High-throughput Assessment of Barrier Function Using Human iPSC-derived Brain Microvascular Endothelial Cells

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Abstract

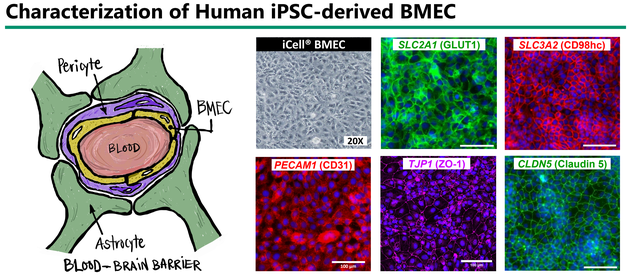
OBJECTIVE/RATIONALE: The blood-brain barrier (BBB) is composed of specialized brain microvascular endothelial cells (BMEC) that serve to regulate the flow of substances into and out of the brain. BMEC have physical, transport, and metabolic properties that are regulated by other vascular, immune, and 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/RESULTS: Human iPSC-derived brain microvascular endothelial cells (iCell BMEC) 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 cell culture inserts (Corning) coated with Collagen-IV and Fibronectin (Sigma). Cryopreserved cells were thawed in the presence of iCell Plating Supplement for the best results. To increase the throughput of TEER measurements, however, BMEC were also plated on CytoView-Z plates and 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. Real-time traces of impedance over time were used to demonstrate lot-to-lot consistency of iCell BMEC with uncorrected TEER resistance values of ~3500 Ω at 140 hours. This assay was used to profile molecules in 384w format (n=4 wells; 8-point dose-response curve) that disrupt the barrier, incl. bupropion, mannitol, prazosin, VEGF, and verapamil.

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.

Keywords: Blood Brain Barrier, iPSC, and TEER

Characterization of Human iPSC-derived BMEC



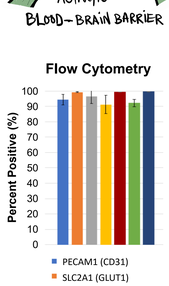
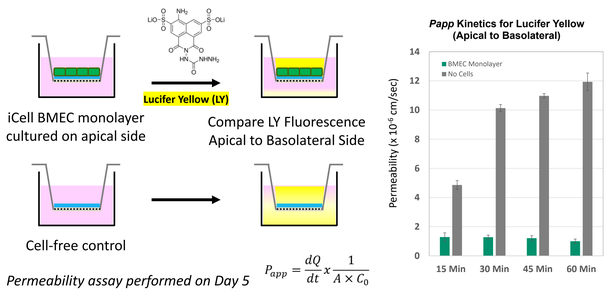


Figure 1. Brain microvascular endothelial cells (BMEC) are a central element of the blood-brain barrier (BBB), together with astrocytes and pericytes, to form an interface between nervous tissue and circulating blood. BMEC are unlike other endothelial cells lining peripheral blood vessels, however, in that they display distinctive morphological, structural, and functional features. iCell BMEC have cobblestone morphology as tightly packed cells with uniform size and clear cell boundaries. Marker expression as measured by ICC reveals the endothelial markers (Claudin 5, ZO-1), transporters (GLUT1, CD98hc), and efflux/ influx proteins (BCRP, P-gp, MRP1, TfRc; data not shown). Analysis of cell purity by flow cytometry indicates that protein expression for markers such as BCRP, GLUT1, MDR1, MRP1, and TfRc are ≥90% positive.

Permeability Assay with Human iPSC-derived BMEC

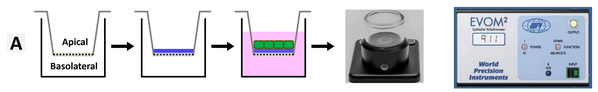


Permeability assay performed on Day 5

$$P_{app} = \frac{dQ}{dt} x \frac{1}{A \times C_0}$$

Figure 2. A permeability assay using transwells was developed to measure fluorescence intensity of a molecule (e.g., lucifer yellow) permeating through a cultured monolayer of iCell BMEC over time. Assay was performed on Day 5 post-thaw and LY fluorescence signal from the apical to basolateral side was compared. The apparent permeability coefficient (*P*app) was calculated to measure integrity of barrier function. Data shows minimal diffusion through the monolayer (green bars) compared to an empty transwell (no cells; gray bars). *P*app measurements below 1 x 10^{-6} cm/sec formation of tight junctions and strong barrier function.

