High-throughput assessment of barrier function using human iPSC-derived brain microvascular endothelial cells (BMEC)

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ABSTRACT:

BACKGROUND & PURPOSE. The blood-brain barrier (BBB) is composed of specialized brain microvascular endothelial cells (BMEC) that serve to regulate the flow of substances into and out of the brain. BMEC have physical, transport, and metabolic properties that are regulated by other vascular, immune, and neural cells to create a tightly controlled microenvironment of the central nervous system. Understanding how BMECs work alone and in concert with these other cell types is essential to understand how the brain functions during health and disease. One of the key functional features of BMEC is barrier formation, and the strength and integrity of this barrier can be evaluated via measurement of the electrical resistance across the cell layer. In this study, the barrier function properties of human iPSC-derived BMEC were measured using different platform technologies, such as impedance and trans-endothelial electrical resistance (TEER).

METHODS. Human iPSC-derived brain microvascular endothelial cells (a.k.a. iCell® Brain Microvascular Endothelial Cells) and all media with supplements were from FUJIFILM Cellular Dynamics. These cells were differentiated similar to previously published protocols from an apparently healthy normal (AHN) male donor 01279. Development of TEER assays to measure the barrier function of BMEC were performed using 24-well or 96-well cell culture inserts (Corning) coated with Collagen-IV and Fibronectin (Sigma). Lucifer Yellow was used to measure permeability in these cell culture inserts. To increase throughput of TEER measurements, cells were also tested on CytoView-Z plates and impedance signal was recorded using the Maestro Z (Axion Biosystems). This technology monitors cell coverage (or confluence) as a resistance at high frequency (41.5 kHz) and a very sensitive TEER at a low frequency (1 kHz). These properties were monitored continuously over the course of 10 days.

RESULTS. Data from culture inserts demonstrated consistent TEER >1500 Ω.cm^2 measured using standard 96-well STX HTS EVOM electrode and low permeability to Lucifer Yellow on day 5 in culture. Culturing BMEC on the CytoView-Z plates enables measuring real-time traces of impedance over time and demonstrated lot-to-lot consistency with uncorrected TEER resistance values of ~3500 Ω at 140 hours. This assay was used to profile compounds in 384-well format (n=4 wells; 8-point dose-response curve) that are known to disrupt the barrier and decrease TEER after exposure to the cells, including, mannitol and VEGF.

CONCLUSIONS. Characterization of and testing with the individual cellular components that make up the BBB provides added insight to the functional aspects of this complex system. Human iPSC-derived BMEC offer a robust and reliable source of cells to interrogate the multiple properties of this specialized cell type.

Build Assays to Predict Drug Permeability Across BMEC Monolayer

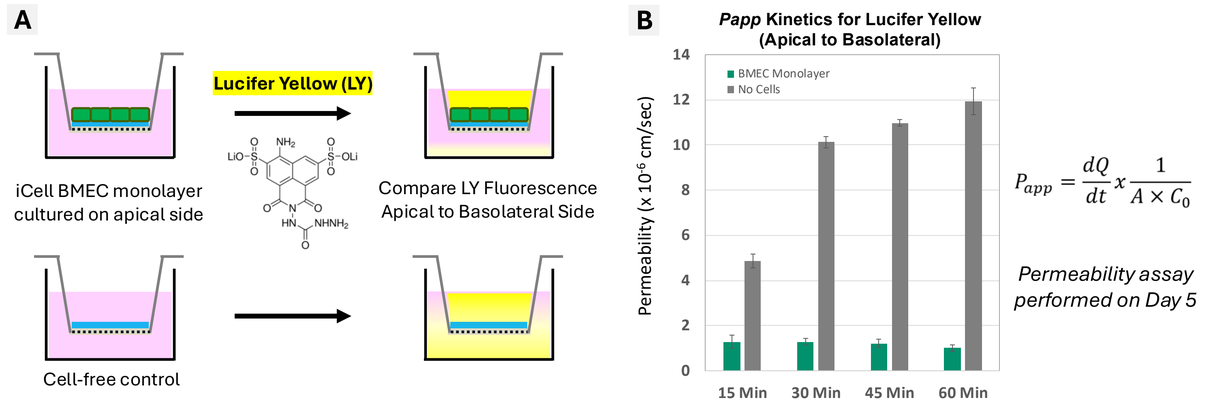

Figure 3. Permeability assay using human iPSC-derived BMEC on Transwell cell culture insert.
(A) iCell BMEC were cultured on the apical side until Day 5 and then movement of lucifer yellow permeating across the cell monolayer (from apical to basolateral side) was compared to an empty, cell-free control.
(B) The apparent permeability coefficient (*P*app) was calculated over time and data shows minimal diffusion across BMEC barrier (green bars) compared to control (gray bars). *P*app measurements below 1 x 10^{-6} cm/sec indicate formation of tight junctions and strong barrier function.

