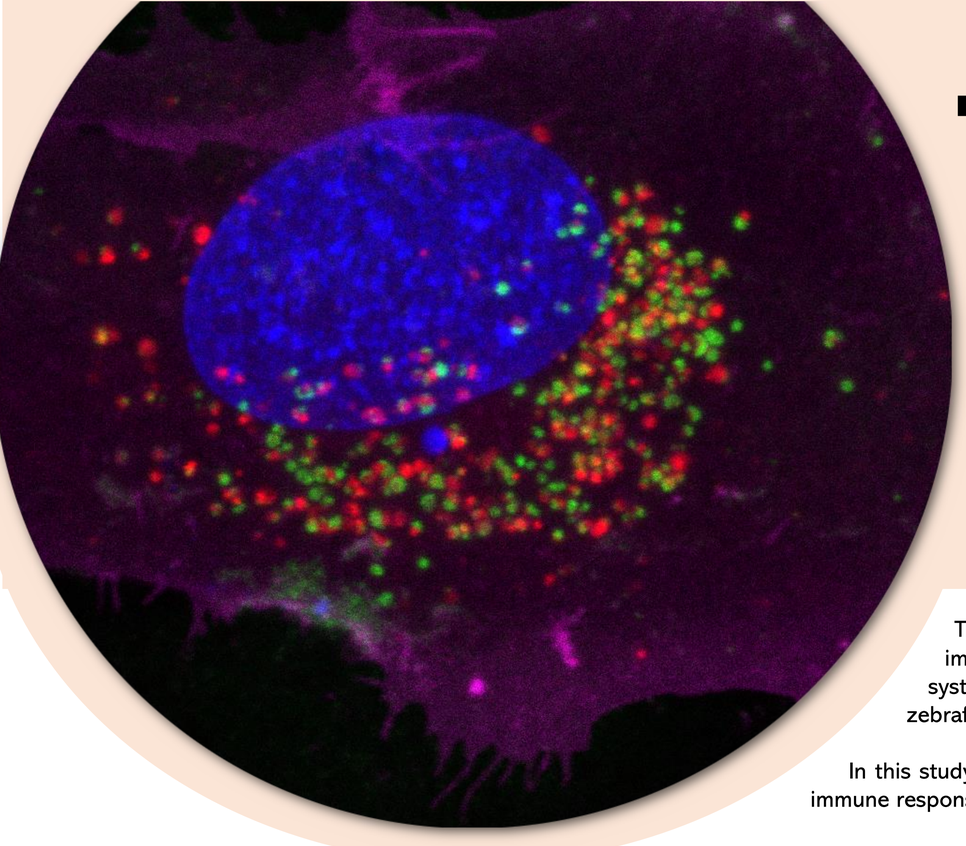

Trout gut cells...

as a model to understand the effects of polystyrene nanoplastics on gut immune function

Universitat Autònoma de Barcelona

Institut de Biotecnologia i de Biomedicina

Departament de biologia cel·lular, fisiologia i immunologia

García, M.; Solà, R.; Brandts, I.; Teles, M.; Roher, N.