Transwell Assay to Measure TEER



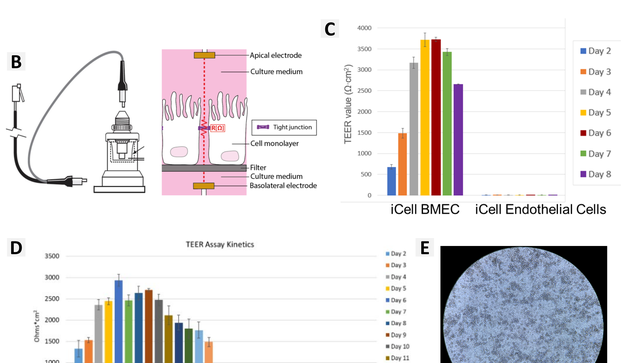


Figure 3. TEER is a widely accepted technique to measure barrier integrity and tight junction dynamics for a cell monolayer. A) iCell BMEC were developed around functional assay on Transwell cell culture inserts. B) The EVOM TEER instrument and chamber cup setup were used to measure TEER. As soon as 2 days post-plating, TEER values typically begin >500 Ω•cm^2. TEER data generated with the iCell BMEC outperformed other models when compared to C) human iPSC-derived endothelial cells or D) primary endothelial cells. For reference, the range for physiological TEER value is typically above 1500 Ω•cm^2. E) Lastly, iCell BMEC can be imaged on the membrane of the Transwell during culture to visualize cell health and morphology.

Impedance Assay as an Alternative to Measure TEER

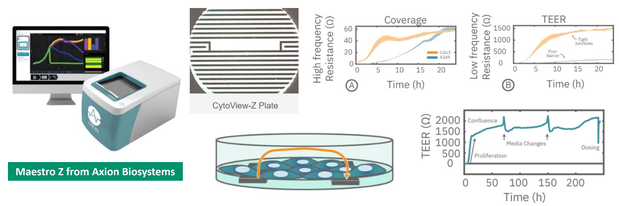


Figure 4. Maestro Z technology enables high-throughput assessment of TEER by detecting the blockage of small AC current passed from one electrode to another. Specialized plates (CytoView-Z) enable real-time measurement of barrier function as well as visualization of the cells. Measuring impedance at a high frequency (41.5 kHz) indicates coverage (or how many cells are there), whereas low frequency resistance is sensitive to the intercellular barrier formed by tight junctions.

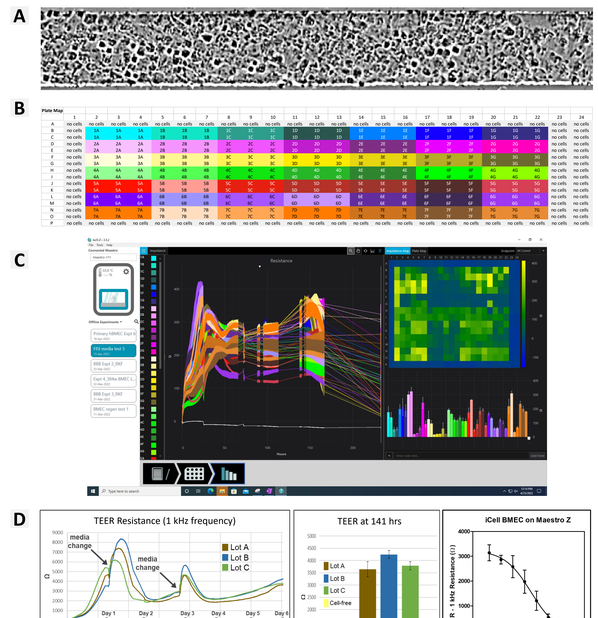
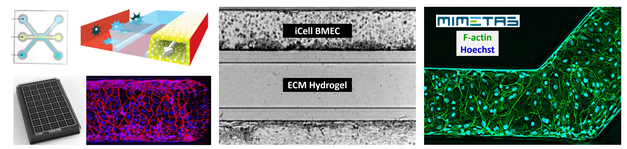