iPSC-derived BMEC Offer a Scalable + Reproducible Human Cell Model

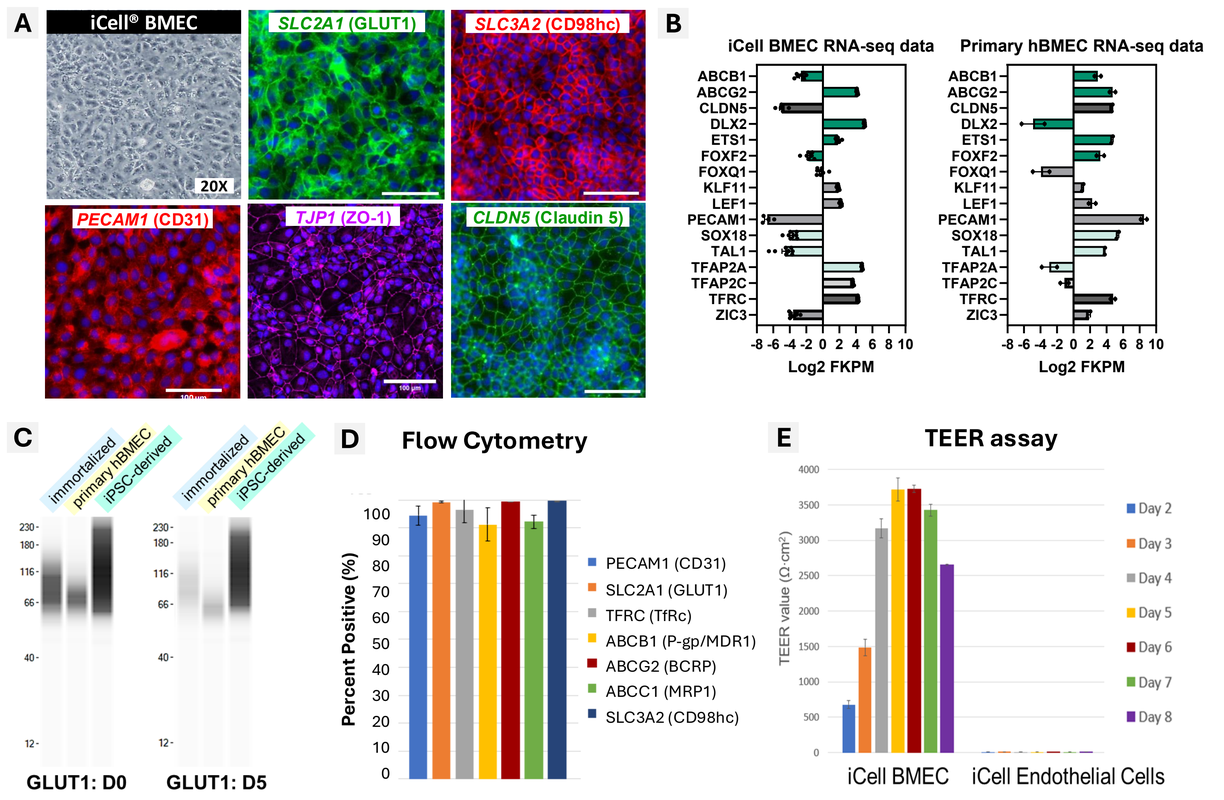

Figure 1. BMEC are a central element of the blood-brain barrier (BBB) and they are unlike other endothelial cells lining peripheral blood vessels in that they display distinctive morphological, structural, and functional features.
(A) Cobblestone morphology of iCell BMEC with tightly packed cells of uniform size and clear cell boundaries. ICC reveals the endothelial markers (Claudin 5, ZO-1) & transporters (GLUT1, CD98hc).
(B) Whole genome RNA-seq analysis was performed on iCell BMEC and compared against primary human BMEC. Select panel of genes listed highlight some similarities and differences between the cell models.
(C) Western blot analysis of GLUT1 protein expression comparing immortalized, primary human BMEC, and iPSC-derived BMEC at thaw (D0) and after 5 days in culture (D5).
(D) Analysis of cell purity by flow cytometry indicates that marker expression for CD31, GLUT1, TfRc, MDR1, MRP1, and CD98hc are ≥90% positive.
(E) Functional testing via TEER assay showcases the differences in endothelial cell types through formation of tight junctions.

