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IN VITRO APPROACH FOR EVALUATING DIGESTED NANOMATERIALS EFFECTS ON INTESTINAL BARRIER PERMEABILITY

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Background and Objectives

Oral ingestion is considered highly relevant for the investigation of biological effects of nanomaterials (NMs). The need to investigate nanomaterials (NMs) dissolution profile in the digestive tract and their eventual internalization and translocation through the intestinal barrier has been emphasized by different regulatory bodies, and it is currently object of a new OECD Guidance Document (GD), led by Italy, under preparation at the WNT (TGP 4.158). This GD is aimed to provide scientific basis to define conceptual framework and procedures for determining the intestinal fate of orally ingested NMs using a two-step *in vitro* approach simulating the intestinal digestion of NMs in the oro-gastrointestinal (OGI) tract and their interactions with the intestinal mucosa.

The proposed approach coupling two *in vitro* steps: the first related to acellular model of NM simulated digestion in the different digestive compartments (mouth, stomach, intestine), the second focused on NM uptake/translocation through an advanced *in vitro* model of intestinal barrier (cellular assay).

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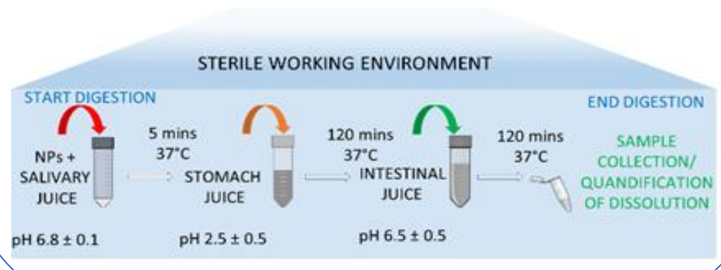
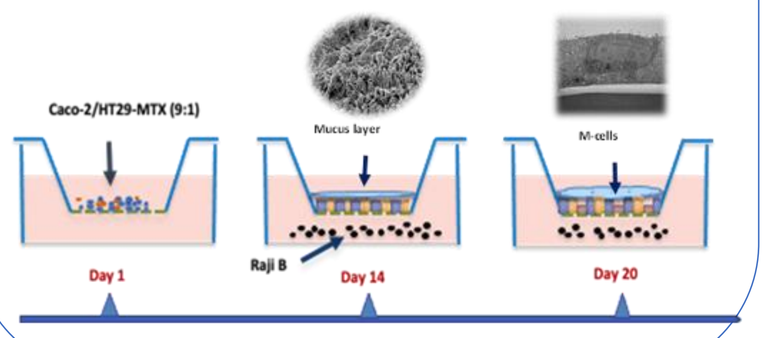
Simulated digestion (acellular assay)

Dissolution is considered the critical step for determining NMs fate in the environment and within the body, representing a useful predictor of NM biodurability *in vivo*. Within the project context a modified version of *Cascade in vitro digestion assay protocol* has been developed. Dissolution assay was carried out in sterile condition using realistic NM concentrations starting from food daily intake values of three selected NM case studies – SiO_2, TIO_2, ZnO - all relevant for oral exposure.



Barrier crossing/uptake (cellular assay)

Due to several limitations of Caco-2 mono-culture model, the use of a tri-culture model was explored for NMs absorption. It includes mucus-secretory cells, HT29-MTX, due to the relevance of mucus defensive properties and its impacts on (nano)particles mobility, and a human lymphocyte B cell, Raji-B, adding at the 14th day of co-culture, able to promote Caco-2 conversion in specialized M cells that are involved in the particulate uptake.



Barrier permeability and cytotoxicity

Tri-culture intestinal barrier model, cultured for 21 days on permeable inserts (3 µm pore size) have been exposed in the apical side at two different concentrations of digested SiO_2-NM203, TiO_2-NM104, and ZnO-NM110 at 500 and 1000 µg/ml, and incubated for 24 and 48h.

Before NM treatments the integrity of the barrier was checked by the Trans-Epithelial Electric Resistance (TEER) measurement.
At the end of both incubation time, 24h and 48h, the effect of digested NMs on barrier permeability was assessed via the paracellular marker Lucifer Yellow (*NANoReg protocol*)
Moreover, cytotoxicity of digested NMs has been evaluated by MTS assay on Caco-2 cells (*NanoValid protocol*).

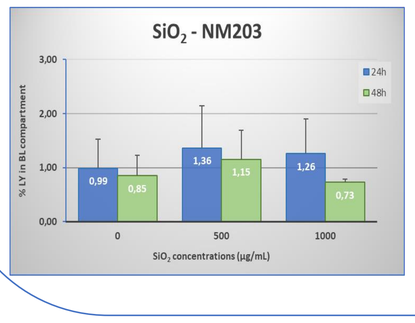
Discussion/Results

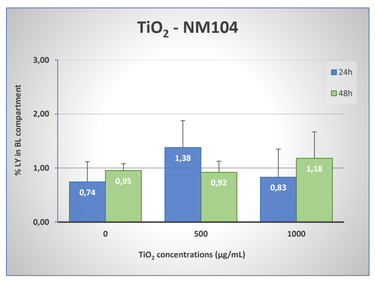
BARRIER INTEGRITY - TEER

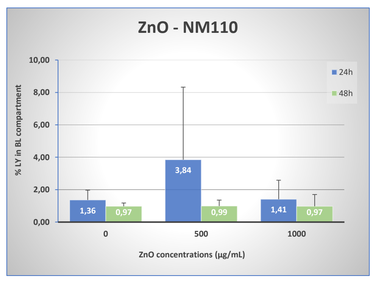
Before treatment with digested NMs, the integrity of mono and tri-culture models were evaluated by TEER measurements to verify insert suitability to run the experiment.
Mean TEER values obtained at ISS on 3 µm pore size inserts are:
Caco-2: 283 ± 45 Ohms x cm²
Caco-2/HT29-MTX/Raji B cells: 250 ± 44 Ohms x cm²

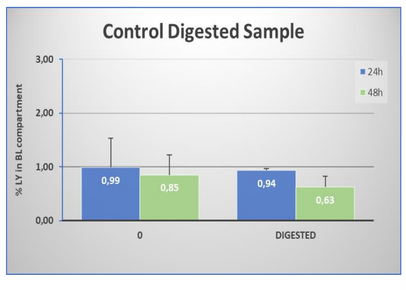
BARRIER PERMEABILITY ON TRI-CULTURE MODEL - LUCIFER YELLOW (LY) ASSAY

- ✓ **Preliminary results show that digested NMs induce an impairment of the barrier more pronounced at 24h, particularly at 500 g/mL. This data suggests that barrier perturbation is not irreversible.**
- ✓ **The digested control behaves very similarly to the untreated control.**









CYTOTOXICITY – MTS

The IC50 values obtained with digested NMs are: ≥100µg/mL for SiO_2 and ZnO, >100µg/mL for TiO_2 and digested control. For SiO_2 and TiO_2 values are in agreement with data obtained at ISS with pristine NMs, while less toxicity was reported for ZnO (mean pristine ZnO IC50– 27 µg/mL).