and biological sciences. From laboratories to industry, NPs have received many positive reviews from their users. However, there are still very few people and researchers assessing the downside of using NPs. Since various kinds of NPs from different materials are currently in use, some scientists still believe in acceptable impact of their side effects. Nevertheless, more risk assessments of NPs on the environments as well as plant and animal health are necessary [1].

Among NPs, silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) receive a lot of concerns in all aspects from synthesis to their applications. Due to their unique physiochemical, electrical, mechanical, optical and thermal properties, the AgNPs can be found in wide range of applications including activities against bacterial and viral threats [2, 3], incorporation in nano-scale sensors for fast response and lower limit detection, diagnosis, drug delivery, wound dressing, chemotherapeutic agent, etc. [4-6]. On the other hands, the AuNPs owing to their unique chemical and optical properties can be found intensively in applications for drug delivery [7, 8] and biomedical imaging and diagnosis [4, 6, 9, 10].

As the use of metal NPs is continually increasing, there is a demand for better understanding the effects of the particles on ecological and biological systems. Several reports recently have pointed out some toxic activities of AgNPs such as: immunotoxicology and cytotoxicity [11, 12] and genotoxicology by chromosomal alterations, nucleus ablation, etc. [5, 12]. Abdelhalim and co-workers [13] have observed various adverse effects of AuNPs on tissue, cellular and subcellular levels of rat liver include cloudy swelling, polymorphism, binucleation, hyaline vacuolation, karyopyknosis, karyorrhexis, karyolysis and necrosis, etc. Another study by Ma and co-workers [14] also pointed out the role of AuNPs on the accumulation of autophagosome and the impairment of lysosome degradation capacity. Since metal NPs, in general, are biopersistent and biodurable, the fast increase in their applications over the past decades has raised concerns on their effects on health and environments [15]. Recent report has pointed out that NPs, which tend to accumulate in the sludge instead of in the effluents of wastewater treatment plants, can have impact on microbial community in agricultural soils as it showed reduction in the fungal component when the sludge was mixed with soils [16]. Another report has suggested that AgNPs affect the growth and induce modifications in nutritional content of radish [17]. Within the scope of this study, I would like to discuss on the size- and shape-dependent cytotoxicity aspect of silver and gold nanoparticles.

2. Materials and Methods

2.1. Materials

The mold strains using in this study was an isolated strain *Aspergillus niger* D15 obtained from the laboratory of Department of Microbiology – Biochemistry - Molecular Biology, School of Biotechnology and Food Technology, Hanoi

* Corresponding author: Tel.: (+84) 4-3869-2764
Email: hieu.dangminh@hust.edu.vn

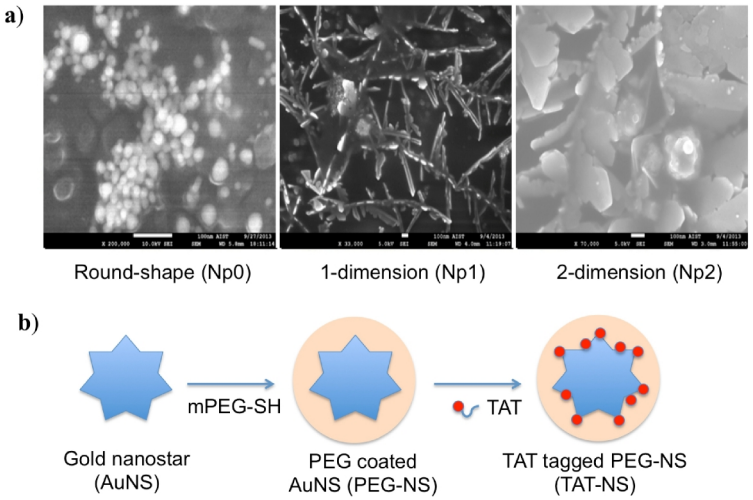

Fig.1. (a) SEM images of three different types of AuNPs. All AuNPs were prepared at concentration of 100 ppm. Scale bars: 100 nm. (b) Schematics for the making of TAT tagged gold nanostars.

University of Science and Technology (HUST). The BT549 breast cancer cell line (ATCC® HTB-122™) was a stable cell line provided by Vo-Dinh Lab. at Department of Biomedical Engineering, Duke University.

