Investigation for The Prediction of Intestinal Drug Absorption in Human Using FUJIFILM human iPS cell-derived Small Intestinal Epithelial like Cells (F-hiSIEC™).

○Yasushi Morohashi[1], Shinji Mima[1], Yuki Imakura[1], Nao Yamazaki[1], Takahiro Iwao[2], Tamihide Matsunaga[2], Kazuya Maeda[3], Hiroyuki Kusuhara[4], Seiichi Mochizuki[1], Setsu Endoh[1] and Ryo Suzuki[1]

1 Bio Science & Engineering Laboratories, FUJIFILM Corporation, 2 Department of Clinical Pharmacy, Graduate School of Pharmaceutical Sciences, Nagoya City University, 3 Laboratory of Pharmaceutics, School of Pharmacy, Kitasato University 4 Laboratory of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Science, The University of Tokyo

Please contact us by e-mail using contact information below for any questions.
yasushi.morohashi@fujifilm .com

Introduction

[Background] FUJIFILM human iPS cell-derived Small Intestinal Epithelial like Cell (F-hiSIEC™) was developed as an alternative cell for better prediction of intestinal absorption. Although the CYP3A4 activity corrected by protein amount in F-hiSIEC™ was comparable to that in the human small intestine, our previous result indicated that the predictability of intestinal absorption for CYP3A4 substrate drugs using F-hiSIEC™ was not high enough[1]. We speculated that one of the causes of this discrepancy is attributed to the morphological differences between F-hiSIEC™ and the human small intestine. As these differences could cause insufficiency of metabolic capacity in the assay system, the metabolic activity through intestinal epithelial cells was much lower than the permeability (**Fig. 1**).

[Purpose] In this study, we aimed to modify cell culture protocol of F-hiSIEC™ and investigated the possibility for the prediction of intestinal drug absorption.

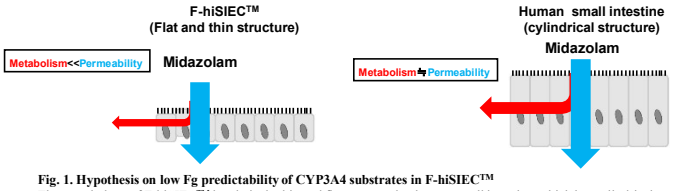


Fig. 1. Hypothesis on low Fg predictability of CYP3A4 substrates in F-hiSIEC™
The morphology of F-hiSIEC™ is relatively thin and flat compared to human small intestine, which has cylindrical structures. Due to this structural difference, metabolic activity of a CYP3A4 substrate, for example Midazolam (Fg = 0.55), was much lower than permeability in the assay system using F-hiSIEC™. Consequently, Fg predictability of F-hiSIEC was not high enough.

Cell culture protocol

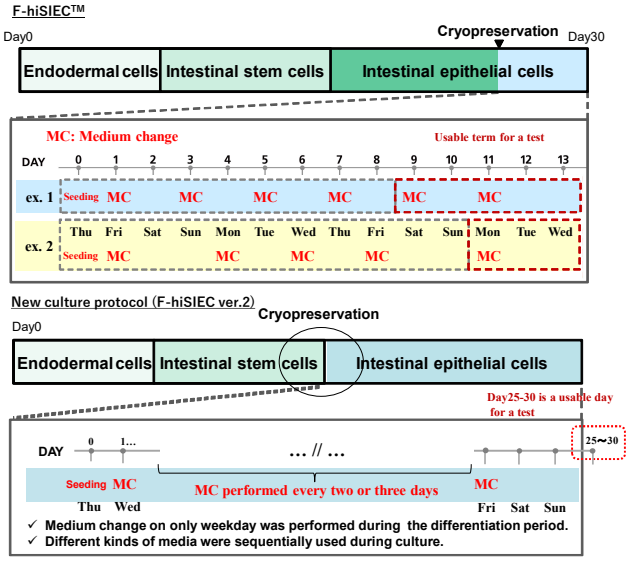


Fig. 2. The representative scheme of culture protocol

New culture protocol was established to differentiate intestinal epithelial cells.

