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Influence of Silver and Selenium Nanoparticles on Mesenchymal Stromal Cells

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Silver consisting drugs are used in the prevention and treatment of bacterial and viral diseases. Selenium consisting drugs is used as antioxidant and antitumor preparations in pharmaceutics. Biological activity and toxicity determine the dose and form of these elements. It means that the particle size reduction increases efficiency. Nanoparticles were obtained by chemical methods. The suspensions were analyzed using the cross-correlation method of photons. It was proved that the increase of concentration of silver nanoparticles 20-45 nm are observed of morphological changes and apoptosis of cells in the cell culture. Selenium particles with a minimum size of 500 nm and a minimum dose of 0.005 mg / ml cause a toxic effect: a decrease in survival rate, single morphology changes.

Keywords: nanotoxicity, influence of nanoparticles, toxicity of nanoparticles, mesenchymal stem cell.

Introduction

Currently, nanoparticles (NP) are widely used in the development of drugs and biologically active additives [Jones S.A., 2004; Elechiguerra et al, 2005].

However, the accelerating production and introduction into commercial products of AgNPs have consequence to release into the environment and affect the environmental concerns and humans health. There are many questions to be understood the positive and negative effects of AgNPs. To elucidate the environmental transformation of AgNPs, the behavior of AgNPs should be thoroughly monitored in complex environmental relevant conditions [Liu et al, 2015; McGillicuddy et al, 2017].

NP successfully transported through the barriers of the body, reached various populations of cells of organs and tissues, including mesenchymal stromal cells (MSC)[Schwartz S., 2009]. The fissions errors of MSCs are fundamental in the cancer development [Wang, 2013]. There are data about toxic effect of NP, the ability to exert negative effects at the cellular level, leading to metabolic disorders, genetic changes, apoptosis and necrosis in the current scientific literature [Foldbjerg et al, 2009]. The aim of this work is to determine the effect of silver and selenium nanoparticles on the morphology and survivability of MSC cultures.

Currently, silver NP are widely used in medicine, pharmacology, as hygienic and packaging, BADS and other applications. Silver NP is used in bone and cartilage transplants due to its antimicrobial and antiviral properties. The studies focus on the study of cytotoxicity, genotoxicity and cell differentiation, depending on the particle size, their concentration and exposure time [Tran et al, 1997].

Selenium hold the potential to bioaccumulation and cryptotoxic to ecosystems. Though selenium is found naturally in the earth's crust, especially in carbonate rocks and volcanic and sedimentary soils, about 40% of the selenium emissions to atmospheric and aquatic environments are caused by various industrial activities such as mining-related operations [Tan et al, 2016].

The role of selenium compounds have been extensively studied as antioxidants, in the prevention and treatment of hepatitis and various types of tumors [Yu et al, 1997]. The dose and form of selenium are important factors determining its biological activity, toxicity and antitumor activity [Beheshti et al, 2013]. Studies show that the action of selenium extends to several types of tumors, including the breast, lung, prostate and colon.

New results of influence of a number of inorganic NP on the viability of mesenchymal precursors were obtained in this research.

Methods

Synthesis and analysis of the nanoparticles

A suspension of silver nanoparticles was prepared by the reduction of tannin salts of AgNO3 or Ag2SO4 in the presence of a buffer solution (LenReaktiv, Russia).

Selenium nanoparticles were synthesized in reaction of selenious acid (LenReactiv, Russia) and ascorbic acid (Biosynthesis, Russia) according to equation:

$$H_2SeO_3 + 2C_6H_8O_6 \rightarrow Se + 3H_2O + 2C_6H_6O_6 \quad (1)$$

and leaded to the formation of a zero-valence - selenium sol Se0 and dehydroascorbic acid. Sol was unstable in solution and precipitates after 24 hours. In this work, BSA of the culture medium (10%, BioSera, Brazil) was used as the stabilizer.

The size of nanoparticles were determinated by the photon's cross-correlation method (Nanophox).

Isolation of MSC and coculture with nanoparticles

Mesenchymal stem cells (MSC) was isolated by enzymatic method. DMEM medium (PanEco, Russia) was used for culture cells, containing fetal bovine serum (FBS, 10%), and the penicillin–streptomycin solution (1%, 10 000 units of penicillin and streptomycin (10 mg in 0.9% NaCl)) in a humidified atmosphere of CO2 (5%) and air (95%) at 37 °C. Production of CD34-, CD45-, CD14-, CD20-, CD44+, CD73+, CD90+, CD105+ was proved by flow cytometry. After 5-7 days, the cell culture was more than 80% confluent, 0.25% trypsin-0.02% EDTA was used for the re-seeding, seeded in a ratio of 1: 2, NP was added on the third passage.

