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ヒトiPS細胞由来腸管上皮細胞(F-hiSIEC™)の新規培養法による、薬物代謝能の改善と毒性評価への展開可能性

Novel culture method of human iPS cell-derived intestinal epithelial cells (F-hiSIEC™) for improvement of drug metabolizing ability and potential application to toxicity evaluation

○望月 清一[1]、今倉 悠貴[1]、諸橋 康史[1]、山﨑 奈穂[1]、岩尾 岳洋[2]、松永 民秀[2]、中村 健太郎[1]

([1]富士フイルム株式会社 バイオサイエンス&エンジニアリング研究所、[2]名古屋市立大学 大学院薬学研究科 臨床薬学分野)

○Seiichi Mochizuki[1], Yuki Imakura[1], Yasushi Morohashi[1], Nao Yamazaki[1], Takahiro Iwao[2], Tamihide Matsunaga[2], Kentaro Nakamura[1]

(1 Bio Science & Engineering Laboratory. FUJIFILM Corporation 2 Dept. Clinical Pharmacy, Graduate School of Pharmaceutical Sciences. Nagoya City Univ.)

ご質問・ご意見ございます方、ポスターPDFをご希望の方は、下記にご連絡ください。ぜひ毒性研究に関わる皆様のご意見をいただきたく存じます。

seiichi.mochizuki@fujifilm.com

1. Abstract

【背景】 我々はヒトiPS細胞由来腸管上皮細胞（F-hiSIEC™）を開発し、この細胞を用いてヒト生体に近い性質を有する*in vitro*細胞アッセイモデルの構築を進めている。これまで、本細胞を用いて薬物動態、毒性、免疫・炎症等の評価モデルを構築し、その有用性を示してきた[1)]。一方で、CYP等による薬物代謝に関しては生体小腸との間に差があり、更なる改善が求められていた。

【方法】 既報[2)]を基に、F-hiSIEC™を新規培養法（新規培地での気液培養）にて培養し、細胞の基本特性および薬物代謝能について従来の培養法と比較した。

【結果・考察】 新規培養法により、小腸マーカー、薬物トランスポーター、薬物代謝酵素の遺伝子発現が上昇した。CYP3A4の代表的な基質であるミダゾラムの代謝を評価した結果、Fg（消化管代謝回避率）予測値が従来法よりもヒトに近い値を示すことがわかった。さらに、複数のCYP3A4基質を用いた評価より、従来法では困難であった化合物間のFg比較が可能となり、ヒトFgと高い相関を示すことがわかった。加えて、新規培養法は、消化管での代謝を考慮した毒性評価にも展開できると考えられる。

Outline of this study

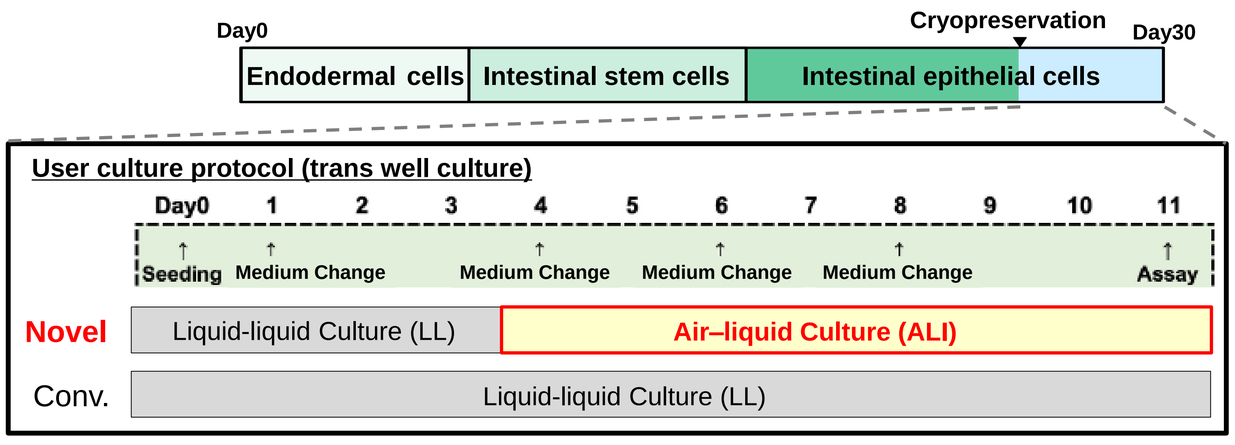
Novel Culture method

- **Improve gastrointestinal drug metabolism**
- **Improve *in vitro* Fg prediction accuracy**
- **Make F-hiSIEC™ a more useful tool for DMPK and TOX**

