Characterization of reproducibility and biological variability in a stem cell-derived human intestinal epithelium model for applications in inflammation

Abstract 488

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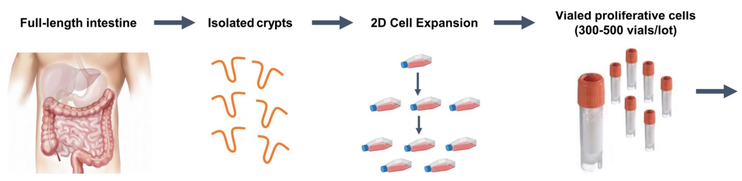
Variability in stem cell-derived culture systems

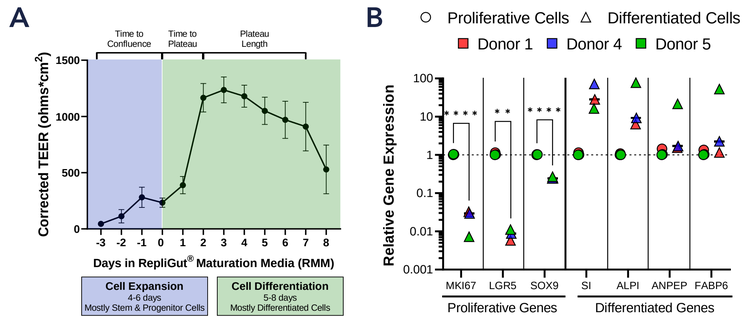
- Human stem cell-derived culture systems offer several advantages for studying gastrointestinal diseases:
  - Reduces need for animal-based preclinical studies
  - Allows for high throughput assay design
  - Reduces downstream timelines and costs
  - Replicates human physiology
- Inherent variability in stem cell-based complex cultures interferes with the ability to build and validate robust assays that query drug responses and pharmacokinetics
- We aimed to identify and minimize sources of variability in the RepliGut® Planar platform in order to better design reproducible inflammation-based assays

RepliGut® Planar

RepliGut® Planar is a unique, stem cell-derived platform that recreates the human transverse colonic 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



10-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 expansion phase (4-6 days) then a cell differentiation phase (5-8 days) that can be monitored via TEER.
(B) Gene expression confirms downregulation of proliferative cell genes and upregulation of differentiated enterocyte genes relative to cells in the proliferative phase. Asterisks denote statistical significance (p**<0.02, unpaired t-tests).

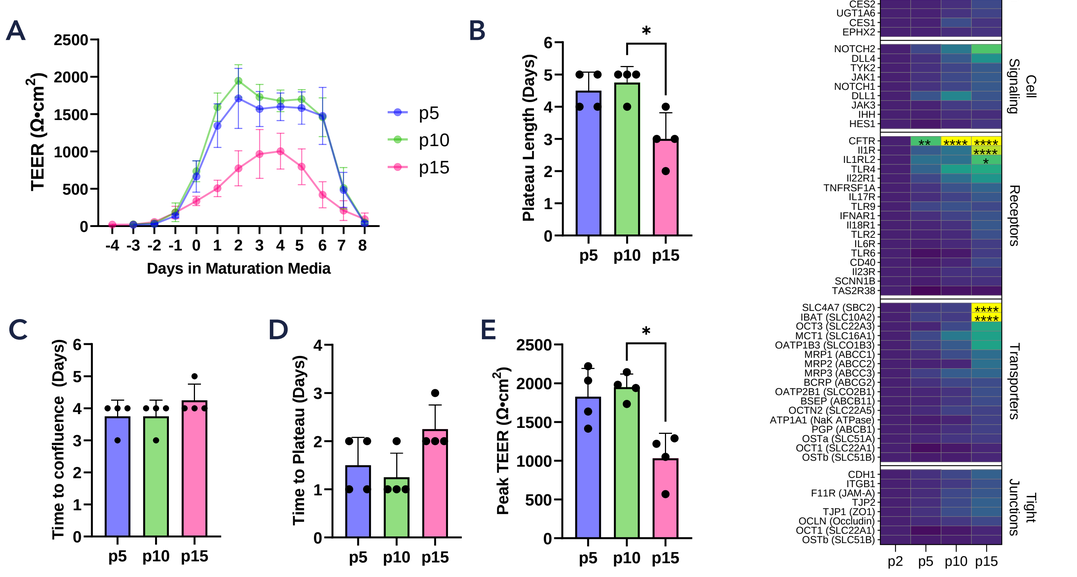
Cell passage number is a source of variability that can be easily controlled

