Human Intestinal Organoid Culture System for Drug-Induced Gastrointestinal Toxicity Screening

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INTRODUCTION

Drug-induced gastrointestinal (GI) toxicity is one of the most common adverse events (AEs) in Phase 1 clinical trials. Symptoms include diarrhea, dehydration and ulceration, which produce epithelial damage and impaired barrier function. Many chemotherapeutic agents have dose-limiting complications due to GI toxicity and result in compromised efficacy in the clinic. Therefore, the early detection of GI liability in novel therapeutics is crucial during preclinical drug discovery. Organoids generated from the human intestinal epithelium recapitulate numerous features of the *in vivo* adult intestine, including self-renewal and differentiation pathways, cellular composition, and cellular organization. Intestinal organoids derived from human biopsy samples can be cultured and the viability of intestinal organoids can be measured following treatment with test compounds. Drug-induced GI toxicity may also result from perturbations to colonic epithelial barrier function. Therefore, *in vitro* models that more accurately represent the physiology of the intestinal epithelium may be more predictive of clinically adverse events compared to immortalized or transformed cell lines. This study focuses on utilizing intestinal organoids for viability and barrier function assays in a 96-well plate format for preclinical drug-induced GI toxicity evaluation.

METHODS

Human Intestinal Culture and Maintenance
Intestinal organoids derived from human biopsy samples from duodenal, ileal and colonic regions were obtained from Hubrecht Organoid Technology (HUB)* as frozen aliquots. The organoids were cultured and passaged in 50% Corning® Matrigel® domes according to manufacturer protocols supplied with the IntestiCult™ Organoid Growth Medium (Human). Cultures were incubated at 37°C and 5% CO_2, for 6 - 8 days before passaging, with medium changes 2 - 3 times per week. Organoids were passaged using Gentle Cell Dissociation Reagent (GCDR) followed by mechanical disruption to break up domes. Suspensions were washed, resuspended in a 50:50 mixture of IntestiCult™ Organoid Growth Medium and Matrigel® and replated in domes.

96-Well Plate Viability Assay
Organoids were harvested as described above and seeded in a 6 µL Corning® Matrigel® dome format in a 96-well plate in IntestiCult™ Organoid Growth Medium and the plates incubated at 37°C and 5% CO_2 for two days. On day 2, the medium was removed and fresh media containing varying concentrations of reference drugs were added at three replicates per dose. The drugs used included gefitinib (EGFR inhibitor with clinical intestinal adverse events), colchicine (a microtubule polymerization inhibitor with clinical intestinal adverse events), and loperamide (an anti-diarrheal drug). Drugs were solubilized in DMSO and added to cultures with a final DMSO concentration of < 0.1%. Cultures were treated with drug for 5 days. After treatment, viability was determined using CellTiter-Glo® 3D cell viability assay following manufacturer's protocols. The viability of each treatment condition was expressed as a percentage of the solvent control.

Organoid-Derived Monolayer Culture
To establish the monolayer cultures, 7- to 10-day-old organoid cultures were harvested from Corning® Matrigel® domes using GCDR. Organoids were washed once with DMEM/F-12 then incubated at 37°C for 10 minutes in 0.05% trypsin-EDTA and mechanically disrupted to dissociate the organoids into single cells. The cells were washed and resuspended in IntestiCult™ Organoid Differentiation Medium (ODM; Human) containing 10 µM Y-27632 and gentamicin. The cell suspension was then seeded onto 24-well or 96-well Transwell® inserts coated with a 2% Corning® Matrigel® solution, and differentiated in IntestiCult™ Organoid Differentiation Medium (Human) for 7 – 10 days. Transepithelial electrical resistance (TEER) readings were taken using a World Precision Instruments EVOM2 voltmeter.

Caco-2 Monolayer Culture Caco-2 cells were cultured and maintained in high glucose DMEM containing 10% FBS, 15mM HEPES, 1X GlutaMAX™, 1X non-essential amino acids, and gentamicin. To establish monolayer cultures, Caco-2 cells were harvested with 0.05% trypsin-EDTA, washed with DMEM/F-12, resuspended in culture medium, seeded onto 24-well or 96-well Transwell® inserts, and differentiated for 21-24 days.

Barrier Integrity Assessment
At the end of the differentiation period, the medium was switched with fresh medium supplemented with increasing concentrations of test drug in both the apical and basal chamber. The apical medium was also supplemented with the fluorescent permeability marker, 4kDa FITC-dextran. The monolayers were exposed to the test drug treatment for 2 days, then the basal medium was collected and the cumulative basal concentration of 4kDa FITC-dextran was quantified. The post-treatment barrier integrity was assessed with the fluorescent permeability marker, Lucifer Yellow. The basal concentration of Lucifer yellow was quantified and the permeability coefficient (P_{app}) for each condition was calculated. Finally, viability was determined using Promega CellTiter-Glo™ cell viability assay following manufacturer's protocols, and the end-point viability of each treatment condition was expressed as a percentage of solvent control.

