PATROLS
Advanced Tools for NanoSafety Testing

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# Intestinal mucin profile and inflammatory response following ENM exposure – an *in vitro/in vivo* comparison

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**Background:**

Oral exposure to engineered nanomaterials (ENM) is increasingly likely due to their frequent use in consumer products. In the context of the Horizon 2020-funded project PATROLS (*Physiologically Anchored Tools for Realistic nanOmateriaL hazard aSsessment*) we investigate the effects of ENM on the intestinal epithelia and its mucus barrier.

The mucus-secreting goblet-like cell line (HT29-MTX-E12 (E12)) serves as an *in vitro* model to study the toxicity and pro-inflammatory effects of ENM on intestinal epithelial cells as well as on the mucus composition and integrity.

In feeding studies with mice a physiological way of GI-tract exposure to ENM was investigated.

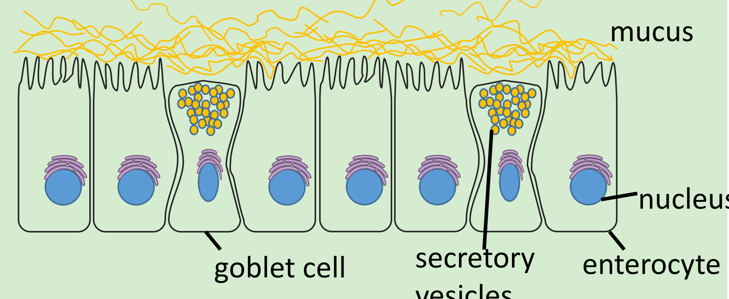


**Fig. 1 Intestinal barrier**

**Conclusions & Outlook**

- The expression of mucin genes and mucus secretion is strongly enhanced in confluent E12 cells.
- In contrast to confluent cells, ENM exposure has relatively strong effects on pre-confluent cells, especially on the IL-8 and MUC2 expression.
- Both *in vivo* and in confluent cells none of the ENM affects the expression of pro-inflammatory genes.
- Both *in vivo* and in confluent cells $CeO_2$ exposure induces a downregulation of MUC1.
- The E12 cell line is a promising model to address potential effects of ENM in the gastrointestinal tract. Confluent E12 cells are more appropriate to investigate intestinal epithelial cellular responses to ENM.
- Complementarily, the gut microbiome of ENM-fed mice was assessed. Preliminary results indicate few, weak ENM-specific effects.

## Model characterisation

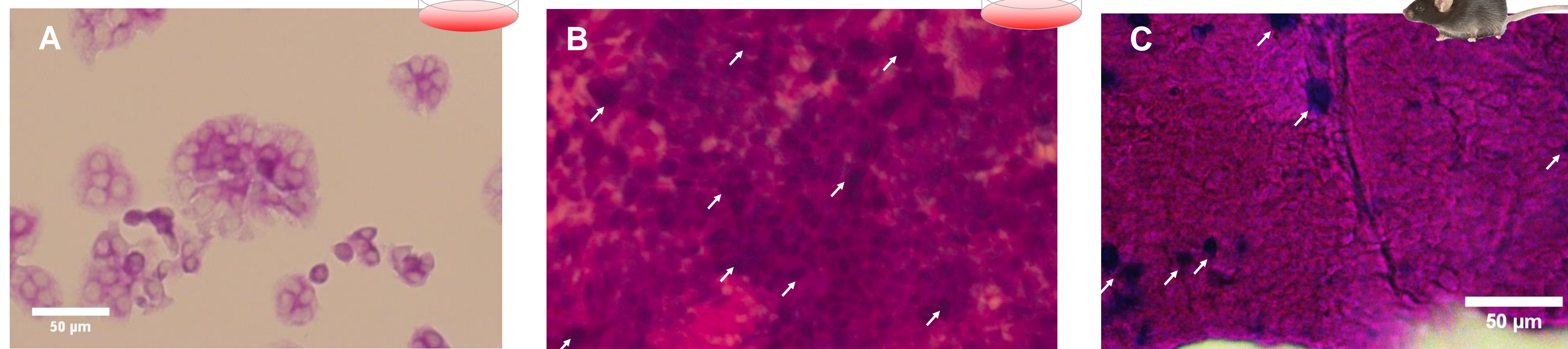


**Fig. 2 Mucus secretion** of E12 cells after 2 (A) and 22 days (B) of culture, as well as of the mouse small intestine. The PAS reaction developed a pinkish-purple colour on neutral – i.e. uncharged – mucus. Acidic mucus was stained dark-blue with Alcian Blue (arrows).

