Characterization of reproducibility and biological variability in RepliGut® Planar – a stem cell-derived model of the intestinal barrier

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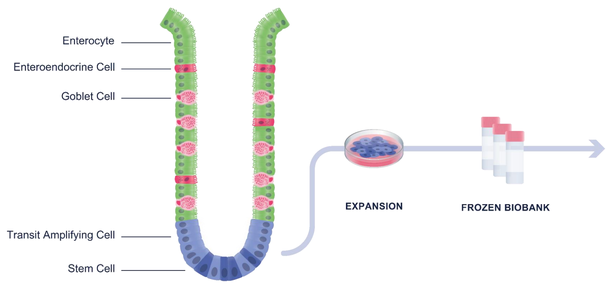
Introduction

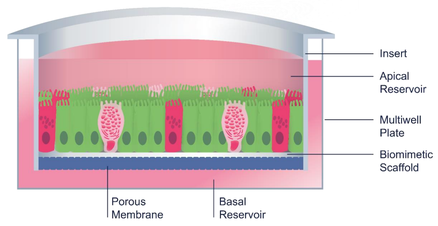
- Human stem cell-derived culture systems offer several advantages for studying gastrointestinal diseases:
  - Allow for high throughput assay design
  - Mimic the continuous cell turnover kinetics in the intestine
  - Replicate human physiology
- Inherent variability in stem cell-based complex cultures interferes with the ability to build and validate robust assays that query drug responses and pharmacokinetics, especially in 3D organolids
- RepliGut® Planar is an adult stem-cell derived intestinal model which, unlike organoids, is initiated by directly thawing cells into a transwell format and differentiating in situ. Start to finish experiments in as little as 7 days
- We aimed to identify and minimize sources of variability in the RepliGut® Planar platform in order to better design reproducible assays assessing barrier integrity and toxicity

Donor demographics used in this study. All tissue was obtained under strict ethical guidelines with donor family consent.

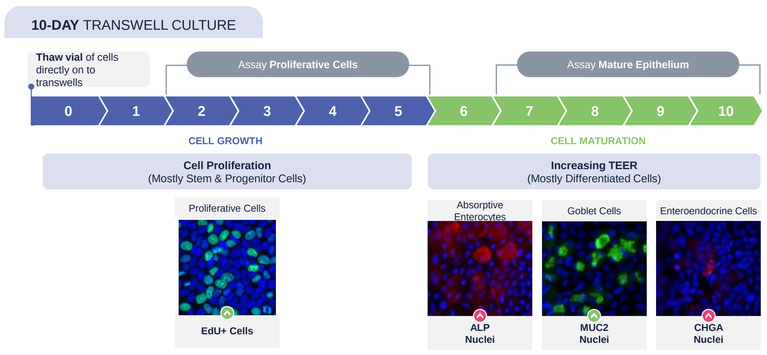
Table 1. Donor Characteristics				
Donor	**001**	**004**	**005**	**006**
Age (years)	23	51	50	51
Sex	Male	Female	Male	Male
Race	Caucasian	Caucasian	African American	African American
Height (in)	75	59	70	60
BMI	22.8	32.0	22.7	31.3
Weight (lbs)	182	158	162	219

RepliGut® Planar





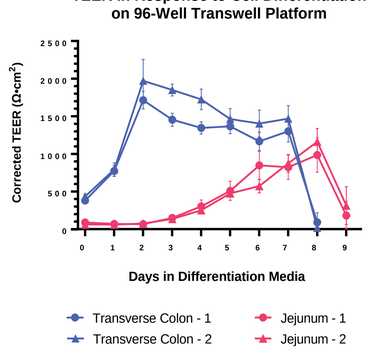
RepliGut® Planar is a stem cell-derived platform that recreates the human intestinal epithelium and enables biologically relevant screening of compounds and disease modeling. The transwell format allows for easy apical and basolateral access for compound addition or supernatant analysis.



The RepliGut® Planar cell culture timeline consists of a cell expansion phase (4-6 days) then a cell differentiation phase (5-8 days) that can be monitored via TEER.

