

Establishment of a Blood-Brain Barrier Model using iPSC-Derived Human Cells

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FUJIFILM



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Abstract

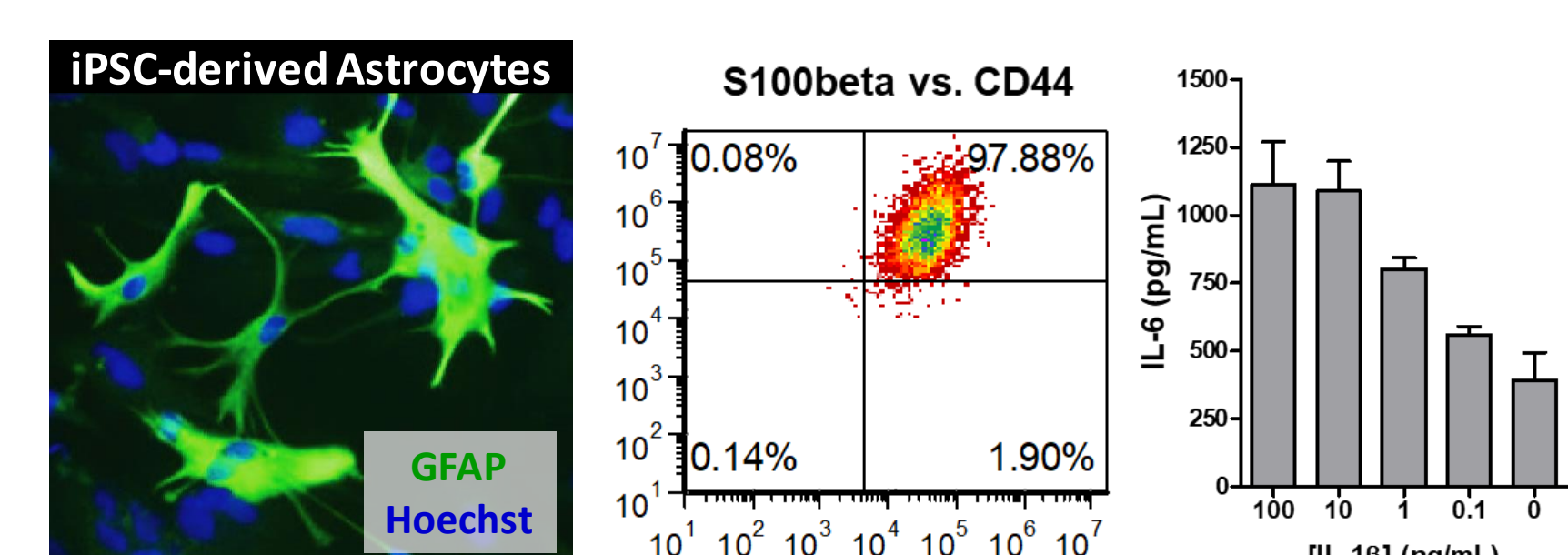
Objective: The blood-brain barrier (BBB) is a specialized network of cells that function to maintain a tightly controlled microenvironment around the brain. Modeling the BBB *in vitro* is needed to evaluate barrier function, test drug permeability, and study the diseases that affect it. Induced pluripotent stem cell (iPSC) technology is a powerful tool to generate the cells that compose the BBB and establish such a model.

Methods: As a leader in iPSC technology and innovation, FUJIFILM Cellular Dynamics, Inc. (FCDI) generated, characterized and utilized three unique human iPSC-derived cell types for use in BBB model development, i.e., astrocytes, brain microvascular endothelial cells (BMEC), and pericytes.

Results: Differentiation of iPSC into BMEC yields a cell type with distinctive cellular structures (tightly packed, cobblestone morphology, proper organization of tight junctions), appropriate marker expression (transporters: GLUT1, CD98hc and efflux/influx proteins: BCRP, P-gp, MRP1, transferrin receptor), and functional assay performance (effective barrier formation, low permeability). These features separate BMEC from other vascular endothelial cells lining peripheral blood cells. iPSC-derived pericytes show a characteristic stellate morphology, appropriate marker expression, and phagocytosis function. Establishment of a reliable BBB model required optimization of media and supplements to enable long-term survival of all three cell types in co-culture and to promote high transendothelial electrical resistance (TEER) signal in assays using Transwell inserts. Preliminary data shows the possibility of integrating this cellular BBB system with emerging organ-on-a-chip (OoC) technologies and other 3D culture platforms.