Interrogate Barrier Formation Dynamics with Impedance-based TEER

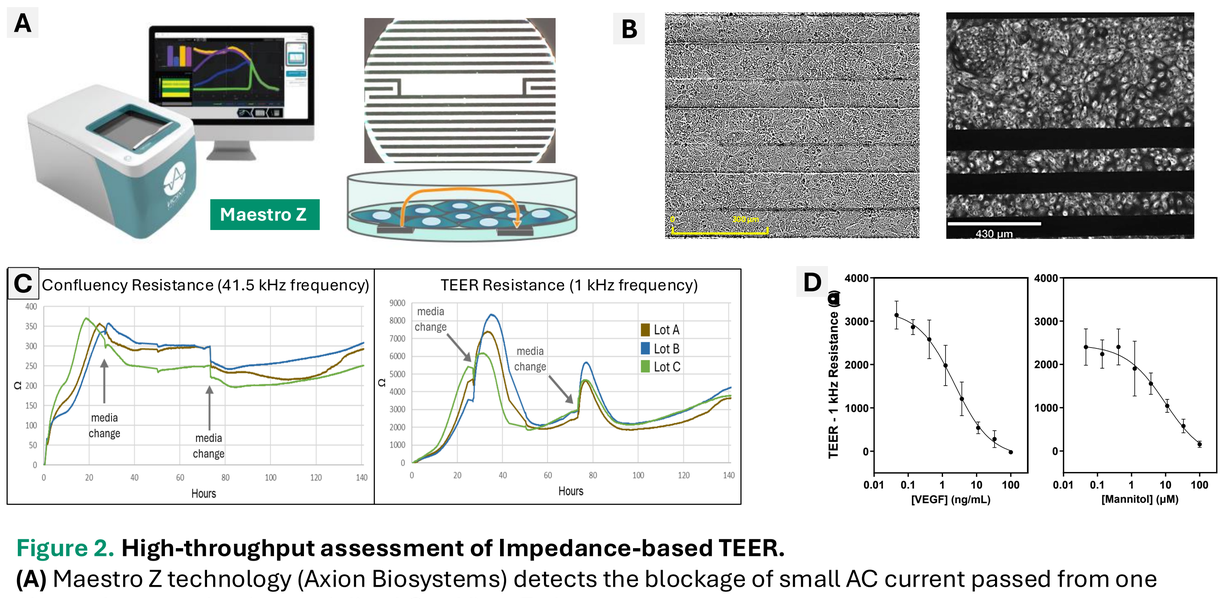

Figure 2. High-throughput assessment of Impedance-based TEER.
(A) Maestro Z technology (Axion Biosystems) detects the blockage of small AC current passed from one electrode to another in specialized CytoView-Z plates.
(B) Not only do these plates enable real-time measurement of barrier function, but also visualization of the cells. iCell BMEC were brightfield imaged on a 384-well plate (left) and live-cell stained with Mitotracker Deep Red in 96-well format (right).
(C) High frequency (41.5 kHz) resistance measurements indicates confluency, and low frequency (1 kHz) impedance signals are sensitive to the intercellular barrier formed by tight junctions (TEER). Three different lots of iCell BMEC were assayed on a 384-well CytoView-Z plate for 6 days.
(D) VEGF and Mannitol (8-pt dose response, n=4 wells at each concentration) yield a robust decrease in TEER after exposure to the cells for 24 h.

