P465 Development of A Novel Transporter "Thaw and Go" Model to Study Regulatory Authority Recommended SLC Transporters

Jie Wang[1], Joanne Bourgea[1], Kirsten Cooper[1], Charles L. Crespi[2], Christopher J. Patten[1], and Na Li[1]
[1]*Two Oak Park Drive, Corning Life Sciences, Corning Incorporated, Bedford, MA 01730, USA*
[2]*836 North Street, Corning Life Sciences, Tewksbury, MA 01876, USA*

Abstract

SLC Transporters are membrane-bound proteins that facilitate the vertical movement of endogenous or exogenous substrates across biological membranes. They have been shown to play a critical role in the pharmacokinetics, safety and efficacy profiles of drugs, and are of emerging clinical importance in the absorption and disposition of drugs. Currently, cell-based assay systems, such as polarized cell line, primary cell, and stable cell line, are the main *in vitro* methods suitable for studying drug interactions with SLC transporters. All these methods have disadvantages such as high cost, intensive cell culture maintenance, or lab-to-lab, donor-to-donor inconsistency. We have developed and validated a new transporter "Thaw and Go" model for studying uptake clearance and drug-drug interaction (DDI) with SLC transporters. The complete portfolio includes all the regulatory authority (USFDA, EMA) and industry recommended SLC transporters: Organic Anion-Transporting Polypeptide 1B1 and 1B3 (OATP1B1 OATP1B3), Organic Anion Transporters 1 and 3 (OAT1, OAT3), Organic Cation Transporters (OCT1 and OCT2). The studies demonstrate that the novel cell-based system has many advantages over the current cell-based assay systems including high uptake activity, high signal-to-noise ratio, ease-of-use, batch-to-batch consistency and flexibility. We have optimized assay conditions with 96 well as well as 24 well plates for the alternative high-throughput methods.

Introduction

It is widely accepted that uptake and efflux transporters play a critical role in the distribution and elimination of drugs. Drug-drug interactions, due to interference of drug transporter activity by co-administered drugs, has been demonstrated both *in vitro* and *in vivo* for a wide range of drug structures. Due to the high cost associated with DDI's, *in vitro* model systems are used as convenient and cost effective tools for identifying compounds with transporter DDI potential in the early stages drug development. Among the *in vitro* methods available for studying drug transporters, cell-based assay systems are the most commonly used for studying drug uptake via the SLC transporters. The cell-based systems for drug uptake studies include a) primary hepatocytes, and b) recombinant cell lines overexpressing individual SLC drug transporters. Primary hepatocytes contain multiple transporters, making the identification of individual transporters important for a specific drug a very complex process. Working with stable cell lines that expresses a single SLC transporter, requires researchers to spend extensive time developing the lines in-house. Or, if the lines are obtained by outside sources, there are typically high associated royalty fees. Further, with cells lines there can be extensive cell culture time investment for maintaining cells. Poor between-lab reproducibility of IC_{50} inhibition data can also be an issue with cell lines, making data comparison across labs difficult.

The newly developed Corning® TransportoCells™ products supply the researcher with an easy "Thaw and Go" model to study SLC transporters. The product is supplied as a vial of cryopreserved cells which can be stored indefinitely. When needed, cells can be thawed, plated and assayed for drug uptake all within 24 hours. The products demonstrate both high uptake activity and uptake ratios (large assay window) for all 6 regulatory required SLC transporters. The cells can be plated and assayed on either 24 well and 96 well plates. Uptake assays can be carried out with cold compounds (LC-MS detection), radiolabeled or fluorescent compounds. The product has been validated for both kinetic and inhibition (DDI) applications. Very high lot-to-lot consistency is also demonstrated.

Material and Methods

DMEM high glucose, MEM non-essential amino acid solution, FBS, HBSS with Ca^{2+}/Mg^{2+} are from Corning cellgro® 24 well and 96 well Corning BioCoat™ Poly-D-Lysine (PDL) plates are from Corning. 500mM sodium butyrate is from EMD-Millipore. M-per mammalian protein extraction reagent and BCA protein assay kit are from Thermo Scientific.