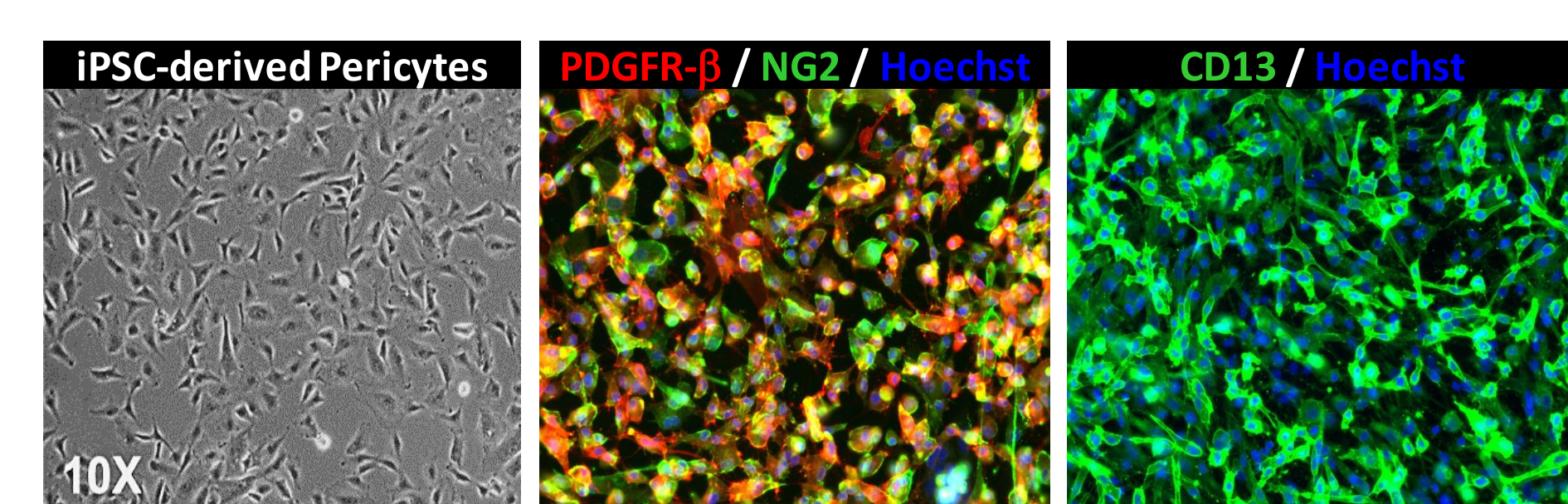
Conclusion: FCDI succeeded in establishing a fully human iPSC-derived BBB model, manufacturing a consistent supply of cells at-scale, and cryopreserving the material for subsequent on-demand use.

Characterization of iPSC-derived Astrocytes



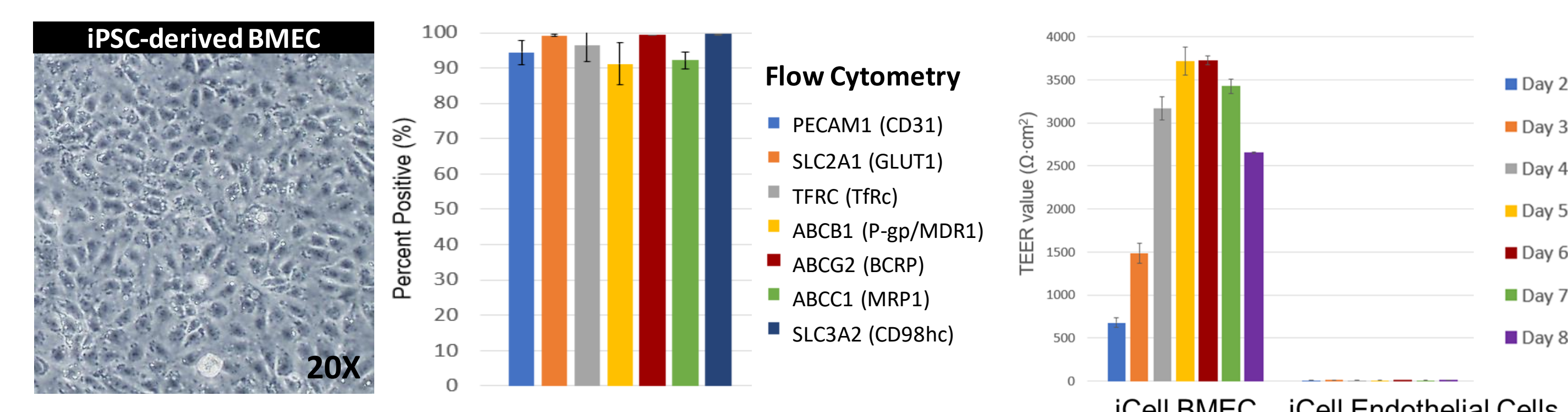
Astrocytes are specialized glial cells and represent the most abundant cell type in the CNS. iCell® Astrocytes are a highly pure population of cells (>95% by flow cytometry) that express relevant markers (S100-beta, GFAP, CD44) and are highly functional (release IL-6). Most importantly, they can be co-cultured with other human iPSC-derived cell types, including neurons and microglia (data not shown here).