Monitor TEER in 24w or HTS-compatible 96w Transwell Devices

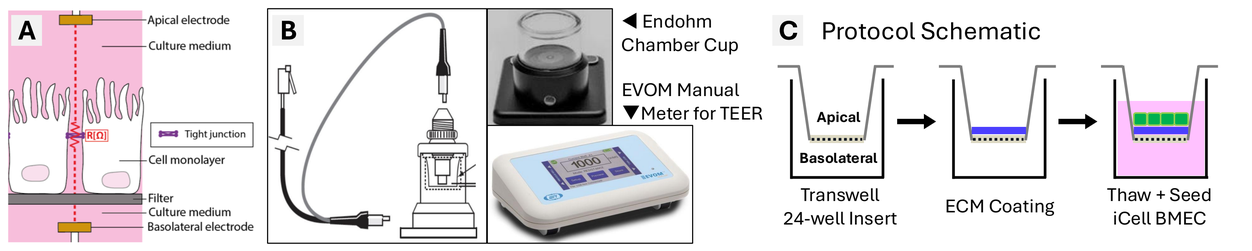

Figure 4. TEER assay is the gold-standard method to quantitatively measure barrier integrity.
(A) Schematic of TEER measurements across a cell monolayer with electrodes on the apical and basolateral sides to probe barrier integrity and tight junction dynamics.
(B) World Precision Instruments (WPI) offers EVOM technology as the trusted brand for TEER equipment.
(C) iCell BMEC were developed using a TEER assay on with cells seeded on the apical side of a 24-well Transwell cell culture insert coated with Fibronectin and Collagen-IV.
(D) TEER values typically exceed >500 Ω•cm^2 after 2 days post-plating, continue to rise above 2000 Ω•cm^2 by DIV 4, and persist for at least a 5-day assay window. For reference, physiological range for TEER is typically >1500 Ω•cm^2. iCell BMEC outperformed primary human endothelial cells (hBMEC).
(E) After formation of a stable cell barrier, this culture setup can be used to assess compounds for toxicity. Staurosporine was shown to decrease TEER values in a dose-depended manner.
(F) Higher throughput methods (24w → 96w format) to evaluate the integrity of the BBB are in high demand. Launching iCell BMEC as a standalone product was intended for initial optimization and onboarding experiments. FCDI recommends HTS Transwell-96 Permeable Support with 0.4 μm PET membrane (Corning #7369) and has implemented a workflow with this system (please inquire for technical support).
(G) TEER assay data from iCell BMEC in mono-culture or the complete iCell BBB tri-culture kit were compared from DIV 3 to 11 in culture. While serviceable TEER values were obtained (from n=8 wells each), the complete tri-culture BBB model yielded more consistent data (less error) overall.
(H) Methods for imaging cells cultured on the Transwell membrane (both live and fixed) has been requested from multiple users. This example image shows iCell BMEC in mono-culture on the Transwell membrane stained for actin with phalloidin (green) and for nuclei with Hoechst 33342 (blue).

Extract High Value with a Full 96w Transwell from just one iCell BBB Kit

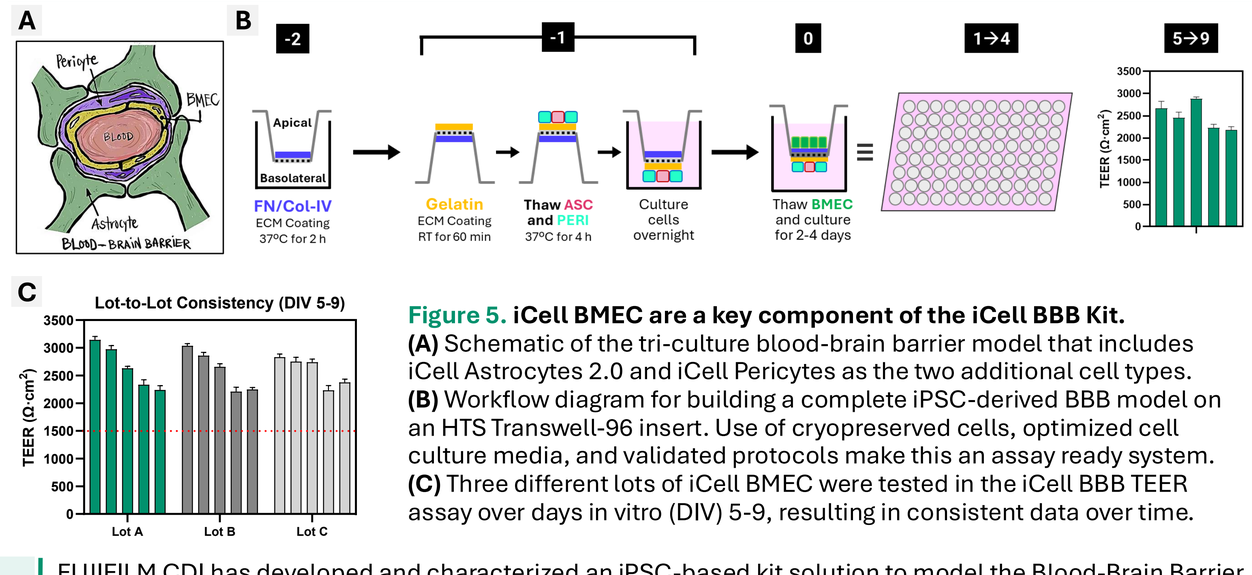

Figure 5. iCell BMEC are a key component of the iCell BBB Kit.
(A) Schematic of the tri-culture blood-brain barrier model that includes iCell Astrocytes 2.0 and iCell Pericytes as the two additional cell types.
(B) Workflow diagram for building a complete iPSC-derived BBB model on an HTS Transwell-96 insert. Use of cryopreserved cells, optimized cell culture media, and validated protocols make this an assay ready system.
(C) Three different lots of iCell BMEC were tested in the iCell BBB TEER assay over days in vitro (DIV) 5-9, resulting in consistent data over time.

Summary

FUJIFILM CDI has developed and characterized an iPSC-based kit solution to model the Blood-Brain Barrier with iCell BMEC as the key cell type forming tight junctions and creating a selective barrier. Multiple platform technologies, incl. Transwell® (24- and 96-well), impedance-based CytoView-Z plates from Axion Biosystems, and organ-chips (data not shown) were used for permeability and TEER measurements to demonstrate the functional performance of this cell type. The advancements to HTS-compatible formats make these assays easier to implement in routine workflows. These findings highlight human iPSC-derived BMEC and the BBB model with astrocytes and pericytes as a reliable model to study BBB properties and support investigations into brain barrier function in health and disease.