Characterization of human iPS cell-derived intestinal epithelial like cells (F-hiSIEC™) and their utility as pharmacological models

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0. Introduction

We have developed small intestinal epithelial cells (F-hiSIEC™) differentiated from human iPS cells. F-hiSIEC™ has properties similar to those of the human small intestine and have been shown to be useful as a surrogate in vitro model of the human small intestine for drug absorption, immune and inflammatory evaluation, intestinal toxicity evaluation etc. [1)]. However, the prediction of drug metabolism in the gastrointestinal tract (Fg value) deviates from that in humans, and improvement in prediction accuracy is needed. In this study, we examined whether a novel method of F-hiSIEC™ (New medium & novel culture method) could improve the accuracy of Fg prediction.

1) Imakura et al., *Biochem. Biophys. Res. Commun.* **692** (2024) 149356.

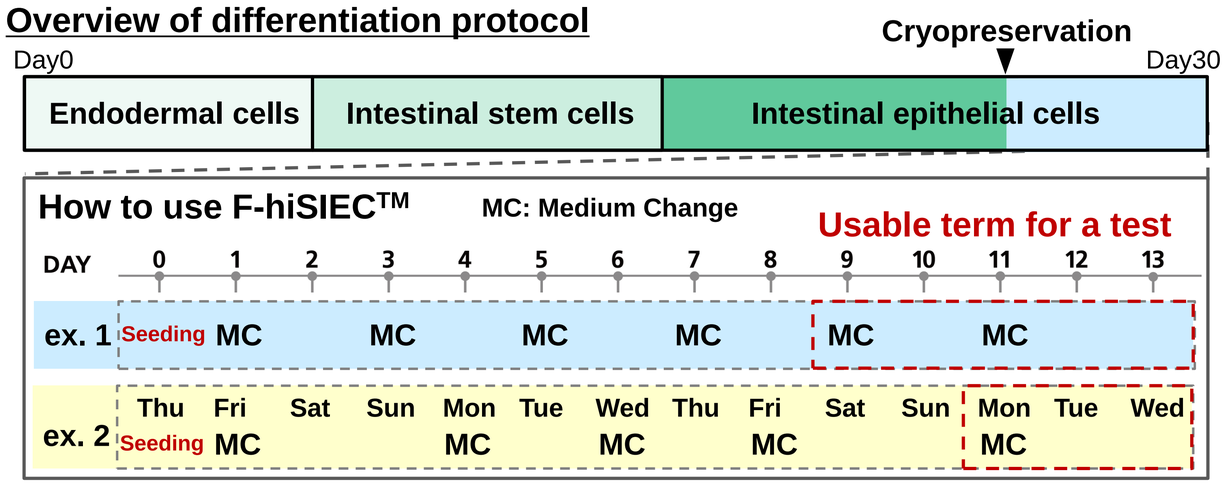
Characteristics of F-hiSIEC™

- **Forms barrier function** on trans wells
- Expression of **transporters and metabolic enzymes** is similar to that of humans
- **Multiple cell type markers** are expressed, including **absorptive epithelial cells**, **goblet cells**, and **M cells**
- Co-culture with **enterobacteria** and culture of **norovirus** is possible

Purpose of this study

1. Method

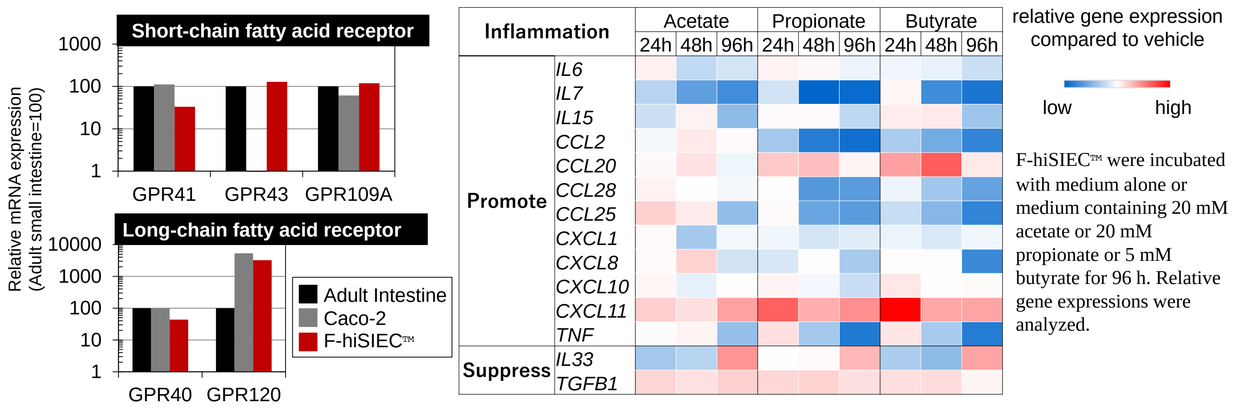
Overview of differentiation protocol



F-hiSIEC™ differentiate into mature small intestinal cells by culturing for 9-11 days after cell seeding.

