Generation of a Blood-Brain Barrier Model using Cryopreserved Human iPSC-Derived Brain Microvascular Endothelial Cells, Pericytes, and Astrocytes

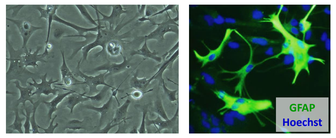
Kat Czysz, Ouissame Filali, Christie A. Munn, Madelyn E. Goedland, Sarah Burton, Megan K. Livingston, Rebecca K. Fiene, Kimihiko Tomotoshi, Makiko Ohshima, Deepika Rajesh, Coby B. Carlson, and Ravi Vaidyanathan

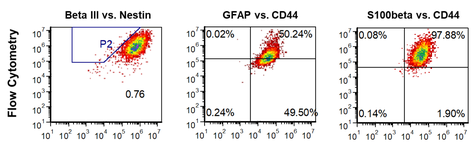
FUJIFILM Cellular Dynamics, Inc., Madison, WI USA

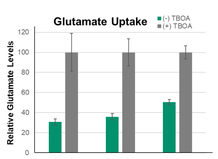
Abstract

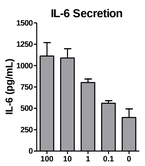
The blood-brain barrier (BBB) is a specialized network of cells that function to maintain a tightly controlled microenvironment around the brain. For many years, the scientific community has needed a robust and human-relevant BBB model to evaluate barrier function and its role in drug permeability *in vitro*, as well as to study diseases that affect it. The power of iPSC technology provides access to specialized cell types of the brain required to assemble such a model system, but the field has been challenged with generating cells that contain the appropriate markers, manufacturing a consistent supply of cells at-scale, and cryopreserving material for subsequent on-demand use. As a leader in iPSC technology and innovation, FUJIFILM Cellular Dynamics has generated and characterized 3 unique human iPSC-derived cell types for use in BBB model development, including astrocytes, brain microvascular endothelial cells (BMEC), and pericytes. Perhaps most notably, the differentiation and cryopreservation of BMEC to yield a cell type with distinctive morphological (cobblestone and tightly packed), structural (proper organization of tight junctions and appropriate expression of transporters), and functional (effective barrier formation) features that differ from other vascular endothelial cells lining peripheral blood cells has made the highest impact. Additionally, optimized media and supplements to enable long-term survival of 3 cell types in co-culture and to promote superior functional performance in transendothelial electrical resistance (TEER) assays is a key factor in the establishment of a reliable BBB model. The potential to integrate this system with emerging organ-on-a-chip technologies and other 3D cell culture systems offers an exciting new capability for the drug discovery community to advance the understanding of BBB function with respect to human health and disease.

Characterization of iPSC-derived Astrocytes



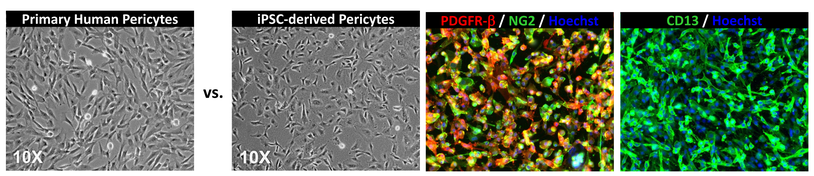


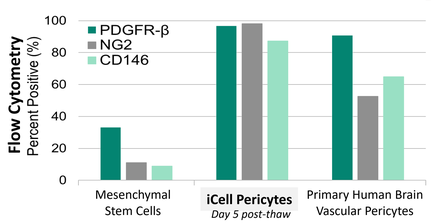




Astrocytes are specialized glial cells and represent the most abundant cell type in the CNS. iCell Astrocytes are human iPSC-derived cell that show typical astrocyte cell morphology by microscopy, are a highly pure population of astrocytes (>95% by flow cytometry) that express relevant markers (S100 beta, GFAP, CD44); they also exhibit robust glutamate uptake (modulated by TBOA) and cytokine-mediated inflammatory responses (i.e., release of IL-6). Most importantly, the cells have been successfully co-cultured with other human iPSC-derived cell types, including neurons and microglia.

