Anal. Bioanal. Electrochem.,Vol. 5, No. 5, 2013,647 - 664

Analytical & Bioanalytical Electrochemistry

Full Paper

Electroanalysis in the Evaluation of Nanoparticle Toxicity

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Received: 29 July 2013/ Accepted: 15 October 2013/ Published online: 31 October 2013

Abstract- An increasing use of nanomaterials requires careful assessment of their toxicity, ways of biological interactions, and possible consequences for living organisms. A variety of nanomaterials and fragmented studies of nanomaterial toxic effects on living organisms have led to contradictory views on their safety. The article proposes a potentiometric method of evaluating antioxidant activity of biological systems, which gives an indication of oxidative stress. Cell lines WI-38 and L20B, introduced with gold and silver nanoparticles, were used as samples. The work demonstrates a correlation between cell viability, cytokine status, the level of antioxidant activity and concentrations of Ag and Au nanoparticles, absorbed by cells. Determination of the quantity of nanoparticles absorbed by cells and antioxidant concentration in cells can serve as optional methods of assessing a negative impact of nanoparticles on living substrates.

Keywords- Potentiometric method, Oxidative stress, Antioxidant activity, Electron microscopy studies, Cell culture, Nanoparticles of noble metals, Stripping voltammetry

1. INTRODUCTION

Rapid developments of nanotechnology pose another level of risk to humans and other living organisms [1]. In this context understanding of interaction of nanomaterials with the living matter becomes essential, in particular, accumulation of nanoparticles in cells, subsequent creation of oxidative stress, and, in the long run, impact of nanomaterials on human health [2-4]. Unfortunately, the issues of nanomaterial toxicity and safety still remain open. The mechanisms of how nanoparticles induce destructive effects in cells and biological systems have not been fully studied yet. One of the reasons for nanomaterial toxicity is considered to be excessive amount of reactive oxygen species (ROS) in response to nanoparticle localization into cells, i.e., occurrence of oxidative stress, which triggers abnormal reactions, irreversibly damaging cells, as well as cell genetically programmed death [5]. Sioutas et al. [6-8] state that nanoparticles generated more free radicals and ROS than particles of larger size, probably due to larger active surface area. A growing number of ROS regulated the intracellular concentration of calcium, and stimulated the production of cytokines [9]. Nanoparticles of different compositions (fullerenes, carbon nanotubes, quantum dots, etc.) contributed to the production of ROS *in vivo* and *in vitro* [5]. In human MonoMac-6 cell line free radicals were produced at a high level after cells were incubated with carbon nanoparticles [10]. Gold nanoparticles induced oxidative damage to the cells of lung fibroblasts [11].

The effect of silver nanoparticles (15 nm (50 μg/ml) on the cells of alveolar macrophages generated a 10-fold rise in the level of ROS and twofold reduction of glutathione, which indicated functional abnormalities in cells. The level of cytokines - substances that signal inflammatory process in cells - changed [12].

A significant increase in ROS was observed in rats' liver cells (BRL 3A line) [13] under the effect of silver nanoparticles (15, 100 nm) at concentrations from 5 μg/ml to 50 μg/ml. The cell morphology (size and shape) changed, and glutathione concentration in the cell culture reduced significantly. An 80% decrease in mitochondrial membrane potential of cells also identified cytotoxicity of silver nanoparticles. The level of impact of silver nanoparticles was directly related to their concentration. The most pronounced effect was observed with a maximum concentration of silver nanoparticles.

Peetscha et al. synthesized spherical silver-doped calcium phosphate in a co-precipitation route from calcium nitrate/silver nitrate and ammonium phosphate in a continuous process and colloidally stabilized by carboxymethyl cellulose [14]. The lethal silver concentrations for human cell lines, i.e. human mesenchymal stem cells, lymphocytes, and monocytes, were in a similar range (1-2.5 μg silver ml^{-1}). Such silver-doped calcium phosphate nanoparticles may be used to achieve a local antibacterial effect, but attention must be paid not to reach cytotoxic silver concentration for the surrounding tissue.

The results of a comprehensive study of the effect of nanoparticles on human cell line (Hep G2) are presented in [15]. In particular, the work shows the process of localization of silver nanoparticles in cells. The particles were found in cytoplasm, lysosomes, and cell nuclei. The impact of nanosilver (0.2µg/ml and0.5µg/ml) on oxidative state of the cell culture was analyzed, and oxidative stress in cells was recorded. In addition, nanosilver caused an increase in concentrations of lactate in the culture medium, which proved the negative effect of nanosilver on living cells.

Katsnels on et al. [16] compared adverse bioactivity of virtually equidimensional gold and silver nanoparticles, administered to rats at equal mass doses either intratracheally (with a single instillation) or intraperitoneally (with repeated injections). It was found out that when equal mass doses of gold and silver nanoparticles were used, silver nanoparticles had a higher level of cytotoxicity than gold nanoparticles. It was also shown that oral administration of a bioprotective complex comprising pectin, some vitamins, glutamate, glycine, acetyl-cysteine, calcium, selenium, and fish oil preparation rich in omega-3 PUFA attenuated toxicity and, especially, genotoxicity of silver nanoparticles.

