Investigation of the Genotoxicity of Some Nanometal Oxide Particles by the Fiber Optic SOS-type Biosensor

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Abstract

In this article the main attention was given to the registration level of the genotoxicity of nano-oxide metals such as: AgO, ZnO, CuO, CdO, TiO₂, and CeO₂. They were chosen at the dimensions in the frame of 50-100 nm. It was shown that these Nano Particles (NPs) at the concentration of 1, 0 µg/ml are characterized by a different level of genotoxicity and according to our assumption, the deviation in this respect is related to the peculiarities of their penetration into the reference cells, fluctuations in the size and inherent to them biological effects. The increasing concentrations of the above mentioned NPs (up to 10 µg/ml) brought about the change of the Photoluminescence (PhL) signal of biosensor. Namely, this signal has appeared and decreased earlier than at lower doses of nano-oxide metals. The obtained results are in a good agreement, where it is demonstrated by other authors on the basis of the use of some different approaches as well as with the existed information about their general toxicity.

Keywords: Determination, Genotoxicity, Nano-dimensions, Oxide metal, SOS-type biosensor

Introduction

In the recent decades, several thousands of varieties of nanomaterials are created, and therefore the possibility of the effect of Nano Particles (NPs) on animals, humans and environment in whole is increasing [1]. A number of anthropogenic sources, such as metallurgical, cement industry, combustion of coal, polymeric compounds, oil, gas, diesel fuel, and other processes have significantly increased contents of NPs in the environment [2]. It is generally recognized that the changes in the physical properties of the materials in the transition to NPs really accompanied by changes in their biological effects, in particular, it concerns to asubstantial their accumulation, in the lung, penetration of the tissue, overcoming the skin barrier, ability to have the so called “inflammatory potential” and to interact with different biological molecules, including nucleic acids as the carrier of genetic information [3,4].The level of the hydrophobic properties and the presence of the electrical charge increase the abilities of NPs to binding with biomolecules and to their accumulation in organisms since the immune system often are not able to recognize the presence of such complexes [5].

Despite the dramatic increase in the use of nanosized materials, little information is available on their potential toxic effects on the environment. Their potential deleterious effects on ecological health should be identified to allow their safe use. Most current literature on the toxicity of NPs comes from mammalian studies that focus on respiratory exposure or from in vitro assays with mammalian cells [6]. Last time it was taken into the attention that it is necessary to estimate the role of NPs in the reproductive function of animals which is under the effect of these substances [7,8]. The Ecotoxicological studies of NPs are much more limited. Only a few reports focus on their acute toxic effects on the aquatic biotas [9].

Especially it is necessary to underline that for an effective as well as safe use of NPs and different composites with their participation, the detailed and comprehensive analysis should be done not only concerning general toxicity of these substances but in respect of their genotoxicity. Since such effects may be realized as cancerogenesis for living organisms and, more significantly as genetic mutations in next generations. This is very important to control the level of genotoxicity of nanomaterials.

Today, more than 100 different methods to assess genotoxicity are proposed, but really not more than 20 test systems are practically used. According to practice demand it is essential to have information not only about total toxicity, but also about the genotoxic effect of the environmental factors. Moreover, there is required to obtain test results in on-line regime. It is possible only on the application of a new generation of the instrumental approaches based on the biosensor technology. The start in the development of such approaches intended for the determination of genotoxicity was done not long ago [10]. Today we have the panel of the bacterial tests based on the induction of the SOS repair system on the DNA damage: SOS-Chromo [11], Umu [12], Lux-Fluoro [13], VitoTOX® [14] and some other variants of the biosensors [15]. The Lux-Fluoro test is a unique combination of two bioassays [16], which coincidently measure genotoxicity (SOS-Lux test) and cytotoxicity (Lux-Fluoro test) of single substances and their mixtures.

Early, we have analyzed the general toxic-ity of the number NPs on the basis of metal oxides and their complexes with other

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substances in the form of the nano composites [17-21]. It was to be done with the involvement of luminescent bacteria, Daphnia magna, Saccharomyces cerevisiae and vegetables with respect to their ability to germinate and functioning photosynthetic system. The main purpose of this work is to analyze the genotoxicity level of the number of the most dispersed NPs.

**Materials and Methods**

The fibre optics biosensor on the basis of cells is combined:

1. The SOS system, indicating on the presence of DNA-damaging agents, as a receptor component.
2. The bioluminescent system as a rapid reporter which acts as a constructor [22,23].

