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Comparative Study of Genotoxicity of Silver and Gold Nanoparticles Prepared by the Electric Spark Dispersion Method

Evgenii Plotnikov[1*], Sergey Zhuravkov[1], Andrew Gapeyev [2], Vladimir Plotnikov[3], Irina Martemianova[1], Dmitrii Martemianov[1]

[1]Tomsk Polytechnic University, Lenin Av. 30, Tomsk, Russia.
[2]Institute of Cell Biophysics of Russian Acad. Sci., Pushchino, Russia.
[3]Polytech ltd, Asinovskaya str. 7, Tomsk, Russia.

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ABSTRACT

Objective: There is plenty of information dedicated to nanomaterial toxicity, but it is often contradictory. This work presents results of the comparative investigation of silver and gold nanoparticles genotoxicity and genotoxic effect of water medium after the spark dispersion process.
Material and methods: The nanoparticles were produced in water by the modified energy-efficient electric spark dispersion method. The comet assay, transmission electron microscopy, DLS spectroscopy and thermal desorption of nitrogen (BET method) were used for the investigation.
Results: The silver nanoparticles showed a genotoxic effect which appeared at concentration of 0.03 mg/ml and above. The application of gold nanoparticles did not lead to a significant DNA damage at concentrations range of 0.01 - 0.03 mg/ml. However, a notable level of genotoxicity was observed at concentrations of gold nanoparticles about 0.1 mg/ml. Supernatant water medium (with trace of Au and Ag metals) following the spark dispersion process showed no genotoxic action.
Conclusion: Accordingly, the testing of the nanoparticles produced by the electric spark dispersion in water revealed more expressed genotoxic effect of silver nanoparticles in comparison with the gold ones.

INTRODUCTION

At nanoscale, usual materials can show different properties, because of an enormously increased surface area. Nanoparticles cause different biological effects that make it potentially more harmful for living organisms (Barar, 2015; Yah, 2013). High reactivity properties can be potentially hazardous to human health and the normal functioning of biological systems. The task to evaluate the genotoxicity of different nanoparticles is important to this day and much scientific effort has been concentrated in this area (Karlsson, 2010). Nowadays, gold and silver nanoparticles are used in a variety of applications, such as bio labeling, catalysis, electrochemistry etc (Perevezentseva *et al.*, 2014). For instance, silver has been widely used for anti-bacterial purposes since the ancient time (Plotnikov *et al.*, 2016). However, reliable scientific sources on potential harmful effects of nanoparticles are insufficient as well as a lack of common standard for nanotoxicology.

This problem has been gradually tackled only recently. Despite common efforts, the results are often contradictory, especially for *in vitro* and *in vivo* toxicity of nanoparticles (Bondarenko, 2013). Nanosilver has been known as a toxic agent for macro- and microorganisms. Some work shows the opposite (Charehsaz *et al.*, 2017).

The hormesis effect of silver nanoparticles has been revealed for cells cultures (Jiao *et al.*, 2013). Gold nanoparticles were also known to exhibit in vitro geno- and cytotoxicity, but for the cell culture showed the absence of dose-dependent genotoxicity (Paino *et al.*, 2012; Schulz *et al.*, 2011). However, some reviews have shown the lack of a genotoxic potential of silver and gold nanoparticles (Nam *et al.*, 2013). The method of synthesis and the presence of residual contaminants could also influence on the nanoparticles toxicity (Samberg *et al.*, 2011).