Risom et al. suggested that the oxidative stress induced by nanoparticles may have several sources: [17]

- ROS can be generated directly from the surface of particles when both oxidants and free radicals are present on the surface of particles. Many compounds hitch-hiking on the surface of nanoparticles (usually present in ambient air) are capable of inducing oxidative damage, including ozone (O_3) and NO_2.
- Nanoparticles of transition metals (iron, copper, chromium, vanadium, etc.) can generate ROS acting as catalysts in Fenton type reactions.
- Altered functions of mitochondrion. As shown in several studies, small nanoparticles are able to enter mitochondria [18,19] and produce physical damage, contributing to oxidative stress [19].
- Activation of inflammatory cells, such as alveolar macrophages and neutrophils, which can be induced by phagocytosis of nanoparticles, can lead to generation of ROS and reactive nitrogen species [17,21]. Alveolar macrophages participate in the initiation of inflammation in the lung.

Unfortunately, the research methods of nanoparticle toxicity are very limited, and the results are contradictory.

The proposed work aims to demonstrate how methods of electroanalysis can be applied to the study of accumulation of nanoparticles in cells and the relation between this parameter and cell viability, occurrence of oxidative stress, and changes in the cytokine status of cells. In this case, electrochemical methods seem to be the most promising as they are sensitive and easy to use. The choice of potentiometry for assessing oxidant/antioxidant (OAO) status of

biological objects in the 'nanoparticle–cell' system is justified by the fact that oxidative stress and signal-generating reactions are of the same nature.

2. EXPERIMENTAL

2.1. Instruments

The following instruments were used to synthesize nanoparticles of gold and silver:

- Magnetic stirrer with controlled heating IKA (Germany);
- Ultratome Leica EM UC6 to obtain ultrathin slices;
- Transmission electron microscope FEI MORGAGNI 286 (USA) to obtain micrographs of cells;
- Ultrasonic disperser Ultrasonic Processor VCX 750 (Sonics, USA) to disperse nanoparticle sols;
- ELISA reader Multiscan EX (Thermo Lab systems) to determine secreting cytokines IL1ß, IL6;
- Potentiometric analyzer MPA-1 ('IVA', Ltd, Russia) with a thick-film platinum electrode ('IVA', Ltd, Russia) to determine antioxidant activity.

2.2. Materials

To cultivate cell cultures: nutrient medium IGLA MEM, with Earle's salts, 10% solution of calf embryonic serum, sterile glutamine and gentamicin (4%) as antibiotic. Cells were removed from the culture flasks with 0.25% tripsin and Versen solution.

To synthesize nanoparticles salt: solutions $HAuCl_4$ (0.001 M) and $AgNO_3$ (0.1 M); sodium citrate ($Na_3C_6H_5O_7$) (0.1 M)

To study antioxidant activity of cells: $K_4[Fe(CN)_6]$ and $K_3[Fe(CN)_6]$, phosphate buffer solution (pH =7.4)

To stain cell cultures: trypan blue solution

For voltammetric studies: solutions of metal ions, prepared from certified reference material of aqueous solutions of silver ions (I) and gold (III) with a certified value of 1 mg/cm^3 and 0.1 mg/cm^3, respectively, by diluting state standard reference sample with 0.1 M HNO_3 (for silver) and 2 M HCl (for gold)

For microscopic studies: 2.5% glutaraldehyde solution; 1% OsO_4 solution; 0.2 M phosphate buffer; a mixture of araldite and acetone (at 1:1 ratio); lead citrate ($[OOCO(OH)(CH_2COO)_2]_2Pb_3*2H_2O$)

To determined quantity of secreting cytokines IL1ß, IL6: interleukin-6- ELISA -Best and interleukin-1ß- ELISA -Best kits (JSC Vector-Best).

2.3. Investigated Subject

- L20B cell culture derived from mouse L-cell line and expressed with human poliovirus receptors;
- WI-38 culture of human embryonic lung cells transformed by SV-40 virus.

2.4. Methods

- potentiometric method: to measure total antioxidant activity [22];
- optical method: to assess cell viability;
- Stripping voltammetry method: to determine quantity of nanoparticles absorbed by cells [23].

2.5. Preparation and characterization of nanoparticles

Nanoparticles were prepared by chemical reduction of gold and silver ions from aqueous solutions of hydrogen tetrahloroaurata ($HAuCl_4$) and silver nitrate ($AgNO_3$) by sodium citrate ($Na_3C_6H_5O_7$) that also functioned as stabilizer [24]. Sol concentrations were expressed in mg/l of a corresponding metal.

2.6. Preparation of biological material

Nanoparticles were introduced into the nutrient medium as suspension in distilled water at two concentrations: starting cytotoxic concentration [25]10 mg/l and 25 mg/l.

Suspension of nanoparticles in the nutrient medium, containing antibiotic (gentomysin, 15 ml) was introduced into the studied cell culture. A mixture of cells with nanoparticles was incubated in culture flasks for 24 h. Then the medium containing nanoparticles was removed and replaced with a new one containing no nanoparticles. The cell culture with nanoparticles was left for 120 h. Cell samples were analyzed after 24 and 120 h of incubation.

Cell viability was assessed by using the standard staining method with trypan blue solution.

2.7 Microscopic analysis

The cellular sediment was placed in 2.5% solution of glutaraldehyde and 1% OsO_4 solution for 2 h and then washed with 0.2 M phosphate buffer solution. The resulting suspension was dewatered using a mixture of spirits with increasing concentrations and acetone. The sample was placed in a mixture of acetone and araldite (at 1:1 ratio) and first incubated for 24 h at 37°C, and then for 2-3 days at 50-60°C. Ultrathin slices were prepared by using ultratome Leica EM UC6, contrasted with lead citrate and examined under an electron microscope Morgagni 268.

2.8. Determination of secreting cytokines IL1ß, IL6

Secreting cytokines IL1β, IL6 in the cultured liquid were determined by using the ELISA method.