Morphology, Gene expression, Barrier function and P-gp activity

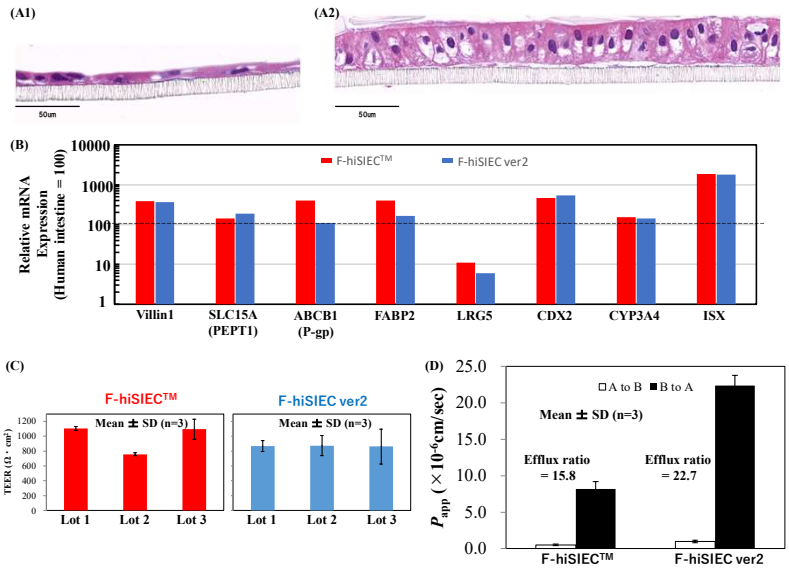


Fig. 3. Comparison of various features between F-hiSIEC™ and F-hiSIEC ver2 at the end of differentiation
(A) Cell morphology stained with HE. (B) The relative mRNA expression levels. (C) Barrier function of cell sheet on cell culture insert by TEER. (D) The apparent permeability coefficient (P_{app}) of Digoxin (10 μM; P-gp substrate) in the apical-to-basolateral (A to B) and the basolateral-to-apical (B to A) direction. Efflux ratio was determined by the ratio of each P_{app} value (B to A / A to B).

-Cell morphology of F-hiSIEC ver2 dramatically was changed compared to F-hiSIEC™ by a new culture protocol.

-Similar features (mRNA, barrier function and P-gp activity) were shown between F-hiSIEC™ and F-hiSIEC ver2.

CYP3A4 metabolic activity and its robustness

Table 1. Comparison of protein amount and metabolite production between F-hiSIEC™ and F-hiSIEC ver2.

	Unut	F-hiSIEC	F-hiSIEC ver2.
Protein amount	μg/well	74 ± 8	228 ± 9
Metabolite production (1-OH MDZ and 4-OH MDZ)	μL/hr/well	1.57 ± 0.07	12.2 ± 0.8

At the end of the differentiation, culture medium was removed and cells on the 24-well culture inserts were lysed. Protein amount of cell lysate was determined by BCA protein assay. Each cell was incubated with 10 μM midazolam, for 1 hr at 37°C. The samples were collected from 3 compartments (apical, basal and cell), and the metabolites, 1-hydroxy midazolam (1-OH MDZ) and 4-hydroxy midazolam (4-OH MDZ), were measured by UPLC–MS/MS. The amounts of metabolites were divided by the initial concentration of Midazolam to calculate the metabolite production clearance. Data represented as the means ± SD (n=3).

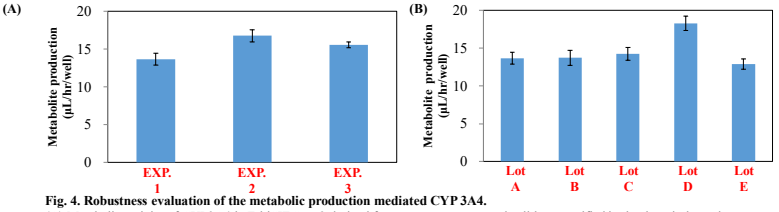


Fig. 4. Robustness evaluation of the metabolic production mediated CYP 3A4.
(A) Metabolic activity of CYP3A4 in F-hiSIEC ver2 derived from same cryopreserved cell lot quantified in the three independent experiments. (B) Metabolic activity of CYP3A4 in F-hiSIEC ver2 derived from 5 different cryopreserved cell lots quantified in the experiment performed on same day. Metabolite production was calculated by quantifying 1-hydroxy midazolam and 4-hydroxy midazolam. Each bar and error bar represent the mean and SD, respectively (n=3).