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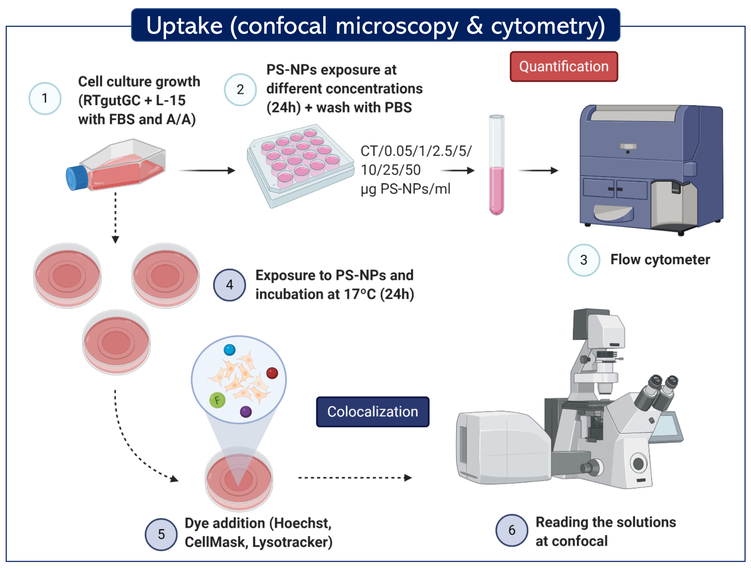
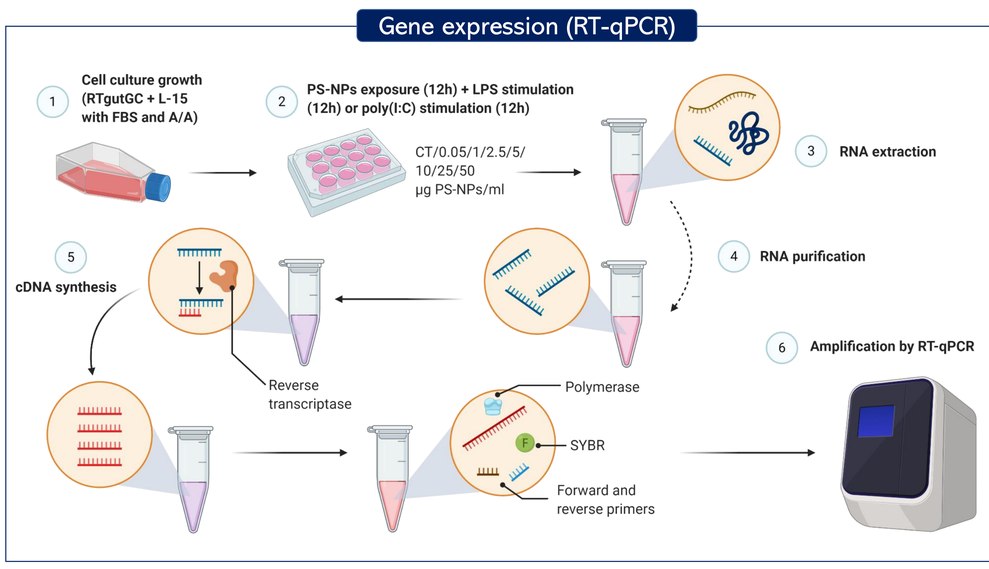
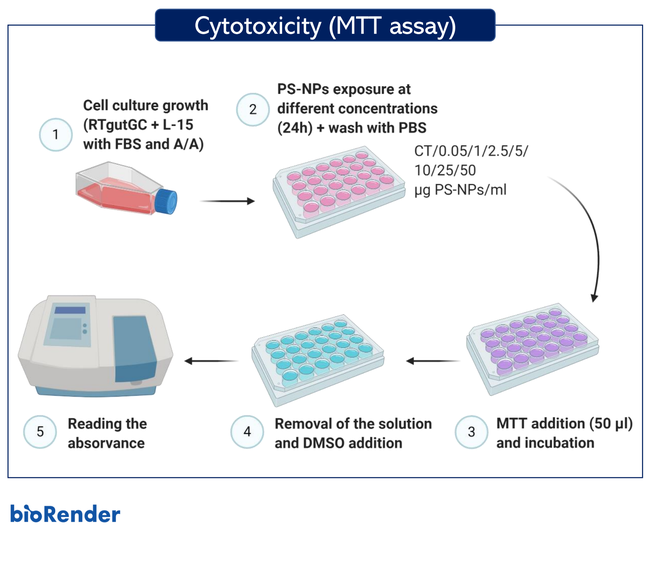
The increasing and ubiquitous presence of nanoplastics (NPs) in the aquatic environment demands urgent assessment in order to understand their impact on ecosystems and human health. An important open question is how the exposure to NPs might interfere with the normal function of the immune system of aquatic organisms. Our previous study using zebrafish as a model demonstrated that polystyrene NPs (PS-NPs) were efficiently taken up by zebrafish liver cells (ZFL) accumulating in lysosomes, possibly in an attempt of the cell machinery to metabolize PS-NPs.