Comparison of TEER kinetics and gene expression across cell passage numbers

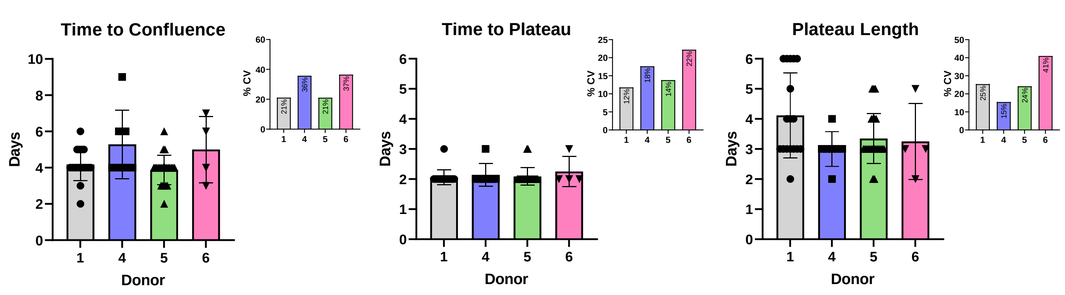
Methods: Transverse colon epithelial cells from passages 2, 5, 10 and 15 were plated on transwell plates and TEER was measured every 24 hours in culture. RNA was isolated from cultures grown to day 3 in RepliGut® Maturation Media.

(A) Error bars represent mean ± SD; n=3 technical replicates ; n= 4 biological replicates. (B-E) Error bars represent mean ± SEM. Each data point represents an individual cell lot. Kruskal-Wallis One-Way ANOVA. (F) Heatmap of gene expression (2-(ΔΔCt)) relative to passage 2 cells. Asterisks denote statistical significance (*p<0.05, Two-way ANOVA with Dunnett's multiple comparison test). n = 5 technical replicates for p2 (across 2 experiments), n = 9 technical replicates for p5, p10, and p15 (across 5 experiments)

Conclusion: p10 cells were selected for further experimentation.



Barrier formation and integrity are consistent across human donors



Comparison of culture metrics across four human donors.
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.
Conclusion: No significant differences were observed between time to confluence, time to plateau or plateau length. Figure insets: %CV of these metrics was variable across donors.