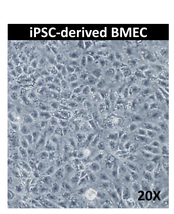
Characterization of iPSC-derived Pericytes

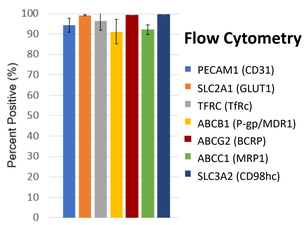


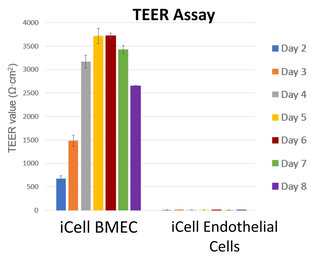


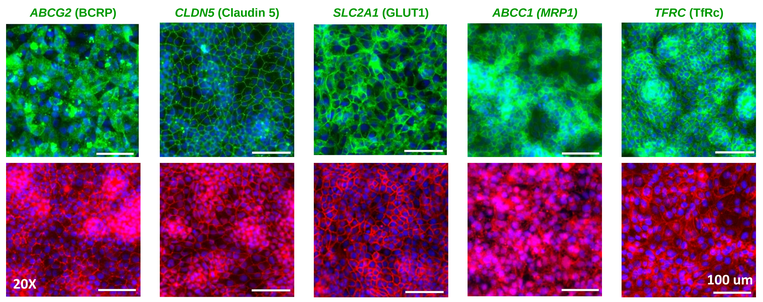
Pericytes are mural cells (like vascular smooth muscle) which ensheathe endothelium throughout the body. They are an important element of the BBB and function to maintain homeostasis of the barrier, regulate blood flow, and facilitate neuro-inflammatory responses. iCell Pericytes display similar morphology to primary cells and express relevant markers (PDGFR-beta, NG2, CD13, and CD146) by ICC and flow cytometry. These cells also display functional phagocytic activity of *S. aureus* bioparticles in mono-culture (data not shown).

Characterization of iPSC-derived Brain Microvascular Endothelial Cells



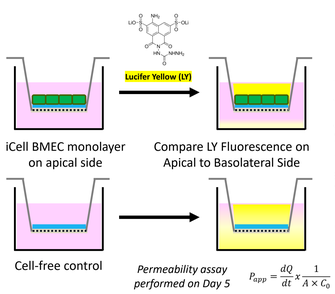


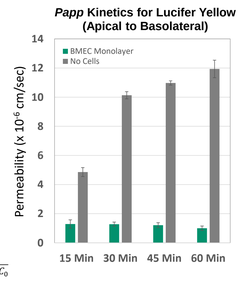




Brain microvascular endothelial cells (BMEC) are unlike other vascular endothelial cells lining peripheral blood vessels in that they display distinctive morphological, structural, and functional features. iCell BMEC have cobblestone morphology as tightly packed cells with uniform size and clear cell boundaries; marker expression (by ICC and flow cytometry) reveals characteristic of endothelial cell markers (CD31, ZO-1, Claudin 5), transporters (GLUT1, CD98hc), and efflux/influx proteins (BCRP, P-gp, MRP1, and TfRc). Perhaps most importantly, there is effective barrier formation with these iPSC-derived BMEC at measured by TEER.

Permeability Assay with Human iPSC-derived BMEC

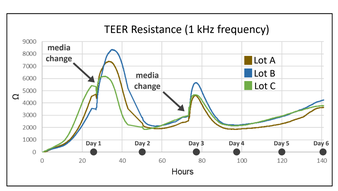


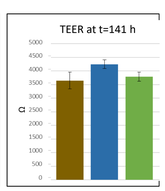


A transwell permeability assay measures fluorescence intensity of a molecule (e.g., lucifer yellow) permeating through a cultured monolayer of iCell BMEC over time. Here, the assay was performed on Day 5 post-thaw and LY signal from the apical to basolateral side was compared and the apparent permeability coefficient (*P*app) was calculated. The data shows minimal diffusion through the monolayer, indicating tight barrier function.