3. RESULTS AND DISCUSSION

The potentiometric method [26] was used to determine OAO activity of cell cultures. The procedure of adapting the method for the study of the interaction between nanoparticles and cells is described below.

3.1. Potentiometric analysis in studying antioxidant activity of cellular structures containing nanoparticles

The information source (analytical signal) with regard to antioxidant activity was the Pt-electrode potential shift in the mediator system $K_3[Fe(CN)_6/K_4[Fe(CN)_6]$, which was observed when the sample was introduced into the solution, containing 10^{-4}M $K_3[Fe(CN)_6]$ and $4\cdot10^{-6}$ M $K_4[Fe(CN)_6]$. This shift resulted from chemical interaction of antioxidants with $K_3[Fe(CN)_6]$, i.e. the changed ratio of oxidized and reduced forms of the mediator system components caused by Reaction 1:

$$a\cdot Fe(III) + b\cdot AO = a\cdot Fe(II) + b\cdot AO_{Ox} \quad (1)$$

where AO : antioxidant, AO_{Ox}: antioxidant oxidation product, a and b : stoichiometric reaction coefficients.

Antioxidant activity (M-eq) was calculated applying Equation 1:

$$AOA = \frac{C_{Ox} - \alpha C_{Red}}{1+\alpha} \qquad (1)$$

where $\alpha = (C_{Ox} / C_{Red})\cdot 10^{(E_1 - E)nF/2.3RT}$

As an antioxidant molecule may contain several functional groups with antioxidant properties, AOA was understood as effective equivalent concentration of antioxidants reacting with $K_3[Fe(CN)_6]$.

This method was successfully applied to study blood and its fractions [27]. The study of changes in the oxidation state of cells, as they interacted with nanoparticles, was affected by some difficulties: low concentrations of antioxidants in cells, possible interaction of nanoparticles with the mediator system components, adsorption of nanoparticles and metal ions on the electrode surface, which may be accompanied by neglected changes in the electrode potential and distortion of measurement results.

3.2. Choice of mediator system for cell culture analysis

The criteria for choice were as follows: maximum value of the electrode potential shift in the system while various concentrations of antioxidants were introduced in the system solution; sufficient rate of chemical reaction between AO and the mediator system component; quick determination of the potential and its stability over time.

Taking into account small quantities of the studied objects and low concentrations of antioxidants in cells (from 20.0 to 80.0 μM-eq /l) it is possible to use the mediator systems given in Table 1.

Table 1. Composition of mediator systems and calculated potential shift after introduction of antioxidants in concentrations 0.02 and 0.08 μM-eq

No.	$C_{K3[Fe(CN)6]}$ / $C_{K4[Fe(CN)6]}$	Δ*E*, mV
System 1	$10^{-4}/2\times10^{-6}$ M	45/85
System 2	$10^{-4}/4\times10^{-6}$ M	32/70
System 3	$10^{-4}/5\times10^{-6}$ M	29/65
System 4	$10^{-4}/10^{-5}$ M	19/50

It is apparent from Table 1 that Systems 1 and 2 had the maximal values of Δ*E*. However, a system, containing less than 4×10^{-6} M $K_4[Fe(CN)_6]$, is unstable due to oxidation of $K_4[Fe(CN)_6]$ with atmospheric oxygen, thus for further studies the system containing 10^{-4} M $K_3[Fe(CN)_6]$ and 4×10^{-6} M $K_4[Fe(CN)_6]$ was used. In this system the potential was determined quite quickly and was stable over time.

3.3. Impact of sol components on electrode potential shift in mediator system

The potential shift which was observed when sol component -sodium citrate- was introduced into the chosen mediator system, was similar to the potential shift which was observed with the introduction of relevant deionized water volume, and didn't exceed 2.5 mV. Ions of silver and gold, in concentration below 2 μg /ml for silver and 5 μg /ml for gold, also might cause a slight shift in the electrode potential. When sols of gold and silver nanoparticles were introduced into the cell at a concentration of 2 μg/ml and 5μg/ml (in terms of metal), respectively, a marked increase in Δ*E* was observed. The increase continued during the subsequent growth in sol concentration. The experimental data indicated that in order to assess correctly changes in cell antioxidant activity, free nanoparticles should be removed from the investigated medium. This was accomplished by centrifugation for 10 min (rate of rotation – 10 000 rpm).

3.4. Preparation of samples for potentiometric measurements

A monolayer of cells, removed from the flask, was placed in 1 ml of buffer solution (pH=7.4). Cells were mechanically destroyed by freezing and thawing three times. Membranes were separated by centrifugation of the sample. Then the resulting super matant was analyzed.

3.5. Interaction within the 'nanoparticle – cell' system

3.5.1. Distribution of Ag, Au nanoparticles in cell cultures

Figs. 1-4 present micrographs of WI-38 and L20B cell lines after their incubation with silver nanoparticles for 24 h and 120 h. For both lines for first 24 h ultra-structures were retained in the standard samples of cells. Cell nuclei were of various sizes and shapes, cell and nuclear membranes and homogeneous chromatin were visible. After 120 h insignificant destructive changes were observed in these standard samples.

