Development of a gut microbiota-host in-vitro model for immunotoxicity

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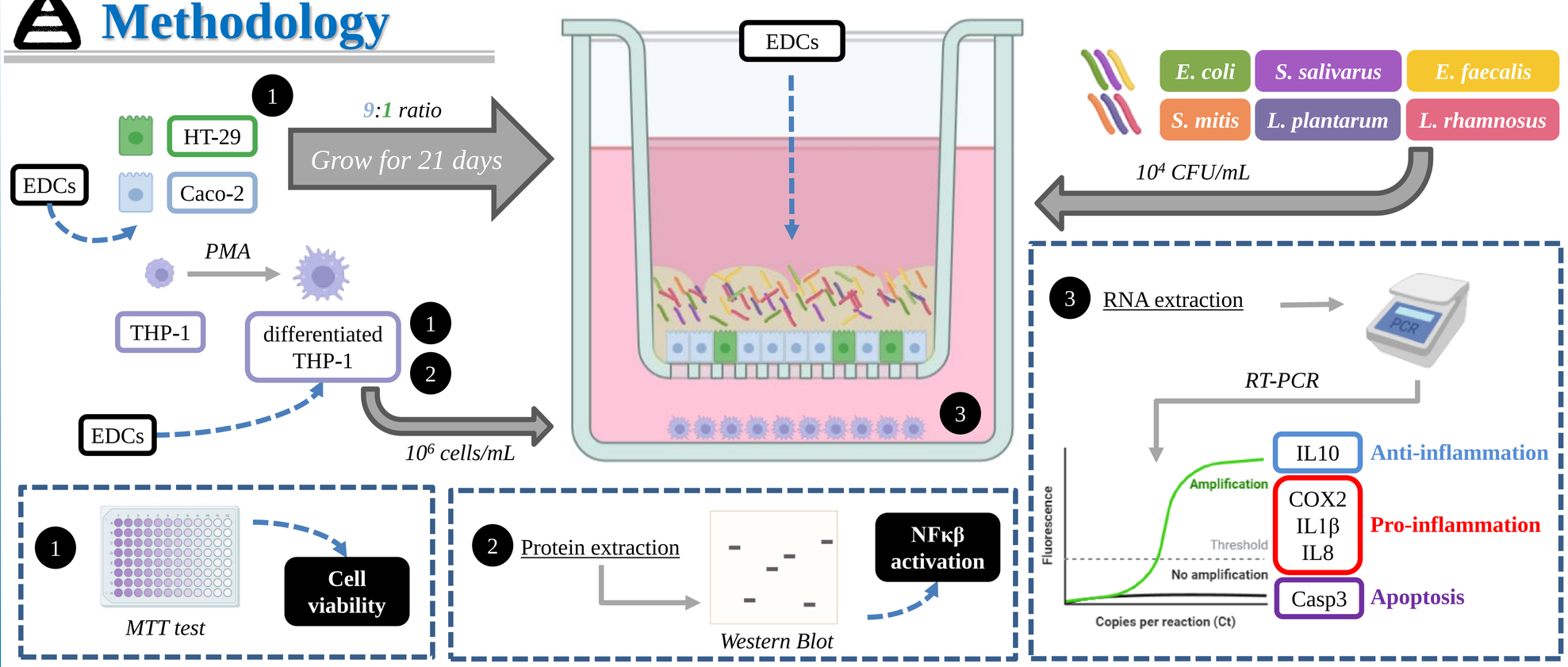
Introduction

Endocrine disruptor chemicals (EDCs) refer to a group of compounds that impair hormone production and cause negative effects in human health [1]. They are present in daily use and consumer products and can be absorbed through the skin, the digestive and the respiratory systems [2–4]. Ingestion of contaminated food, drinking water or house dust are major sources and routes of exposure of some common EDCs such as bisphenols, flame retardants (FRs) and perfluoroalkyl chemicals. [2–6] Those EDC have been found to negatively affect microbiota, which plays a central role in regulating host immune system [7-8]. Specifically, altered microbial metabolism and dysbiosis has been found after EDC exposure, leading to a disruption of the immune system [8].

