Interrogating Human Gut Biology with iPSC-derived Intestinal Epithelial Cells

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ABSTRACT:

Intestinal epithelial cells (IEC) make up the single layer lining of the small intestine, acting as a barrier to prevent entry of harmful products and regulate uptake of nutrients and fluid. This layer is composed of numerous cell types with varied roles. Human iPSC-derived IEC, such as iCell Intestinal Epithelial Cells from FUJIFILM Cellular Dynamics, offer a renewable source of cellular material with consistent intestinal function for use in FDA-recommended New Approach Methodologies (NAM). These cells were characterized by flow cytometry, ICC, and RNA expression using the canonical identity markers CDX2, EpCAM, VIL1, and KRT20. iCell IEC express CDX2, EpCAM, and VIL1 at >80% with KRT20 around 70%. Cells were also tested for proliferation by flow cytometry, where cells were nearly 50% positive for Ki-67 at thaw but decreased to less than 20% after 7 days of culture. Intestinal subsets of Goblet (MUC2), Paneth (LYZ), Enteroendocrine (CHGA), Tuft (AVIL), and Microfold (GP2) cells were identified by ICC at physiologically relevant levels. Nutrient and drug absorption and metabolism of the small intestine is critical to understanding drug efficacy and toxicity. P-gp activity was assessed with iCell IEC exhibiting an efflux ratio >2, indicating functional transporter activity. Intestinal CYP enzyme activity was measured by both P450-Glo™ Assays and LC-MS/MS, and CYP3A4 activity from iPSC-derived IEC was equivalent to primary intestinal cells. Moreover, the estimated in vitro intestinal availability (Fg) for Felodipine, Midazolam, Verapamil, and Sildenafil were highly correlated to human in vivo Fg. Finally, to assess barrier function, cells were cultured on Transwell® Inserts in both 24- and 96-well format and after a 5-day maturation period post-thaw, TEER values above 600 ohms•cm^2 were stably maintained for at least 5 more days. This application was extended further to evaluate the beneficial effects of short-chain fatty acids (SCFA) on iCell IEC under inflammatory conditions induced by cytokines (100 ng/mL TNFα and 50 ng/mL IFNγ). Taken together, these data underscore how human iPSC-derived IEC provide a biologically relevant alternative to immortalized cancer lines without the inherent variability of primary tissue. Implementation of iCell IEC into assays and microphysiological systems for drug metabolism, uptake, and inflammatory response provides a novel platform for interrogating intestinal function in toxicology workflows with more human relevance.

