Interaction of biologically relevant proteins with ZnO nanomaterials: A confounding factor for in vitro toxicity endpoints

The results of in vitro toxicological studies for manufactured nanomaterials (MNs) are often contradictory and not reproducible. Interference of the MNs with assays has been suggested. However, understanding for which materials and how these artefacts occur remains a major challenge. This study investigated interactions between two well-characterized ZnO MNs (NM-110 and NM-111) and lactate dehydrogenase (LDH), and two interleukins (IL-6 and IL-8). Particles (10 to 640μg/mL) and proteins were incubated for up to 24h in routine in vitro assays test conditions. LDH activity (ODLDH), but not interleukins concentrations, decreased sharply in a dose-dependent manner within an hour after exposure (ODLDH<60% of ODref for both MNs at 10μg/mL). A Freundlich adsorption isotherm was successfully applied, indicating multilayer adsorption of LDH. ZnO MNs and LDH had neutral to slightly negative surface charges in dispersion, precluding electrostatic attachment. Particle sedimentation was not a limiting factor. Fast dissolution of ZnO MNs was shown and Zn2+ could play a role in the ODLDH drop. To summarize, ZnO MNs quickly reduced ODLDH due to concentration-dependent adsorption and LDH inhibition by interaction with dissolved Zn. The control of particle interference in toxicological in vitro assays should become mandatory to avoid misleading interpretation of results.