Human organoid lines for modelling the intestinal epithelial barrier in vitro

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Introduction

Pre-clinical intestinal barrier models

When developing new drugs for oral administration, it is crucial to evaluate absorption, metabolism and excretion of candidate therapeutics in reliable intestinal experimental models.

In vivo animal models are often used for drug pharmacokinetic studies, but they are costly, low in throughput and, due to species differences, frequently fail to replicate many features of the human intestine with respect to drug metabolism and bioavailability. For several years human intestinal cancer cell lines cultured as monolayers in vitro have served as an alternative model system. Even though established cancer lines are readily available and easy to expand, they lack important physiological features, such as appropriate tissue cytoarchitecture and expression of transporters/enzymes in comparison with the normal intestine.

Organoid-derived intestinal monolayers

A major boost to modelling the human intestinal epithelium ex vivo has come with the advent of organoid cultures. However, intestinal organoids are typically grown within a hydrogel as 3D structures with a lumen not easily accessible, thus making this system challenging for evaluating transport of molecules across the epithelium. Numerous studies have proposed the culture of cells derived from intestinal organoids as a 2D monolayer on a permeable support, where media with test compounds can be readily added to or sampled from either side of the epithelium. These organoid-derived monolayers provide the opportunity of examining the interaction between drugs and the intestine ex vivo whilst overcoming major caveats imposed by animal models or established tumor cell lines. However, limitations regarding accessibility and number of human intestinal organoids can halt their widespread use in the drug development process.

3D Ready intestinal organoids

Here, epithelial monolayers were derived from two 3D Ready™ non-cancerous human duodenal organoid lines (DP8N3 and DP41N2) from Molecular Devices. Morphological and functional analyses were conducted to assess the resulting monolayers for their ability to model the human intestinal barrier. Molecular Devices proprietary bioprocess technology enables the expansion of reproducible and validated batches of 3D Ready organoids, allowing for easier implementation of relevant in vitro barrier models.

Methods

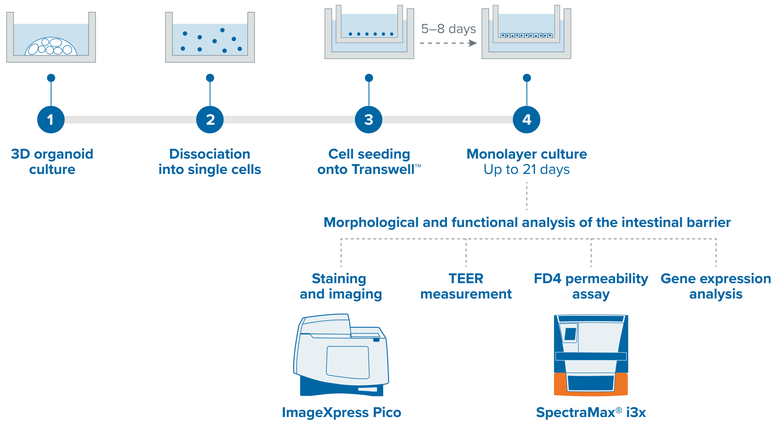

Figure 1. Organoid-derived monolayer formation and characterization workflow.

Methods

Organoid-derived monolayer set-up

DP8N3 and DP41N2 organoids were dissociated into single cells and seeded onto 6.5 mm Transwell® inserts (Costar®) coated with 1:50 diluted Matrigel® (10^5 – $2x10^5$ cells per well) in IntestiCult™ Organoid Differentiation Medium (STEMCELL™ Technologies).

Monolayers reached confluency within 5–8 days and were kept in culture for up to 21 days. Over time monolayers were subjected to:

- Staining for analysis of intestinal barrier markers via the ImageXpress Pico imaging system
- Trans-Epithelial Electrical Resistance (TEER) measurement
- FITC-dextran 4kDa (FD4) permeability assays using the SpectraMax® i3x platform
- Expression analysis of drug transporters via qRT-PCR

Results

Analysis of monolayer morphology

To morphologically characterize the monolayers derived from DP8N3 and DP41N2 we conducted a series of analyses (Figure 2). Cells from either DP8N3 or DP41N2 organoids plated onto Transwell® inserts formed epithelia consisting of a single layer of cells and characterized by the presence of inter-cellular tight junctions.

Figure 2. (A) Representative TL images of cells derived from DP8N3 (left) or DP41N2 (right) organoids after plating onto Transwell® inserts. Cells reached 100% confluence after Day 4. 4x magnification, scale bar, 100 µm. (B) Hematoxylin & Eosin-stained transverse sections of Transwell® inserts seeded with cells from DP8N3 (top) or DP41N2 (bottom) 9 days after monolayerization. Scale bar, 100 µm. (C) Alexa Fluor™ 594 Phalloidin staining of monolayers from DP8N3 (left) or DP41N2 (right) 7 days after seeding shows filamentous (F) actin localization. 10x magnification, scale bar, 25 µm. (D) ZO-1 immunofluorescence staining of Day 14 monolayers from DP8N3 (left) or DP41N2 (right) organoids shows presence of inter-cellular tight junctions. DAPI was used as a counterstain. 20x magnification, scale bar, 25 µm.