The effect of silver NP was evaluated in cultures with doses of NP 0.004, 0.008, 0.016 mg / ml from $AgNO_3$ and Ag_2SO_4 for 2 weeks. Further experience with silver content of 0.008, 0.016, 0.024, 0.032 mg / ml was carried out with NP from AgNO3 also for 2 weeks.

The effect of selenium on MSC was studied according to the scheme: 0.005 and 0.01 mg / ml Se^0 6000 nm, 0.005 and 0.01 mg / ml Se^0 700-1200 nm, 0.005 and 0.01 mg / ml Se^0 500-1000 nm during of the week.

Analysis of morphological changes and toxicity

Morphological evaluation was performed using the method of phase-contrast microscopy using criteria such as the typical/ untypical cell morphology, the presence of apoptotic bodies, and the confluence of the culture. The cytotoxicity assay was performed by using Goryaev's camera with Trypan blue staining.

The nanoparticles synthesis, exposure them to cultures and analysis were repeated more than 10 times, the results of the counting in Gorjaev's chamber were counted using the Student's ratio (p = 0.05) using the Excel Office software.

Results and Discussion

Silver NP

Suspensions of 4 species were obtained: 0.001% of the AgNO3 salt, 0.002% of the AgNO3 salt, 0.001% of the Ag2SO4 salt, 0.002% of the Ag2SO4 salt. The resulting suspensions were analyzed using the cross-correlation method of photons (Nanophox): the suspension contained nanoparticles from 25 to 60 nm, the peak in the distribution curve was in the range of 30 to 50 nm (Fig. 1).

As a result of the experiment to determine the influence of silver NP depending on the precursors, it was found that low doses of nanoparticles (0.004 mg / ml, 0.008 mg / ml, 0.016 mg / ml) from nitrate and silver sulfate salts do not significantly affect on the morphology of the cells for three weeks of cultivation. Explicit differences in the morphology of cells with prolonged exposure of NP obtained from different salts have not been detected.

The control culture of cells without exposure to them by nanoparticles is shown in Fig.2., which reached full confluency on the 14th day of passage (2.1). In the photo, a spindle-like fibroblaste-like cell form (2.2.b.) characteristic of MSC, nuclei with nucleoli (2.2.a.) are clearly visible.

It was possible to establish that increase silver-NP dose caused the decrease of proliferative activity, cell apoptosis becomes more frequent: in Fig. 3. photo of the cell culture after cultivation with 0.032 mg / ml silver nanoparticles is presented. There was cells in the apoptosis in the culture in Fig. 3 (a), that the changes follows it in their morphology: cell loss of mass, detachment of membranes from plastic, the formation of apoptotic bodies, and, as a result, an increase the debris in culture. Counting cells in Goriayev's chamber confirms that the number of living cells decreases