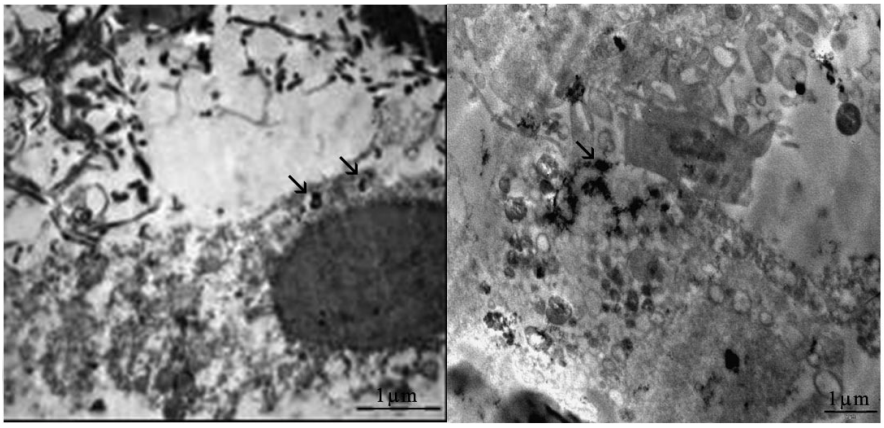

Fig. 1. Micrographs of WI-38 cell lines after their incubation with silver nanoparticles (10 µg /ml (left) and 25 µg/ml (right)) for 24 h

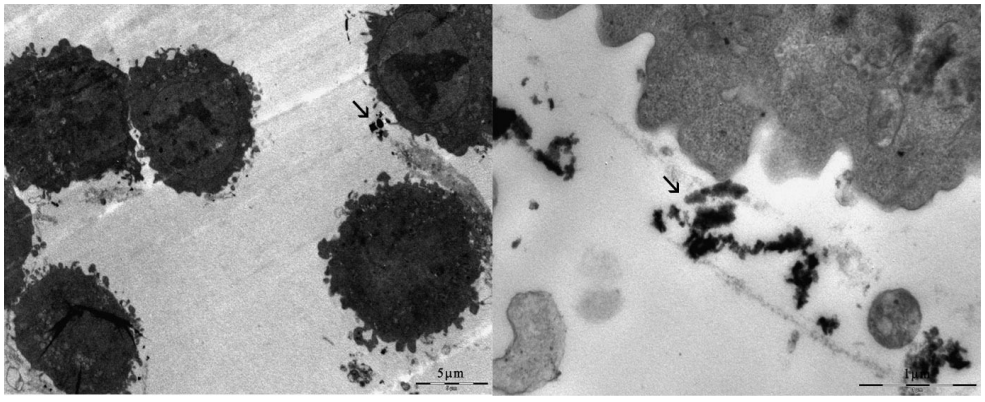

Fig. 2. Micrographs of L20B cell lines after their incubation with silver nanoparticles (10 µg /ml (left) and 25 µg/ml (right)) for 24 h

After incubation of WI-38 cell culture with suspension of silver nanoparticles (10 µg/ml and 25 µg/ml) for 24 h, irregular absorption of nanoparticles was observed. The micrographs

show cells, cell organelles, and dark areas (optically more dense) of nanoparticles (marked with arrows). After first 24 h of incubation, almost all absorbed nanoparticles were localized in cells and their organelles. Nanoparticles were not observed on membranes. With higher concentration of nanoparticles in the starting suspension, the quantity of nanoparticles absorbed by cells increased. After 120 h the micrographs recorded complete destruction of cell membrane and inhibition of the cell line.

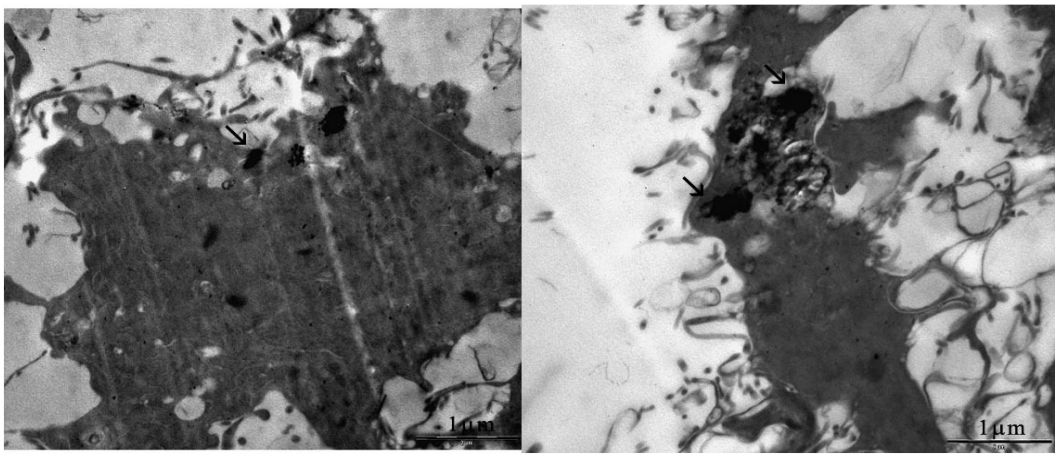

Fig. 3. Micrographs of WI-38cell lines after their incubation with silver nanoparticles (10 µg /ml (left) and 25 µg/ml (right)) for 120 h

