**INTRODUCTION**

Drug-induced gastrointestinal (GI) toxicity is one of the most common adverse events (AEs) in Phase I clinical trials. Symptoms include diarrhea, abdominal pain, and vomiting, and may result in dose reductions in clinical trials and death in rare cases. Modulation of gut commensal bacteria may reduce GI toxicity, although the mechanisms are not well understood. To address this, intestinal organoids derived from human biopsy samples can be cultured and used to study the effects of drugs on GI tissues. This study focuses on utilizing intestinal organoids for viability and barrier function assays in a high-throughput format for preclinical drug testing.

**METHODS**

**Human Intestinal Organoid 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 (OM). Organoids were collected at 7-10 days of culture and resuspended in OM to yield proliferative intestinal organoids. The organoids were seeded in IntestiCult™ Organoid Growth Medium and maintained for 2-3 days before adding test compounds or treatment.

**10-Well Plate Viability Assay**

Intestinal organoids were exposed to test compounds and incubated for 4 days. The organoids were then seeded onto 24-well or 96-well Transwell® inserts and differentiated for 21-24 days. A viability assay was performed following manufacturer’s protocols, and the end-point viability of each treatment condition was expressed as a percentage of solvent control.

**Intestinal Organoid Barrier Integrity Assay**

The permeability coefficient was calculated using the following equation:

\[
P_{\text{app}} = \frac{\Delta C \times d}{V \times \Delta t}
\]

Where

- \(P_{\text{app}}\) = permeability coefficient (cm/s)
- \(\Delta C\) = concentration gradient (mM)
- \(d\) = membrane thickness (mm)
- \(V\) = volume of receiver chamber (mL)
- \(\Delta t\) = time of measurement (min)

**RESULTS**

**Intestinal Organs Exhibited Changes in Morphology and Size after 5 days Gefitinib Treatment**

Intestinal organoids were treated with gefitinib (1 μM) for 5 days and imaged using a high-content imaging system. The organoids showed significant changes in morphology and size compared to control cultures.

**Barrier Integrity Assessed**

Differentiated organoid monolayers were assessed for barrier integrity using fluorescein isothiocyanate (FITC)-labeled dextran (20,000 MW) and lucifer yellow as probes. The following were determined:

- TEER values in Caco-2 and IntestiCult™ ODM
- Relative gene expression in IntestiCult™ ODM
- Caco-2 and differentiated organoid monolayers

**DISCUSSION**

Intestinal organoids derived from human biopsy samples can be cultured in vitro and used to study the effects of drugs on GI tissues. This study focuses on utilizing intestinal organoids for viability and barrier function assays in a high-throughput format for preclinical drug testing.

**CONCLUSION**

Intestinal organoids derived from human biopsy samples can be cultured and used to study the effects of drugs on GI tissues. This study focuses on utilizing intestinal organoids for viability and barrier function assays in a high-throughput format for preclinical drug testing.

**SUMMARY**

- Human intestinal organoids can be used in 96-well viability and barrier function assays for preclinical drug-induced GI toxicity evaluations.
- Organoids grown in IntestiCult™ Organoid Growth Medium (OM) incorporate a functional luminal barrier composed of a polarized intestinal epithelium layer. Drug-induced changes in organoid viability demonstrate the capability of organoids to identify compounds that induce GI toxicity among the compounds tested.
- Organoid-derived monolayers display GI toxicity profiles comparable to those of GI50 and TGI50, and demonstrate the capability of organoids to identify GI toxicity among the compounds tested.