Gold(III) chloride trihydrate ($HAuCl^4 \cdot 3H_2O$), L (+)-ascorbic acid (AA), trisodium citrate dehydrate, 1 N hydrochloric acid solution (HCl), O-[2-(3-Mercaptopropionylamino)ethyl]- O′-methylpolyethylene glycol (mPEG-SH, MW 5000), silver nitrate ($AgNO_3$, 99.995%) were purchased from Sigma-Aldrich (St. Louis, MO). Cystein-terminated TAT peptide (residues 49–57, sequence Arg-Lys-Lys-Arg-Arg-Arg-Gln-Arg-Cys-$CONH_2$) was purchased from SynBioSci (Livermore, CA).

AgNPs of Np0 (round shape), Np1 (1-dimension shape) and Np2 (2-dimension shape) with longest diameter of 30 to 40 nm, 10 to 100 nm and 10 to 100 nm, respectively (Fig. 1a) were obtained from International Training Institute for Material Science (ITIMS) at HUST, and all preserved in deionized water at 100 ppm of concentration.

2.2. Silver and gold nanoparticle synthesis

Three types of AuNPs: AuNS (AuNPs with star-shape), PEG-NS (the AuNS coated with polyethylene glycol) and TAT-PEG-NS (the PEG-NS tagged with TAT peptide, a human immunodeficiency virus type 1 (HIV-1) encoded TAT peptide) were prepared following methods described by Yuan et al. [18] and Fales et al. [19]. Briefly, citrate gold seeds were prepared by adding 15 ml of 1% trisodium citrate to 100 ml of boiling HAuCl4 (1 mM) under vigorous stirring for 15 minutes. The solution was cooled and filtered by 0.22 µm nictrocellulose membrane. AuNS (~60 nm diameter) were prepared using a seed mediated method by quickly mixing AgNO3 (100 µl, 2-3 mM) and ascorbic acid (50 µl, 0.1 M) together into 10 ml of HAuCl4 (0.25 mM) with 12 nm citrate gold seeds (100 µl, OD520: 3.1) followed by filtration using 0.22 µm nictrocellulose membrane. PEG-NS were prepared by adding final 5 µM of PEG-SH to freshly synthesized gold nanostars for 10 minutes followed by one centrifugal wash then resuspending in pure ethanol. TAT-NS were prepared by mixing final 100 µM of TAT peptide in 1 nM of PEG-NS for 48 hours followed by two centrifugal washes in ethanol. Fig. 1b shows schematics for the three different types of gold nanostars. The characteristics of AuNPs including zeta potential, diameter and concentration were assessed by nanoparticle tracking analyzer NanoSight NS500 (Malvern Instruments Ltd., UK).

2.3. Growth inhibition test for A. niger

A. niger D15 was cultivated on PDA (Potato dextrose agar) medium at 30°C. After 72 hours of incubation, spores were harvested and preserved in physiological saline medium followed by determination of the spore density with hemocytometer equipment. The spore solution was then kept at 4°C in refrigerator until use.

The inhibition tests were conducted in glass tubes. Each tube was prepared with 5ml of PDB (Potato dextrose broth) medium containing AgNPs at a certain concentration. The test concentrations for NPs were 50, 25 and 12.5 ppm. Spore solution was added to each test tube to the spore density of 10^4 spores / ml. The control tube contained spores suspended in 5 ml of PDB without NPs. Tubes were then incubated at 30°C with shaking for 24 hours. 100

µl of suspension from each tube was spread on plate containing PDA medium follow with incubation at 30°C. The growth of *A. niger* can be justified on the culturing plates at 24 and 48 hours of incubation.