* *Corresponding Author*
Email: plotnikov.e @ mail.ru

The behavior of nanoparticles inside the cells and metabolic and immunological responses induced by these particles are still not clear enough. One of the way occurrences of silver nanoparticles genotoxicity is the mitochondrial damage followed by the increase of reactive oxygen species (ROS) that enhance DNA damage in a dose-dependent manner (AshaRani *et al.*, 2009). Oxidative stress plays an important role in nanoparticles effects, including oxidative damage to protein and DNA (Xia *et al.*, 2006). Antioxidants have a strong influence on cytotoxicity of nanoparticles that proved ROS-induced mechanism of genotoxicity (Foldbjerg *et al.*, 2011). One of the main factors of nanoparticles toxicity is a particles size (Park *et al.*, 2011). Even narrow differences in nanoparticle sizes may cause significantly different overall biological response (Coradeghini *et al.*, 2013). Here we applied a comet assay for comparative investigation of genotoxicity of gold (Au) and silver (Ag) nanoparticles, prepared by the modified electric spark dispersion method in water.Despite of plenty different method of nanoparticles production, including even laser ablation (Stašić, et al, 2016), modified electric spark dispersion allowed to produce nanoparticles of different shapes and properties by changing experimental medium and parameters of electric impulses (Zhuravkov *et al.*, 2014). The comet assay is a convenient and informative method for genotoxicity studies (Vandghanooni *et al.*, 2011). The main aim of this work is to evaluate the possible genotoxicity of obtained nanoparticles.

MATERIALS AND METHODS

Nanoparticles preparation

The device for an electric spark pulse dispersion of metals (Figure 1) was used to produce nanoscale silver and gold powder. This device was developed and produced in Tomsk Polytechnic University (Tomsk, Russia).

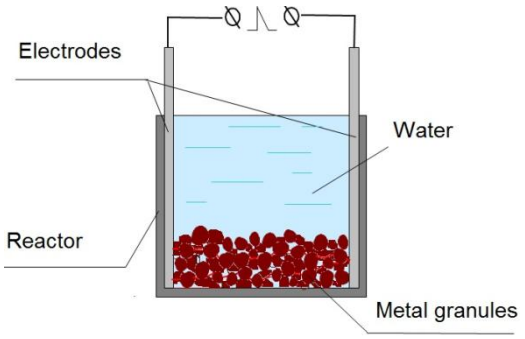

Fig. 1: Schematic illustration of the device for nanoparticle preparation. The silver and gold are shown as metal granules.

The nanoparticles preparation procedure is described at length in our previous work (Zhuravkov *et al.*, 2014; Plotnikov *et al.*, 2014). In brief, the reactor was filled with gold or silver granules of different forms and diameters. Under the impact of the current pulse (duration of 15 μs with the voltage amplitude of 500 V, the current amplitude 250 A, and frequency of pulses in the range from 300 to 1000 s^{-1}) a lot of micro discharges were generated, that led to metal melting, evaporation and spraying of the small metal parts. The obtained precipitates were dried.

Methods for characterization of nanoparticles

Surface characteristics (including specific surface area, pore volume etc) were determined by the nitrogen thermal desorption (BET method) using a surface area and porosity analyzer Sorbtometr M (CJSC «Catacon», Russia, Novosibirsk). The method provides a measurement of a specific surface area at different partial pressures of the adsorbate gas (nitrogen) and allows to determine the micropore volume and the total volume of meso- and macropores in test samples.

The morphology of the nanoparticles was studied by a transmission electron microscope JEM-2100 (JEOL, Japan). The dynamic light scattering spectroscopy was applied for nanoparticle size determination by Zetasizer Nano (Malvern Instruments Ltd, UK). Gold and silver nanopowders were dispersed in water by sonication, prior to testing.