Fig. 1

Graph of the silvernanoparticles size distribution in suspension

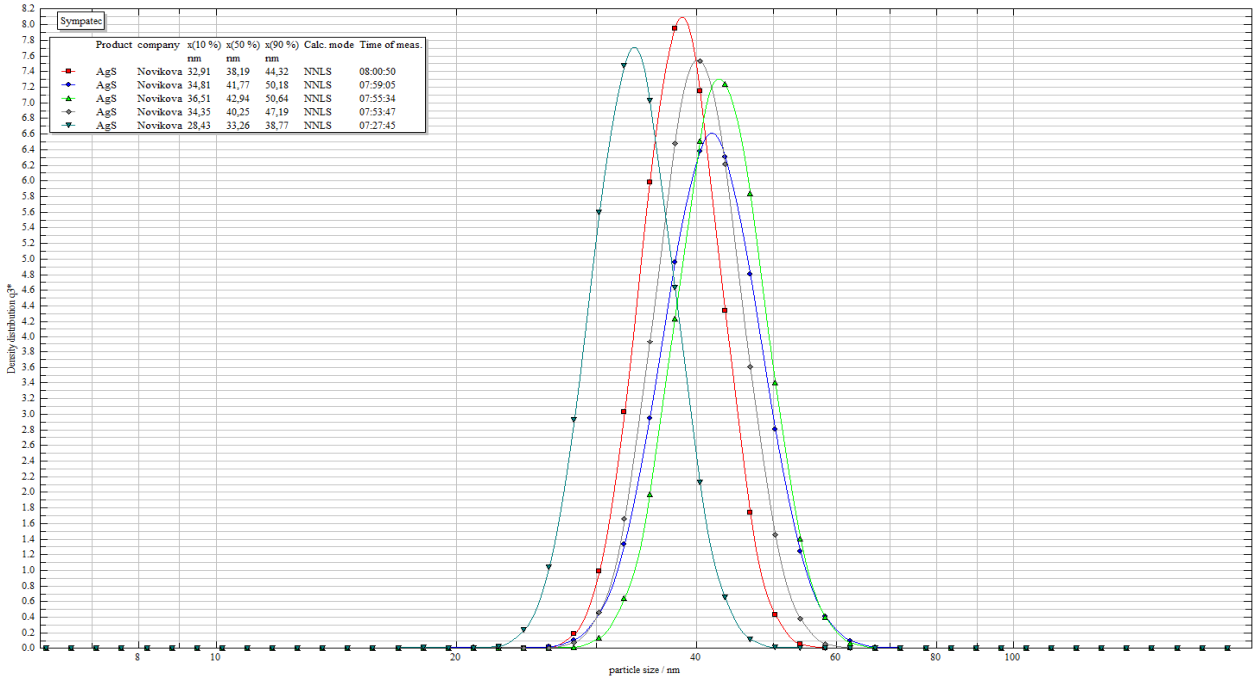


Fig. 2

Control cell culture low-power and high-power fields: 2.1. 40× magnification general view of the monolayer of cells, 2.2. 400× magnification: a-core and nucleoli, b-cell, c-debris

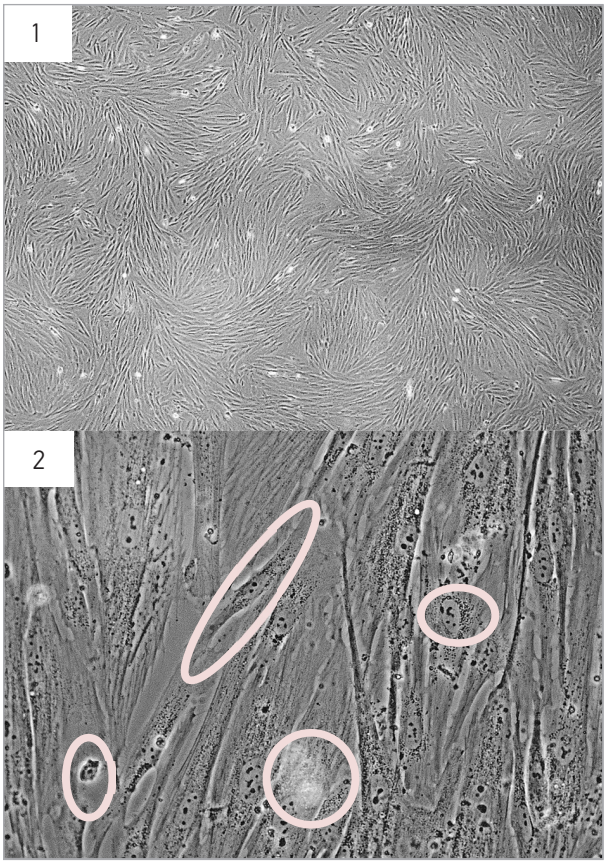
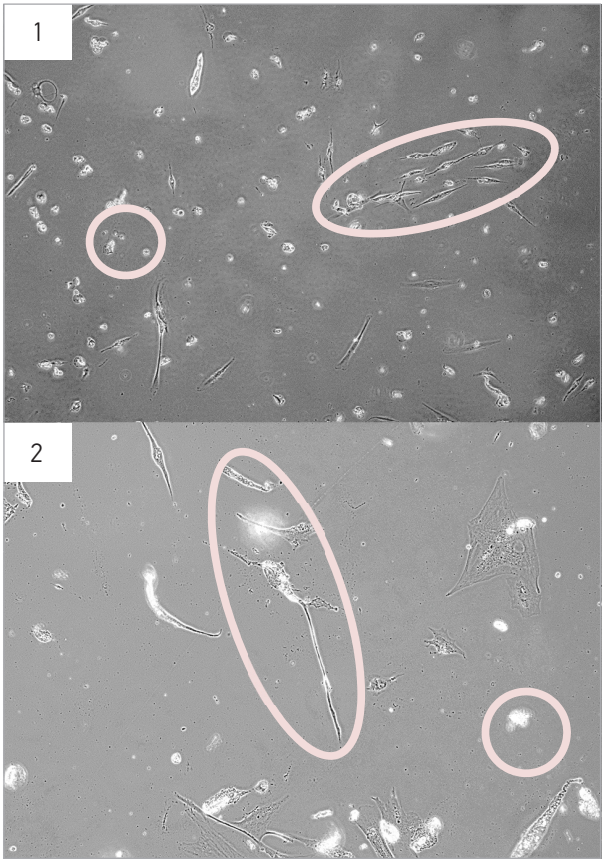


Fig. 3

Culture of cells with silver-NP concentrations of 0.032 mg / ml on the 14th day: 3.1. 40× magnification 3.2. 400× magnification where a - cells in the apoptosis stage, b - debris



in the culture, the survival rate decreases with an increase in the dose of silver nanoparticles in culture.