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

TEER in Response to Cell Differentiation on 96-Well Transwell Platform

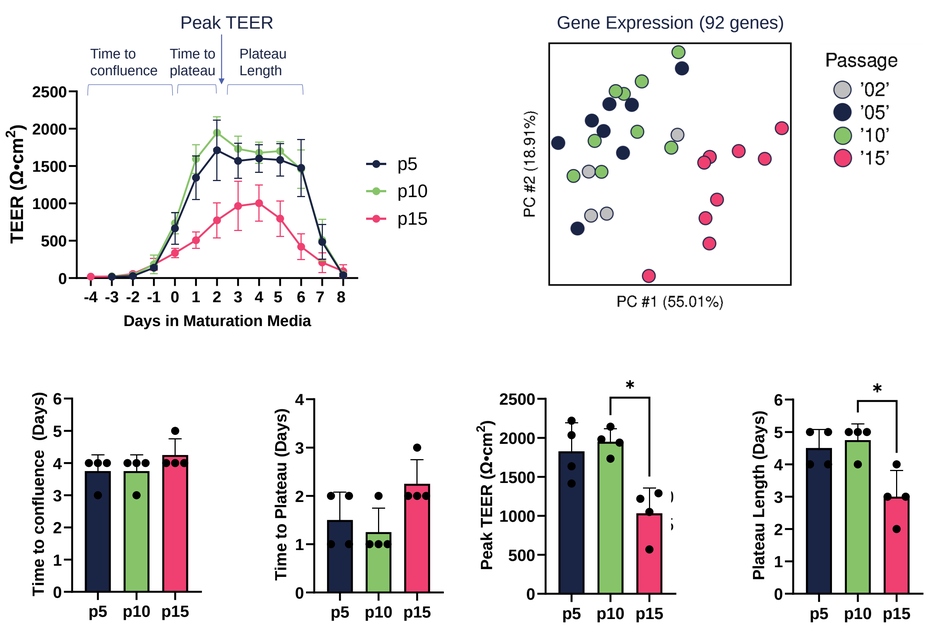


Regional specificity is retained in the cell culture barrier formation and integrity kinetics

Characterizing sources of variability

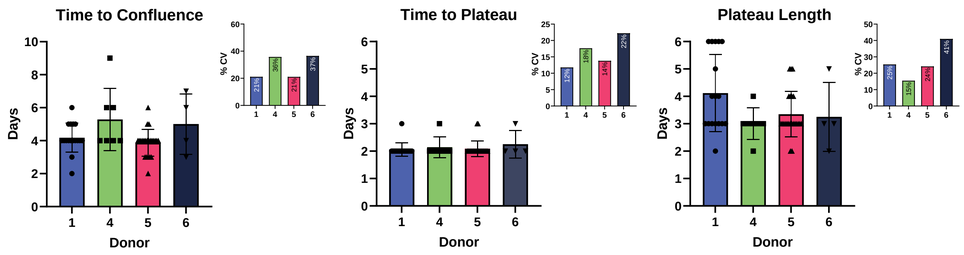
Comparisons between stem cell passage numbers

Methods: Transverse colon epithelial cells from passages 2, 5, 10 and 15 were plated on transwell plates and TEER was measured every 24 hours in culture. RNA was isolated from cultures grown to day 3 in RepliGut® Maturation Media.



- Significant changes in transverse colon TEER kinetics were observed at passage 15, as compared to passages 5 and 10
- Principal component analysis of a 96 gene Biomark dataset shows clustering of passage 2, 5, and 10 gene expression patterns.