Permeability coefficient (P_{app}) Determination
The permeability coefficient was calculated using the following equation: $P_{app} = \frac{dQ}{dt} x \frac{1}{A \times C_0}$

Where dQ = [compound] in receiver chamber (µM) x receiver chamber volume (cm³); dt = assay duration (s);
A = membrane surface area (cm²) ; C_0 = [compound] in donor chamber (µM)

IC_{50} Determination
The concentration of 50% inhibition (IC_{50}) of intestine organoid growth for each drug was calculated by plotting the log of the drug concentration against the percent of maximum viability from solvent control cultures using GraphPad Prism® 5. To generate a curve fitting these data points the following equation was used: log(inhibitor) vs. response – variable slope (4 parameters): Y=bottom + (top-bottom)/[1+10^((LogIC50-X)*HillSlope)]

Intestinal Organoid Characterization
Intestinal organoids were characterized by immunocytochemistry (ICC) and quantitative real-time PCR (qPCR) for intestinal proteins and genes, respectively.

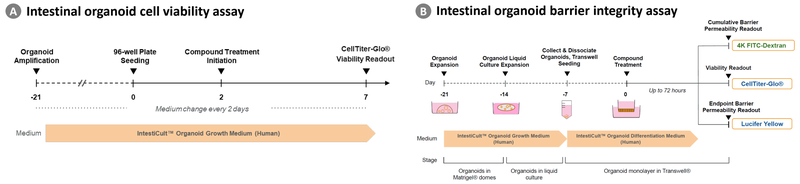


FIGURE 1: Schematic of 96-well Human Intestinal Organoid Cell Viability and Barrier Integrity Assay Protocols

(A) Human intestinal crypts were seeded in IntestiCult™ Organoid Growth Medium to yield proliferative intestinal organoids. Intestinal organoids were cultured in the presence of varying concentrations of small molecule drugs for 5 days, after which cell viability was assessed by Promega® CellTiter-Glo® 3D. (B) Human intestinal crypts were seeded in IntestiCult™ Organoid Growth Medium to yield proliferative intestinal organoids. To establish the monolayer cultures, organoids were harvested, resuspended in IntestiCult™ ODM, seeded onto 96-well Transwell® inserts, and maintained in IntestiCult™ ODM for 7 days. On the day of drug treatment, the medium was replaced with fresh medium supplemented with varying concentrations of test drug in both the apical and basal chambers. The monolayers were exposed to test drug treatment for 2 days. Barrier integrity was determined by measuring the basal concentration of 4kDa FITC-Dextran (cumulative readout) and Lucifer Yellow (post-treatment endpoint readout). Viability was assessed by Promega CellTiter-Glo™.

RESULTS

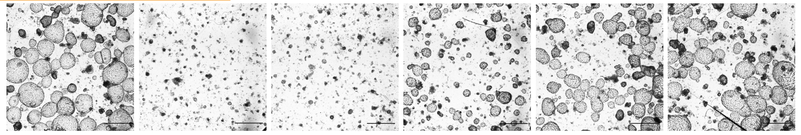
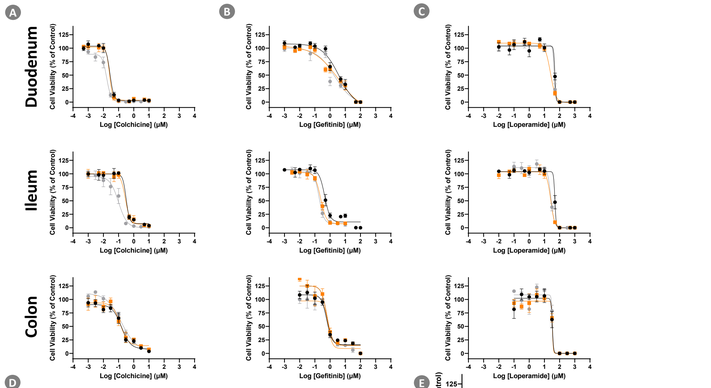


FIGURE 2: Intestinal Organoids Exhibited Changes in Morphology and Size after 5 days Gefitinib Treatment

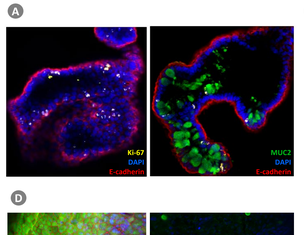
Human duodenal organoid cultures were cultured in IntestiCult™ Organoid Growth medium (OGM; Human) in the presence of varying concentrations of gefitinib for 5 days. Brightfield images shown are representative images of triplicate wells. Scale bar = 200 µm. Results demonstrated an inhibition of organoid growth and compromised culture morphology with gefitinib treatment starting from 0.1 µM.