Uptake Assay on 24 well and 96 well PDL plates
Day 1: Thaw cells in prewarmed plating media containing DMEM (high glucose), MEM non-essential amino acid, and 10% FBS. Centrifuge down the cell suspension and resuspend the cell pellet in plating media to reach 1x10^6 viable cells per mL. For OATP1B1*1a, OATP1B3 and their controls, 5 mM sodium butyrate needs to be supplemented. Plate 400 µL cell suspension into each well of 24 well Poly-D-Lysine plate (100 µL for 96 well plate), gently rock the plate to evenly distribute the cells. Incubate the plate at 37ºC with 8% CO_2. After 3-4 hours refeed the cells with the pre-warmed plating media (supplement sodium butyrate if applicable). Incubate the cells overnight at 37ºC with 8% CO_2.

Day 2: Wash the cells 3 times with pre-warmed assay buffer (HBSS with Ca^{2+} and Mg^{2+}), leave the last wash with the cells for 10min in the incubator allowing the cells equilibrate with the buffer. Make the substrate solution in the assay buffer, incubate the cells with the substrate solution for desired time. To stop, wash the cells three times with cold assay buffer at the end of the incubation, lyse the cells and ready for analysis and protein analysis.

Conclusions

- Corning TransportoCell products include all six SLC transporters recommended by regulatory agencies: OATP1B1, OATP1B3, OAT1, OAT3, OCT1 and OCT2.
- All transporter cells demonstrate high signal-to-noise with their probe substrates.
- Assay parameters, such as assay time post plating, seeding density, assay buffer, solvent concentration, sodium butyrate, have been optimized for optimal performance.
- The assay protocol is optimized for both 24 well and 96 well formats, with all three assay detection systems (cold compound with LC-MS, radiolabeled compound with scintillation counter, and fluorescent compound with fluorescent reader)
- Product consistency has been demonstrated with very minimal lot-to-lot variation.

Related Posters

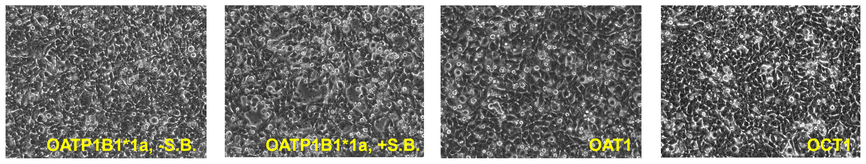
P434 Characterization of a Novel Transporter Model for Studying Drug Interaction with Organic Anion-transporting Polypeptide (OATP)
P435 Validation of a Novel Transporter Model to Study Organic Anion/Cation Transporters
P464 Development of High-Throughput Cell-Based Fluorescence Assays to Study Regulatory Authority Recommended SLC Transporters

Figure 1. Summary of Corning® TransportoCells™ SLC Transporter Cells Performance

Transporters	Catalog #	Post Thaw Viability	Post Thaw Recovery (X10^6/vial)	Probe Substrate	Incubation Time (min)	Uptake Activity in Transporter Cells (pmol/mg/min)	Uptake Activity in control Cells (pmol/mg/min)	Uptake Ratio
OATP1B1*1a (WT)	354859	90%	13.7	2 µM E17βG	5	42.2	0.46	**92***
OATP1B3	354851	91%	14.6	2 µM CCK-8	5	201.3	0.87	**232***
OAT1	354857	93%	12.4	3 µM PAH	10	141.0	0.38	**372**
OAT3	354858	88%	13.4	2 µM E3S	5	121.1	0.91	**133**
OCT1	354852	88%	13.5	30 µM TEA	10	253.0	4.8	**53**
OCT2	354853	89%	13.5	30 µM TEA	10	171.5	4.8	**36**
Control Cells	354854	96%	11.5	NA	NA	NA	NA	NA

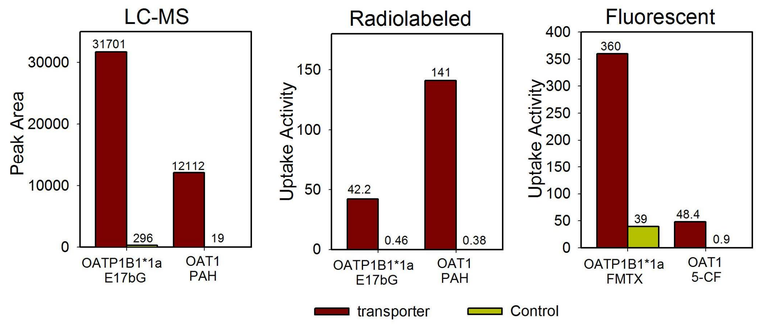
* 5 mM sodium butyrate is supplemented at plating for OATP1B1*1a and OATP1B3. See Figure. 6.