2. Examples of F-hiSIEC™ applications

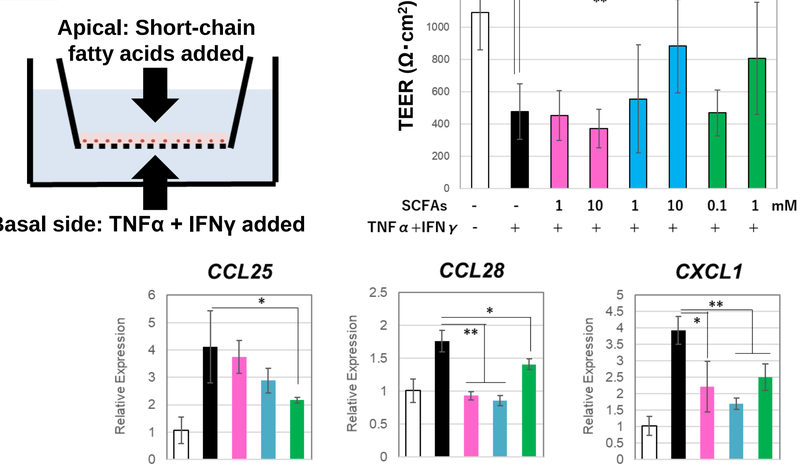
Fatty acid receptor expression and response



F-hiSIEC™ were incubated with medium alone or medium containing 20 mM acetate or 20 mM propionate or 5 mM butyrate for 96 h. Relative gene expressions were analyzed.

It was shown that fatty acid receptor-mediated immune responses can be assessed.

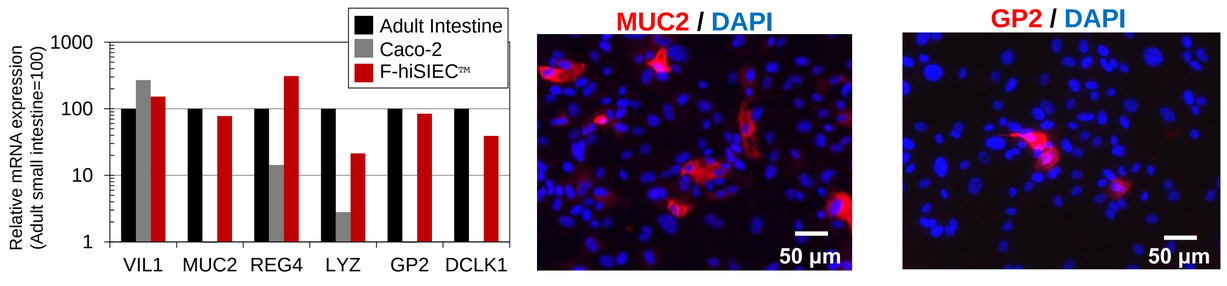
Intestinal inflammation model



On day 8 of cell seeding, inflammatory cytokines (100 ng/mL TNFα, 50 ng/mL IFNγ) were added to the basal side of the cell culture insert and short-chain fatty acids to the apical side. The gene expressions and TEER values were evaluated 24 hours after addition. *$p < 0.05$, **$p < 0.01$.

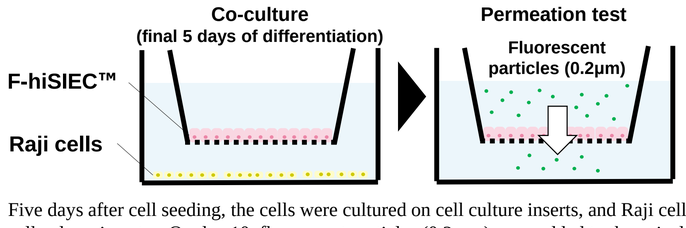
Decreased TEER and increased inflammatory cytokine gene expression due to inflammatory conditions were suppressed by the addition of short-chain fatty acids.

