

## **Cytotoxicity And Genotoxicity Induced In Human Cells Incubated With Commercial Silica Nanoparticles**

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Nanotechnology is a highly promising molecular technology which may present a variety of hazards for environmental and human health. In fact, the same properties which make nanoparticles so attractive in industry and medicine, such as small size, chemical composition, structure, large surface area and shape, may contribute to the toxicological profile in biological systems.

In this work we investigated about the cytotoxicity and the mechanisms of action of Ludox<sup>®</sup> nanoparticles (commercial colloidal silica nanoparticles in aqueous phase) with two different diameters (20 nm and 7 nm), purified and characterized at the Department of Chemical Sciences (University of Padova). Various cell lines were treated for different incubation times: fibrosarcomeric cells HT-1080, lung cancer cells A-549 and normal human lung fibroblasts, CCD-34Lu. At the end of treatments performed with increasing concentration of nanomaterials in culture medium with or without foetal calf serum (FCS), we measured cell viability with MTS (CellTiter 96<sup>®</sup> AQueous One Solution Cell Proliferation Assay, Promega) and clonogenic assays. Our results show that smaller Ludox<sup>®</sup> (SM30, 7 nm) are more toxic than Ludox<sup>®</sup> AS30 (20 nm) and that A-549 and CCD-34Lu cell lines are the more sensitive cells. Moreover, the presence of FCS in the culture medium during the treatments with Ludox<sup>®</sup> reduces cell toxicity, by promoting nanoparticle aggregation. In A-549 and HT-1080 cells we measured apoptotic index by DAPI staining and caspase-3 activity to investigate the mechanism of cell death induced by Ludox<sup>®</sup> nanoparticles: apoptosis induction was higher in HT-1080 after treatment with Ludox<sup>®</sup> SM30 nanoparticles. Finally, to study the genotoxicity induced in human cells incubated with commercial nanoparticles, we analysed the production of reactive oxygen species (ROS) in A549 and CCD-34Lu cells and gene expression alteration through microarray analysis after treatment with Ludox<sup>®</sup> AS30 and SM30: treatment of 2 h without FCS causes production of ROS with a dose-dependent relationship. This treatment does not induce DNA-double strand breaks, analysed by immunofluorescence of the phosphorylated form of the histone H2AX ( $\gamma$ H2AX), but microarray analysis showed a relevant gene expression alteration respect to control, with a significant number of genes up or down-regulated involved in different cellular pathway, such as MAPK signalling pathway, cell cycle and regulation of actin cytoskeleton.