Human Intestinal Organoids and Derived Barrier Models for High-Efficiency Assessment of Drug-Induced Gastrointestinal Toxicity

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Introduction

Drug-induced gastrointestinal (GI) toxicity is one of the most frequent adverse events in Phase I clinical trials, mainly due to damage to the intestinal epithelium and functional cells and finally to barrier integrity. There is a critical need for highly physiologically relevant *in vitro* intestinal models to improve preclinical safety assessment.

Compared to traditional 2D cell lines, human intestinal organoids (HIOs), which recapitulate the *in vivo* intestinal architecture, self-renewal capability, and cellular function, represent a promising model.

This study demonstrates the high accuracy and unique advantages of HIOs and HIO-derived barrier models in predictive drug safety evaluation.

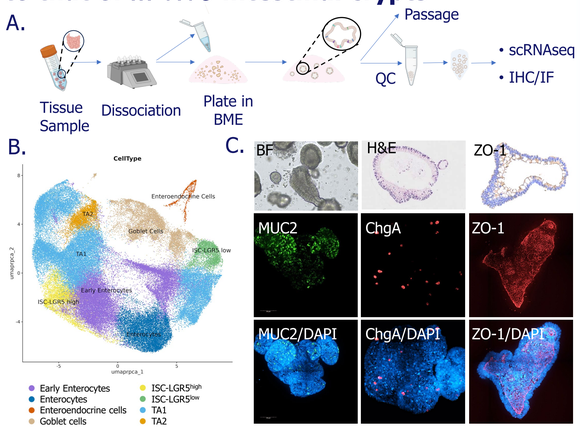
Methods

- 3D-cultured HIOs and HIO-derived gut barrier models were established and validated via single-cell RNA sequencing (scRNAseq) and immunostaining (IHC/IF).
- Systematic *in vitro* evaluation methods were developed to assess the GI toxicity of drugs:
  - 3D cell viability assay (CellTiter-Glo 3D, CTG)
  - Histopathology staining (H&E, Periodic Acid-Schiff)
  - IHC and IF staining for tight-junction and functional markers
  - Barrier-integrity measurement, including transepithelial electric resistance (TEER) and Lucifer Yellow (LY) permeability

Conclusion

- HIOs and their derived barrier models recapitulate the crypt-villus architecture and contain all major intestinal epithelial cell lineages.
- Compared with the Caco-2 model, HIOs exhibit significantly greater sensitivity to GI-toxic drugs in cell viability assays (78% vs. 22%).
- HIO-based static barrier and gut-on-a-chip models accurately simulate the tight junction-equipped intestinal barrier and show high predictive value for drug-induced barrier damage.
- The gut-on-a-chip model offers greater physiological relevance than the static barrier and was more sensitive to drug-induced barrier dysfunction.
- In summary, this platform provides a reliable and predictive tool for de-risking gastrointestinal toxicity in drug development.

1 HIOs preserve cellular diversity comparable to that of *in vivo* intestinal crypts



HIO model construction process and quality control data. A, Flowchart of the HIO model construction. **B,** scRNAseq data revealed that HIOs maintain self-renewal and multi-differentiation capacity and encompass nearly all cell types in the intestinal crypts. **C,** IHC/3D-IF staining results demonstrated the expression and localization of tight junctions (ZO-1$^+$), enteroendocrine cells (ChgA$^+$), and goblet cells (MUC2$^+$).

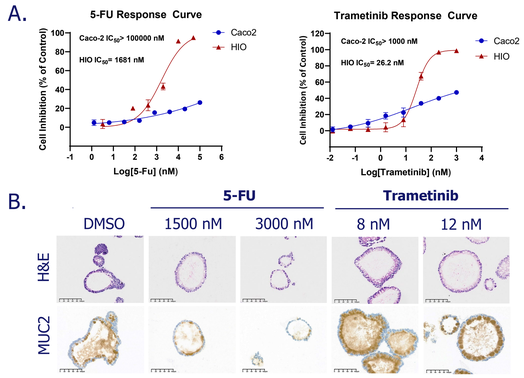
2 HIOs as a promising model for intestinal toxicity assessment

Drug Name	Caco-2_IC_{50} (nM)	HIO_IC_{50} (nM)	Diarrhea Incidence(%)	Literature C_{max} (nM)
Colchicine	156	31	77	17
Idarubicin	48	4	73	88
5-Fu	> 100,000	1,681	50-80	~100,000
Trametinib	> 1000	26	60-70	36
Imatinib	22,676	18,295	60	3,200
Axitinib	> 10,000	495	54	56
Mycophenolate Mofetil	3,113	566	48	57,000
Docetaxel	> 10	1	42	3,700
Paclitaxel	> 10	3	10-20	9-17
Dofetilide	>1000	>1,000	3	9
Fondaparinux	> 100000	>100,000	<3	840
Verapamil	> 10,000	>10,000	2	99
Acetaminophen	> 500,000	>500,000	1	140,000
Triamcinolone	> 5,000	>5,000	Not noted	27
Alfuzosin	> 5,000	>5,000	Not noted	32
Fomepizine	> 1,000,000	>1,000,000	Not noted	210,000

HIOs and Caco-2 Responses to a Panel of Diarrhea Drugs. We selected 9 drugs with high and 7 drugs with low clinical diarrhea incidence. In CTG assays, HIOs achieved 78% sensitivity and 100% specificity in GI toxicity prediction, versus Caco-2's 22% sensitivity and 100% specificity. Successfully predicted data are shown in blue.