Thus, it has been established that silver nanoparticles have a proportional concentration of negative impact on MSCs. There is an oppression of proliferative activity, a change in morphology, apoptotic cells appear.

Selenium NP

As a result of the production of selenium nanoparticles, the suspension was analyzed by cross-correlation of photons, which showed that the particles reached a size of more than 6000 nm (Fig. 4D). The suspension obtained after the centrifugation was also analyzed using the cross-correlation method of photons: it contained particles ranging in size from 700 nm to 1200 nm (Fig. 4C). After the inclusion of an additional filtration step through a 0.22 μm filter, the analysis showed that particles were obtained from 500 nm to 1000 nm (Fig. 4B). After further centrifugation and filtration, we obtained NP from 50-100 nm (Fig. 4.A.). Thus, the particles obtained in groups B, C and D was not nanoparticles. But their influence on MSC was still appreciated.

Morphological changes in cell cultures with selenium in groups with NP around 500-1000 nm and 700-1200 nm caused similar effects: the cells changed their characteristic form (5.a), cell proliferation slowed down, the number of cells decreased, which is indicated by low confluency cultures (Fig.5.) in comparison with the monolayer of the control (Fig.2.), large conglomerates of particles with proteins were found (5.b.).

Fig. 4

Graph of the selenium nanoparticles size distribution in suspension

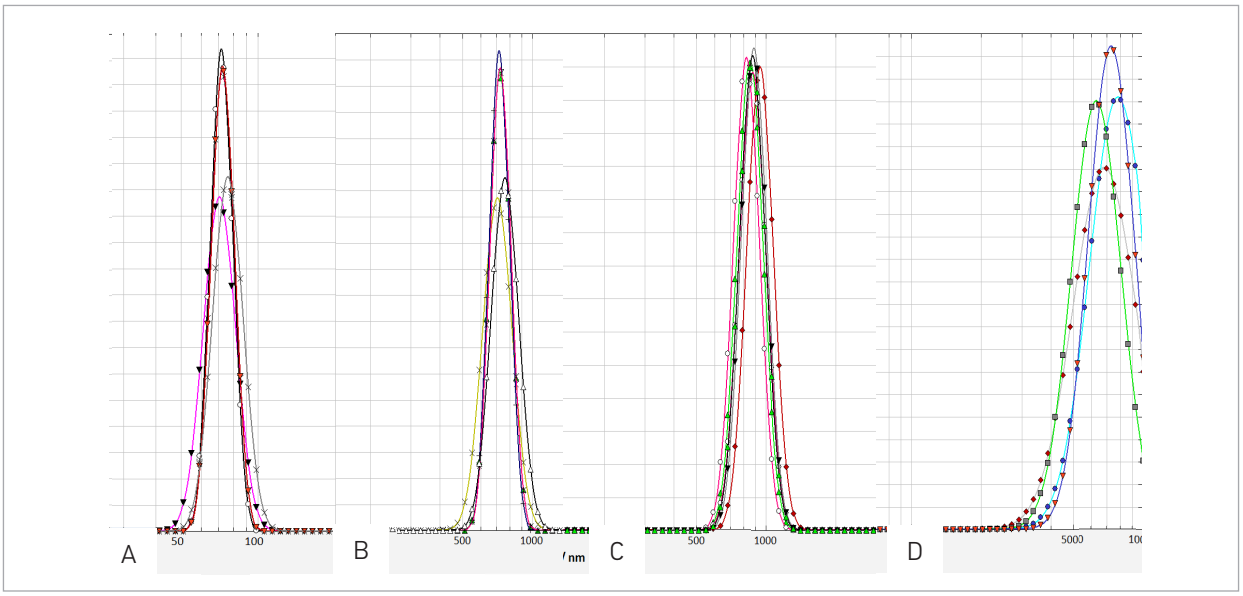


Fig. 5

Culture of cells with selenium particles with diameters 500-1000 nm (5.1.) and 700-1200 nm (5.2.); A - the cell, B -conglomerate of particles with proteins, C - conglomerate of particles 200× magnification