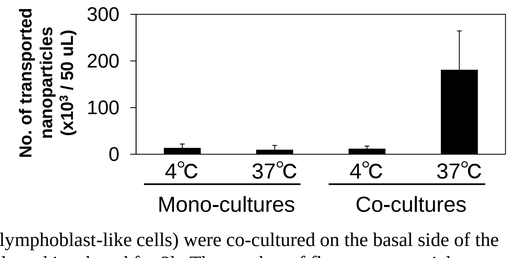
Presence of various intestinal epithelial cells



The presence of goblet cells (MUC2), endocrine cells (REG4), Paneth cells (LYZ), M cells (GP2), and tufted cells (DCLK1) was demonstrated.

M-cell (Microfold cell) model

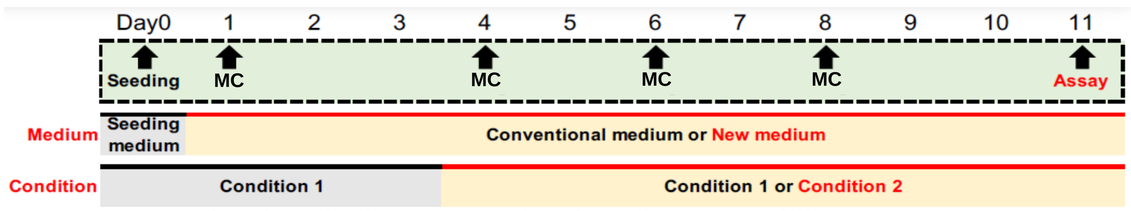




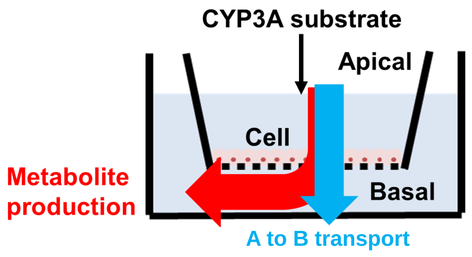
Five days after cell seeding, the cells were cultured on cell culture inserts, and Raji cells (lymphoblast-like cells) were co-cultured on the basal side of the cell culture inserts. On day 10, fluorescent particles (0.2 μm) were added to the apical side and incubated for 2h. The number of fluorescent particles passed through to the basal side was counted by flow cytometry.

Co-culturing with Raji cells suggested that uptake by M cells could be assessed.

3. Novel method for drug metabolism evaluation



Conventional method: **Conventional medium & Condition 1**
Novel method: **New medium & Condition 2**

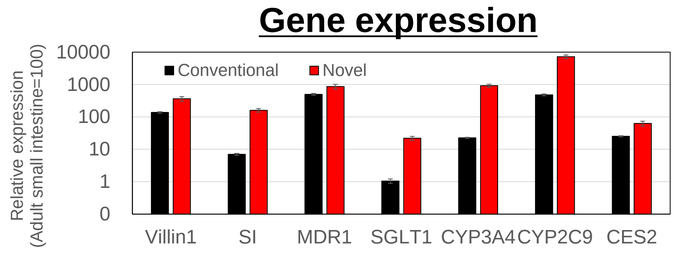


$$\text{Estimated Fg}^{2)} = \frac{\text{A to B transport}}{\text{A to B transport + Metabolite production}}$$

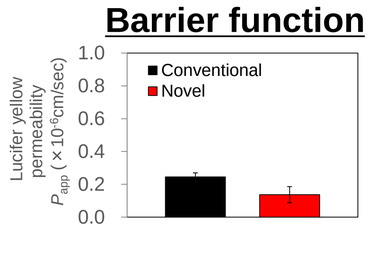
Estimated Fg values of each compound were calculated by the above equation. A to B transport represents the transcellular transport clearance in the apical-to-basolateral direction. Metabolic production was calculated by quantifying metabolite in apical, cell and basal compartment.

2) Michiba et al., *Drug Metab Dispos* 50: 204-213 (2022)

Gene expression



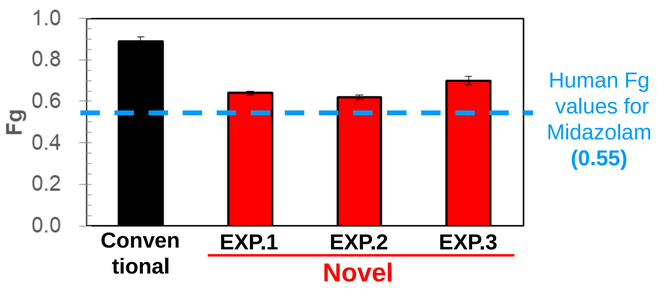
Barrier function



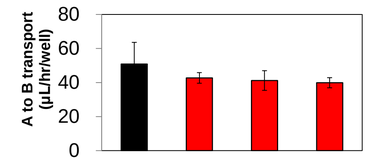
The novel method increased the expression of pharmacokinetic-related genes. Barrier function was maintained in the novel method as in the conventional method.