Characterization of iPSC-derived Pericytes

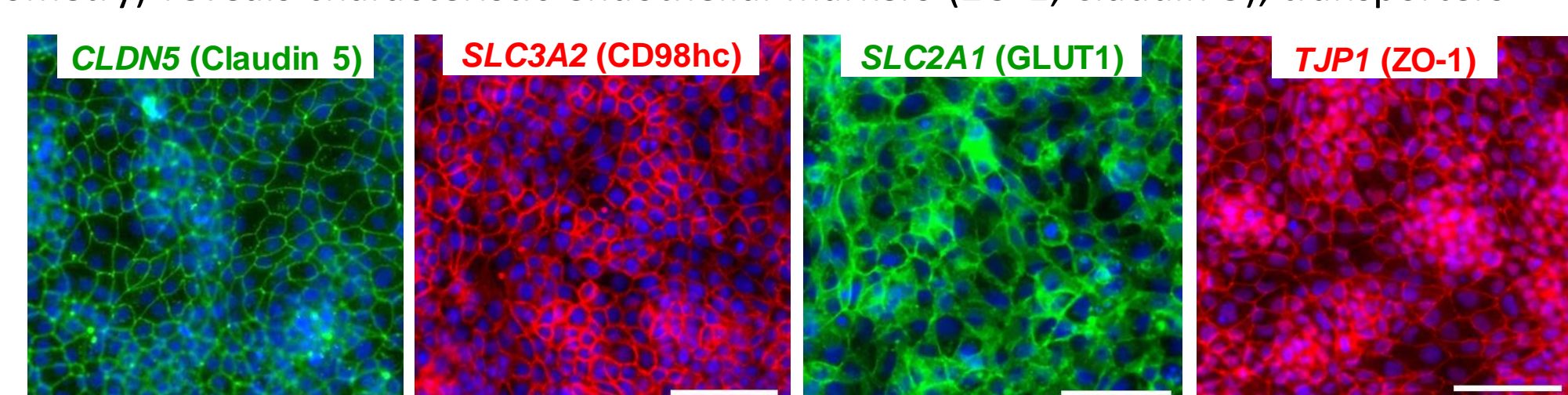


Pericytes are mural cells (like vascular smooth muscle) which ensheath endothelium throughout the body. They are an important element of the BBB and function to maintain homeostasis of the barrier, regulate blood flow, and facilitate neuro-inflammatory responses. iCell® Pericytes display similar morphology to primary cells and express relevant markers (PDGFR-β, 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 here).