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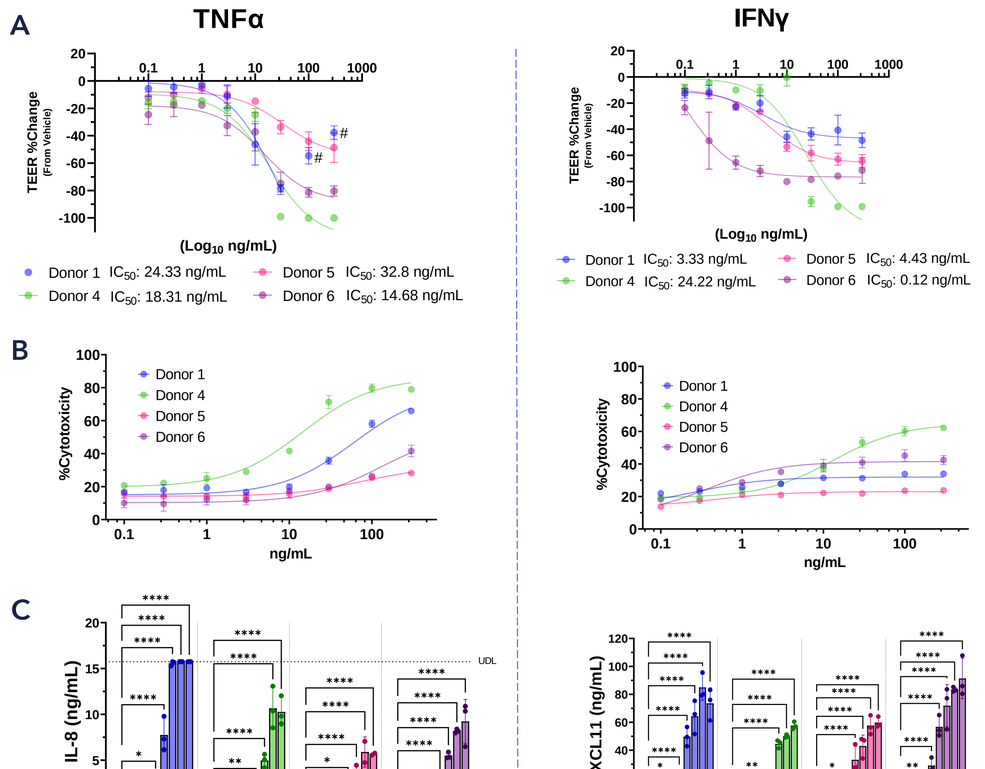
Donor variability is highlighted in distinct responses to inflammatory stimuli

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

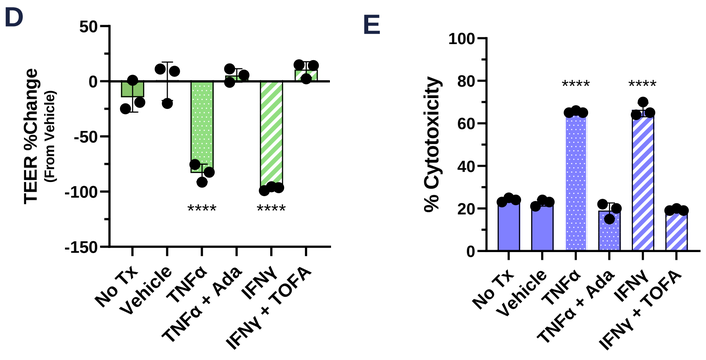
Methods: Transverse colon epithelial cells (p10) from 4 human donors were plated on 96-transwell plates. On day 2 in RepliGut® Maturation Media, cells were treated with multiple doses of TNFα or IFNγ.



(A) Dose response curves from TEER measurements at t = 48 hr with corresponding IC50 values.

(B) LDH Cytotoxicity at t = 48 hr post-treatment. Data are graphed as a percentage relative to a max lysis control. Error bars represent mean ± SEM. n = 3.

(C) Release of cytokines IL-8 in response to TNFα (left) and CXCL11 in response to IFNγ (right); n=3 per treatment group. TNFα and IFNγ doses shown are in ng/mL. (p*<0.01, Two-way ANOVA with Dunnett's multiple comparisons test)



(D) Barrier integrity and (E) cytotoxicity were assessed in Donor 5 for 300 ng/mL TNFα or 300 ng/mL IFNγ treatment in the presence or absence of 3 ug/mL of adalimumab (Ada) or 100 μM tofacitinib (TOFA) for 48 hours. (****p<0.0001, One-way ANOVA with Tukey's multiple comparison test.) All points represent mean ± SD. n = 3.

Conclusions

- When sources of variability are well controlled, donor-specific differences in response to inflammatory stimuli persist, which is reflective of patient populations.
- The RepliGut® platform recreates critical features of the human intestinal epithelium and represents a physiologically relevant system to study inflammatory responses in vitro across different human populations
- Clinically validated anti-TNFα-and IFNγ antagonists can protect from cytokine-induced barrier disruption and cytotoxicity, demonstrating the utility of the RepliGut® Planar as a tool to explore drug pharmacology associated with TNFα-and IFNγ pathways in IBDs.

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