Key Characteristics of iPSC-IEC: Multicellular Diversity

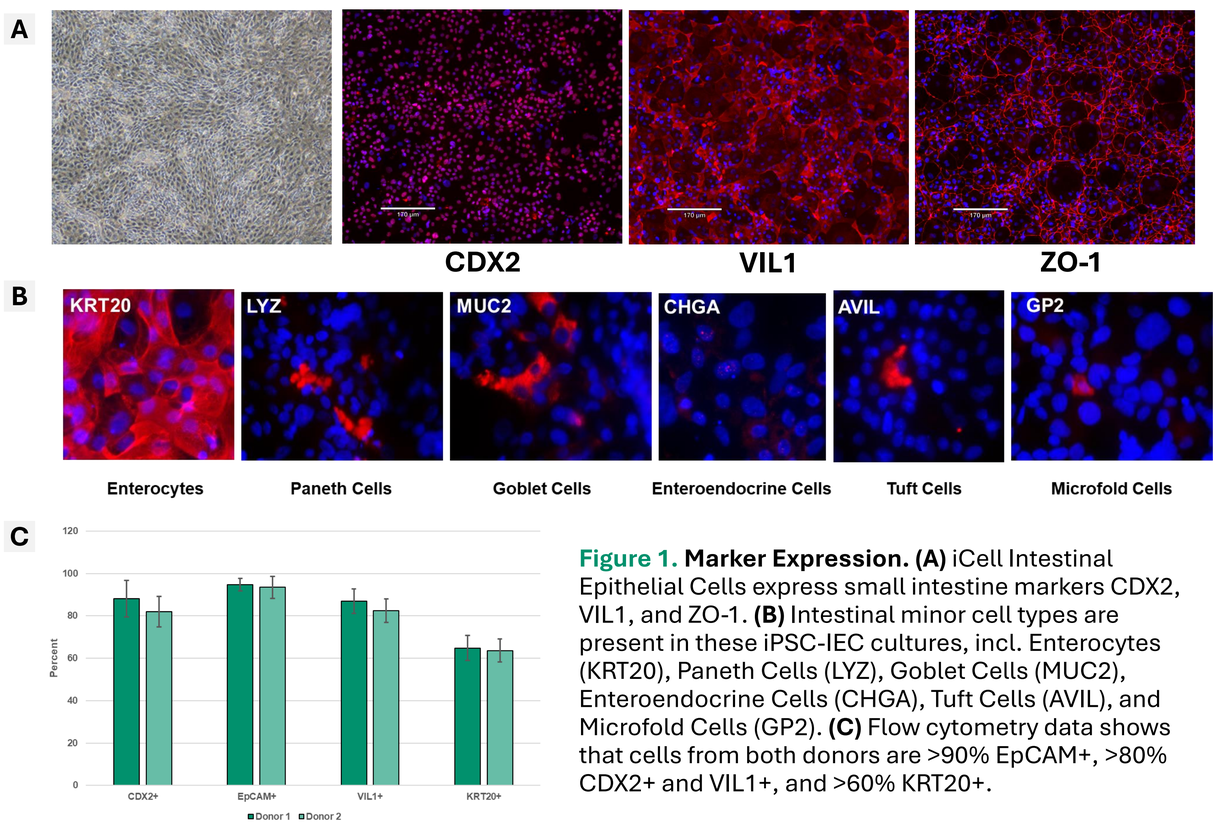

Figure 1. Marker Expression. (A) iCell Intestinal Epithelial Cells express small intestine markers CDX2, VIL1, and ZO-1. **(B)** Intestinal minor cell types are present in these iPSC-IEC cultures, incl. Enterocytes (KRT20), Paneth Cells (LYZ), Goblet Cells (MUC2), Enteroendocrine Cells (CHGA), Tuft Cells (AVIL), and Microfold Cells (GP2). **(C)** Flow cytometry data shows that cells from both donors are >90% EpCAM+, >80% CDX2+ and VIL1+, and >60% KRT20+.