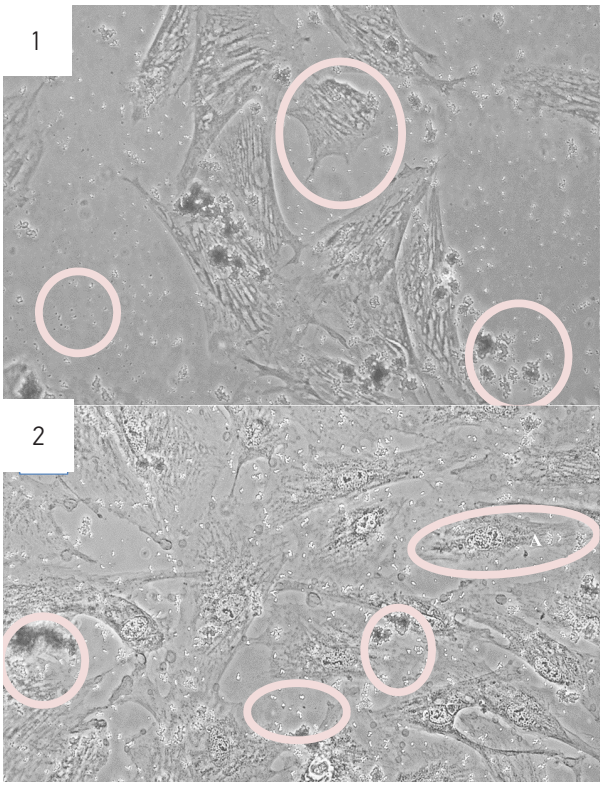
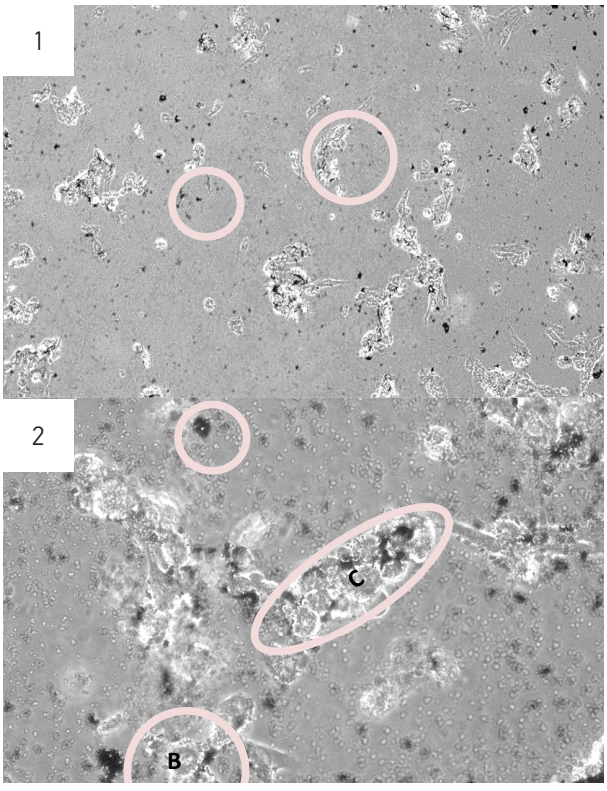


Fig. 6

Cell culture after 30 min of selenium 6000 nm exposure: 6.1. 40× magnification, 6.2. 400× magnification, A - conglomerates of particles with debris, B - apoptotic cell, C - apoptotic vesicles formation



But the groups of cells exposed with selenium particles over 6000 nm died within half an hour (Fig. 6). In the process of apoptosis (6.a.), the cells were detached from the plastic, formed apototic vesicles (6.c.), All this was accompanied by a conglomeration of particles with proteins and debris (6.b.). This picture was observed regardless of the selected doses of 0.005 and 0.01 mg / ml.

A graph of regularity in the survival of the culture against the particle size and dose was constructed showing that: the percentage of dead cells increases with increasing dose and the percentage of survivors decreases with increasing particle size.

Thus, it has been proved that increase of the selenium particles dose caused morphological changes in MSC, cell differentiation, cell apoptosis. It is proved that increasing particle size increases caused the toxic effect. It was found that selenium particles with a minimum size of 50 nm and a minimum dose of 0.005 mg / ml caused a toxic effect: a decrease in proliferative activity, few changes in morphology.

Conclusions

Thus, silver and selenium nanoparticles have a negative effect on mesenchymal stromal cells. From the results obtained, it be assumed that the effect on the entire organism will be negative and it is necessary to create conditions for monitoring and protecting the environment and man from the accumulation of silver and selenium nanoparticles.{Gurauskiene, 2006, Eco-design methodology for electrical and electronic equipment industry}

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