Bridging the Gap Between *In Vitro* Models and Human Physiology
The Advantages of HUB's Intestinal Patient-Derived Organoids in Toxicology

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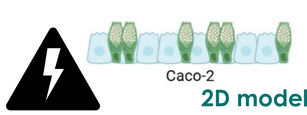
HUB ORGANOIDS is now part of MilliporeSigma

Background

Gastrointestinal (GI) toxicity is among the most frequently observed drug-induced adverse effects, occurring both in clinical trials and after market approval. Although generally non-life-threatening, GI toxicity often requires dose adjustments or co-medication, which can compromise therapeutic efficacy and patient quality of life.

Current challenges

- Limited physiological relevance (static conditions, monocultures)
- Low human predictivity of traditional 2D cultures
- Poor scalability and/or reproducibility
- High cost, ethical concerns and translational gaps in animal models
- Regulatory pressure to reduce animal testing (3Rs)



2D models

Such as Caco-2 cells - which overrepresent Paneth and goblet cells - or other cancer-derived lines are not suitable for assessing toxicity in healthy GI epithelial cells. **Troglitazone**, a PPARγ ligand, was considered safe when tested in Caco-2 cells[2,3] but later showed significant GI toxicity.

Animal models

Often fail to predict human GI toxicity, as illustrated by drugs like alosetron and benoxaprofen, both **appeared safe in preclinical animal studies yet were withdrawn post-marketing** due to severe GI adverse effects.

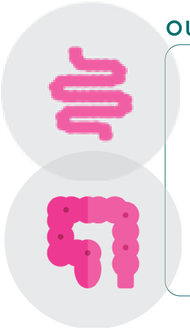
Why does it matter

- Poor translation from 2D preclinical models leads to compound attrition
- Late-stage toxicity findings are costly, time-consuming and ethically challenging
- Improving human relevance reduces risk, cost and uncertainty
- Regulatory and societal interest to reduce animal testing

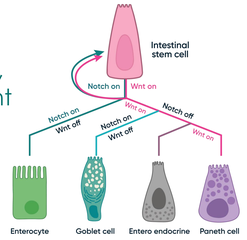
References

- [1]Sato et al; Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium;Gastroenterology. 2011; 141(5): 1762-1772
- [2]Klopotek et al; PPARγ Ligand Troglitazone Lowers Cholesterol Synthesis in HepG2 and Caco-2 Cells via a Reduced Concentration of Nuclear SREBP-2. 2004; Volume 231, Issue 8
- [3]Hosokawa et al; Fucoxanthin induces apoptosis and enhances the antiproliferative effect of the PPARγ ligand, troglitazone, on colon cancer cells. 2004; Volume 1675, Issues 1-3, 113-119
- [4]Kourula et al; Intestinal organoids as an in vitro platform to characterize disposition, metabolism, and safety profile of small molecules. 2023; Volume 188, 1 September 2023, 106481
- [5]Lin and Will; Evaluation of Drugs With Specific Organ Toxicities in Organ-Specific Cell Lines. 2012; Volume 126, Issue 1, Pages 114–127

OUR SOLUTION: ORGANOID-BASED DRUG DEVELOPMENT



Intestinal organoids provide a 3D, multicellular, and physiologically relevant model that more closely resembles native human intestinal tissue



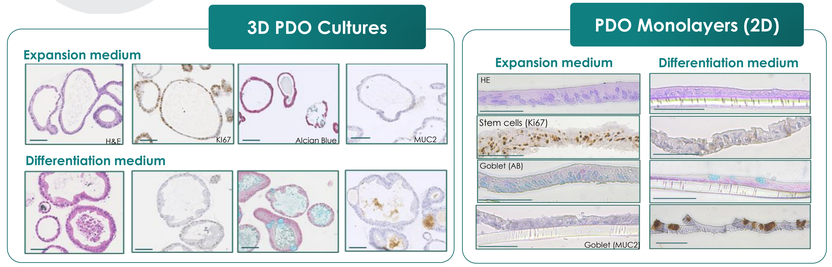


Figure 1. Specialized media conditions support the enrichment of distinct epithelial cell lineages.

Aim of this study: Provide a combination of **human-relevant, highly specific and adaptable** assays to predict GI toxicity with intestinal organoids in high-throughput format.