Comet assay for genotoxicity assessment

The genotoxicity of nanoparticles was assessed by the alkaline comet assay with modification, as described in previous work (Plotnikov *et al.*, 2014; Gapeyev *et al.*, 2011). The applied method was based on the analysis of cells with stained DNA (Ostling *et al.*, 1984; Tice *et al.*, 2000). For each experiments mouse leukocytes were used as a test model. Experiment included the following steps. Blood samples were collected in tubes containing phosphate buffered saline (PBS, 136.7 mMNaCl, 2.7 mMKCl, 8.1 mM Na_2HPO_4, and 1.5 mM KH_2PO_4; pH=7.2) and anti-coagulating agent. Then the blood was diluted to the final concentration of leukocytes 1×10^6 cells/ml. Tested nanoparticles in concentrations range of 0.01 - 0.1 mg/ml were incubated with blood cells at 37°C for 30 min. Distilled water was used as a negative control. The positive control contained cells with hydrogen peroxide (0.07 μg/ml). The slides for microscopy were made of 0.5% low-melting agarose (Serva, Germany). The cells were immobilized in the middle layer of the slide. Then, the samples were treated in lysing and stayed at 37°C for 25 min and after that treated by alkaline solution at 4°C for 20 min both in dark places. Electrophoresis of all slides were carried out at the electric filed intensity 2 V/cm, current 300 mA. After the electrophoretic procedure, the slides were washed and stained with ethidium bromide. All samples were stored in dark place. The calculation procedure was provided by a Comet Expert System (Gene Expert, Russia). The DNA damage was registered in at least 50 cells per sample and represented as olive tail moment (tail DNA, %×μm).

Statistical analysis

All experiments were repeated three times. The results are presented as mean values and standard errors of the mean (SEM). Different groups of data were compared by Mann-Whitney *U*-test.

RESULTS

Characterization of tested nanoparticles.

Many scientists have suggested that the size and shape are critical factors for nanoparticle-induced toxicity. The potency of nanoparticles to induce cell damage is size-dependent. Nanoparticles were characterized by TEM, DLS and BET methods to determine size and morphology.

Figure 2 (a, b) shows the TEM-images of the gold and silver powder samples. The results of nanoparticles size determination are shown in figure 2 (c, d). It was found that 96% of the particles have a size in the range from 25 to 200 nm. The evaporation of the metal in the area of micro-discharges leads to subsequent condensation of the vapors and the particle formation. According to figure 2, gold and silver particles have rather similar morphology.

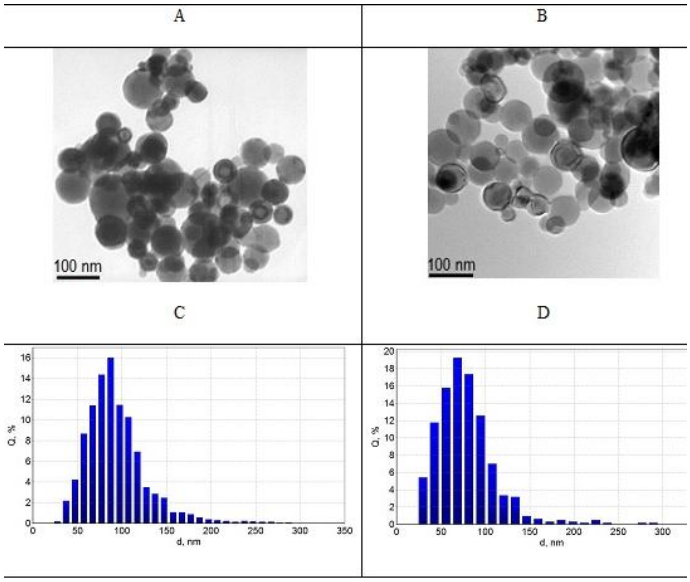

Fig. 2: TEM-images and size distribution of the tested gold (a, c) and silver (b, d) nanoparticles.

Detailed characteristics and properties of gold nanoparticles of this type were shown previously (Plotnikov *et al.*, 2014).The estimated specific surface of powder equals to 5.49 m^2/g and 5.75 m^2/g for gold and silver particles respectively.

Assessment of In Vitro genotoxicity of gold and silver nanoparticles

The incubation of white cell culture in the presence of gold nanoparticles caused notable growth of DNA damage level only at concentrations of 0.1 mg/ml or above, as it is shown in figure 3. According to the data on figure 3, tested nanoparticles expressed dose-dependent genotoxicity. However, gold nanoparticles caused less DNA damage in all concentration range. At high tested concentration (0.1 mg/ml) gold and silver nanoparticles revealed DNA damage comparable to hydrogen peroxide (0.07 µg/ml). The comparison of cell distribution by the level of the DNA damage (tail DNA) revealed heterogeneous results (Figure 4).