1) Imakura, et al. *Biochem Biophys Res Commun*. 692:149356, 2024
2) Shirai, et al. *Drug Metab Pharmacokinet*. 55:100994, 2024

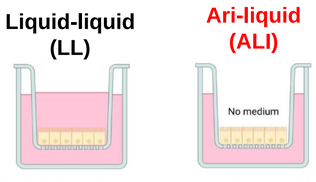
2. Method

Overview of differentiation protocol



Difference between novel and conventional methods

	Cell	Medium		Culture method
		Seeding	Culture	
Novel [2)]	F-hiSIEC™ Cell	F-hiSIEC™ Seeding	New	Day0-4: LL Day4-11: ALI
Conv.			F-hiSIEC™ Culture	Day 0-11: LL

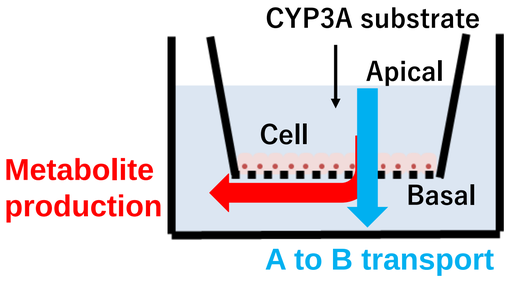


2) Shirai *et al*., *Drug Metab Pharmacokinet* **55**: 100994 (2024)

Formula for calculating *in vitro* Fg[3)]

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

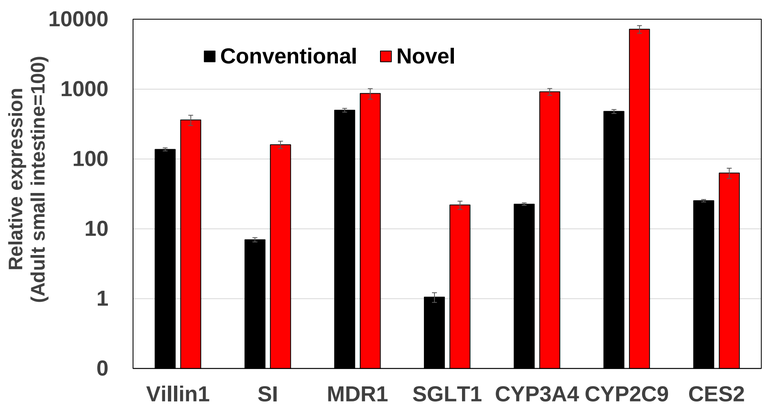
3) Michiba et al., *Drug Metab Dispos* 50: 204-213 (2022)



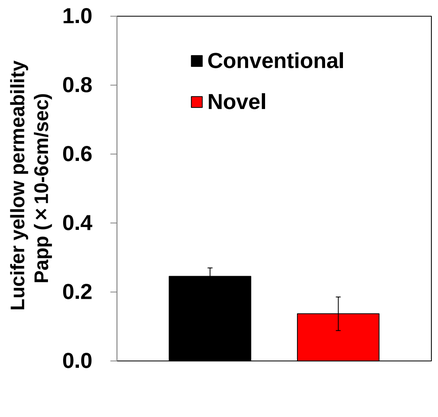
- Estimated Fg value of each compound were calculated by the equation described above.
- A to B transport represents the transcellular transport clearance in the apical-to-basolateral direction.
- Metabolic production was calculated by quantifying metabolite in apical, cell and basal compartment.