Tight Barrier Formation Enables Physiologically-relevant Assays

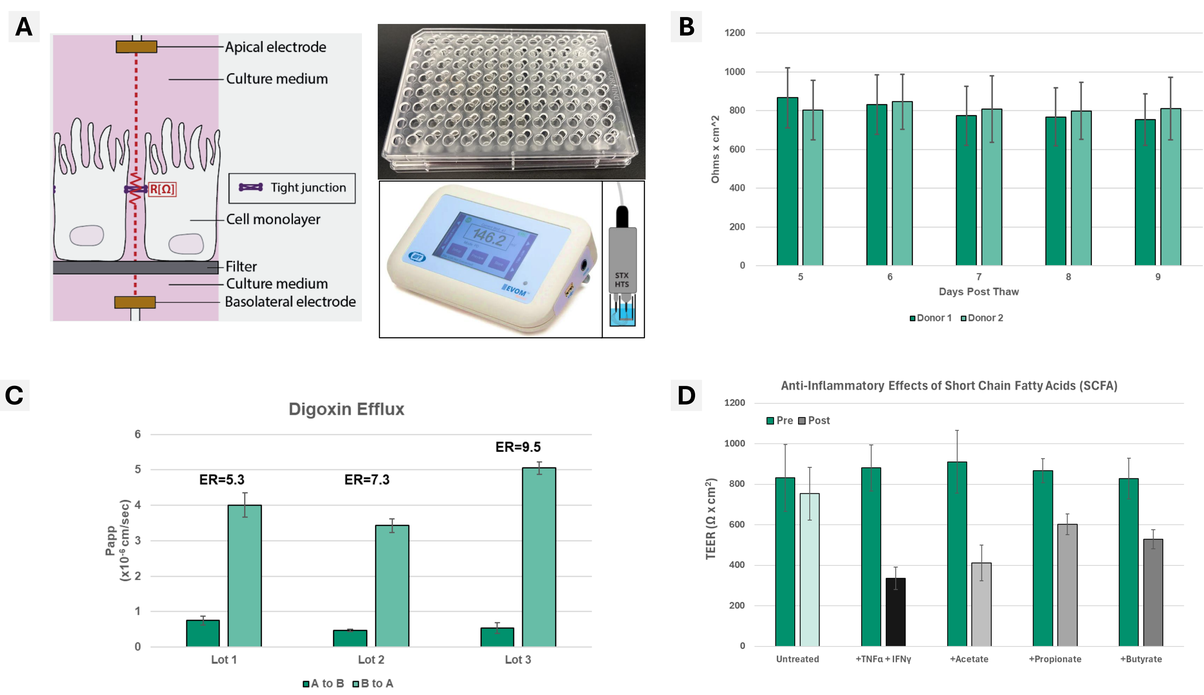

Figure 2. Barrier Formation. (A) Transendothelial Electrical Resistance (TEER) is a widely accepted technique to measure barrier integrity and tight junction dynamics for a cell monolayer. HTS Transwell-96 Permeable Support (Corning) were used and measurements taken with STX HTS electrode and an EVOM meter (WPI). **(B)** iCell IEC were shown to maintain a stable, high-quality cell barrier (>600 ohms x cm^2) over multiple days (≥5) in culture. Establishment of a functional barrier enables several physiologically-relevant assays downstream. **(C)** Digoxin efflux assay is an in vitro test that measures the transport of digoxin (a high affinity substrate of P-gp/MDR1) by P-glycoprotein (P-gp), which is a major efflux pump. High efflux ratios (ER) were consistently determined across 3 different lots of cells. **(D)** Short-chain fatty acids (SCFA) from gut bacteria, such as acetate, propionate, and butyrate, can benefit intestinal epithelial cells by modulating an inflammatory response (triggered here by TNFα and IFNγ) and reinforcing tight junctions between cells, strengthening the intestinal barrier integrity.

Correlation to *in vivo* Availability of Substrate

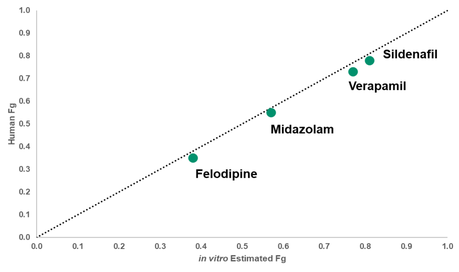

Figure 3. Availability of Substrate Correlation. The intestinal availability (Fg) of CYP3A substrates (Felodipine, Midazolam, Verapamil, and Sildenafil) in iCell Intestinal Epithelial Cells was measured on Day 11. The results were compared with reported *in vivo* human intestinal Fg for these compounds, and a strong *in vitro* correlation was observed.