-Protein amount and CYP3A4 activity of F-hiSIEC ver2 were higher than these of F-hiSIEC™.

-Robustness of CYP3A4 activity was confirmed.

Estimation of intestinal availability

Table 2. Summary of the estimated intestinal availability (Fg) for CYP3A substrates

<F-hiSIEC™>

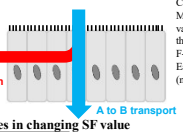
Substrate	Metabolite	A to B transport (μL/hr/well)	Metabolite production (μL/hr/well)	Estimated Fg value	In vivo human Fg value[5,6]
Midazolam	1-hydroxy midazolam 4-hydroxy midazolam	51.1 ± 2.7	1.57 ± 0.07	0.97 ± 0.00	0.55

<F-hiSIEC ver2>

Substrate	Metabolite	A to B transport (μL/hr/well)	Metabolite production (μL/hr/well)	Estimated Fg value	In vivo human Fg value[5,6]
Felodipine	Dehydrofelodipine	3.28 ± 0.22	3.32 ± 0.48	0.50 ± 0.02	0.35
Midazolam	1-hydroxy midazolam 4-hydroxy midazolam	40.3 ± 3.5	16.8 ± 0.8	0.71 ± 0.02	0.55
Verapamil	Norverapamil D617	6.36 ± 1.44	1.33 ± 0.28	0.83 ± 0.01	0.73
Sildenafil	N-desmethyl sildenafil	18.4 ± 0.3	1.21 ± 0.20	0.94 ± 0.01	0.78

Prior to the addition of the substrates, the cell on 24-well inserts were preincubated for 20 min in transport buffer. The transport study was initiated by replacing the buffer in the apical compartment with the substrate-containing buffer (10 μM; Midazolam, Verapamil and Sildenafil, 25 μM; Felodipine). A to B transport represents the transcellular transport clearance in the apical-to-basolateral direction.
Estimated Fg value of each compound were calculated by the following equation (1)[4].

$$Fg = \frac{\text{A to B transport}}{\text{A to B transport} + \text{Metabolite production} * \text{Scaling factor (SF)}} \quad \ldots(1)$$



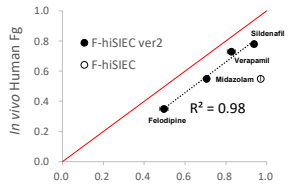


Fig 5. Correlation between *in vitro* estimated Fg values and *in vivo* human Fg values of CYP 3A4 substrate drugs.
Comparison of *in vitro* estimated values (SF=1) of Felodipine, Midazolam, Verapamil and Sildenafil and those *in vivo* human Fg values. The red line and the dashed line represent the identity line and the regression line, respectively. Closed circle represents Fg value from F-hiSIEC ver2 and open circle represents Fg value from F-hiSIEC™. Each symbol and error bar represent the mean and SD, respectively (n=3).

Table 3. Summary of the effect on estimated Fg values in changing SF value

Substrate	In vivo human Fg value	Estimated Fg value SF=1.5	SF=2.0	SF=2.5
Felodipine	0.35	0.40 (-14%)	0.33 (6%)	0.28 (19%)
Midazolam	0.55	0.62 (-14%)	0.55 (0%)	0.49 (11%)
Verapamil	0.73	0.76 (-4%)	0.71 (3%)	0.66 (10%)
Sildenafil	0.78	0.91 (-17%)	0.88 (-13%)	0.86 (-10%)
rmspe	-	0.124	0.0741	0.131

Estimated Fg values were calculated by equation 1 and the value of SF was changed from 1.5 to 2.5. 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.

