Multiparametric Toxicity Assessment of Consumer-Relevant Nanomaterials in Intestinal Epithelial Caco-2 Cells

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The rapid progress in the development and application of nanomaterials (NM) in various areas such as industry, environment, agriculture, and biomedicine raised serious concerns regarding their safety to the human health and the environment [1]. The gastrointestinal tract is a likely route of entry for many NM, both by intentional ingestion or via nanoparticle (NP) dissolution from food containers or by secondary ingestion of inhaled particles [2].

In this study, our objective was to evaluate the influence of the chemical composition [Ag, Au, TiO_2, SiO_2 and graphene oxide (nano_GO)], primary size (10, 30 and 60 nm for AgNP and AuNP), crystal structure (rutile/anatase and anatase for TiO_2NP) and surface coating (citrate and PEG) for NM toxicity in a human intestinal barrier model. Caco-2 cells were exposed to various concentrations of NM (0.8-48 μg/cm^2) for 24 h and cytotoxicity was investigated by determining changes in cell morphology, cellular metabolic activity, plasma membrane integrity, intracellular levels of reactive oxygen species (ROS) and ATP. Changes in DNA integrity (strand breaks and oxidative damage) were also assessed.

No evident changes in Caco-2 cells morphology have been detected after exposure to any of the tested NM. However, large clusters of NM aggregates/agglomerates were visible, particularly at high concentrations. Our data showed a concentration-dependent reduction in the metabolic activity of Caco-2 cells exposed to the AgNP (AgNP_10 > AgNP_30 >AgNP_60) but no changes in plasma membrane integrity compared to control cells were observed. At the same time, citrate-coated AuNP also decreased cell metabolic activity of Caco-2 cells regardless of size, while PEG capping was effective in preventing changes in metabolic activity induced by AuNP_10. Regarding TiO_2NP, only the highest tested concentrations of rutile/anatase were able to induce a slight decreased in the metabolic activity of cells. Among the non-metallic NM, nano_GO proved to be more cytotoxic than SiO_2NP, inducing a greater decrease in cellular metabolic activity, 48% *vs* 19%, respectively. Only exposure to AgNP significantly increased intracellular ROS of Caco-2 cells to levels of 103% relative to the control, whereas all tested NM induced an increase in intracellular ATP levels. At the same time, only AgNP significantly increased DNA strand breaks.

Based on the obtained cytotoxicity profile, the tested NM can be ranked for cytotoxicity as AgNP > nano_GO > AuNP > TiO_2NP ~ SiO_2NP. The cytotoxic effects of the tested NM were more visible at higher concentrations and the smallest particles were the most cytotoxic. Additional research is needed to unravel the mechanisms of action and properties responsible for NM-mediated toxicity in human intestinal barrier models.

References

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