Figure 5. TEER assay development on the Maestro Z platform. A) Visualization of iCell BMEC monolayer on the CytoView Z plate enables verification of cell viability, attachment, and morphology. Assay multiplexing is also possible, if desired. B) 96- or 384-well format allows for screening of multiple conditions with high replicates, including ECM, culture media, cell densities, etc. In this example, 49 different conditions at n=6 and numerous blank/cell-free control wells. C) The easy-to-use AxIS Z™ software provides a simplified approach to the setup, execution, and analysis of experiments. D) Real-time impedance assays were monitored on the Maestro Z for 3 different lots of iCell® BMEC cultured in a 384-well CytoView-Z plate for 6 days. The data indicate similar traces of impedance over time and essentially the same endpoint TEER value (3600-4200 Ω at 141 hours) across all 3 lots. Importantly, this application can be used to profile molecules that disrupt the BBB, including VEGF (8-pt dose response, n=4 wells at each concentration).

Organ-on-a-Chip Models with Human iPSC-derived BMEC



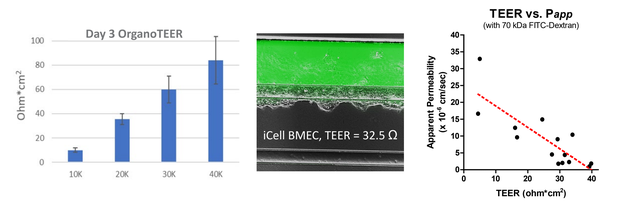


Figure 6. Expanding beyond 2D culture or Transwell inserts to any microphysiological system is of high importance, but it is not a seamless transition. Here, iCell BMEC are used with an OrganoPlate 3-lane 40 microfluidic device (MIMETAS). Increasing cell densities of iCell BMEC (in complete triculture) result in increasing TEER values (ohm*cm^2) recorded on Day 3 with the OrganoTEER™ system. TEER data acquired on Day 5 (x-axis) were plotted against the Apparent Permeability coefficient (n=14; y-axis) as calculated from the permeation rate of FITC-labeled dextran (70 kDa) which resulted in a linear negative correlation. As expected, when barrier integrity is high, TEER values are also high, and permeability is low. R^2=0.6775, with significant correlation P value = 0.0003 .

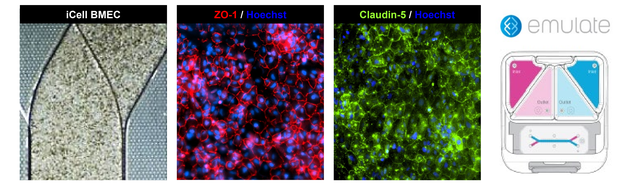


Figure 7. Emulate Organ Chip (S-1) was used and iCell BMEC were maintained under flow culture for 7 days. Tight junction formation was observed through the whole bottom channel as indicated by robust staining for ZO-1 and Claudin-5 . Initial testing of iCell BBB kit on the Emulate OoC platform posed some challenges (i.e., cell detachment, bubbles), but we did observe improved cell marker staining (ZO-1 and Claudin-5) after culturing on the chips with dynamic flow. Additional work is needed.

Summary and Future Directions

FUJIFILM CDI has developed a kit-based solution to model the BBB with human iPSC-derived cell types. The unique features of iCell Brain Microvascular Endothelial Cells (BMEC) have inspired us to investigate the properties of this specialized cell type in mono-culture. iCell BMEC can form tight junctions that limit the passive diffusion of molecules across the barrier and exhibit extremely robust and reproducible TEER values in different assay formats, including a traditional Transwell assay, impedance-based Maestro Z platform technology, as well as the organ-on-a-chip OrganoTEER system. These high throughput screening platforms will be important for screening compounds in a simple in vitro model before testing compounds on complex 3D in vitro iCell BBB model. We will launch iCell BMEC Kits later this year, until then you can find more information on the iCell BBB kits at the link below.

www.fujifilmcdi.com/icell-blood-brain-barrier-isogenic-kit-01279-r1241