RECAPITULATION OF NATIVE HUMAN EXPRESSION PATTERNS OF KEY TRANSPORT AND DRUG METABOLISM GENES IN AN IN VITRO HUMAN DONOR-DERIVED INTESTINAL EPITHELIUM

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Background & Objectives

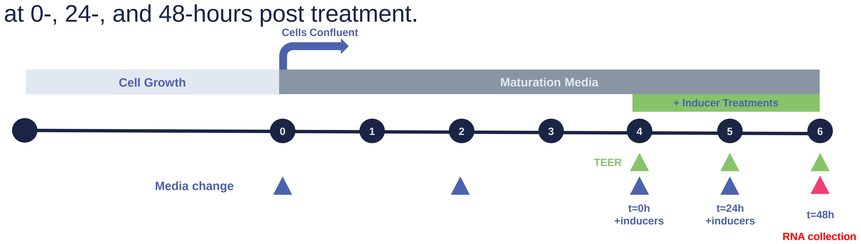
Orally administered therapeutics must pass through the single-cell thick intestinal epithelium before they can enter systemic circulation. The intestinal epithelium forms a barrier comprised of absorptive enterocytes and other cells which express various apical and basolateral transporters to facilitate absorption of intestinal contents and excretion of waste. The intestinal epithelium contains phase I and II metabolism enzymes, particularly in the jejunum, which can significantly impact drug bioavailability and result in drug-drug interactions. Therefore, understanding transport across and metabolism within the intestinal epithelium is important for successful development and formulation of orally delivered medications.

The objectives of this study were:

- Evaluate if Repligut® Planar-Jejunum could function as a model for intestinal drug ADME for orally formulated drugs and evaluating drug-drug interactions
- Determine impact to barrier function from known inducers of drug metabolism
- Assess capacity for induction of drug metabolism and transport genes in primary human intestinal cells

Methods

- RepliGut® Planar-Jejunum cultures were cultured to confluence then assessed for expression of key transport and metabolism genes following induction by transcription factors agonists
- RepliGut® Planar-Jejunum cultures were treated with a range of concentrations of model gene expression inducers, including, Rifampin, 3-methycholanthrene (3-MC), Calcitriol, and CITCO.
- Following 48 hours of drug treatment, RNA was collected for bulk RNAseq.
- Barrier integrity was evaluated with Transepithelial Electrical Resistance (TEER) at 0-, 24-, and 48-hours post treatment.



Conclusions

- Chemical induction was well-tolerated by RepliGut® Planar-Jejunum cultures and produced no significant changes in epithelial barrier function.
- RepliGut® Planar-Jejunum cultures demonstrated induction of major pathways associated with steroid and cholesterol metabolism and transport, with specific induction of key enzymes and transporters
- This model of the human gut epithelium could serve to study the contribution of the intestine to drug metabolism and clearance for orally-formulated medications.

References

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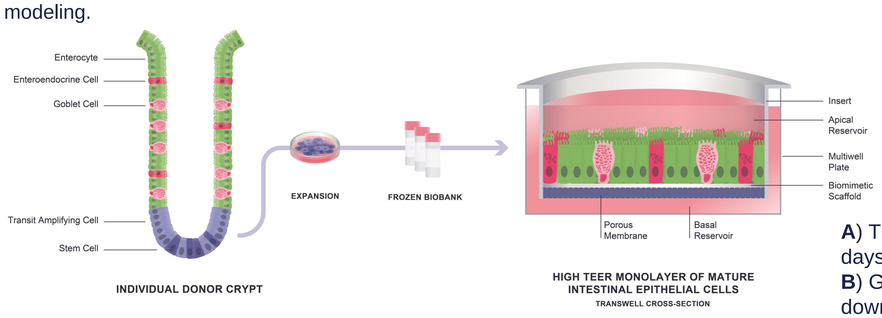
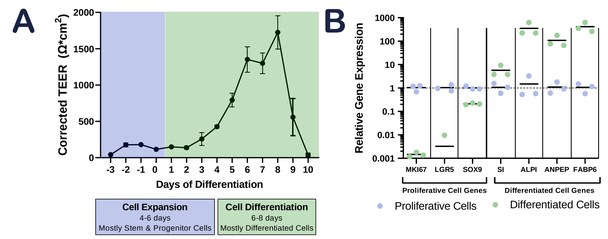
RepliGut® Planar - Jejunum

RepliGut® Planar is a human stem cell-derived platform that recreates the intestinal epithelium and enables biologically relevant compound screening and disease modeling.



