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# Rapid Bioluminescent Enzymatic Assay for Toxicological Assessment of Metal Nanoparticles

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## Abstract

The properties of many materials change as their size approaches the nanoscale. There is increasing scientific evidence that physical and chemical properties of manufactured NPs lead to an increase in their bioavailability and toxicity. As all changes in living organisms induced by toxic substances originally occur at the lowest, molecular, level of organization, enzyme inhibition based assays have great potential to assess safety of nanoparticles. The bioluminescent enzymatic bioassays for assessment of nanomaterial biotoxicity using the soluble or immobilized coupled enzyme system of luminous bacteria NAD(P):FMN-oxidoreductase + luciferase (Red + Luc) as a test system were employed in this study. This method specifically detects the toxic properties of substances based on their effect on the parameters of the bioluminescent enzyme reactions. The commercially available metal nanoparticles (MNPs), including silver nanoparticles (Ag), nanoparticles of silicon dioxide (SiO<sub>2</sub>), and titanium dioxide (TiO<sub>2</sub>), of different sizes were tested in the study. A protocol based on the optical properties of MNPs for correcting the results of the bioluminescent assay was also developed. The inhibitory effects of MNPs on the bioluminescent Red + Luc enzyme system were measured. Results indicated that the soluble Red + Luc coupled enzyme system was more sensitive to the inhibition effect of MNPs than its immobilized form. The inhibitory activity of MNPs decreased in the following order: Ag > TiO<sub>2</sub> > SiO<sub>2</sub>. That correlated well with results of other biological methods. Due to substantial advantages such as technical simplicity, short response time and high sensitivity to analysis, this bioluminescent enzymatic bioassay has the potential to be developed as a general bioassay for safety assessment of a wide variety of nanomaterials. This study was supported by the Russian Science Foundation (project no. 16-14-10115).

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