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Validating intestinal effects of food-grade titanium dioxide using a murine gut organoid model as alternative to *in vivo* models

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Background: Nanoparticles (NPs) found in the human diet mainly originate from inorganic food additives, often used *quantum satis* in common foodstuff, which raises public health concerns due to daily exposure. The whitener and opacifying agent titanium dioxide (TiO_2, E171 in EU) is one of the most studied nanomaterial, evoking inflammatory responses and precancerous lesions in the rodent intestine. Investigating the potential hazards of chronic oral exposure to NPs is often time-consuming and requires animal models, specific spaces and skills. However, recent technical advances in stem cells and three-dimensional cultures allowed the use of organoids as an alternative model to *in vivo* experiments. Herein we used murine intestinal organoids to characterize intestinal impacts of food-grade TiO_2 in comparison to already reported *in vivo* data, and to validate organoids as a reliable model for studying the effects of foodborne NPs in the gut.

Methods: Three different wild-type C57bl/6 mice were used for small intestine collection. Intestinal crypts were purified, dissociated, and cells were cultured for organoid growth. After 4 passages, organoids were dissociated and seeded as a 2.5D culture, then exposed to 0.1, 1, 10 or 100µg/ml of E171 for 24h. Supernatants were collected, and cytotoxicity assessed by LDH release quantification. Total RNA was extracted from samples and analyzed for cell proliferation and differentiation, genotoxicity, antimicrobial peptides, permeability, oxidative stress, Toll Like Receptors (TLR), NFκB, cytokine and chemokine gene expressions by qPCR. Cell apoptosis was also evaluated by cleaved Caspase-3 quantification using immunofluorescence.

Results: Gut organoids exposed to E171 showed a dose-dependent up-regulation of the cell proliferation marker *Mki67* together with increased protein expression of cleaved-Caspase-3, suggesting epithelium renewal or restructuring. This occurred in parallel to a decreased expression of the enterocyte differentiation markers *Alpi* and *Krt20* as well as up-regulation of the neuroendocrine marker *Chga*. Moreover, food-grade E171 decreased gene expression of antimicrobial peptides (*Lyz*, *Reg3b*, *S100a8*) and tight junction proteins (*F11r*, *Tjp1*, *Ocln*, *Cldn7*, *Cldn15*), suggesting altered epithelial secretion and permeability. We also showed that the TLR4-NFκB pathway was negatively impacted in a dose-dependent manner, while oxidative stress, cytokine and chemokine gene expressions remained unaltered. Although E171 exposure was not cytotoxic, TiO_2 increased expression of *gadd45a* at low dose (i.e. 1µg/ml), suggesting DNA damage.

Conclusions: Taking together, a 24h-exposure of murine intestinal organoids to food-grade TiO_2 impacts epithelial barrier integrity (cell proliferation and differentiation, gut permeability, genotoxic effect) and antimicrobial defenses as reported *in vivo* in rodent models, hence validating the use of intestinal organoids for toxicological studies of foodborne NPs.