4. Fg estimation of CYP3A4 substrates

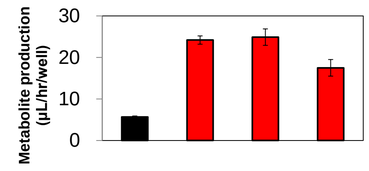
Fg estimate for Midazolam



Permeation clearance

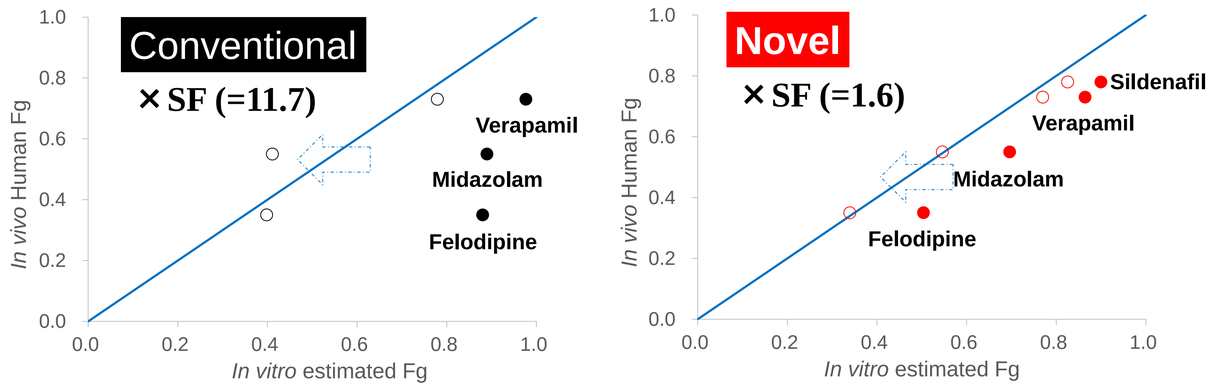


Metabolic clearance



The estimated Fg values of Midazolam in the novel method ranged from 0.62 to 0.70, closer to human Fg values than in the conventional method.

Estimated Fg values for typical CYP3A4 substrates



Substrate	*In vivo* human Fg value[3)4)]	Estimated Fg values [(1)] Without SF (SF=1.0) Conventional	Without SF (SF=1.0) Novel	With SF Conventional (SF=11.7)	With SF Novel (SF=1.6)
Felodipine	0.35	0.88 (+153%)	0.42 (+19%)	0.40 (+14%)	0.31 (-12%)
Midazolam	0.55	0.89 (+62%)	0.70 (+27%)	0.41 (-25%)	0.59 (+7%)
Verapamil	0.73	0.98 (+34%)	0.86 (+18%)	0.78 (+7%)	0.80 (+10%)
Sildenafil	0.78	Not tested	0.90 (+15%)	-	0.85 (+9%)
rmspe	-	-	-	0.17	0.09

Parentheses represent difference between estimated Fg value and *in vivo* human Fg value. These differences and root mean squared percentage error (rmspe) were calculated by the following equation (2) and (3), respectively.

$$\text{Estimated Fg} = \frac{\text{A to B transport}}{(\text{A to B transport}) + (\text{Metabolite production}) \times \mathbf{SF}} \quad \ldots(1)$$

$$\text{Difference (\%)} = \frac{(\text{Estimated Fg} - \textit{In vivo} \text{ human Fg})}{\textit{In vivo} \text{ human Fg}} \quad \ldots(2) \qquad \text{rmpse} = \sqrt{\frac{\sum(Difference)^2}{N}} \quad \ldots(3)$$

3) Verma *et al.*, *J Med Chem* **53**: 1098-1108 (2010)

4) Gertz *et al.*, *Curr Drug Metab* **9**: 785-795 (2011)

The estimated Fg values showed good correlation with the measured values. The SF for absolute value prediction was also smaller for the new method, and the accuracy of the predicted values after correction by SF was higher.

Conclusion：**F-hiSIEC™ has characteristics similar to the human small intestine and is a useful pharmacological model.**
The novel culture method improved the metabolic function of F-hiSIEC™ and the accuracy of Fg prediction for CYP3A substrate drugs.