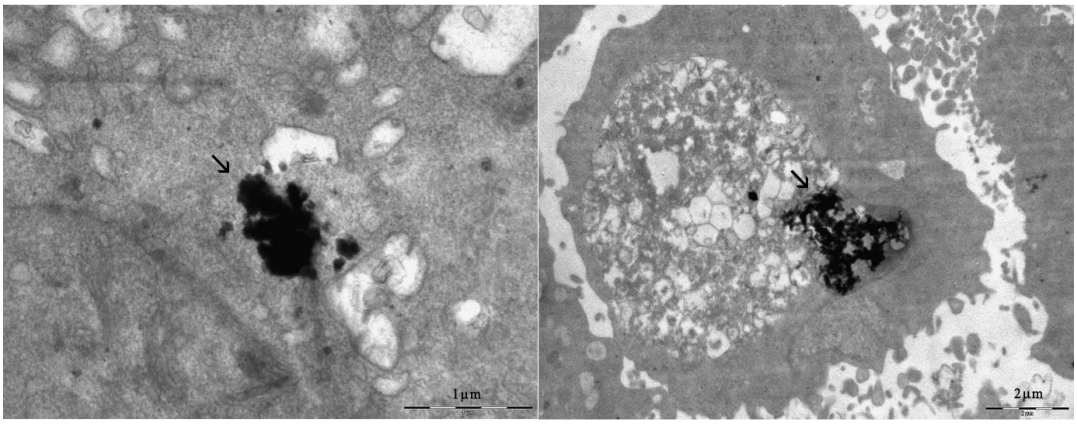

Fig. 4. Micrographs L20B (bottom row) cell lines after their incubation with silver nanoparticles (10 µg /ml (left) and 25 µg/ml (right)) for 120 h

Fig. 5 demonstrates that after 120 h the quantity of nanosilver in WI-38 cell culture, where suspensions of gold nanoparticles (10 µg/ml and 25 µg/ml) had been introduced, was almost the same. The quantity of nanosilver in cells was much lower than the quantity of introduced nanoparticles. It is also apparent from Fig. 5 that the proportion of died cells in both samples

exposed to nanoparticles was almost identical, but twice as much as the value for the control line, where nanoparticles were not introduced.

Fig. 4 presents the cell micrographs after introduction of suspension of silver nanoparticles (10 µg/ml and 25 µg/ml) into L20B cell culture during first 24 h. The micrographs show cells, cell organelles, and optically more dense areas of nanoparticles Small single osmiophil formations localized inside cells were observed. After 120 h osmiophil inclusions were visible in cytoplasm of individual cells containing large autophagosomes. Zooming in allows to observe conglomerates of 40-50 nm. These samples bore the following destructive changes:

- in cytoplasm: profuse vacuolization and formation of autophagosomes;
- In mitochondria: clarification of mitochondrial matrix, destruction of mitochondrial cristas, and irregular shapes of nuclear membrane.

Fig. 5 shows that after 120h the quantity of nanosilver in the L20B cell culture sample after introduction of suspension of silver nanoparticles (10µg/g) increased slightly, as compared to the L20B cell culture sample, which was exposed to the suspension containing 25µg/ml of silver nanoparticles. However, as it was the case of WI-38 cell culture, the quantity of nanosilverinL20Bcellswas much lower than the quantity of introduced nanoparticles. The proportions of diedL20Bcells resulting from the exposure to nanosilver (in the two samples) and in the standard sample were almost identical.

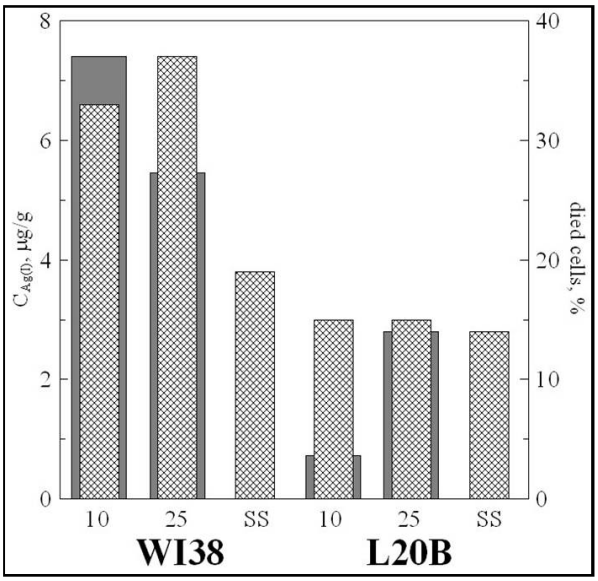

Fig. 5. Chemical analysis (fill area) and viability (hatched area) of WI38 и L20B cells after introduction ofsuspension containing 10µg/g and 25µg/ml of silver nanoparticles (in terms of Ag). SS – standard sample. Incubation time: 120 h

Figs. 6-9 present micrographs of WI-38 and L20B cell lines after their incubation with gold nanoparticles for 24 h and 120 h. For both lines for first 24 h ultra structures were retained in the standard samples. The observed cell nuclei were of various sizes and shapes, cell and nuclear membranes and homogeneous chromatin were visible. After 120 h insignificant destructive changes were observed in these standard samples.

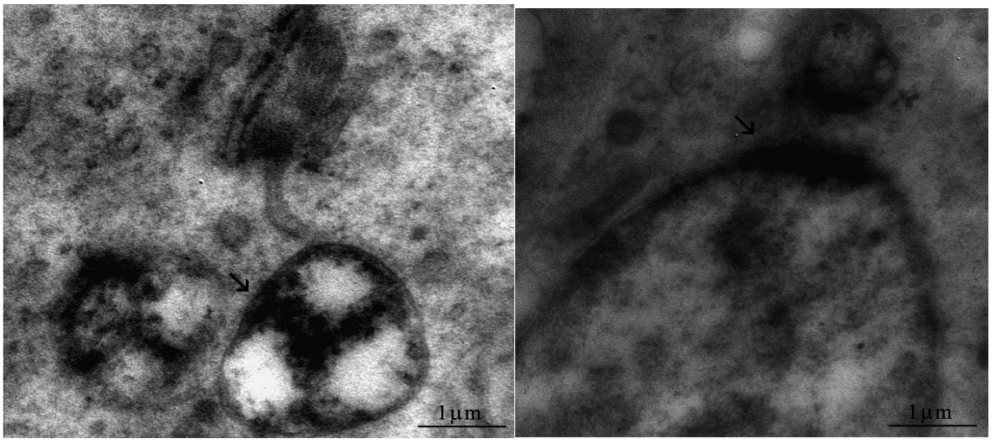

Fig. 6. Micrographs of WI-38cell lines after their incubation with gold nanoparticles (10 µg /ml (left) and 25 µg/ml (right)) for 24 h