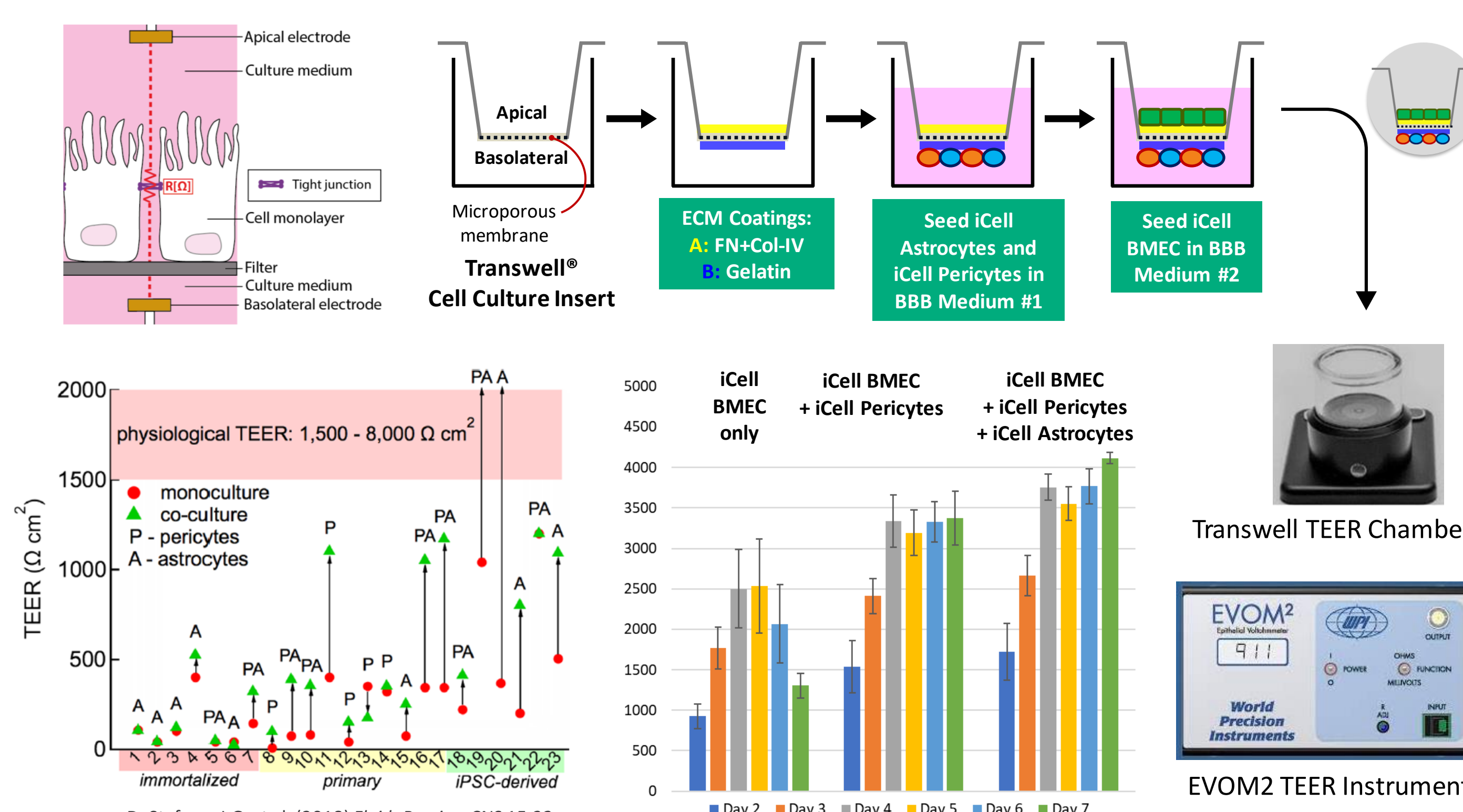
Characterization of iPSC-derived Brain Microvascular Endothelial Cells