2.4. Cell viability test for human breast cancer cells

The BT549 cells were cultured in RPMI-1640 growth media (10% fetal bovine serum (FBS); Invitrogen, Carlsbad, CA), in an incubator with a humidified atmosphere (5% CO_2) according to the ATCC's protocol. The viability of the cells was measured using the CellTiter-Glo® luminescent Cell Viability Assay (Promega, Wyoming, USA). In principle, the amount of ATP molecules formed will be proportional to the number of cells alive in medium. By measuring the amount of ATP through the luciferase reaction which catalyzes the transform of beetle luciferin into oxyluciferin in the presence of Mg^{2+}, ATP and molecular oxygen and creates luminescent light, the number of cells in culture can be estimated. The detailed protocol for the Assay can be found on Promega website: www.promega.com. Briefly, BT549 cells in exponential growth phase were prepared in 96-well plates, 100 µl per well. Blank wells contained only RPMI-1640 medium without cells. AuNPs of three different types were added to experimental wells to three different final concentrations: 0.1, 0.2 and 0.3 nM, and incubated in the incubator with a humidified atmosphere (5% CO_2). Control wells contained 100 µl of cell suspension without AuNPs. At certain time points, plates were taken out and 100 µl of CellTiter-Glo® Reagent was added to each well. Contents were mixed for 2 minutes on an orbital shaker to induce cell lysis. Plates were then allowed to incubate at room temperature for 10 minutes to stabilize luminescent signal before recording with a FLOUstar® OMEGA multi-mode microplate reader (BMG Labtech, Germany).

3. Results and discussion

3.1. Shape-dependent inhibitory effects of AgNPs on the growth of A. niger

In these experiments, the inhibitory effects of AgNPs on *A. niger* growth were tested with three different kinds of NPs: round-shape, two- and one-dimension as described above in the Materials. Results indicated in Fig. 2 shows the growth of *A. niger* at 24 hours of incubation on PDA plates. Spores treated with different AgNPs at different concentrations showed different growth capacity. Data clearly indicated the inhibitory effects of NPs on the spore growth, which showed increased with the concentrations of NPs (Fig. 2a).

Comparing images taken at 48 hours of incubation, at 50 ppm of concentration, one- and two-dimensional AgNPs (Np1 and Np2) showed more efficient at growth inhibition than the three-dimensional NPs in term of retardation in spore formation when comparing the color of colonies formed. In case of Np0, from 24 hours to 48 hours of incubation, all colonies developed into totally dark colored colonies from the white ones indicated high level of spore formation while in the case of the other NPs, the white colonies developed into partly dark colonies indicated uncompleted spore formation. Several toxicity mechanisms for AgNPs have been

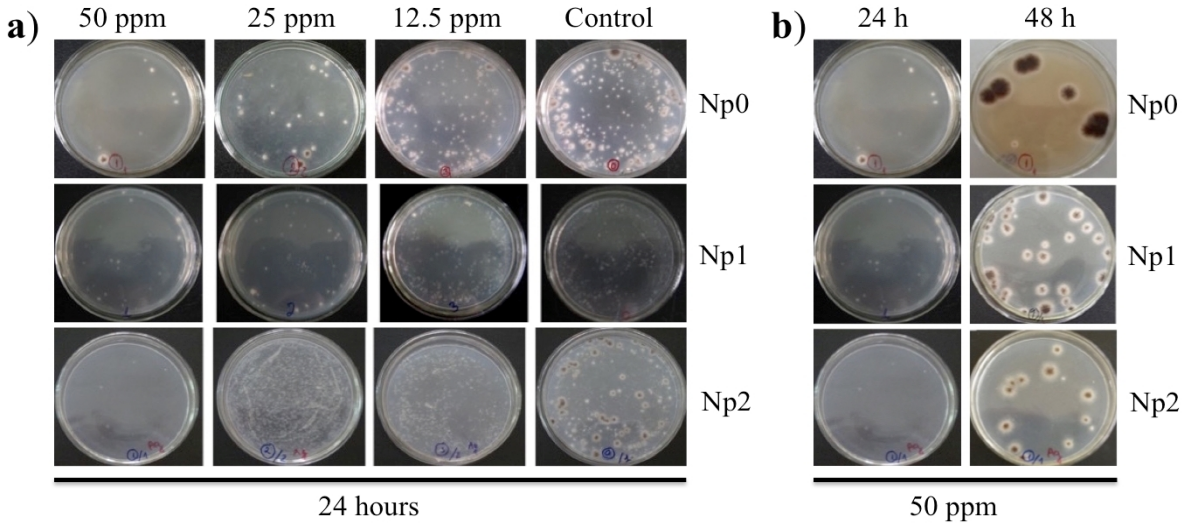

Fig. 2. Shape-dependent inhibitory effects of AgNPs on the growth of *A. niger*. (a) Mold growth at 24 hours of incubation after treated with different AgNPs and at different concentrations. (b) Mold growth at 24 and 48 hours of incubation after treated with different AgNPs at 50 ppm of the particles' concentration.

