Caco-2 cells as an *in vitro* model to determine detrimental effects on the intestinal barrier. Studies with SiO_2- and ZnO-NPs at sub-toxic doses

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Abstract

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[1],[2],[3]. This increased use of NPs and the lack of complete knowledge on their potential hazard in the gastrointestinal tract, as a main protection barrier, require further investigations.

ZnO-NPs are already used in food packaging products due to their antimicrobial and UV-absorbent properties[4]. Furthermore, amorphous SiO_2-NPs are used as food additive and as a component in milk powders, instant soups, etc. to improve flow-ability[4] [5] [6].

The human Caco-2 cell line is derived from colonic epithelial adenocarcinoma cells. This cell line has the capability to differentiate into small intestine enterocyte after reaching confluence, when it is grown under normal cell culture conditions. After 21 days of differentiation the cells become to be polarized and acquire tight junctions, microvilli and membrane transporters[7] [8]. 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 barriers.

Intestinal cell toxicity of ZnO and SiO2-NPs were evaluated in differentiated Caco-2 cells. Moreover, intestinal integrity and paracellular permeability were also evaluated after 24 hours of NPs incubation at sub-toxic doses in order to mimic a realistic environmental exposure. The nanomaterials were characterized for their morphology and size by TEM (Fig. 1) and DLS/LDV.

The analysis of cytotoxicity show non-toxic effects of amorphous silica in differentiated Caco-2 cells, while ZnO-NPs led to significant reduction of Caco-2 cells viability. The use of sub-toxic doses of both NPs demonstrates that the integrity and permeability remain properly after 24 hours of exposure (Fig. 2-3). Hence, neither of these two NPs at sub-toxic doses were able to damage our system of differentiated Caco-2 model.

References

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Figure 1. TEM images of SiO_2-NPs (A) and ZnO-NPs (B) in dried form.

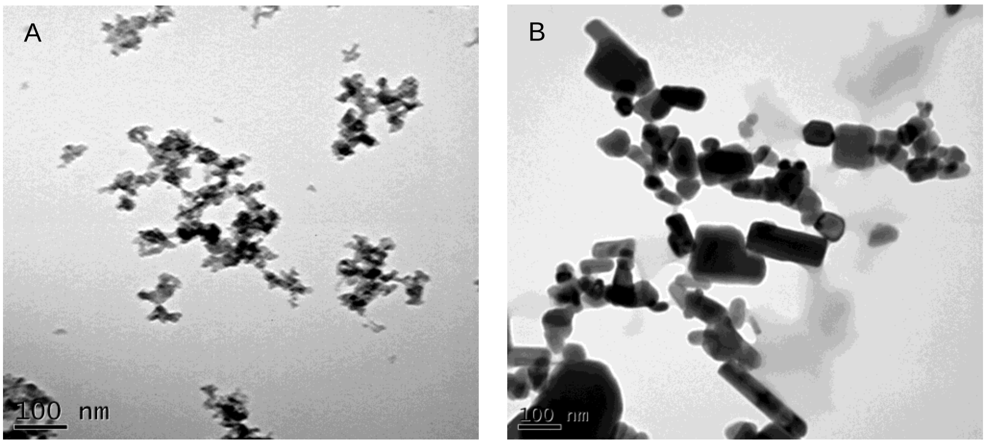

Figure 2. Integrity and permeability analysis of SiO_2-NPs. Integrity was evaluated using trans epithelial electrical resistance (TEER; A). Permeability was evaluated analyzing the concentration of paracellular compost named Lucifer Yellow (LY) in basolateral medium (B)

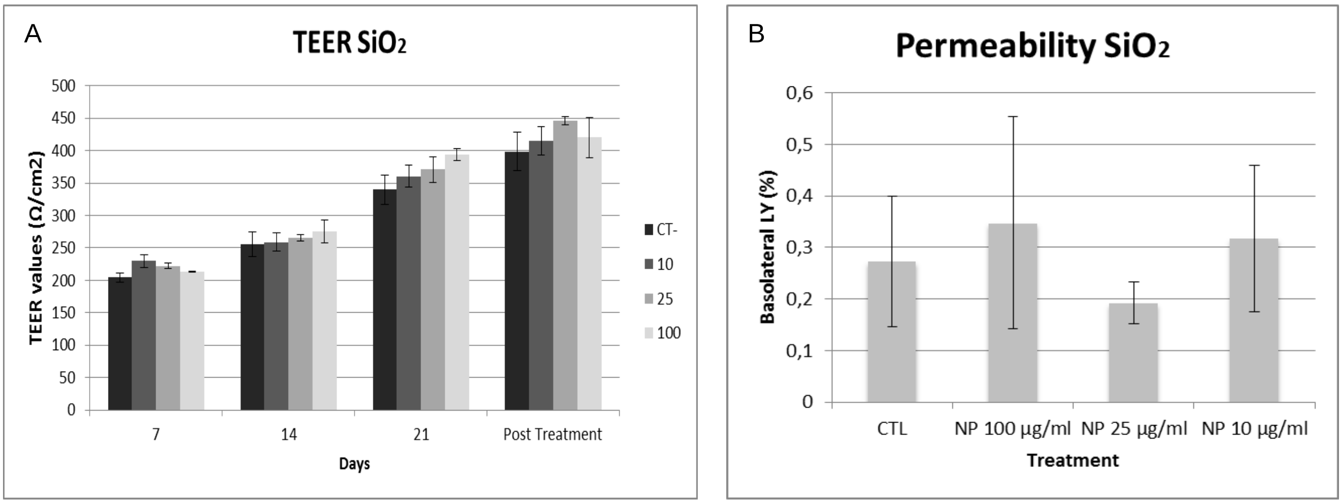

Figure 3. Integrity and permeability analysis of ZnO-NPs. Integrity was evaluated using trans epithelial electrical resistance (TEER; A). Permeability was evaluated analyzing the concentration of paracellular compost named Lucifer Yellow (LY) in basolateral medium (B)