- **Objective: to develop an in-vitro system that simulates the small intestine, containing microbiota and immune cells, to determine how exposure to BPA, FRs (TDCPP) and PFOA affects intestinal immunity.**

Methodology



The numbers refer to the experiments performed at each step of the workflow:

1) Viability analysis by MTT test on Caco-2/HT cells and differentiated THP-1 after exposure to EDCs.

2) NFκβ activation measured by phospho- NFκβ detection by WB after protein extraction from exposed THP-1 cells.

3) Gene expression measured by RT-PCR after RNA extraction from THP-1 cells exposed to EDCs, with or without microbiota.

Figure 1: *schematic representation of the methodology and workflow.*

Results and discussion

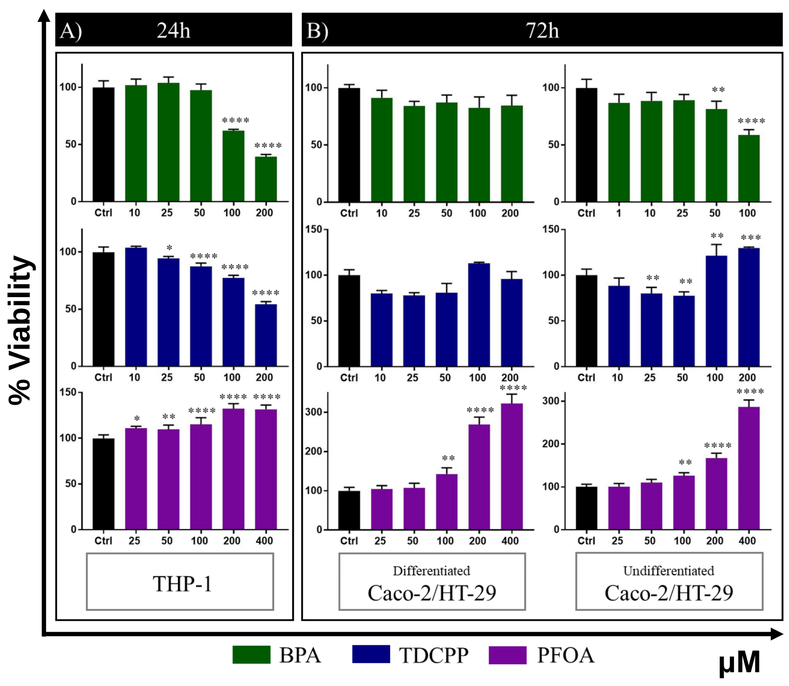


Figure 2: cell viability by MTT analysis: A) THP-1 cells were exposed to BPA (green), TDCPP (blue) or PFOA (purple) for 24h. B) differentiated and undifferentiated Caco-2/HT-29 cells were exposed for 72h. One-way ANOVA was performed for statistical analysis using Bonferroni's correction. *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001.

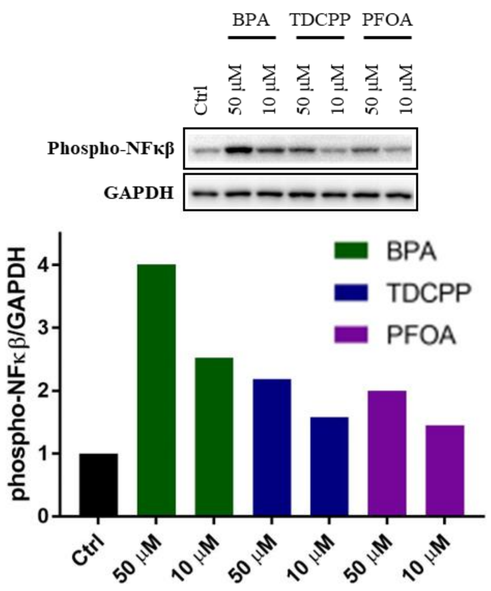


Figure 3: NFκβ activation by Western Blot in THP-1 cells after exposure to BPA 10-50 μM, TDCPP 10-50 μM and PFOA 10-50 μM for 24h. Phospho-NFκβ signal was corrected using GADPH.