reported. The intensively discussed mechanism is that AgNPs can interact with cell membrane proteins, disrupt the integrity of cell membrane, activate signaling pathway, leading to inhibition in cell proliferation [20]. Another mechanism which has also been discussed involves the cellular uptake of AgNPs by diffusion or endocytosis that cause mitochondrial dysfunction, generation of Reactive Oxygen Species (ROS), leading to damage of proteins and nucleic acids inside the cells, and finally inhibition of cell proliferation or causing cell death [20-23]. A previous review has discussed the toxic effect of AgNPs to a broad spectrum of common fungi and a possible toxic mechanism of disruption of cell membrane, inhibition of normal building process [5]. The real underlying mechanism of action of AgNPs against fungi, however, so far has not been unveiled

3.2. Shape-dependent toxicity of gold nanoparticles on human breast cancer cells

Three types of AuNPs, which are nanostars (AuNS), PEG coated nanostars (PEG-NS) and the PEG-NS tagged with TAT peptide, the HIV-1 encoded peptide that were well studied for its function as facilitating the cell penetration, (TAT-NS) were tested for their toxicity to the human breast cancer cells, BT549. Results of the viability test, as shown in Fig. 3, indicated that BT549 cells were affected by all types of AuNPs. While the bare particles (AuNS) and the TAT-NS showed little impact on the cell viability with relatively unchanged amount of ATP after 26 hours of incubation (Fig. 3a and 3c), the PEG coated one showed remarkable impact since it indicated reductions in the ATP contents (Fig. 3b). In all cases, the impact of AuNPs on the cells did not indicated correlation with the concentrations of the nanoparticles. The control case showed growing trend of the ATP content correspond to increasing number of the cells which indicates normal cell functions.

Due to their physical and chemical properties, AuNPs recently have become attractive for biological and biomedical applications, especially as delivery vehicles for drugs, diagnostic tools and optical nanomaterials [24]. In general, the toxicity of nanomaterials can occur in several different mechanisms in the body, which are the induction of oxidative stress by free radical formation, interact with cellular components, disrupt or alter cell