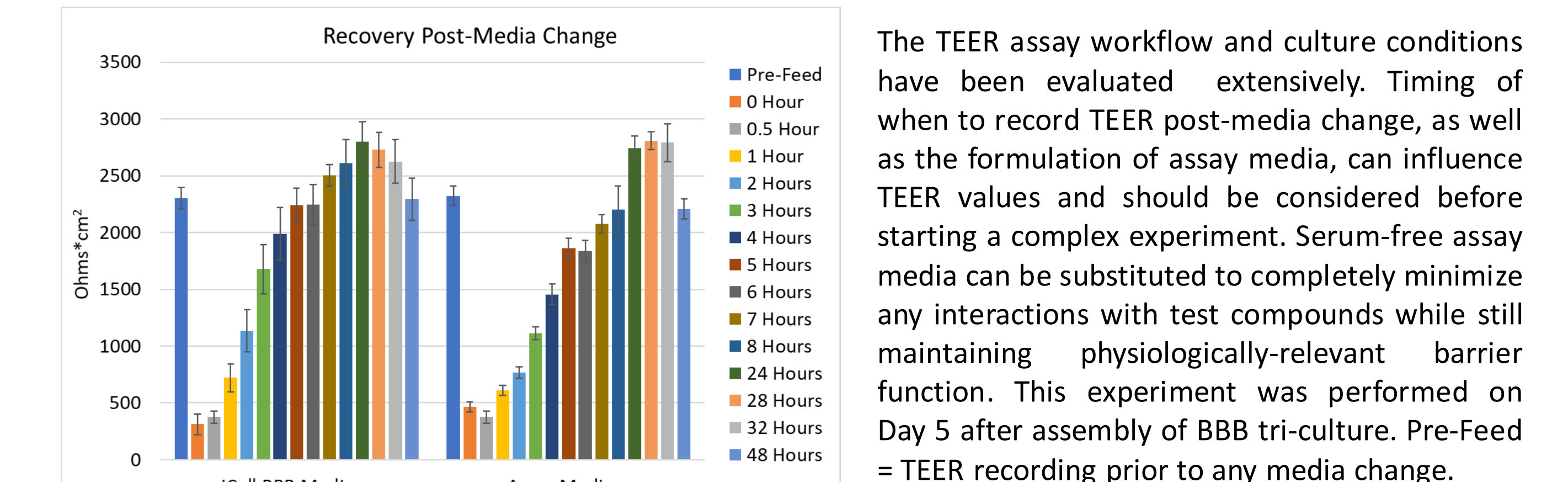
Brain microvascular endothelial cells (BMEC) are unlike other vascular endothelial cells lining peripheral blood vessels in that they display distinctive morphological, structural, and functional features. iCell® BMEC have cobblestone morphology as tightly packed cells with uniform size and clear cell boundaries. BMEC marker expression (by ICC and flow cytometry) reveals characteristic endothelial markers (ZO-1, Claudin 5), transporters (GLUT1, CD98hc), and efflux/influx proteins (BCRP, P-gp, MRP1, TfRc; data not shown here). iCell BMEC exhibit much higher functionality in a TEER assay compared to iCell® Endothelial Cells.



TEER Assay with Human iPSC-derived BBB Model

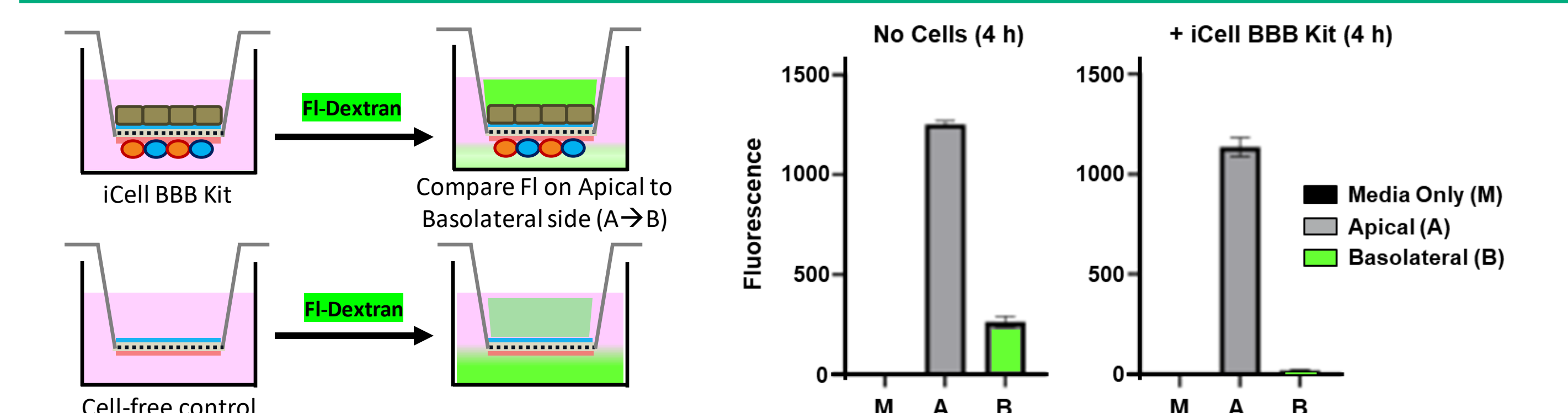


Transendothelial Electrical Resistance (TEER) is a widely accepted technique to measure barrier integrity and tight junction dynamics for a cell monolayer. The iCell BBB Kit was developed and optimized using a sandwich assay on Transwell cell culture inserts. On Day (-2) of the TEER assay, the apical side of the microporous membrane is coated with fibronectin and collagen-IV, while the basolateral side is coated with gelatin. On Day (-1), iCell Astrocytes and iCell Pericytes are seeded together on the basolateral side in BBB Medium #1. On Day 0, 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·cm². TEER data generated with the iCell BBB Kit outperformed other models using immortalized, primary, or iPSC-derived BMEC cells reported in the literature and is expected to exceed the minimum range for physiological TEER (1500 Ω·cm²).