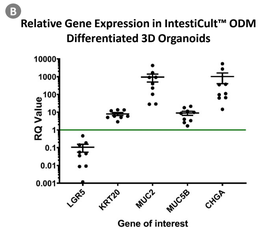


	Colchicine	Gefitinib	Loperamide
	Average IC_{50} ± SD (µM)		
Duodenum	0.0231 ± 0.00679	0.329 ± 0.165	40.2 ± 12.1
Ileum	0.240 ± 0.128	0.709 ± 0.0128	34.8 ± 12.9
Colon	0.170 ± 0.0370	0.800 ± 0.0503	33.7 ± 0.918

FIGURE 3: Human Intestinal Organoids Have High Sensitivity to Toxic Effects of Gefitinib and Colchicine.

Cell viability in human duodenal, ileal, or colonic organoids treated with (A) colchicine, (B) gefitinib, and (C) loperamide for 5 days. (D) Summary of IC_{50} values and standard deviation generated from three experiments. (E) IC_{50} values of Caco-2 cells treated with colchicine and gefitinib.





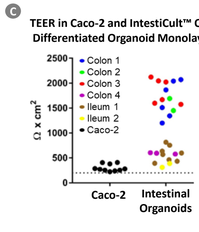


FIGURE 4: Intesticult™ Organoid Differentiation Medium Promotes Differentiated Cell Types

Human intestinal organoids were cultured and expanded in IntestiCult™ OGM and differentiated in IntestiCult™ ODM. (A) ICC of intestinal organoids stained for proliferation marker Ki-67 and goblet cell marker MUC2 (n = 3). (B) Gene expression analysis of immature organoids (OGM; RQ = 1) versus mature organoids (ODM) by qPCR. The reduction in LRG5 and increases in KRT20, MUC2, MUC5B, and CHGA suggest IntestiCult™ ODM promoted differentiation into goblet and enteroendocrine cell types. (C) Human intestinal organoid monolayers were differentiated in IntestiCult™ ODM on Transwell® membranes as a submerged monolayer. Organoid monolayers show higher TEER values compared to Caco-2 cultures ($p < 0.0001$). (D) ZO-1 and MUC2 staining indicate tight junction formation and presence of goblet cells in organoid monolayers.

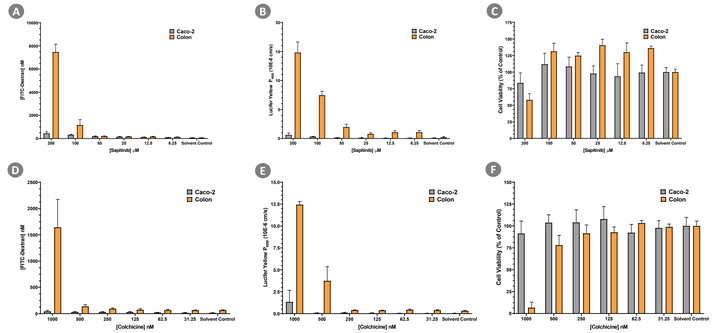


FIGURE 5: Effect of Sapitinib and Colchicine on the Barrier Integrity of Caco-2 and Organoid Monolayers

Caco-2 and colon-derived organoid monolayers cultured in 96-well Transwell® inserts were exposed to sapitinib or colchicine for 48 hours. Sapitinib and colchicine produced a dose-dependent response on the barrier integrity in organoid monolayers but not Caco-2 monolayers when assessing (A, D) 4kDa FITC-dextran permeability, (B, E) Lucifer yellow permeability, and (C, F) total cell viability. Representative results from 3 independent experiments. These results suggest the organoid monolayers are more susceptible to barrier integrity disruption compared to Caco-2, and may be more predictive of adverse clinical events for GI toxicity.

SUMMARY

- Human intestinal organoids can be used in 96-well viability and barrier function assays for preclinical drug-induced GI toxicity evaluation.
- Organoids grown in IntestiCult™ Organoid Growth Medium (Human) incorporate a functional lumen enclosed by a polarized intestinal epithelial cell layer. Drug dose-response curves of organoid viability demonstrated superior sensitivity to GI toxicity inducing agents compared to those observed using Caco-2 cells.
- Diverse cell types are present in 3D intestinal organoids and organoid-derived monolayers, including stem cells, enterocytes and functional goblet cells.
- Organoid-derived monolayers display ZO-1 expression and higher TEER values compared to Caco-2 cell monolayers; suggesting the formation of tight junctions indicative of intact barrier function, while greater sensitivity to GI toxicity-inducing agents compared to Caco-2 cells may be more predictive of adverse clinical events.

*STEMCELL Technologies Inc. has licensed the worldwide, non-exclusive rights to use organoid lines from HUB Organoids for the purpose of providing routine organoid assay-based services.