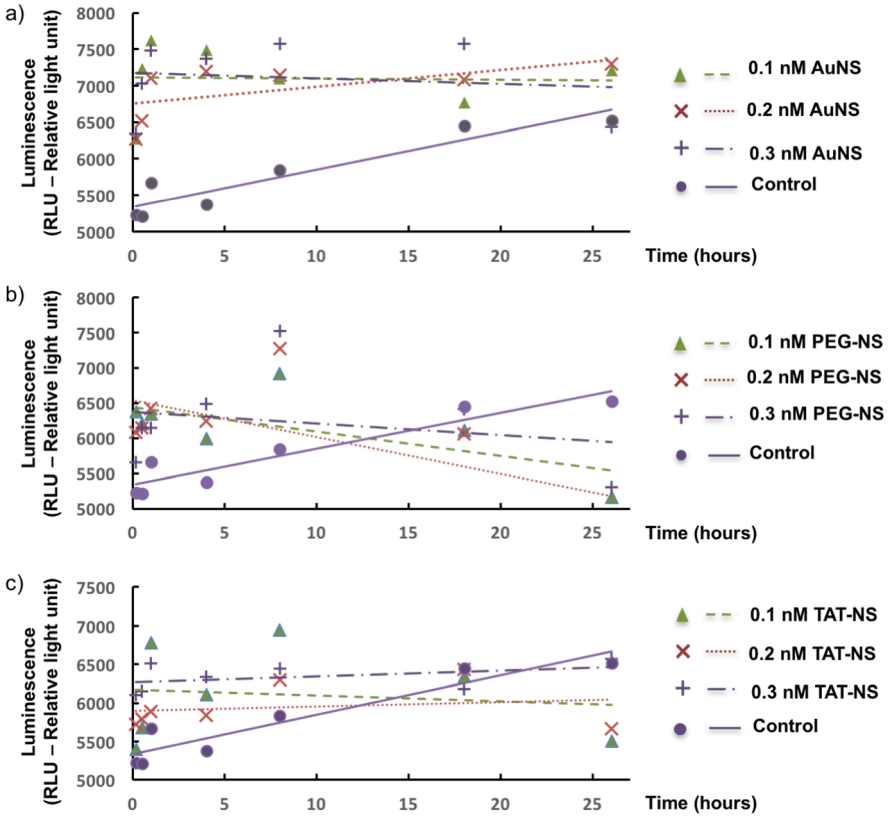

Fig. 3. Shape-dependent toxicity of (a) gold nanostars (AuNS), (b) PEG coated nanostars (PEG-NS), and (c) TAT tagged nanostars (TAT-NS) on BT549 human breast cancer cells. Lines represents the trend-lines.

functions, and cell/tissue accumulation, etc. [25]. Due to their unique properties, the toxicity of nanomaterials can be unique from xenobiotics and may be driven by the size, shape, chemical composition and surface characteristics. These could affect the mode of endocytosis, cellular uptake, efficiency of particle processing in the endocytic pathway, distribution and accumulation, interaction with other molecules or cell components, formation of free radical or decide short- or long-term toxicity. In this study, there are three types of AuNPs have been used in which the AuNS (around 60 nm diameter) could be the smallest in size and the other two could be similar in size. PEG was used for coating the AuNS to improve its stability, and thus increase the size of the particles. PEG, a common pharmaceutical excipient, is used extensively in commercial quantum dots (QDs) for stabilizing QDs in acidic environment inside the cell after endocytosis. Previous study on PEG-QDs found no significant toxicity on cells, but differences in accumulation and clearance [26]. However, another study by Zhang and co-workers [27] has observed size-dependent in vivo toxicity of PEG coated AuNPs on mice at a dose of 4000 µg/kg body-weight. Although the study could not conclude the smaller particles have greater toxicity, the authors suggested the further metabolism of the particles should be considered as an important issue. On the other hand, TAT peptide is a well-studied member of the cell-penetrating peptides (CPPs) family, which facilitates the transfer of the nanoparticles across cell boundary [24]. Although there is no direct evidence, the toxicity of AuNPs in this study could come from the accumulation and/or interaction of the particles with cell components, and that the toxicity of PEG-NS was highest in compare to the others might correlate to the ease of the transportation across cell membrane which may decide how the integrity of the membrane could remain.

4. Conclusions

In conclusion, the study has assessed the health and environmental risks of silver and gold nanoparticles. It pointed out that AgNPs can inhibit the growth of the mold *A. niger* and the one- and two-dimension particles shows more effective than the round (none-dimension) ones. Three types of AuNPs shows affected the viability of the BT549 human breast cancer cells. However, the PEG-NS indicates highest toxicity to the cells in compare to the two other AuNPs. Future study should focus on the interaction, accumulation, distribution of the nanoparticles in order to unveil the mechanism of their toxicity.

Aknowledgements

The author thanks to Dr. Ho Phu Ha, School of Biotechnilogy and Food Technology and the International Training Institute for Material Science, HUST for generous providing silver nanoparticles. The author also thanks to Prof. Vo Dinh Tuan, Fitzpatrick Institute for Photonics, Duke University, USA for providing chance to visit and practice the making of gold nanoparticles. The author acknowledges kind support from the members of Vo-Dinh Lab during the visit stay.

References

[1] Ai J. et al. Nanotoxicology and nanoparticle safety in biomedical designs. *Int J Nanomed.* **6** (2011): 1117-1127.

[2] Lara H. H. et al. Silver nanoparticles are broad-spectrum bactericidal and virucidal compounds. *J Nanobiotechnology* **9** (2011):30.

[3] Taylor E. and Webster T. J. Reducing infection through nanotechnology and NPs. *Int J Nanomed.* **6** (2011): 1463-1473.

[4] Austin L. A. et al. The optical, photothermal, and facile surface chemical properties of gold and silver NPs in biodiagnostics, therapy and drug delivery. *Arch Toxicol.* **88** (2014): 1391-1417.

[5] Ge L. et al. Nanosilver particles in medical applications: synthesis, performance and toxicity. *Int J Nanomed.* **9** (2014): 2399-2407.

[6] Srisam M. et al. Single Nanoparticles Plasmonic Sensors. *Sensors* **15** (2015):22774-22792.

