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Cytotoxic Effects of Ag/TiO2 Nanoparticles on the Unicellular Organism Paramecium tetraurelia

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Abstract : Introduction and Objective: Ag-TiO2 nanoparticles (NP) have been characterized as effective antibacterial compounds against E. aureous, E. coli, Salmonella and others. Because these nanoparticles have been used in plastic-food containers, there is a concern about the toxicity of Ag-TiO2 NP for higher organisms from protozoan, invertebrates, and mammals. The objective of this study is to evaluate the cytotoxic effect of Ag-TiO2 NP on the survival and swimming behavior of the unicellular organism Paramecium tetraurelia. Material and Methods: Preparation of metallic silver on TiO2 surface was based on chemical reduction route of AgNO3. Aqueous suspension of TiO2 nanoparticles was preparing by adding 5 g of TiO2 to 250 ml of deionized water and followed by sonication for 10 min. The required amount of AgNO3 solutions was added to TiO2 suspension, maintaining heating and stirring. Silver concentration was 0.5, 1.5, 5.0, 25, 35 and 45 % w/w versus TiO2. Paramecium tetraurelia (Carolina Biological, Cat. # 131560) was used as a biological preparation. It was cultured in artificial culture media made as follows: Stigmasterol 5 mg/ml of ethanol, Caseaminoacids 0.3 gr/lt.; KCl 4mM; CaCl2 1mM; MgCl2 100uM and MOPS 1mM, pH 7.3. This media was inoculated with Enterobacter-sp. Paramecium was concentrated after 24 hours of incubation by centrifugation. The pellet of cells was resuspended in 4.1.1 solution prepared as follows (in mM): KCl, 4 mM; CaCl2, 1mM and Trizma, 1mM; pH 7.3. Transmission electron microscopy (TEM) studies were performed to evaluate the appropriate dispersion and topographic distribution AgNPs deposited on TiO2. The experimental solutions were prepared as follows: 50 mg of Polyvinyhlpirolidone were added to 5 ml of 4.1.1. solution. Then, 50 mg of powder 25-Ag-TiO2 was added, mixing for 10 min and sonicated for 60 min. Survival of Paramecium and possible toxic effects after 25-Ag-TiO2 treatment was observed through an inverted microscope. The Paramecium swimming behavior and possible dead cells were recorded for periods of approximately 20-50 seconds by using a digital USB camera adapted to the microscope. Results and Discussion: TEM micrographs demonstrated the topographic distribution of AgNPs deposited on TiO2. 25Ag-TiO2 NP was efficiently dissolved and dispersed in 4.1.1 solution at concentrations from 0.1, 1 and 10 mg/ml. When Paramecium were treated with 25Ag-TiO2 NP at 100 ug/ml, it was observed that cells started swimming backwards. This backward swimming behavior is the typical avoiding reaction of the ciliate in response to a noxious stimulus. After 10 min of incubation, it was observed that Paramecium stopped swimming backwards and exploited. We can argue that this toxic effect of 25Ag-TiO2 NP is probably due to the calcium influx and calcium accumulation during the long-lasting swimming backwards. Conclusions: Here we have demonstrated that 25Ag-TiO2 NP has a specific toxic effect on an organism higher than bacteria such as the protozoan Paremecium. Probably these toxic phenomena could be expected to be observed in a higher organism such as invertebrates and mammals.

Keywords : Ag-TiO2, calcium permeability, cytotoxicity, paramecium
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