Perform Phase I / Phase II Drug Metabolism Studies with iPSC-IEC

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Donor	Genotype	Phenotype
Donor 1	CYP3A4 *1B/*1B	Homozygous Enhanced Expression
	CYP3A5 *3/*3	Homozygous Nonfunctional
Donor 2	CYP3A4 *1B/*1B	Homozygous Enhanced Expression
	CYP3A5 *1/*3	Heterozygous Non-Functional Carrier

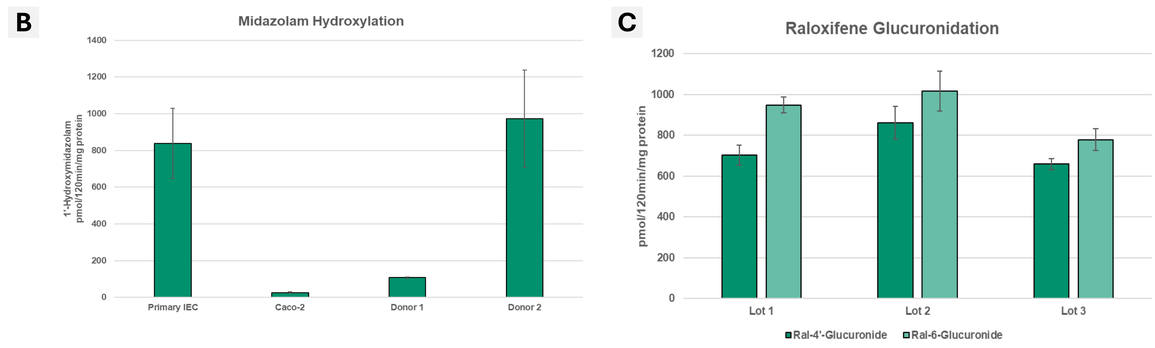


Figure 4. Assessing CYP Activity in iCell IEC. Phase I / Phase II drug metabolism studies are essential biotransformation processes that convert lipophilic drugs into hydrophilic, excretable compounds primarily by cytochrome P450 enzymes. While Caco-2 cell monolayers are widely used to estimate intestinal permeability and absorption, they have significant limitations when used for comprehensive drug metabolism studies. **(A)** iPSC-derived intestinal epithelial cell donors have different CYP3A5 genotypes. **(B)** iCell IEC have a genotype-phenotype relationship to Midazolam metabolism, with Donor 1 characterized as a Low Metabolizer and Donor 2 having activity equivalent to an average of primary IEC. Both donors have CYP3A activity significantly higher than that of Caco-2. **(C)** UGT1A raloxifene glucuronidation is equivalent and consistent across both iCell IEC donors.

Microfold Cell Mediated Transcytosis

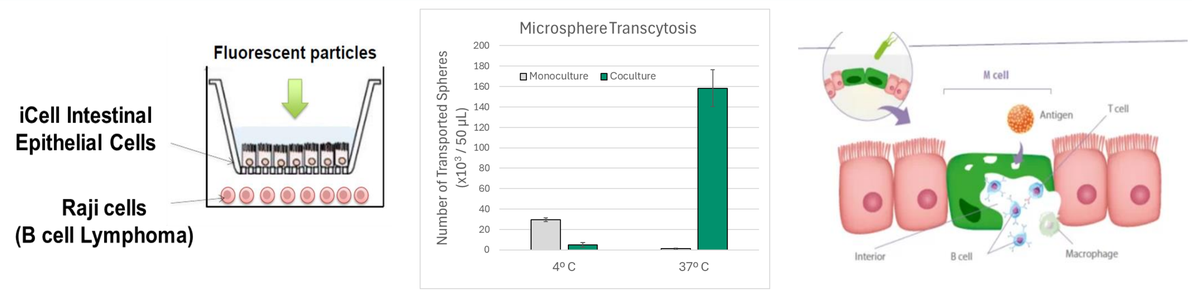


Figure 5. Microsphere Transcytosis. M-cell activity can be induced in iCell Intestinal Epithelial Cells. After plating iCell IEC on Transwell plates, Raji cells are cultured in the basal compartment to mimic an intestinal Peyer's Patch. When added to iCell IEC monoculture, Carboxylate-modified Microspheres (ThermoFisher) are transported across the intestinal barrier at a low rate. In contrast, coculture with Raji cells induces M-cell mediated transcytosis.