[7] Calixto G. M. F. et al. Nanotechnology-Based Drug Delivery Systems for Photodynamic Therapy of Cancer: A Review. *Molecules* **21** (2016):342.

[8] Mocan L. et al. Advances in cancer research using gold nanoparticles mediated photothermal ablation. *Clujul Medical* **89**(2) (2016):199-202.

[9] Vo-Dinh T. et al. Plasmonic Nanoprobes: From Chemical Sensing to Medical Diagnostics and Therapy. *Nanoscales* **5**(21) (2013):10127-10140.

[10] Bucharskaya A. et al. Towards Effective Photothermal/Photodynamic Treatments Using Plasmonic Gold Nanoparticles. *Int. J. Mol. Sci.* **17** (2016):1295.

[11] Fröhlich E. Value of phagocyte function screening for immunotoxicity of NPs in vivo. *Int J Nanomed.* **10** (2015): 3761-3778.

[12] Katsnelson B. A. et al. Some inferences from in vivo experiments with metal and metal oxide NPs: the pulmonary phagocytosis response, subchronic systemic toxicity, genotoxicity, regulatory proposals, searching for bioprotectors (a self-overview). *Int J Nanomed.* **10** (2015): 3013-3029.

[13] Abdelhalim M. A. K. et al. Gold nanoparticles induced cloudy swelling to hydropic degeneration, cytoplasmic hyaline vacuolation, polymorphism, binucleation, karyopyknosis, karyolysis, karyorrhexis and necrosis in the liver. *Lipids in Health and Disease* **10** (2011):166.

[14] Ma X. et al. Gold Nanoparticles Induce Autophagosome Accumulation through Size-Dependent Nanoparticle Uptake and Lysosome Impairment. *ACS Nano* **5**(11) (2011):8629-8639.

[15] Utembe W. et al. Dissolution and biodurability: Important parameters needed for risk assessment of nanomaterials. *Particle and Fibre Toxicology* **12** (2015): 11.

[16] Durenkamp M. et al. NPs within WWTP sludges have minimal impact on leachate quality and soil microbial community structure and function. *Environ. Pollut.* **211** (2016): 399-405.

[17] Zuverza-Mena N. et al. Effect of silver NPs on radish sprouts: Root growth reduction and modifications in the nutritional value. *Front. Plant Sci.* **7** (2016): 90.

[18] Yuan H. et al. TAT Peptide-Functionalized Gold Nanostars: Enhanced Intracellular Delivery and Efficient NIR Photothermal Therapy Using Ultralow Irradiance. *J. Am. Chem. Soc.* **134** (2012): 11358-11361.

[19] Fales A. M. et al. Development of Hybrid Silver-Coated Gold Nanostars for Nonaggregated Surface-Enhanced Raman Scattering. *J. Phys. Chem.* **118** (2014): 3708-3715.

[20] McShan D. et al. Molecular Toxicity Mechanism of Nanosilver. *J Food Drug Anal.* **22**(1) (2014): 116-127.

[21] Rinna A. et al. Effect of silver nanoparticles on mitogen-activated protein kinases activation: role of reactive oxygen species and implication in DNA damage. *Mutagenesis* **30** (2015): 59-66.

[22] Kaba S. I. and Egorova E. M. In vitro studies of the toxic effects of silver nanoparticles on Hela and U937 cells. *Nanotechnology, Science and Applications* **8** (2015): 19-29.

[23] Bao H. et al. New Toxicity Mechanism of Silver Nanoparticles: Promoting Apoptosis and Inhibiting Proliferation. *Plos ONE* **10**(3) (2015): e0122535.

[24] Lévy R. et al. Gold nanoparticles delivery in mammalian live cells: a critical review. *Nano Reviews* **1** (2010): 4889.

[25] Aillon K. L. et al. Effects of nanomaterial physicochemical properties on in vivo toxicity. *Adv Drug Deliv Rev* **61**(6) (2009): 457-466.

[26] Ballou B. et al. Noninvasive imaging of quantum dots in mice. *Bioconjug. Chem* **15** (2004):79–86.

[27] Zhang X-D. et al. Size-dependent in vivo toxicity of PEG-coated gold nanoparticles. *Int J Nanomed.* **6** (2011):2071-2081.