In this study, we aim to understand whether the gut immune homeostasis could be disturbed by exposure to NPs. Hence we evaluate the effect of PS-NPs on the immune response of RTgutGC cells, since fish gastrointestinal epithelium is one of the principal portals of entry for both NPs and pathogens in teleosts.

Methodology







Results

1 Uptake

Flow cytometry analysis showed a dose-dependent uptake of PS-NPs by RTgutGC cells after 24 h of exposure. (Fig.1). Confocal microscopy confirmed cellular accumulation of PS-NPs and showed colocalization in lysosomes (Fig.2).

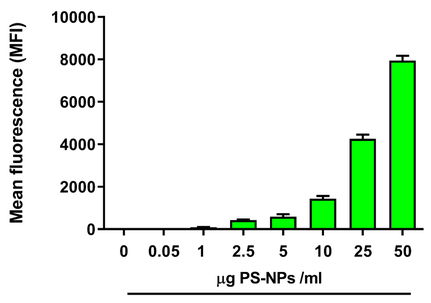


Fig.1: Flow cytometry analysis (mean fluorescence intensity in RTgutGC).

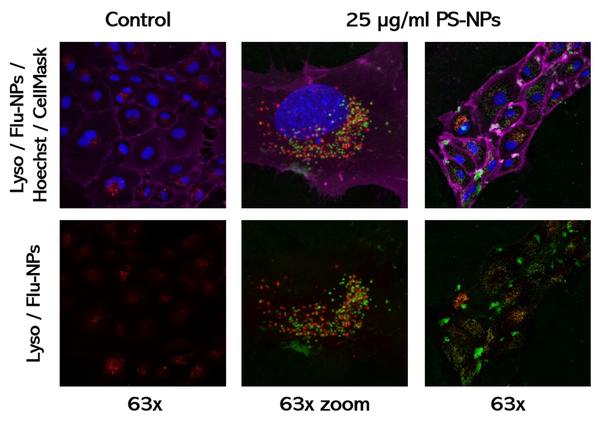


Fig.2: Confocal images of RTgutGC cells. Cells were stained with Lysotracker, Hoechst and CellMask. Fluorescent PS-NPs incorporate Dragon Green as a dye.

2 Cytotoxicity

The exposure to PS-NPs did not cause toxicity to RTgutGC cells, after 24h incubation for the testes concentrations (Fig.4).

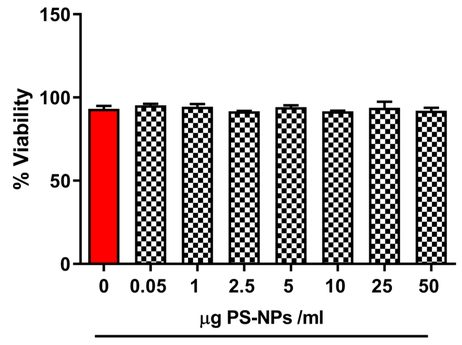


Fig.4: Viability of RTgutGC cells assessed with the MTT assay.

3 Gene expression

Exposure of RTgutGC cells to PS-NPs (up to 50 mg/l) did not induce expression of proinflammatory genes. Nevertheless, when we co-exposed cells to PS-NPs and a viral (poly(I:C)) or bacterial challenge (LPS), this triggered a synergistic immune response, with the up-regulation of the expression of antiviral genes or pro-inflammatory cytokines (Fig.3).

Fig.3: Gene expression of antiviral genes (vig1, mx) and pro-inflammatory cytokines (il1β, il12) after co-exposure to PS-NPS and PAMPs.

Conclusions

- There is a dose-dependent uptake and accumulation of PS-NPs in RTgutGC cells.
- PS-NPs are not cytotoxic to RTgutGC cells at concentrations of up to 50 mg/l.
- As in ZFL, in RTgutGC cells PS-NPs colocalized in lysosomes.
- Co-exposure of RTgutGC cells to PS-NPs and PAMPs (Poly(I:C)) or LPS) triggers a synergistic response, modulating the expression of pro-inflammatory cytokines and antiviral genes.

Bibliography

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