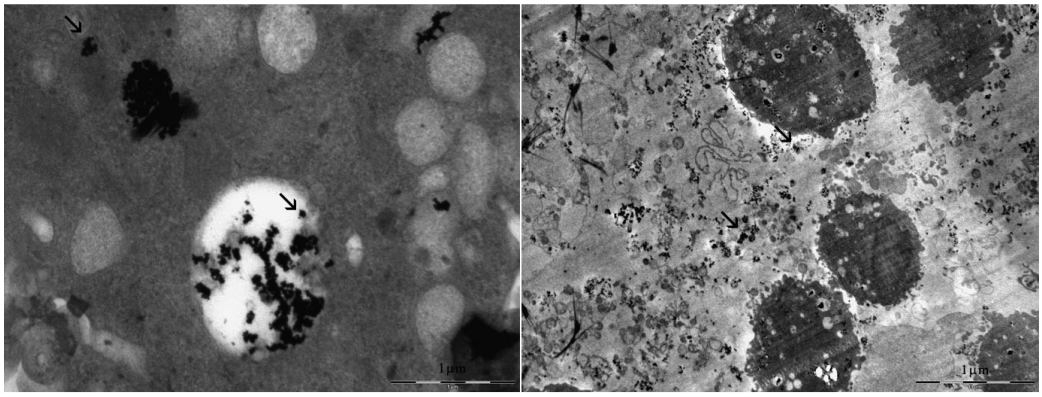

Fig. 7. Micrographs of L20B cell lines after their incubation with gold nanoparticles (10 µg /ml (left) and 25 µg/ml (right)) for 24 h

Fig. 6 shows that, after incubation of WI-38 cell culture with suspension of gold nanoparticles (10µg/ml) for 24 h, cells, cell organelles, and darker areas (optically more dense) of absorbed clusters of nanoparticles (both inside and outside cells) became visible. If gold nanoparticles were introduced in a concentration of 25 µg/ml for first 24 h, they localized outside cells and inside cell organelles, and some destruction changes in cells were already observed. Higher concentration of nanoparticles in the starting suspension led to

higher quantity of nanoparticles absorbed by cells. After incubation for 120 h, complete destruction of cell membrane and inhibition of the cell line were observed.

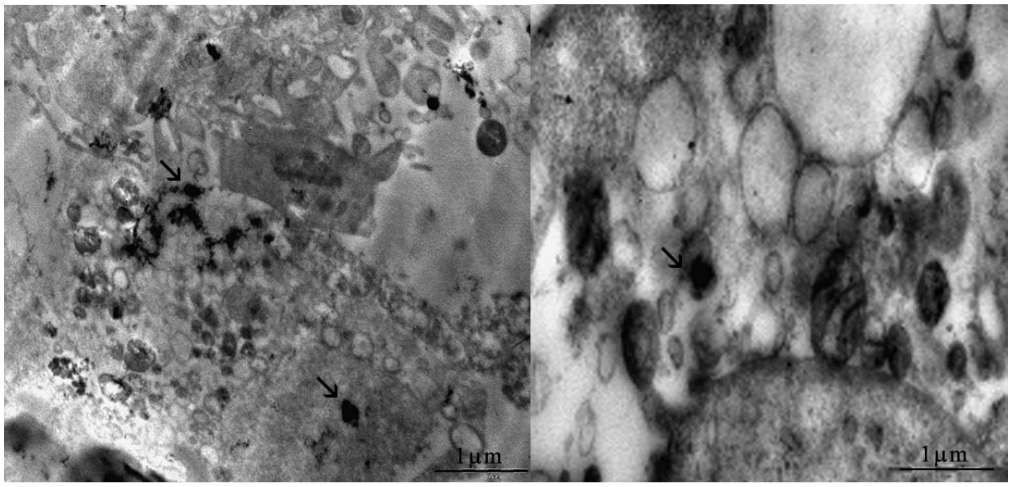

Fig. 8. Micrographs of WI-38 cell lines after their incubation with gold nanoparticles (10 µg /ml (left) and 25 µg/ml (right)) for 120 h

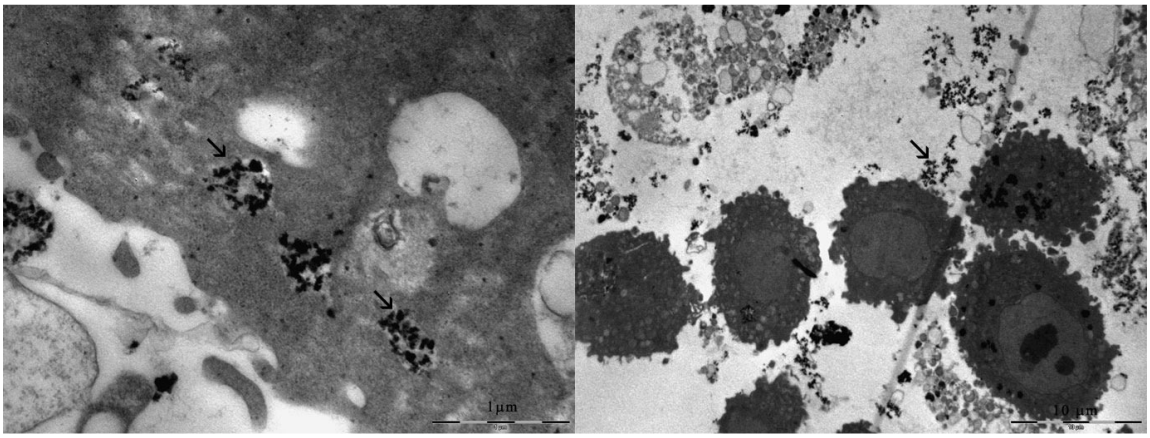

Fig. 9. Micrographs L20B cell lines after their incubation with gold nanoparticles (10 µg /ml (left) and 25 µg/ml (right)) for 120 h