Evaluation of barrier function

The ability of DP8N3- and DP41N2- monolayers to act as a barrier to the movement of ions, small and larger molecules was determined by measuring the Trans-Epithelial Electrical Resistance (TEER) over a period of 21 days (Figure 3).

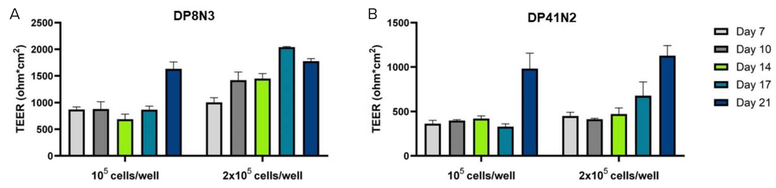

Figure 3. TEER values of monolayers derived from (A) DP8N3 or (B) DP41N2 organoids were measured 7, 10, 14, 17 and 21 days after seeding. Values were determined taking into account blank resistance from inserts with no cells and the culture insert area. Data are expressed as means ± SD (n=3).

Results

Barrier permeability assay

To evaluate the paracellular permeability of the monolayers, translocation of FITC-conjugated dextran (4 kDa, FD4) from the top to the bottom compartment of transwell inserts was measured (Figure 4).

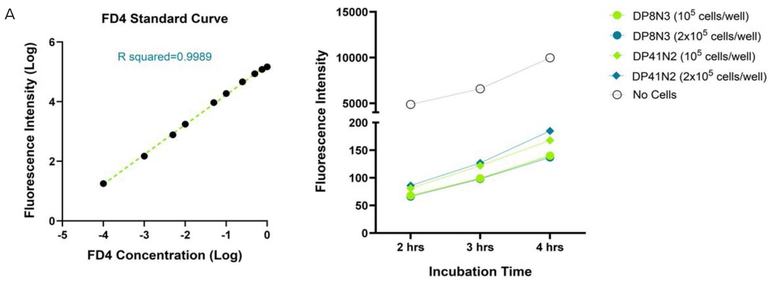

Figure 4. Monolayer cultures were washed with Hanks' Balanced Salt Solution (HBSS) without phenol-red and FD4 in HBSS (1 mg/ml) was added to the top Transwell® compartment. (A) Fluorescence intensity was determined in the bottom Transwell® compartment 2, 3 and 4 hours (hrs) after the addition of FD4 to the top compartment. A coated Transwell® insert with no cells was used as a control and a FD4 standard curve was run in parallel. (B) Equation used to determine the apparent permeability (Papp) of organoid-derived monolayers. (C) Graphs showing Papp (4 hrs) values determined for monolayers derived from DP8N3 (left) or DP41N2 (right) at Day 7, Day 14 and Day 21 days of culture.

Expression analysis of drug transporters

Expression levels of genes encoding key intestinal molecular transporters were analyzed via qRT-PCR. When cultured as monolayers, cells derived from both DP8N3 and DP41N2 organoids markedly up-regulated ABCB1, ABCG2 and SLC15A1 transcripts (Figure 5).

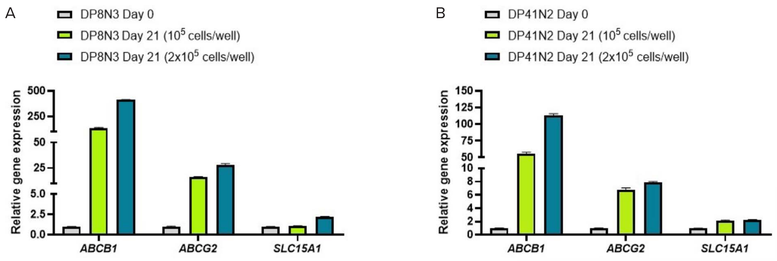

Figure 5. qRT-PCR analysis of ABCB1, ABCG2 and SLC15A1 transcript levels in monolayers from (A) DP8N3 or (B) DP41N2 organoids at Day 0 and Day 21 of the monolayer culture. Cells derived from organoid dissociation prior to seeding are shown as 'Day 0'. Gene expression levels on organoid-derived cells at Day 0 were set at 1. The Ct values of the target genes were normalized by those of the housekeeping gene, beta-Actin and the $2^{-\Delta\Delta Ct}$ method was adopted for the relative quantification. Data are expressed as means ± SD (n=3).

Conclusion

Sourcing organoids for in vitro intestinal barrier

- Both 3D Ready non-cancerous intestinal organoid lines (DP8N3 and DP41N2) developed by Molecular Devices are suitable for the set-up of in vitro intestinal barrier systems.
- DP8N3 and DP41N2 organoids can be expanded at scale by Molecular Devices allowing for easier implementation of in vitro barrier models in drug pharmacokinetic and safety studies.

HUB Organoid Technology used herein was used under license from HUB organoids.