24-hour Confluency



Corning TransportoCells SLC transporter products consist of HEK293 cells transiently overexpressing a single SLC transporter protein including OATP1B1*1a (wild-type), OATP1B3, OAT1, OAT3, OCT1, OCT2, and the empty expression vector (control cells). The post thaw viability all exceed 80%. The post thaw recovery is over 10×10^6 per vial, enough for plating one 24 well or 96 well plate. Cells form a confluent morphology 24 hrs after plating on Poly-D-Lysine plate. Uptake activity are evaluated by incubating the cells with listed prototypical substrates at indicated concentration and incubation time. The uptake ratio is calculated by dividing the uptake activity measured in the SLC transporter cells by that in control cells.

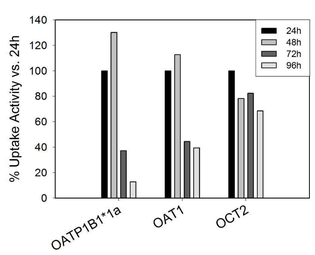
Figure 2: Assay with Cold Compound (LC-MS), Radiolabeled Compound and Fluorescent Compound



Assay protocols are developed for SLC transporter cells with cold compounds (LC-MS), radiolabeled compound, and fluorogenic compound. All methods demonstrate high uptake activity and uptake ratio with probe substrates.

Figure 3: Optimization of the Uptake Assay Plating Time and Seeding Density

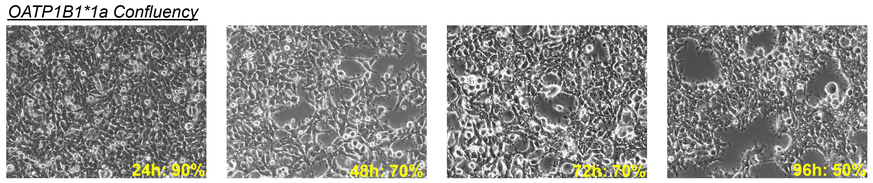
A. 24 – 96 Hours post Plating (Expression Level Peak Time)



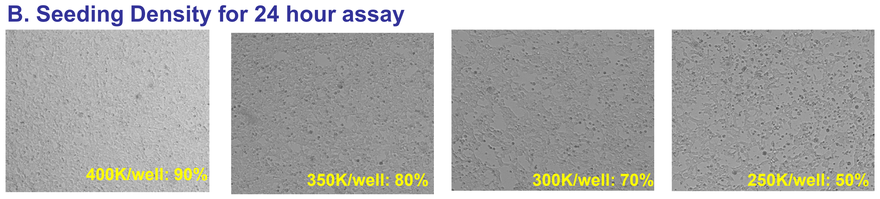
Corning TransportoCells products do not require long-term cell culture and do not support long-term usage. Transporter protein expression (Uptake Activity) peaks at 24-48 hours post plating, then starts diminishing with time. It is recommended to perform the assay at 24 hours post plating.

Cells were seeded onto 24 well Poly-D-Lysine plates at 400K/well (24h assay), 300K/well (48h assay), 200K/well (72h assay) and 150K/well (96h assay). The cells are re-fed at 3-4h post plating and then every 24h. With initial seeding density of 400K/well, cells will become over-confluent after 48h (outside of the cell monolayer starts peeling off the plate) (data not shown).

*OATP1B1*1a Confluency*

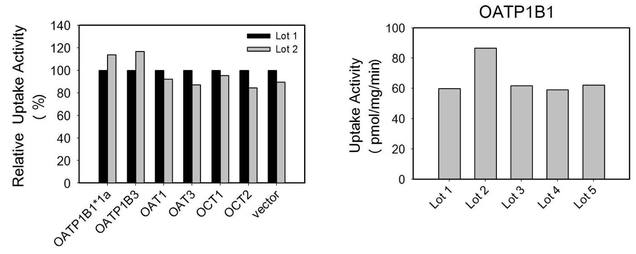


B. Seeding Density for 24 hour assay



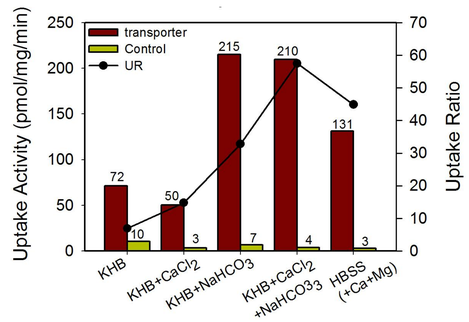
A seeding density of 400K/well is recommended for 24 well Poly-D-Lysine plates for the 24h Uptake Assay. OATP1B1*1a cells are plated at 400K - 250K/well on 24 well PDL plate. The cells are re-fed at 3-4h post plating and assayed at 24h. Uptake activity (pmole/mg/min) is comparable despite the varying confluency (90% to 50%) for all 4 seeding densities (data not shown).