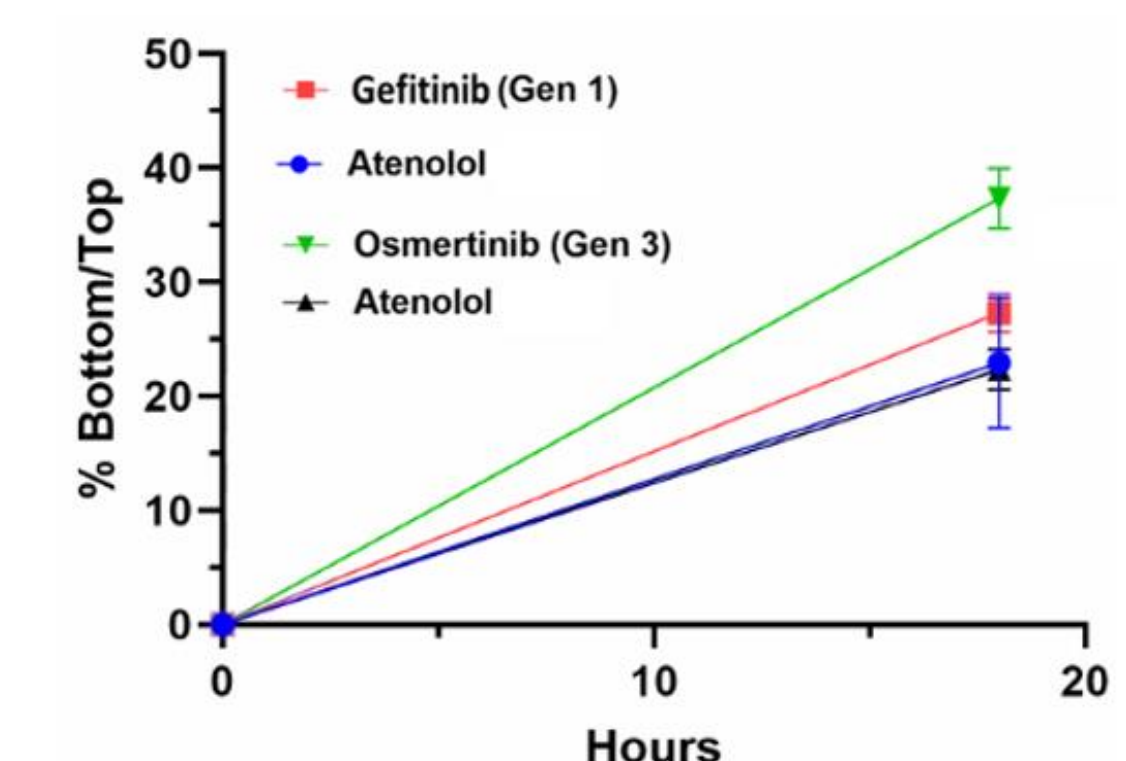


The TEER assay workflow and culture conditions have been evaluated extensively. Timing of when to record TEER post-media change, as well as the formulation of assay media, can influence TEER values and should be considered before starting a complex experiment. Serum-free assay media can be substituted to completely minimize any interactions with test compounds while still maintaining physiologically-relevant barrier function. This experiment was performed on Day 5 after assembly of BBB tri-culture. Pre-Feed = TEER recording prior to any media change.

Permeability Assays with Human iPSC-derived BBB Model



Permeability assays measure the intensity of a fluorescently-labeled dextran molecule (FITC-Dextran) passing across the BBB kit cultured on a cell culture insert / Transwell device. In this example, movement of FITC-Dextran (3 kDa) across the BBB from the apical to basolateral side (A → B) on Day 5 was assessed. The fluorescent conjugate diffuses freely across the membrane when cells are not present (control condition) but cannot cross when cells from the iCell BBB kit are in tri-culture, indicating tight barrier function.