## Gene expression pre-confluent vs. confluent E12 cells

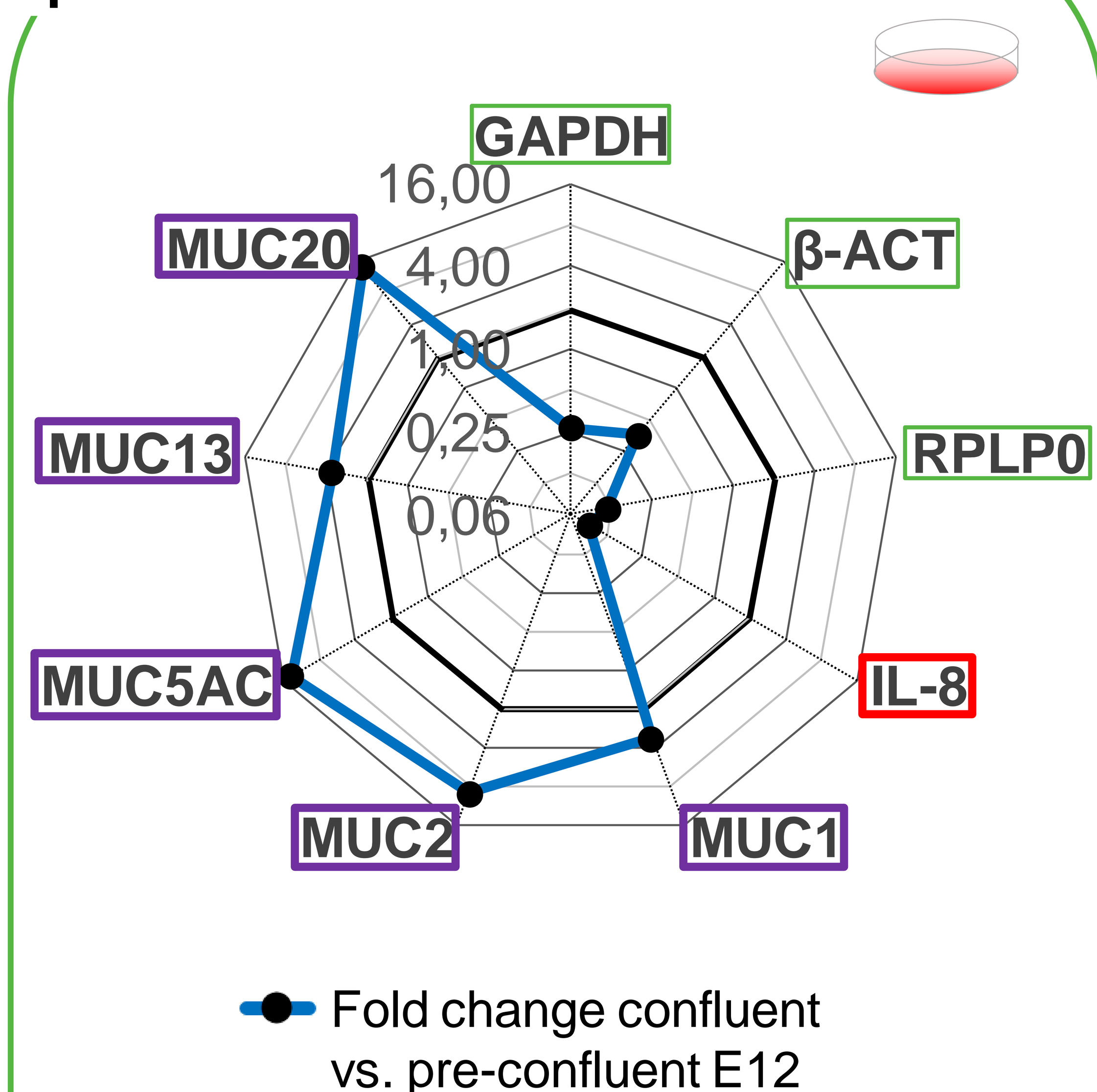


**Fig. 3 Gene expression in confluent E12 cells** depicted as fold change compared to pre-confluent E12 cells. Threshold cycles measured for untreated E12 cells were averaged and then used for calculation of fold changes. Expression of all investigated mucins (purple) is upregulated with confluency while expression of reference genes (green) and pro-inflammatory IL-8 (red) is downregulated.