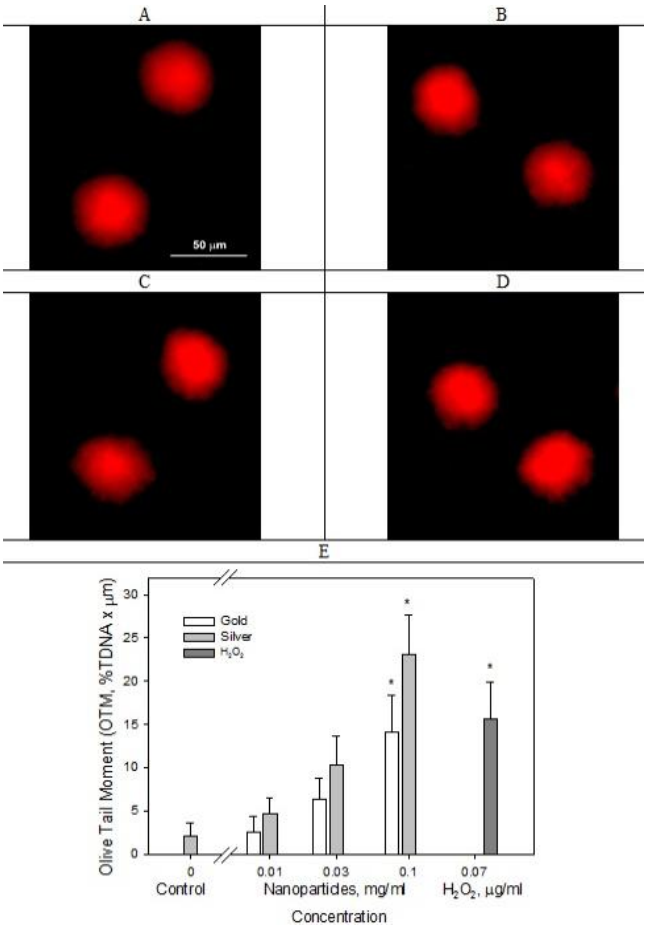

Fig. 3: The DNA damage detection by comet assay in white blood cells. Images of comets in the negative control samples (A), the samples after the incubation of whole blood leukocytes with nanoscale gold (B) and silver (C) particles at a concentration of 0.1 mg/ml, the positive control (0.07 µg/ml H_2O_2 for 10 min at 37°C) samples (D), scale bar is 50 µm. The DNA damage level (Olive tail moment) in white blood cells under the influence of gold and silver nanoparticles (E). The cells were incubated in the presence of nanoparticles for 30 min at 37°C. * P <0.02 versus corresponding control by Mann-Whitney test.

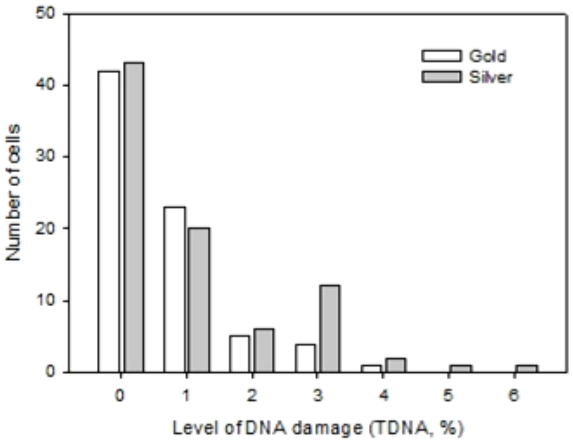

Fig. 4: Cell distribution histogram by the level of DNA damage (Tail DNA, %) After incubation of whole blood leukocytes with nanoscale gold and silver particles at a concentration of 0.1 mg / ml.