Figure 4: Batch-to-Batch Consistency



A minimum of two batches have been manufactured for all six transporter cells and the control. The uptake activity of the two batches are all less than 20% with an average of 12%. The average E17βG uptake activity in five batches of OATP1B1 is 65.8 with a standard deviation of 11.6 (pmol/mg/min).

Figure 5: Assay Buffer Optimization

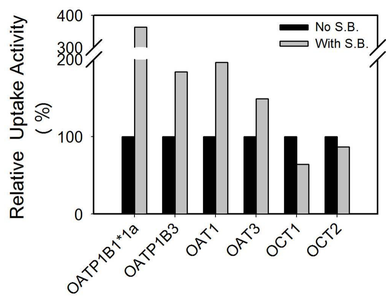


HBSS	KHB	(mg/L)
100	-	$MgCl_2$
100	141	$MgSO_4$
400	350	KCl
60	160	KH_2PO_4
8000	6900	NaCl
48	-	Na_2HPO_4
1000	2000	D-Glu
140	**373 (sup.)**	$CaCl_2$
350	**2100 (sup.)**	$NaHCO_3$

Five assay buffers were tested for OCT2 uptake assay. HBSS with Ca^{2+} and Mg^{2+}, is recommended for use in the uptake assay. OCT2 cells showed the highest uptake activity and uptake ratio with complete KHB buffer, however dramatic precipitation has been observed in the complete KHB buffer, especially when the buffer is warmed up. 10 mM HEPES can be added into HBSS to stabilize the pH.

KHB powder is from Sigma KHB (Cat. No. K3753), HBSS with Ca^{2+} and Mg^{2+} is from Corning Life Sciences (Cat. No. 21-023-CV).

Figure 6: Sodium Butyrate Effect

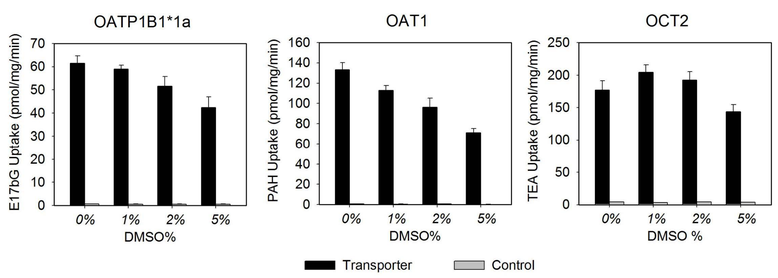


24h Confluency

Transporters	No S.B.	With S.B.
OATP1B1*1a	100%	90%
OATP1B3	100%	90%
OAT1	90%	70%
OAT3	90%	60%
OCT1	90%	50%
OCT2	90%	70%

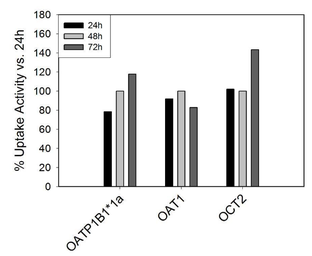
Sodium butyrate is known to induce gene expression in mammalian cells but inhibit cell proliferation. 5 mM sodium butyrate is recommended to be supplemented at plating for OATP1B1*1a and OATP1B3 cells only, to boost uptake activity with minimum impact on the confluency (~10% decrease) (see Figure 1)

Figure 7: Solvent Effect (DMSO)



No more than 1% of DMSO is recommended in the uptake assay to minimize the impact of DMSO on assay performance.

Figure 8: Uptake Assay Time: 18, 24 and 30 hours (Assay Flexibility)



18h: Plate in the afternoon, assay next morning

24h: Plate in the morning, assay next morning

30h: Plate in the morning, assay next afternoon

Comparable assay results were observed for assay times of 18-30 hours post plating. Confluency of 18h, 24h and 30h are all 90-95% (data not shown).

2 µM E17βG, 3 µM PAH, and 30 µM TEA are used in the assay for OATP1B1*1a, OAT1 and OCT2 respectively.

Financial disclosure: This abstract was sponsored by Corning Incorporated.