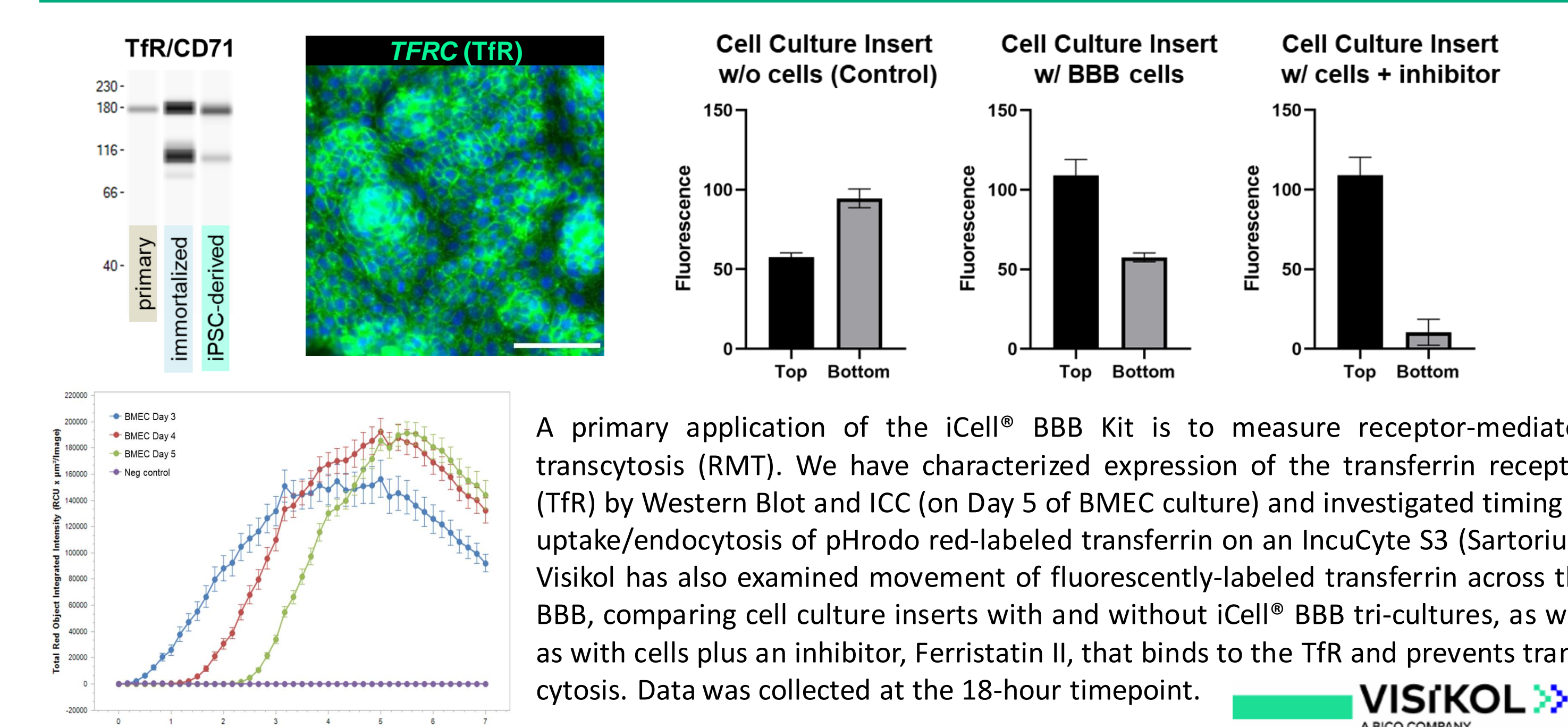


LC-MS permeability data with the iCell BBB Kit directly measures molecules in the apical and basolateral compartments of Transwell inserts. In this example, Osimertinib (green; Gen 3 EGFR inhibitor) shows better penetration across the BBB when compared to both Gefitinib (red; Gen 1 EGFR inhibitor) and the negative control Atenolol (blue and black).

These permeability data were generated and kindly shared with FUJIFILM CDI by Visikol.