Inflammatory Response and Immune Component Coculture

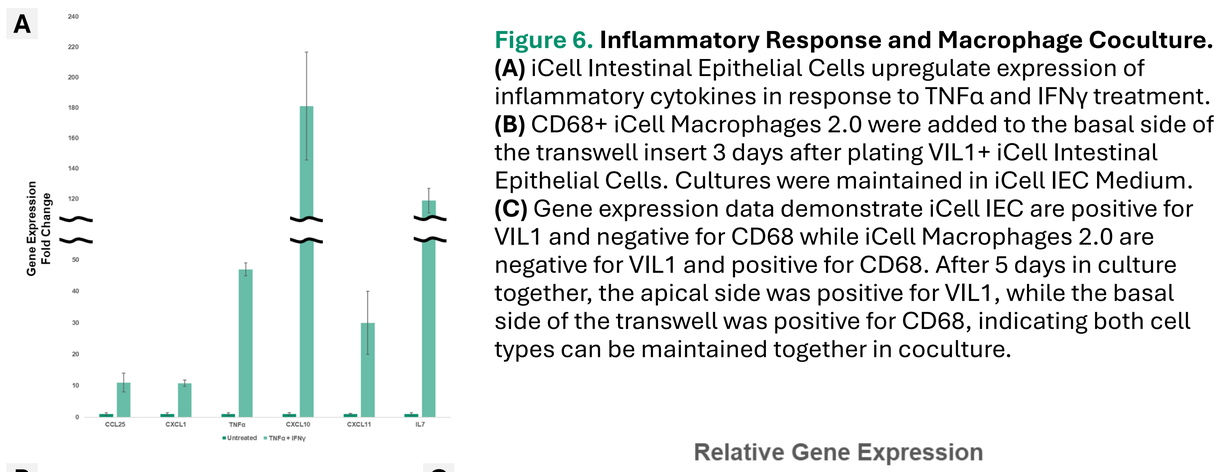


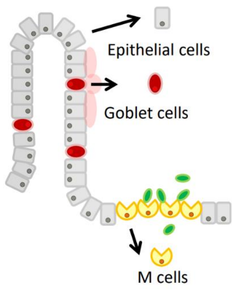
Figure 6. Inflammatory Response and Macrophage Coculture. **(A)** iCell Intestinal Epithelial Cells upregulate expression of inflammatory cytokines in response to TNFα and IFNγ treatment. **(B)** CD68+ iCell Macrophages 2.0 were added to the basal side of the transwell insert 3 days after plating VIL1+ iCell Intestinal Epithelial Cells. Cultures were maintained in iCell IEC Medium. **(C)** Gene expression data demonstrate iCell IEC are positive for VIL1 and negative for CD68 while iCell Macrophages 2.0 are negative for VIL1 and positive for CD68. After 5 days in culture together, the apical side was positive for VIL1, while the basal side of the transwell was positive for CD68, indicating both cell types can be maintained together in coculture.

Summary and Future Directions

iPSC-derived IEC provide a biologically relevant alternative to immortalized cancer lines without the inherent variability of primary tissue. Implementation of iCell IEC into assays for drug metabolism, uptake, and inflammatory response provides a novel platform for interrogating intestinal function in toxicology workflows with more human relevance. Next steps include culture in microphysiological systems, expanded testing of intestinal M cell assays, and co-culture experiments with iPSC-derived macrophages and sensory neurons to evaluate the contribution of inflammation and innervation on IEC function.

Key features:

- Stable, High Quality Barrier Function
- Presence of Small Intestine Minor Cell Types
- Physiological Inflammatory Response
- Phase I / Phase II Drug Metabolism
- Correlation to *in vivo* Human Fg



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FUJIFILM Cellular Dynamics, Inc is the leading provider of iPSC-derived cell types and disease models. We are helping to advance the field with 3D and co-culture systems to better replicate human tissue and biology. FCDI continues to be a leader in new products, manufacturing capacity and quality, and custom services.