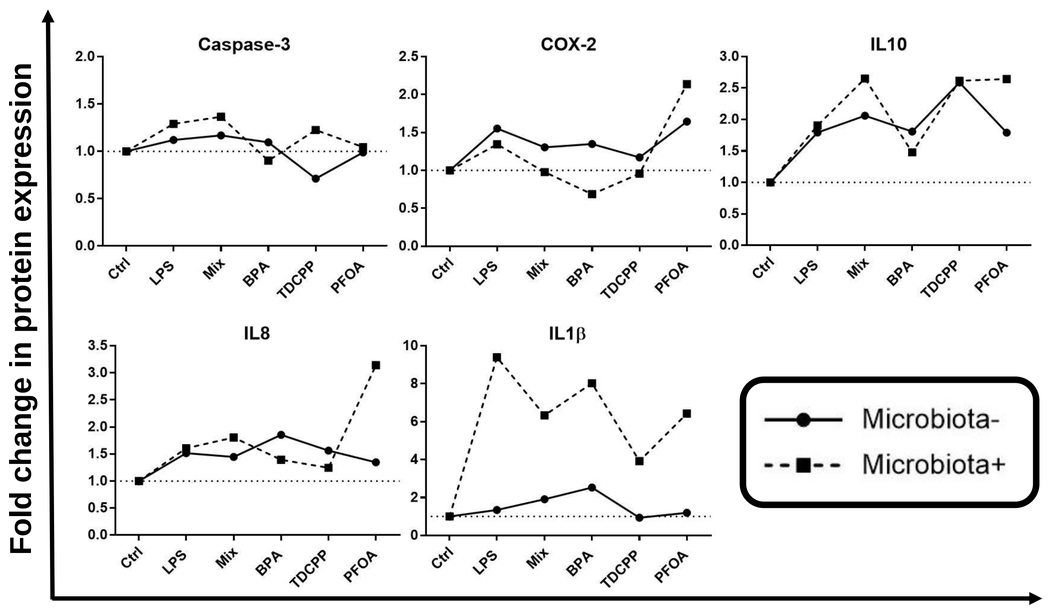


Figure 4: Relative expression of Caspase-3, COX-2, IL1β, IL8 and IL10 after exposure of THP-1 cells to LPS 1 μg/mL, BPA 50 μM, TDCPP 50 μM, PFOA 50 μM and a mix (Mix) of BPA, TDCPP and PFOA (final concentration of 50 μM) for 4h. THP-1 cells were exposed in presence (Microbiota+) and in absence (Microbiota-) of microbiota: *E.coli, S. salivarus, S. mitis, E. faecalis, L. plantarum and L. rhamnosus*.

- Differentiated Caco-2/HT-29 seem to be less affected by EDCs cytotoxicity, since a significative descent on viability can only be observed in undifferentiated cells (Fig. 2B).
- PFOA apparently cause increased viability both in THP-1 and Caco-2/HT-29, most likely by increasing mitochondrial metabolism interfering with the MTT assay. (Fig 2A and B).
- Although all three EDCs increase NFκβ phosphorylation and activation, BPA is by far the most efective (Fig 3).
- BPA, TDCPP and PFOA are related to an increased expression of proinflammatory genes (Fig. 4).
- TDCPP is related to the upregulation of IL10, while BPA induce IL1β and PFOA induce IL8 and COX2, specially in the presence of microbiota (Fig 4).

Conclusions

- Differentiated Caco-2/HT-29 cells seem to be less affected by EDCs than undifferentiated cells.
- BPA, TDCPP and PFOA induce NFκβ activation in THP-1 cells, which is related with an increased inflammatory response in macrophages [10].
- BPA appears to be the most effective of the three EDCs in inducing proinflammatory genes.
- Overall, the microbiota seems to positively contribute to THP-1-mediated inflammation induced by EDCs, specially by BPA and PFOA.

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