Table 1. GI toxic compounds

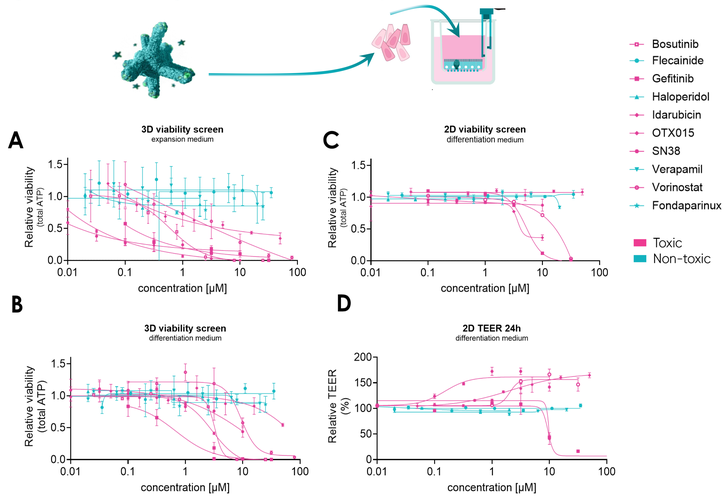
Compound	Compound mode of action
Idarubicin	DNA topoisomerase II inhibitor
SN-38	DNA Topoisomerase I inhibitor
Bosutinib	BCR-ABL kinase inhibitor
Gefitinib	EGFR inhibitor
Vorinostat	HDAC inhibitor
OTX-015	BET inhibitor
Troglitazone	PPARγ ligand
Indomethacin	COX inhibitor

Table 2. Non-GI toxic compounds

Compound	Compound mode of action
Haloperidol	Postsynaptic dopamine receptor blocker
Verapamil	Ca^{2+} channel blocker
Fondaparinux	Inhibitor of factor Xa
Flecainide	Na^{+}/K^{+} ion channel inhibitor
Ribavirin	Guanosine analog

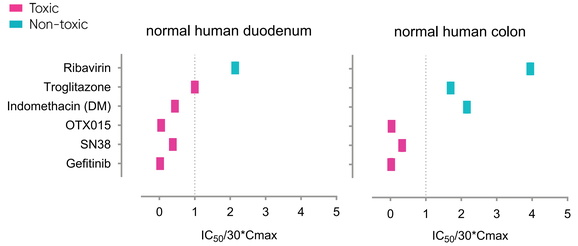
RESULTS

Figure 2. Assessing different aspects of intestinal toxicity using 3D organoids and 2D organoid-derived monolayers.



Our intestinal organoid platform allows the evaluation of different aspects of drug-induced GI toxicity. Duodenal organoids cultured in 3D were exposed for 3 days to compounds listed in **Table 1** and **2** and cell viability was measured via an ATP-based assay (CellTiter-Glo®) to assess toxicity on (A) stem cells and trans-amplifying cell types or (B) fully differentiated enterocytes. Further, 2D organoid-derived enterocyte-enriched epithelial monolayers enable differential assessment of basolateral or apical drug toxicity on (C) differentiated epithelial cell viability as well as (D) barrier integrity (transepithelial electrical resistance, TEER). Cytostatic drugs, such as Idarubicin, are only detectable in 3D expansion conditions and not in 2D monolayers. Non-linear fit, N = 3; mean ± SD.

Figure 3. Normal human duodenal and colon 3D organoid screening as tool to assess GI-toxicity.



Using patient-derived organoids from different anatomical locations, such as duodenum and colon, allows the targeted evaluation of specific toxicological compound profiles. Here, normal (expanding) human (A) duodenal and (B) colon organoids where exposed to a concentration range of 6 compounds for five days (adapted from [4]). A ratio of $IC_{50}/30*C_{max} \leq 1$ was used to identify toxic compounds, as described before.[5] All compounds tested could be correctly identified as toxic for duodenal or colonic organoids in the 3D viability screen. While non-toxic in Caco-2 assays, Troglitazone induced duodenal toxicity in normal human organoids, demonstrating the superior predictive power of organoid models for detecting drug-induced GI toxicity.

IC_{50} – half-maximal inhibitory concentration. C_{max} – maximal determined (human) plasma concentration.

The Guide to Better Decisions: Patient Derived Organoids to de-risk pre-clinical development

Summary and Conclusions

- 3D organoid viability screening correctly identified all GI toxic compounds, including **Troglitazone** which was deemed safe in Caco-2 2D models.
- Expansion and differentiation conditions allow estimation of toxicity towards stem cells versus specialized enterocytes
- 2D monolayer TEER assessment gives insight into mucosal disruption and permeability
- **Further studies with a higher number of compounds are required to confirm the potential of intestinal organoids for standardized toxicological applications.**

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