3. Gene expression, Barrier function

Gene expression



Barrier function

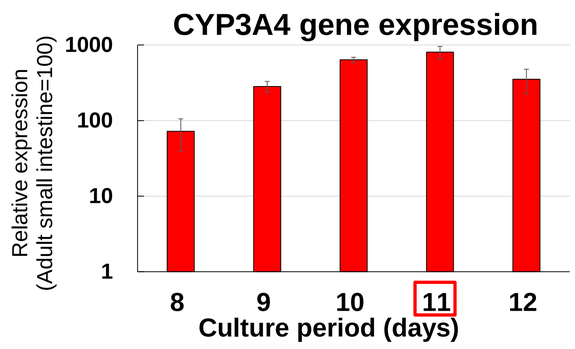


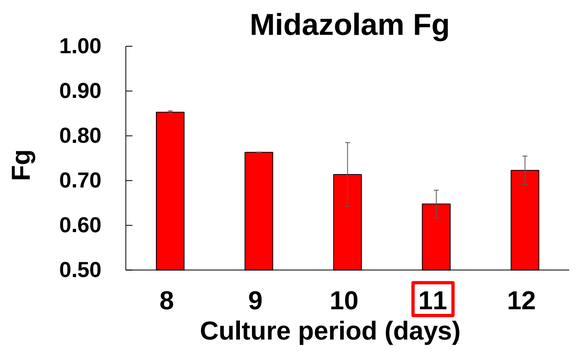
✓**The novel method increased the expression of small intestinal markers, transporters, and metabolizing enzymes.**

✓**Barrier function was maintained in the novel method as in the conventional method.**

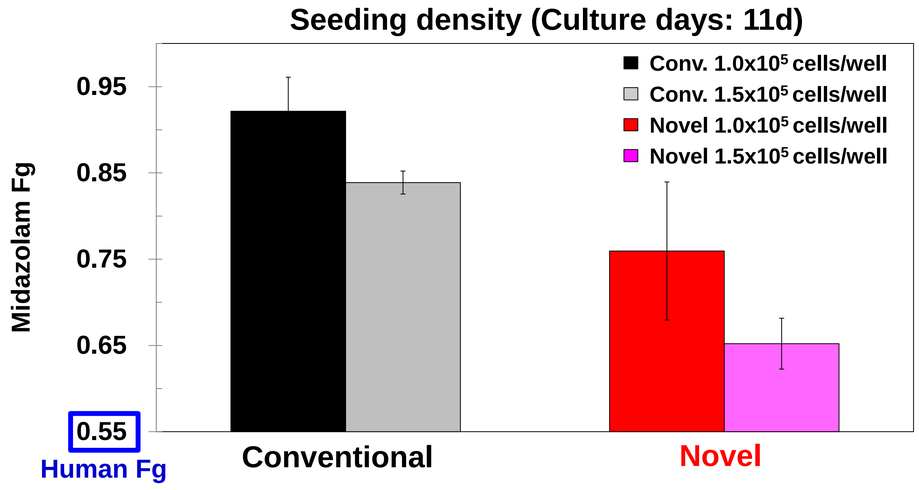
4. Evaluation of Fg in the novel culture method

Optimization of Fg evaluation timing





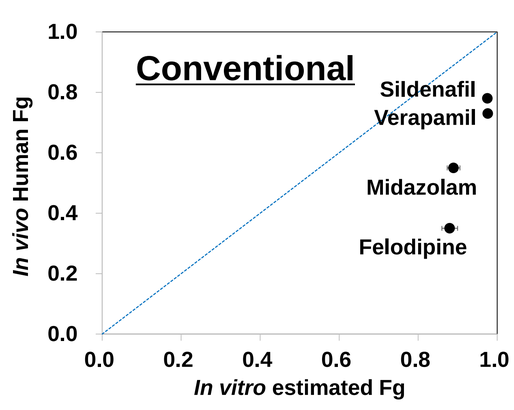
Seeding density (Culture days: 11d)