DISCUSSION

The testing of the working solution (in which nanoparticles were prepared) allowed to determine the genotoxic effect of trace metals due to the electric spark dispersion and other influences on water medium during the process. It should be noted, that metal nanoparticles release metal ions, which can lead to oxidative stress and the consequent DNA damage. Another possibility might be a genotoxic mechanism that depends on the electrical charge of the particle surface (Klien *et al.*, 2012). The conducted experiment revealed that the application of supernatant water on the leukocytes cell culture after the electric spark dispersion of metals did not cause any DNA damage. The genotoxic effect of the supernatant water was not significantly different from the control sample (Plotnikov *et al.*, 2014). It means that there is no influence on the genotoxicity by trace metal and residual changes of water medium after the complex electromagnetic and thermal impact.

The representative images of comets in control samples and after incubation with nanoscale gold and silver particles at a concentration of 0.1 mg/ml are presented in figure 3. The DNA damage represented in Olive tail moment for all samples revealed low gene damage under influence of both type of nanoparticles up to 0.1 mg/ml concentration (Figure 3E). The level of the DNA damage by a silver concentration of 0.1 mg/ml was close to the damage induced by hydrogen peroxide at concentration of 0.07 µg/ml, as standard oxidative agent. At lower concentration of gold nanoparticles (0.01 mg/ml) there was no significant increase in DNA damage compared to the control sample. The growth in gold toxicity observed in the experiment at 0.03 mg/ml, but it cannot be considered as significant. Silver nanoparticles showed slightly more pronounced genotoxicity in all comparative points. However, the difference between gold and silver genotoxicity is not as critical. According to figure 4, silver and gold nanoparticles could cause similar DNA-damage at high concentrations. Moreover, the intact and differently damaged nucleoids were detected in the same sample for both metals.

Supposedly, the incubation of the leukocytes with nanoparticles lead to phagocytosis activation and eventually to the rise of the DNA damage level. This was indirectly confirmed by earlier results of the nanoparticles genotoxic testing and scientific literature (Jena *et al.*, 2012; Plotnikov *et al.*, 2015). Most researchers agree with the opinion that the leading factor of the DNA damage is the oxidative stress induced by the nanoparticles for *in vivo* and *in vitro* applications. It is presumably the main factor of low nanoparticles genotoxicity. Similar mechanisms were found in both macroorganism and in cell cultures (Rim *et al.*, 2013). Big particles exhibited less DNA damage compared to the small ones. The cell vulnerability is presumably explained by not only the oxidative stress, but also by other mechanisms which were likely involved, including the alteration of their proliferation, differentiation, or cell-to-cell signaling (Liu *et al.*, 2012; Eustaquio *et al.*, 2012). The catalytic properties of metals could also directly increment the oxidative chain reactions. The gold nanoparticles rapidly catalyze the decomposition of hydrogen peroxide, leading to the formation of hydroxyl radicals that in turn caused excessive oxidation damage (He *et al.*, 2013).Toxicity of AgNPs was decreased by the antioxidants; that also proved major role of oxidation as the main mechanism (Kim *et al.*, 2011).The molecular way of this mechanism include the disruption of the mitochondrial respiratory chain leading to the production of reactive oxygen species and the interruption of ATP synthesis in cells (AshaRani *et al.*, 2009).However, one study shows the results of much higher toxicity of silver compared with gold *in vivo* for zebrafish embryos, meaning that the revealed DNA damage is not a main factor of overall biotoxicity of silver (Bar-Ilan *et al.*, 2009).In this regard, it is important to take into account the particle size. The bio impact of nanoparticles is highly size-dependent (Barar, 2015).

CONCLUSION

The silver and gold nanoparticles prepared by electric spark dispersion induced a significant augment in DNA damage only in high concentrations about 0.1 mg/ml and more. Presumably, the increase in the DNA damage is related to the contact of nanoparticles with phagocytes and subsequent cell activation. Supernatant water after the spark dispersion of metal has shown the absence of genotoxic properties which means there has been no influence of residual amounts of metals and other physicochemical factors. The gold and silver nanoparticles exhibited dose-dependent genotoxicity on blood leukocytes. The silver nanoparticles showed relatively more expressed genotoxic effect at low dosage, compared to the gold ones.

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CONFLICT OF INTEREST

The authors declare that they all have no conflict of interest.

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