Caco-2 cells as an *in vitro* model to determine detrimental effects on the intestinal barrier. Studies with Ag-NPs at sub-toxic concentrations

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Engineered nanoparticles (NPs) are used in many commercial products due to their desirable characteristics for many industrial applications. In addition, some of them are being used as additives in food and packaging, increasing human exposure. This increased use of NPs and the lack of complete knowledge on their potential hazard in the gastrointestinal tract, as a main protection barrier, requires further investigations. Ag-NPs are already used in many fields, including food packaging products due to their antimicrobial and antifungical properties.

The human Caco-2 cell line is derived from colonic epithelial adenocarcinoma cells. This cell line has the capability to differentiate into small intestine enterocytes after reaching confluence, when it is grown under normal cell culture conditions. After 21 days of differentiation the cells become polarized and acquire tight junctions, microvilli and membrane transporters. This is considered a very useful model to observe uptake of nutrients and pharmaceuticals and, for these reasons, differentiated Caco-2 cells have become a model for *in vitro* studies related with the uptake and transport through intestinal barrier.

Intestinal cell toxicity of Ag-NPs was evaluated in differentiated Caco-2 cells. Moreover, intestinal integrity and paracellular permeability were also evaluated after 24 hours of NPs incubation at sub-toxic concentrations in order to mimic a realistic environmental exposure. Uptake and crossing of Ag-NPs were evaluated by confocal microscopy, TEM-EDX and ICP-MS. Furthermore, genetic damage on Caco-2 monolayer was analyzed using the COMET assay and real-time PCR. In addition, the nanomaterial was characterized for morphology and size by using TEM and DLS/LDV.

The analysis of cytotoxicity shows significant reduction of Caco-2 cells viability after 24 hours of Ag-NPs exposure. The use of sub-toxic concentrations of NPs demonstrates that the integrity and permeability remain properly after 24 hours of exposure. Ag-NPs are able to internalize in Caco-2 monolayer but only few of them are able to cross the barrier. Nevertheless, no genotoxic neither global nor oxidative damage was observed after exposure. Hence, Ag-NPs at sub-toxic doses were not able to damage our system of differentiated Caco-2 model.