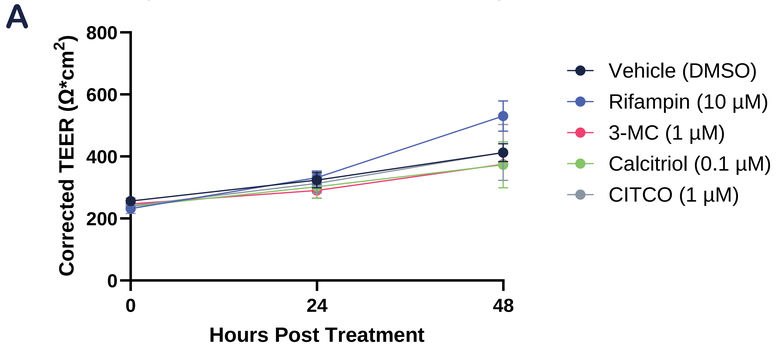


14-day culture timeline allows for investigation and analysis of proliferative or differentiated cell populations

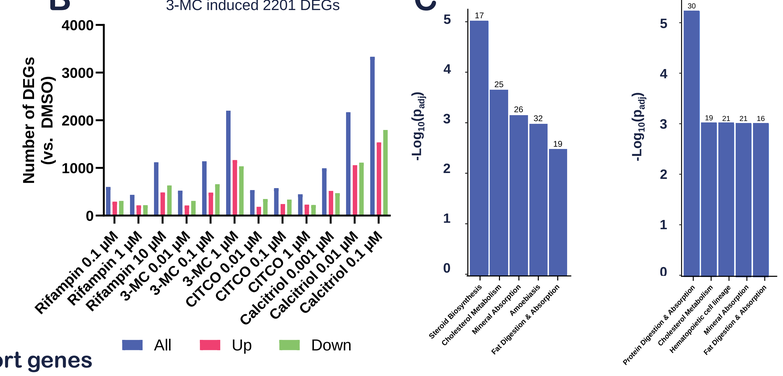
A) The RepliGut® Planar cell culture timeline consists of a cell growth phase (4-6 days) then a cell maturation phase (5-8 days) that can be monitored via TEER.
B) Gene expression of 3 human donors shows cells in the maturation phase have downregulated proliferative cell genes and upregulated differentiated enterocyte cell genes relative to cells in the proliferative phase. D = Donor ; PROLIF = proliferative cells ; DIFF = differentiated cells.

Results

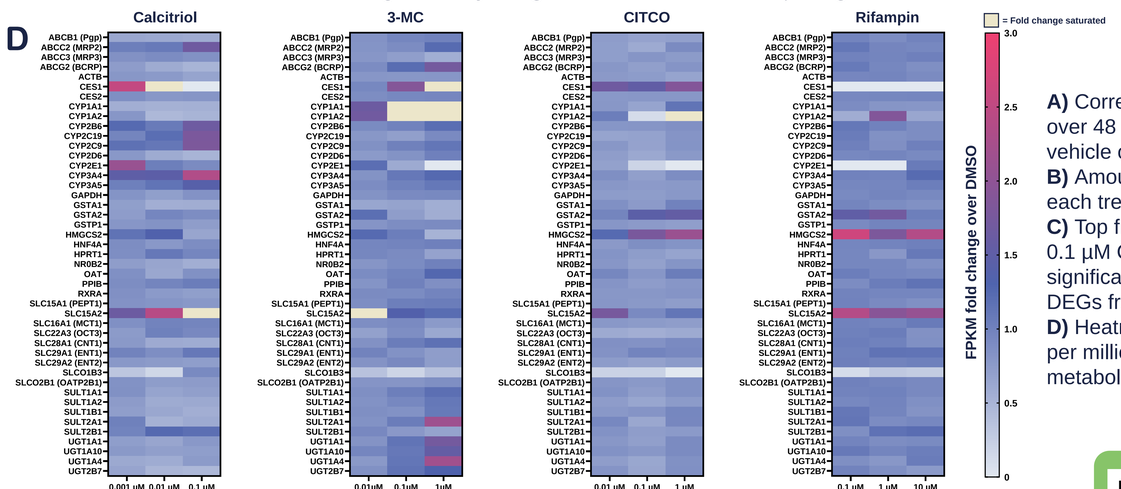
Barrier function of RepliGut® Jejunum cultures is minimally impacted by treatment with inducers of drug metabolism



Calcitriol and 3-MC induce largest changes in gene expression profile



Calcitriol and 3-MC induce changes in key drug metabolism and transport genes



Calcitriol and 3-MC significantly upregulated genes associated with steroid and cholesterol metabolism KEGG pathways

A) Corrected TEER from Repligut® Planar-Jejunum cultures over 48 hours post treatment with highest concentrations of vehicle or chemical inducers.
B) Amount of differentially expressed genes after 48 hours of each treatment tested against Repligut® Planar-Jejunum.
C) Top five enriched KEGG pathways from DEG analysis from 0.1 µM Calcitriol and 1 µM 3-MC. Y-axis represents significance and counts above bars represent number of DEGs from each condition present in the enriched term.
D) Heatmaps depicting mean FPKM (fragment per kilobase per million) fold change enrichment over vehicle for key drug metabolism and transport genes.

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