As can be seen from Fig. 10, after 120 h the quantity of nanoparticles in the cell culture increased along with increasing concentration of gold in the starting suspension. Incubation with nanoparticles at their maximum concentration caused a threefold increase in the proportion of died cells in WI-38 line as compared with the standard line culture.

Introduction of gold nanoparticles (10 µg/ml in terms of gold) in cell cultures led to the formation of nanoparticle clusters inside and outside cells during 24 h after incubation (Fig. 7). Zooming in allows to see that nanoparticles were located between cell processes and in

cytoplasmic vacuoles. In the clusters individual particles were clearly visible. If gold nanoparticles were introduced in cell cultures at a concentration of 25 μg/ml (in terms of Au) during first 24 h, nanoparticles localized inside and outside cells and in damaged mitochondria. Zooming in allows to observe individual particles. After 120 h average-size accumulations of rounded osmiophilic particles localized in vacuoles and cell cytoplasm were observed. However, the highest concentration of cells was observed in damaged mitochondria, where nanoparticles formed aggregates containing separately located particles of 10 nm.

Chemical analysis showed that after 120 h the quantity of nanoparticles in the cell culture increased along with increasing concentration of gold in the starting suspension. Cell viability of this culture decreased - the proportion of died cells grew 2.5-times in comparison with the standard line, moreover it was not related to the quantity of introduced nanoparticles (Fig. 10).

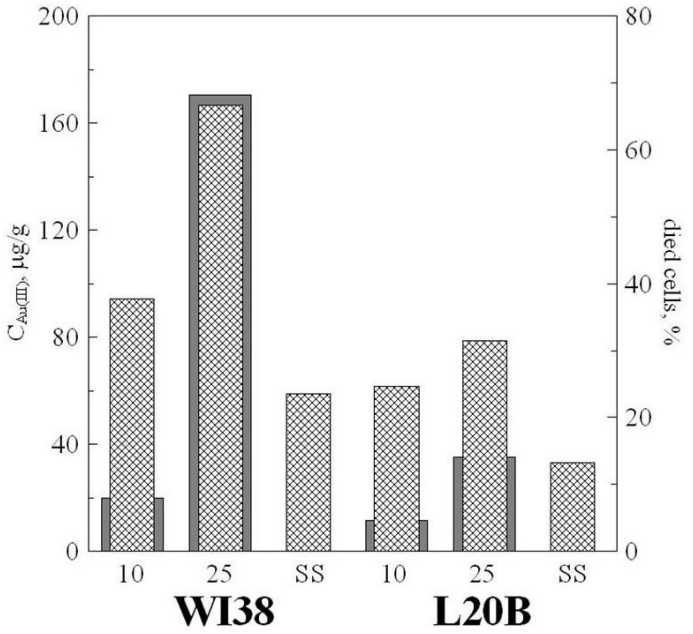

Fig. 10. Chemical analysis (fill area) and viability (hatched area) of WI38 и L20B cells after introduction of suspension containing 10μg/g and 25μg/ml of gold nanoparticles (in terms of Au). SS-standard sample. Incubation time: 120 h

Thus, electron-microscopic examination demonstrated apparent disruption in cells caused by nanoparticles of noble metals, with nano gold making a higher impact than nanosilver. Incubation witha maximum concentration of nanoparticles resulted in a threefold increase in the proportion of died WI-38 cells. Correlation between the absorption of gold nanoparticles and their impact on cell viability was also observed in mouseL20B cells. When exposed to

silver nanoparticles in the same concentration, human epithelial WI-38cellsabsorbed much more metal than mouseL20B culture. Both cell cultures absorbed more gold than silver nanoparticles. Less significant impact of nanoparticles on mouse L20Bcell line as compared to humanWI-38 cell line might be due to differences in their structure, and, consequently, their ability to be involved in endocytosis.

3.5.2. Effect of nanoparticles on cytokines status of cell culture

Inflammatory processes (in our case, toxic effect) resulted either in higher concentration of cytokines or their severe inhibition [27].Fig. 11 demonstrates how chemical nature of nanoparticles and their quantity affected concentration of interleukin-6 (IL-6 cytokine) in WI-38cells. A sit is apparent from Fig. 11,higher concentration of nanoparticles in the suspension, introduced in cell cultures, resulted in more significant changes in cytokine concentration. This confirms high cytotoxicity of silver and gold nanoparticles.