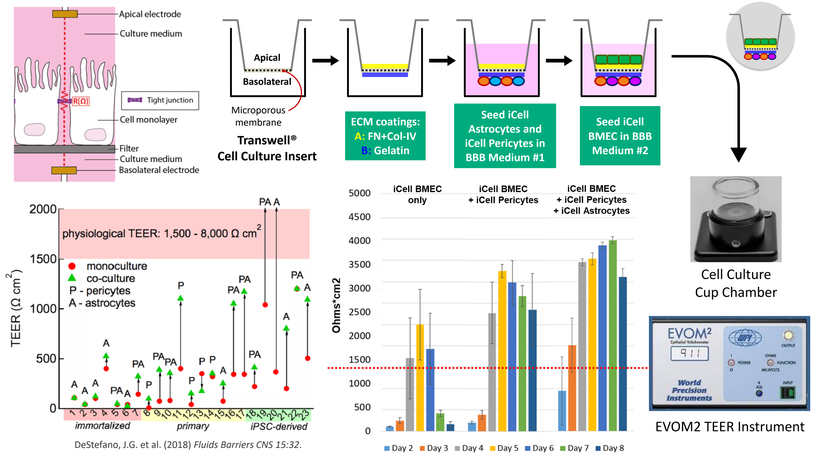
Impedance Measurements are Consistent Across Lots of iCell BMEC

Impedance assays indicating how much electrical current is blocked by a cellular monolayer in an alternate form of TEER. Using the Maestro Z (Axion Biosystems), 3 different lots of iCell BMEC were cultured in a 384-well Cytoview Z plate for 6 days. The data indicate similar real-time traces of impedance over time and essentially the same endpoint TEER value across all 3 lots.



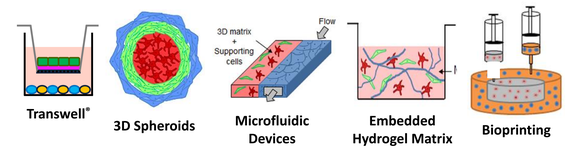


TEER Assay with Human iPSC-derived BBB Kit



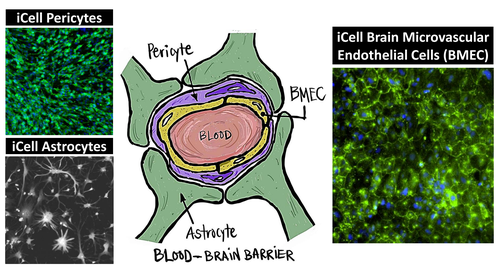
Transendothelial Electrical Resistance (TEER) is a widely accepted technique to measure barrier integrity and tight junction dynamics for a cell monolayer. The iCell BBB Kit was developed and optimized using a sandwich assay on Transwell cell culture inserts. On Day (-1) of the TEER assay, the apical side of the microporous membrane is coated with fibronectin and collagen-IV, while the basolateral side is coated with gelatin. On Day 0. iCell Astrocytes and Pericytes are seeded together on the basolateral side in BBB medium #1. On Day 1, iCell BMEC are thawed and plated on the apical side in BBB medium #2. Testing of the functional BBB tri-culture system is ready as soon as Day 2 with values typically beginning >500 Ω•cm^2. TEER data generated with the iCell BBB kit outperformed other models with immortalized, primary, or iPSC-derived BMEC cells reported in the literature and is expected to exceed the minimum range for physiological TEER (1500 Ω•cm^2).

Next Steps: Wide Range of Possibilities for *in vitro* BBB Models



There are numerous options available to generate an *in vitro* BBB model. Recently, there has been great interest in the development of organ-on-a-chip (OoC) systems for the BBB, incl. devices from MIMETAS and Emulate. Preliminary work is shown here in this panel. The transitioning of a Transwell assay protocol to an OoC microfluidic device, however, does require some effort and a different skill set. Watch out for BBB Application Protocols with the iCell BBB kit on these (and other) systems in the future.

Summary of Human iPSC-derived BBB Model



FCDI's solution for modeling the BBB is to use human iPSC-derived cell types. Here we have presented characterization data for astrocytes, pericytes, and brain micro-vascular endothelial cells (BMEC). Unique features of specialized iCell BMEC enable the formation of tight junctions that limit the passive diffusion of molecules across a monolayer and exhibit extremely high TEER values in different assay formats. Future work includes pharmacological validation of drug permeability and transporter activity, as well as expansion into "brains-on-a-chip".