## Gene expression after ENM treatment *in vitro*

Fold change: 10 / 1.0 / 0.10

| | pre-confluent | | | | confluent | | | |
|---|---|---|---|---|---|---|---|---|
| | $TiO_2$ | Ag | $CeO_2$ | $SiO_2$ | $TiO_2$ | Ag | $CeO_2$ | $SiO_2$ |
| IL-8 | | ** | * | * | | | | |
| MUC1 | | | | | | | * | |
| MUC2 | | | * | | | | * | |
| MUC5AC | | | * | | | * | * | |
| MUC13 | | | ** | * | | | | * |
| MUC20 | | | * | * | ** | | | |

**Fig. 4 Changes in gene expression** following the treatment with 80 µg/cm² ENM for 4 h and 24 h depicted as fold change compared to untreated cells, assessed in pre-confluent and confluent E12 cells (Average of N=3; *p < 0.05; **p < 0.01).

## Gene expression after ENM treatment *in vivo*

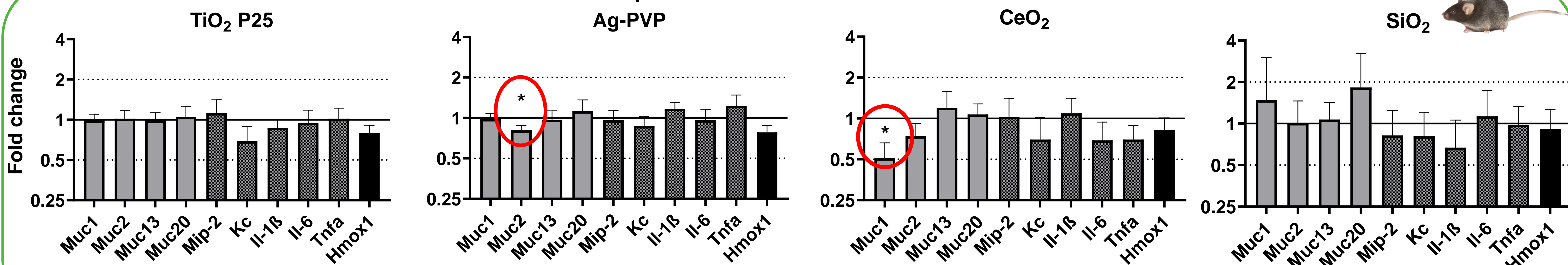


**Fig. 5 Changes in ileal gene expression** following ENM exposure of mice depicted as fold change compared to control mice. Male and female C57BL6/J mice were fed with feed pellets containing either no additive, 1.0% TiO2 P25, or 0.2% Ag-PVP for 28 days. Female C57BL6/J mice were fed with feed pellets containing either no additive, 1.0% CeO2 or 1.0% SiO2 for 21 days.(Average of N≥5; *p < 0.05)

## *Materials & Methods*

$TiO_2$ (P25 Evonik, 26.2 nm ± 10.7 nm), Ag-PVP (Sigma-Aldrich, 40.2 nm ± 17.6 nm), $CeO_2$ (NM-212, 35.4 nm ± 17.0 nm) and $SiO_2$ NP (Sigma-Aldrich, 12.9 nm ± 4.9 nm) were suspended in $H_2O$ (4 mg/mL) and sonicated according to the NanOxiMet suspension protocol (www.nanoximet.eu).

HT29-MTX-E12 cells were grown in DMEM containing 10% FCS for 48h (pre-confluent) or 22 days (confluent) before treatment. 16 – 20h before treatment, the cell culture medium was changed to DMEM containing 1% FCS.

Ileal tissues were obtained from two independent feeding studies that had been performed before the start of this study. Male and female C57BL6/J mice were fed with feed pellets containing either no additive, 1.0% TiO2 P25, or 0.2% Ag-PVP for 28 days. Female C57BL6/J mice were fed with feed pellets containing either no additive, 1.0% CeO2 or 1.0% SiO2 for 21 days.

Gene expression was analysed via **qRT-PCR**. RNA was isolated via Roche High Pure Tissue Kit. Subsequently, RNA was digested with DNase I (Sigma) and cDNA synthesised with the BioRad iScript Kit. Ultimately, cDNA was quantified by qPCR using BioRad SYBR Green.

Mucus secretion was examined with **Alcian Blue staining and PAS reaction** after cell fixation with 4% PFA. The PAS reaction developed a pinkish-purple colour on neutral – i.e. uncharged – mucus. Acidic mucus was stained dark-blue with Alcian Blue.

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