$$\text{Difference (\%)} = \frac{(\textit{In vivo} \text{ human Fg} - \text{Estimated Fg})}{\textit{In vivo} \text{ human Fg}} \quad \ldots(2)$$

$$\text{rmspe} = \sqrt{\frac{1}{4}\Sigma(\text{Difference})^2} \quad \ldots(3)$$

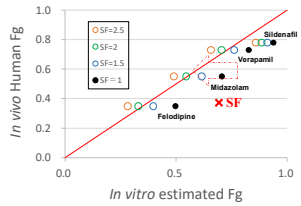


Fig 6. Effect of changing SF value on correlation between *in vitro* estimated Fg values and *in vivo* human Fg values.
Comparison of *in vitro* estimated values of Felodipine, Midazolam, Verapamil and Sildenafil with SF=1 (closed circle) or with SF=1.5, 2.0 or 2.5 (blue, green or orange, respectively).

-Estimated Fg values well correlated with *in vivo* values in human.

-An empirical scaling factor that corrects metabolite production was estimated to be ca. 2 to account for *in vivo* human Fg values.

Discussion

Although F-hiSIEC™ has comparative metabolic activity with the human intestine, the metabolic capacity on culture insert was not high enough due to its cell morphology (**Fig. 1**). In this study, we successfully established a new culture protocol to generate intestinal epithelial cells with cylindrical structures (**Fig.2** and **Fig. 3A2**). The metabolic activity mediated CYP3A4 of F-hiSIEC ver2 was approximately 8-fold higher than F-hiSIEC™ (**Table 1**). Robustness of metabolic activity indicated that our new culture protocol is highly reproducible (**Fig. 4A** and **4B**).

In this presentation, we showed the potential as evaluation model to estimate of Fg values of CYP3A4 substrates. The rank order of Fg values was identical to that of *in vivo* human Fg (**Table 2** and **Fig. 5**). As difference was still observed between estimated values and *in vivo* human values, optimum SF was estimated to be around 2 to fill the gap (**Table 3** and **Fig. 6**).

Michiba *et al.*, reported by using CYP3A4 inhibitor, Fg values of CYP3A4 substrate drugs was successfully estimated in the human jejunal spheroid-derived differentiated intestinal epithelial cells without quantifying their metabolites[4]. As for new drugs, information on whole metabolic pathways is limited. So, we are now challenging to estimate Fg values with F-hiSIEC ver2 in a similar approach.

In addition to CYP3A4, other intestinal enzymes (ex. other CYPs, UGTs, SULTs and CES and so on.) also affect Fg values of some drugs. As F-hiSIEC™ demonstrated that their gene expression (the metabolic function of some enzymes was also confirmed[1)5)6)]) and the protein amount on culture insert was approximately 3-fold increased by this new culture protocol (**Table 1**), the *in vivo* function of multiple enzymes in the small intestine may also be preserved in F-hiSIEC ver2.

References

1) Kabeya *et al.*, *Drug Metab Pharmacokinet* **35**: 374-382 (2020), 2) Verma *et al.*, *J Med Chem* **53**: 1098-1108 (2010)
3) Gertz *et al.*, *Curr Drug Metab* **9**: 785-795 (2011), 4) Michiba *et al.*, *Drug Metab Dispos* **50**: 204-213 (2022)
5) Kitaguchi *et al.*, *Drug Metab Dispos* **50**: 17-23 (2022), 6) Agustina *et al.*, *Drug Metab Dispos* **49**: 972-984 (2021)

37th JSSX annual meeting COI disclosure information

Authors: Yasushi Morohashi, Shinji Mima, Yuki Imakura, Nao Yamazaki, Takahiro Iwao, Tamihide Matsunaga, Kazuya Maeda, Hiroyuki Kusuhara, Seiichi Mochizuki, Setsu Endoh, Ryo Suzuki

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[Conclusion] F-hiSIEC ver2 represented preferable cell structure ,high CYP3A4 activity compared to F-hiSIEC™ and estimated Fg values showing a good correlation for CYP3A4 substrates. Therefore, it is expected that F-hiSIEC ver2 can be used to evaluate intestinal drug absorption in humans.