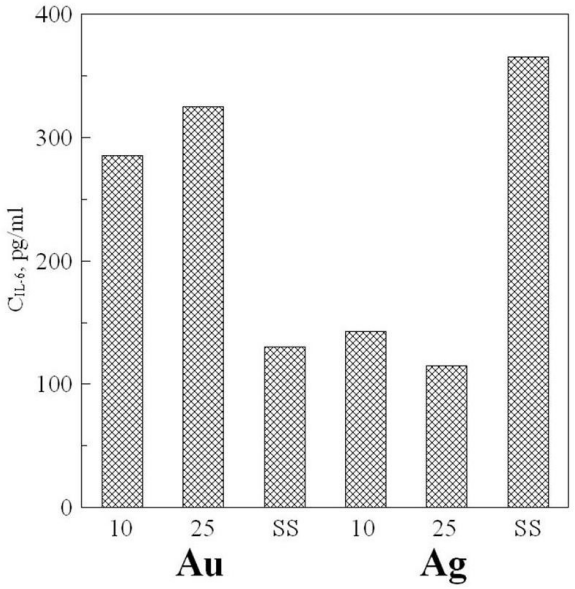

Fig. 11. Concentration of interleukin-6 (IL-6 cytokine) in WI-38cells after introduction of suspension containing 10µg/g and 25µg/ml of gold and silver nanoparticles (in terms of Au and Ag respectively). Incubation time: 120 h

3.5.2. Impact of nanoparticles on oxidant/antioxidant activity of cells

Figs. 12 and13 present the results of the study of antioxidant status of WI-38 and L20Bcellsafter their interaction with gold and silver nanoparticles.Fig.12 shows that 24 h after introduction of nanoparticles inWI-38cell culture, its antioxidant activity increased sharply, which was probably due to intensive generation of antioxidants by cell compensatory mechanism. However, after120 h, antioxidant activity decreased considerably, which,

apparently, was a consequence of the reduced level of antioxidant protection, which, in turn, might lead to oxidative stress.

As for L20B cell lines, the reduction in antioxidant level was observed immediately after first 24 h of incubation.

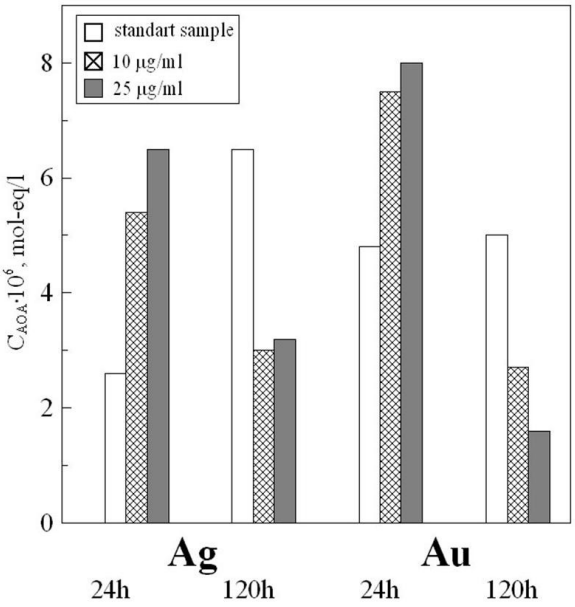

Fig. 12. Impact of nanoparticles on antioxidan activity of WI-38 cells

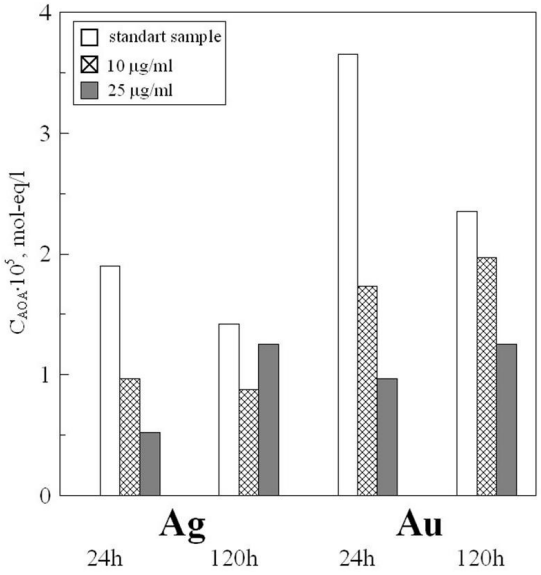

Fig. 13. Impact of nanoparticles on antioxidan activity of L20B cells

Impacts of noble metals nanoparticles on cells lead to changes in the concentrations of cytokines IL-6 (the more, the higher the content of nanoparticles in cell culture). The last correlates with decrease of antioxidant activity and cell viability, which suggesting the occurrence of oxidative stress and confirms the earlier results on the toxicity of silver nanoparticles and gold.

4. CONCLUSION

The current trends in production and use of nanosystems will continue upward, probably due to nanobiotechnologies. Information about the structure of nanoparticles and their impact on living organisms is essential for assessing and reducing risks associated with production and use of nanomaterials, particularly in medicine. The results of the studies have proved toxic effects of silver and gold nanoparticles on metabolic processes in the cell: they damage mitochondria, cristas, nuclear membrane, and, eventually, cause cell apoptosis. One of the mechanisms inducing destructive effects in the cell by nanoparticles is the production of ROS that generate oxidative processes in the cell. Evaluation of oxidative stress using AOA as a source of information provides the possibility to judge about impact of nanoparticles action as toxic agent. The proposed potentiometric method of assessing OAO activity (oxidative stress) of biological objects can be used as a valuable and accessible source of information about threats to human health arising from the impact of nanoparticles on human body. Thus, the proposed alternative method of determining OAO activity of biological systems can serve as a diagnostic tool as well as a tool for assessing toxicity of nanomaterials.

In general, when researching the metabolic fate of nanoparticles in biological and ecological systems two key factors should be taken into account: (1) the structure of the ‘hosting’ cell; and (2) physical and chemical properties of nanoparticles. Useful information about thermodynamic and kinetic properties of nanoparticles, as shown above, can also be obtained by using electrochemical methods.

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