Quality control metrics from 51 independent runs

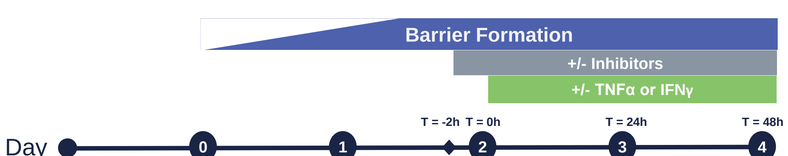


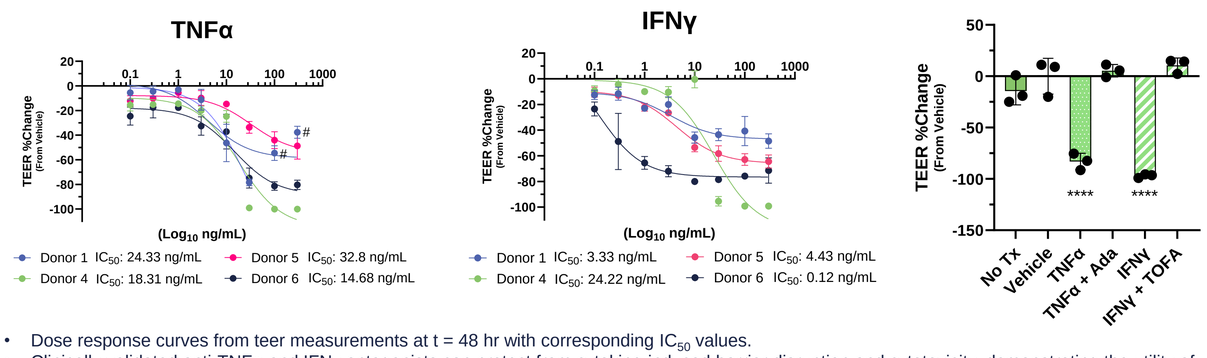
Methods: Passage 10 transverse colon epithelial cells from multiple donors were plated on 96-transwell plates and TEER was measured every 24 hours. **Data shown comprises 51 experiments across 19 cell lots**. Error bars represent mean ± SEM.

Applications of RepliGut® Planar

Inflammation effects on barrier integrity

InflammaScreen™ Assay design

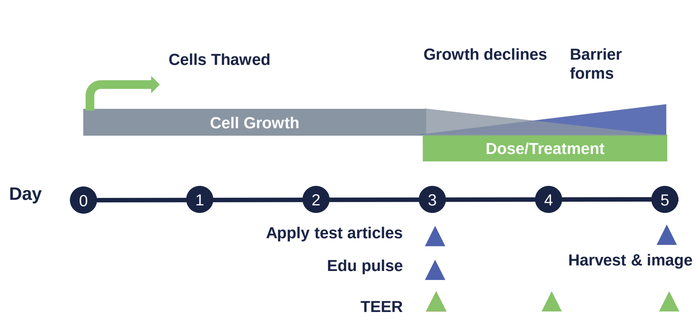




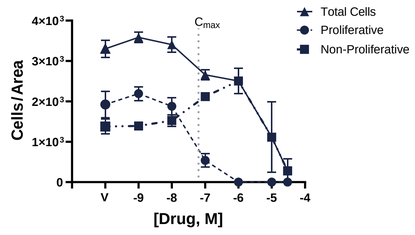
- Dose response curves from teer measurements at t = 48 hr with corresponding IC_{50} values.
- Clinically validated anti-TNFα-and IFNγ antagonists can protect from cytokine-induced barrier disruption and cytotoxicity, demonstrating the utility of RepliGut® Planar as a tool to explore drug pharmacology associated with TNFα-and IFNγ pathways in IBDs.

Stem-cell selective toxicity

StemTox™ Assay design



Idarubicin (DAPI/EdU)



Investigating the dose response when intestinal stem cells are exposed to Idarubicin showing selective toxicity towards the proliferative cell population (●) compared to differentiated cell population (■)

Conclusions

- RepliGut® Models recreate critical features of the human intestinal epithelium and represent a physiologically relevant system to study inflammatory and toxicity responses in vitro across different human populations.
- The direct to Transwell® culture and planar monolayer format has certain advantages over 3D organoids including well controlled differentiation kinetics and ability to monitor barrier function
- By identifying and controlling sources of experimental variability, true donor difference in response to stimuli be detected.

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