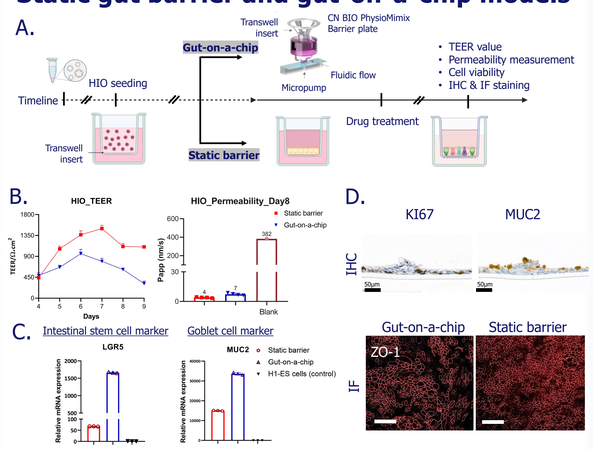
The results reflect HIOs' enhanced physiological relevance and diverse epithelial lineages, enabling more efficient detection of multiple toxicity mechanisms for multiple cell lineages. Imatinib and Axitinib, the two VEGFR inhibitors for which the prediction failed, cause diarrhea primarily through mucosal capillary injury rather than direct epithelial cytotoxicity.

3 HIO models exhibited superior toxicity predictability in cell viability and functional cell differentiation



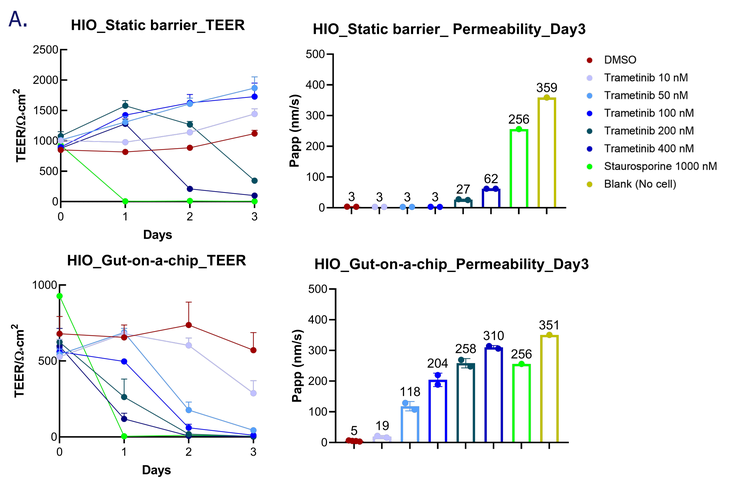
Cell viability detection and IHC staining in 3D HIOs. A, Cell viability curves showed that HIOs exhibited greater sensitivity to 5-FU and Trametinib versus Caco-2. **B,** H&E staining revealed that both drugs impaired bud formation; MUC2-IHC staining showed that 5-FU depleted goblet cells differentiation, while Trametinib induced aberrant hyperplasia.

4 Static gut barrier and gut-on-a-chip models



HIO-derived barrier and gut-on-a-chip model construction and quality control. A, Flowchart of the barrier model construction. **B,** TEER and permeability assay results showed that both models formed an intact intestinal barrier. Results from qPCR **(C)** and IHC/IF staining **(D)** confirm the intestinal stem cell (ISC) self-renewal (KI67), goblet cell differentiation (MUC2+) and the presence of intact tight junctions (ZO-1). Scale bar=50μm.

5 Gut-on-a-chip model: A physiologically biomimetic system with enhanced sensitivity for drug toxicity prediction



Trametinib compromised the gut barrier in static and gut-on-a-chip systems. A, Decreased TEER values and increased LY permeability indicated that Trametinib could induce damage to both barrier models. Compared with the static model (top), the gut-on-a-chip model (bottom) exhibited TEER values closer to a physiological barrier and demonstrated higher sensitivity to Trametinib, showing significant barrier impairment at the clinically relevant C_{max} (36nM, reference to PMC4243903). **B,** Similarly, IF staining revealed that identical Trametinib treatment caused more severe tight-junction disruption (ZO-1) and goblet cell dysregulation (MUC2) in the gut-on-a-chip model compared to the static model, further supporting its physiological relevance and GI-toxicity predictive sensitivity.