This device works in the differential regime which allows registering the comparative level of PhL between the presence of the analysed substance in the measuring cells and buffer solution instead of control probe. For the contact a transducer with the referenced cells, it was used, the approach based on the application of the cellophane films [23]. This material was preliminary boiled in the distillate water for 15 min. Then, the small cylinders with the diameter about 5-6 mm were formed from the cellophane films. These cylinders were filled by the prepared suspension in LB medium at the concentration of 10^7-10^8 cells/ml and equipped with optrodes. Such complex of the optrode with cell suspension was introduced into the measuring cell of the biosensor system. The light emission was measured for some time (from 10 to 90 min) after incubation of the optrodes or the complexes of the optrodes, with cell suspension in the measuring cell filled with the solution to be analysed at the room temperature. The signal was presented in the units relative to the control value.

It was analysed that NPs such as: AgO, CuO, CeO, CdO, ZnO and TiO\(_2\). All these reagents were from Sigma-Aldridge (USA). Appropriate amount of NPs was dispersed in the distillate water and were sonicated to prepare a stock solution. The working solution was made by serial dilutions, followed by sonication and vigorous vortexing with the working cell system. Size and surface topography of the drop coated film of appropriate NPs were investigated by SEM as it is demonstrated for ZnO NPs on figure 1. As a rule, in general the dimensions of the used NPs were in the frame of 50-100 nm. The NPs were not faceted or have any prominent shape; the average particle size estimated from SEM analysis was 50 ± 25 nm.

**Results and Discussion**

At the first experiments were carried-out with the control of the kinetics of the induced PhL in the case of application of the maximal concentration of used chemical substances. These concentrations of NPs were chosen according to literature data about their biological effects [24-27]. At first it was taken the concentration in the frame of 1-10, 0 µg/ml. As a control, samples of bacterial suspension (50-100 µL) in LB medium were used. The obtained results are presented in figure 2. At first there is necessary to mention that the start of the increasing PhL was observed not early as 30-40 min the exposition of SOS cell culture with NPs. Next, it was observed that some fast and more intensive reaction was in the case of the application of AgO, ZnO and CuO NPs in the comparison with the other ones and especially with the CeO.

**Figure 1:** SEM image of ZnO NPs on Si substrate.

**Figure 2:** Dynamics of changes of the PhL level of biosensor after adding the NPs of AgO, ZnO, CuO, CdO, TiO\(_2\), and CeO\(_2\) (curve from above to bellow, respectively) to the measuring cell in the concentration 1.0 µg/ml.

In the analysis of the obtained data there is necessary to pay attention that the level of the genotoxicity decreases in the series of NPs such as: AgO, ZnO, CuO, CdO, TiO\(_2\), and CeO\(_2\). It is maybe as results on the number of reasons. At first, do not exclude that the used NPs had a some variance in the dimensions, or they had a different contact with the referee cells and ability in the penetration of them. At last, the observed difference may be as a reflection of their real biological effects. At the increasing concentration of the individual NPs (up to one order) the dynamic of the PhL signal the of biosensor is changed (Figure 3).

**Figure 3:** Dynamics of changes of the PhL level of biosensor after adding the NPs of AgO, ZnO, CuO, CdO, TiO\(_2\), and CeO\(_2\) (curve from above to bellow, respectively) to the measuring cell in the concentration 10.0 µg/ml.
In particular, the observed changes are concerned to more early appear of the PhL signal (up to 10-15 min) and of course, simultaneous to the time of its decreasing (through 150 min after beginning NPs effect instant of 180 min at the dose of 1.0 µg/ml). Such effects are connected with more effective penetration of NPs to referent cells, stimulation of some reconstruction in their genetic structure and then inhibition of metabolite processes. The overall intensity of the PhL signal in both cases is similar and it is caused by the overall quantity of the referenced cells involved in experiments.

Conclusion

On the application of the fiber optic SOS-type biosensor it was shown the genotoxicity of NPs such as: AgO, ZnO, CuO, CdO, TiO2, and CeO2. Their effect was registered at the concentration started from 1, 0 µg/ml and was raised in the case of its increasing up to 10 µg/ml. The obtained data indicate on the considerable biological effect of the NPs with the dimension from 50 nm up to 100 nm. This effect is realized not in the total toxicity only as it was demonstrated by us [17-21] and others investigators [5-9] early. Moreover, the results obtained by us with the ap-plication of the fiber optic SOS-type of biosensor are in good agreement with that which were demonstrated by others authors [24-27] for the genotoxicity of CuO, AgO, CeO and TiO2 on the basis of the use of some different approaches.

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References


