Cross-Modality Validation of an iPSC-Derived Blood–Brain Barrier Model Using Small Molecules, Antibodies, and Antisense Oligonucleotides

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Introduction

The blood–brain barrier (BBB) is a critical selective interface that shields the central nervous system (CNS) from blood-borne toxins and pathogens. However, this protective function also represents a major challenge for CNS drug development, as the BBB restricts the entry of most small molecules and biopharmaceuticals into the brain. As a result, otherwise promising therapeutics may fail to achieve effective CNS concentrations, limiting their clinical efficacy. There is therefore a strong need for predictive and physiologically relevant in vitro models that can reliably assess brain penetration potential early in drug development.

In this study, we evaluated the iCell® Blood–Brain Barrier (BBB) in vitro model, a novel Transwell-based co-culture system comprising human induced pluripotent stem cell (iPSC)-derived brain microvascular endothelial cells, astrocytes, and pericytes. To assess its predictive performance, the permeability of a panel of small molecules was evaluated and compared with data generated using a widely established MDR1-overexpressing MDCK cell model, as well as with available in vivo unbound brain-to-plasma partition coefficients (Kp,uu,brain). In addition, monoclonal antibody transport was investigated to further characterize the model and assess its applicability across different therapeutic modalities.

Methods

A commercially available iCell® Blood–Brain Barrier Isogenic Kit was used to establish a functional iPSC-derived BBB model in a transwell co-culture system (Figure 1). Barrier integrity and permeability were assessed by TEER measurements and FITC-dextran (40 kDa) permeability. Following establishment in 24-well format, the model was miniaturized to 96-transwell plates by adapting cell seeding densities and confirming assay performance (Table 1).The miniaturized model was validated using small-molecule controls with zero, low, and high permeability (1 µM, 60 min), with results compared with established KO cell lines (Table 1). Functional efflux transporter activity was evaluated using MDR1 substrates (Figure 2A) and BCRP and BCRP/MDR1 dual substrates (Figure 2B) in the presence and absence of specific transporter inhibitors.

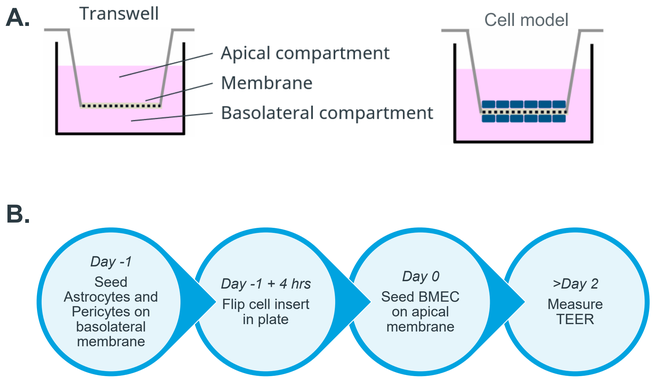


Figure 1. Assay setup of the BBB model. A. Schematic representation of the transwell system part of the iCell® Blood-Brain Barrier Isogenic kit (Fujifilm Cellular Dynamics). **B.** Timelines for cell seeding and readout. Briefly, Astrocyes and Pericytes are seeded on day -1 onto the basolateral membrane of the cell insert previously coated with 0.1% Gelatin, after 4 hours the cell culture insert is flipped and added to the plate containing medium. On day 0 Brain microvascular endothelial cells (BMECs) are seeded onto the apical membrane of the cell insert. Cells are then cultured until TEER measurement (> day 2).

Results

Assay validation of iPSC derived BBB model in 96-transwell format

	iCell® BBB	Abcb1KO-MDCKII-MDR1	Abcb1KO-MDCKII-Mock	MDCKII-BCRP	C2BBe1 Caco-2
FITC-dextran 40kDa	0.00001±0.0		0.17-0.30		0.02±0.002
Mannitol	2.83±0.81	4.47±0.18	4.28±0.15	1.08±0.17	0.54±0.02
Metoprolol	12.95±0.001	20.86±0.30	46.65±3.31	47.86±7.40	12.33±0.59
Caffeine	35.47±15.8	57.92±2.82	64.41±0.67	46.90±0.49	26.25±2.97
TEER (Ωcm²)	~ 1700		~ 100		~ 1000

Table 1. Validation of the iCell® BBB model in 96-transwell format. Permeability characteristics of the iCell® BBB model were assessed using FITC-dextran, a panel of small-molecule controls spanning low to high permeability (mannitol, metoprolol, and caffeine) at 1 µM for 60 min., and transepithelial electrical resistance (TEER). Permeability values are presented as 10^{-6} cm/s (mean ± SD). The model demonstrated expected barrier properties, including near-zero FITC-dextran flux, TEER values exceeding 1500 Ω·cm², and control compound permeability consistent with their known profiles. Historical benchmark data from KO cell lines and Caco-2 cells are included for comparison

Small molecule permeability

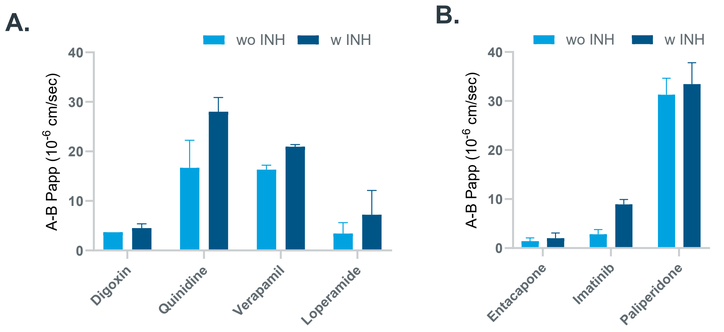


Figure 2. Small molecule permeability in the iCell® BBB model. Small molecule permeability of known MDR1 substrates (digoxin, quinidine, verapamil and loperamide) (**A**) and BCRP substrate (entacapone) plus BCRP/MDR1 dual substrates (imatinib and paliperidone) (**B**) is determined in the iCell® BBB 96-transwell model, with and without efflux transport inhibitors (zosuquidar and Ko143 mixture). Compounds (at 1 µM) were co-incubated for 60 min. either with the inhibitors (at 1 µM each) or with appropriate solvent after which the apparent permeability (Papp value) is determined.

Conclusions

The iCell® BBB model was successfully set up in 96-transwell format using automated cell seeding, and verified by a high physiological TEER measurement, minimal permeability of the FITC-dextran permeability marker and with confirmation of permeability of several small molecules. The assay is still under development to be able to determine iCell® lot to lot variability and consistency of the assay performance. Additionally, the antibody transcytosis assay will be further developed to validate antibody permeability in the model as well.

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