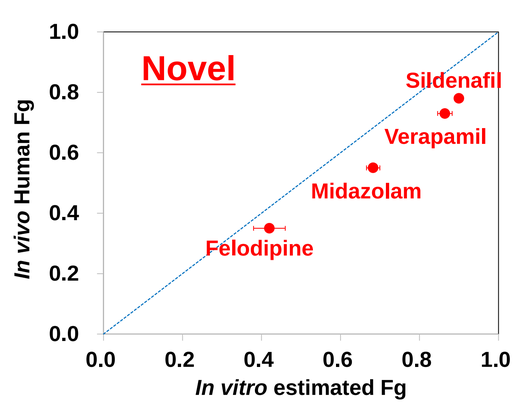


✓**After 11d of culture and a seeding density of 1.5x10^5 cells/well, the *in vitro* Fg of midazolam was close to the human value.**

5. Fg prediction of CYP3A substrates

Estimated Fg values for typical CYP3A4 substrates





Substrate	Method	A to B transport (μL/hr/well)		Metabolite production (μL/hr/well)		Estimated Fg value		*In vivo* human Fg value[4)5)]
		Mean	SD	Mean	SD	Mean	SD	
Felodipine	Conventional	16.5	1.4	2.13	0.36	0.88	0.02	0.35
	Novel	7.22	1.3	**10.1**	1.70	**0.42**	0.04	
Midazolam	Conventional	60.6	8.7	7.43	0.54	0.89	0.02	0.55
	Novel	39.9	3.0	**18.6**	2.17	**0.68**	0.02	
Verapamil	Conventional	26.4	7.9	0.64	0.24	0.98	0.00	0.73
	Novel	30.0	1.6	**4.71**	0.62	**0.86**	0.02	
Sildenafil	Conventional	16.9	7.1	0.390	0.01	0.98	0.01	0.78
	Novel	17.7	0.4	**1.97**	0.20	**0.90**	0.01	

4) Verma et al., *J Med Chem* **53**: 1098-1108 (2010) 5) Gertz et al., *Curr Drug Metab* **9**: 785-795 (2011)

✓**The Fg values predicted by the novel method using F-hiSIEC™ showed good correlation with measured human values.**

6. Potential for expansion into toxicity assessment

Various applications for gastrointestinal research have been developed in F-hiSIEC™.

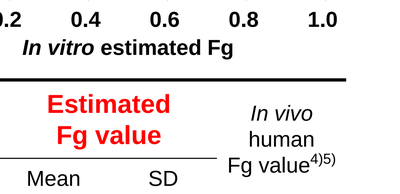
① Intestinal toxicity evaluation *Report at 48th and 49th JSOT
- Evaluation of gastrointestinal cytotoxicity (e.g., 5-FU and its prodrugs)
- Evaluation of differentiation abnormalities (e.g., increase in goblet cells with γ-secretase inhibitor)
- Decreased barrier function in inflammatory conditions (e.g., addition of inflammatory cytokines)

② DMPK evaluation
- **Fg prediction (Fg prediction for CYP3A substrate) ⇒ Novel culture method reported here**
- Fa prediction (P_{app} evaluation on various drugs)
- Enzyme induction (CYP3A4 gene induction by RIF and VD3).

③ Immune and inflammatory evaluation
- Evaluation of substance uptake via M cells
- Evaluation of inflammation suppression by short chain fatty acids

④ Co-culture with intestinal bacteria
- Co-culture with B. fragilis, Lactobacillus[6)], and Salmonella[7)] and evaluation of interactions

⑤ Norovirus culture, disinfectant evaluation



6) Sen, A. et al., *Front Microbiol*. 2023 Apr 13:14:1155438
7) Fuka, Y. et al., *FEMS Microbiology Letters* , 2024, 371 , fnae006

✓ F-hiSIEC™ could be used to assess toxicity through drug metabolism and to predict systemic exposure for toxicity assessment.

✓ Evaluation of drug toxicity via intestinal bacteria using F-hiSIEC™ is also expected.

Conclusion：The novel culture method improved the metabolic function of F-hiSIEC™ and the accuracy of Fg prediction of CYP3A substrate drugs. This evaluation system may be useful as a model for evaluating gastrointestinal absorption. In addition, the ability to accurately predict gastrointestinal metabolism and Fg may be applicable to toxicity assessment.