Building an Assay for Receptor-Mediated Transcytosis

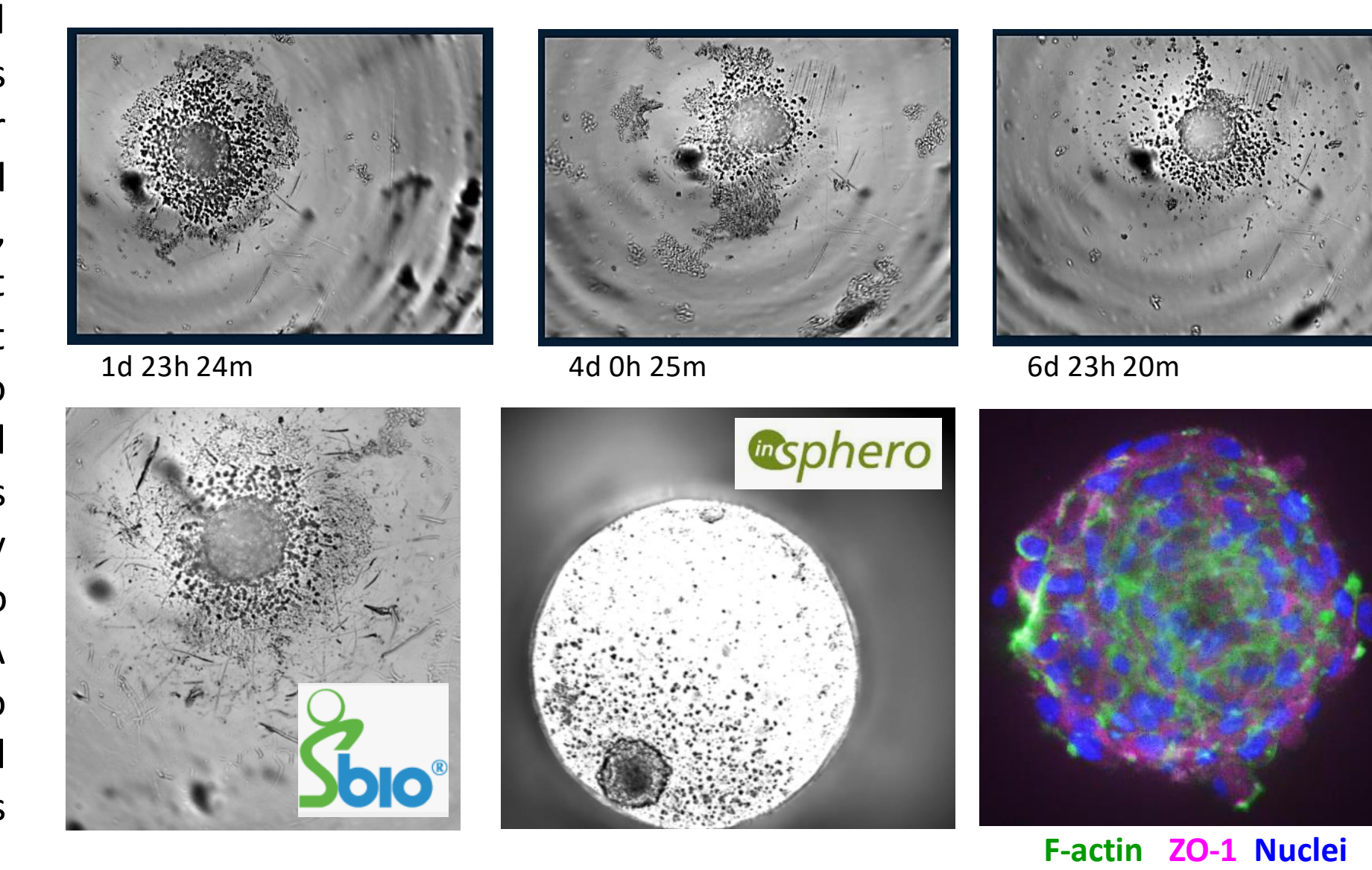


A primary application of the iCell® BBB Kit is to measure receptor-mediated transcytosis (RMT). We have characterized expression of the transferrin receptor (TfR) by Western Blot and ICC (on Day 5 of BMEC culture) and investigated timing of uptake/endocytosis of pHrodo red-labeled transferrin on an IncuCyte S3 (Sartorius). Visikol has also examined movement of fluorescently-labeled transferrin across the BBB, comparing cell culture inserts with and without iCell® BBB tri-cultures, as well as with cells plus an inhibitor, Ferristatin II, that binds to the TfR and prevents transcytosis. Data was collected at the 18-hour timepoint.

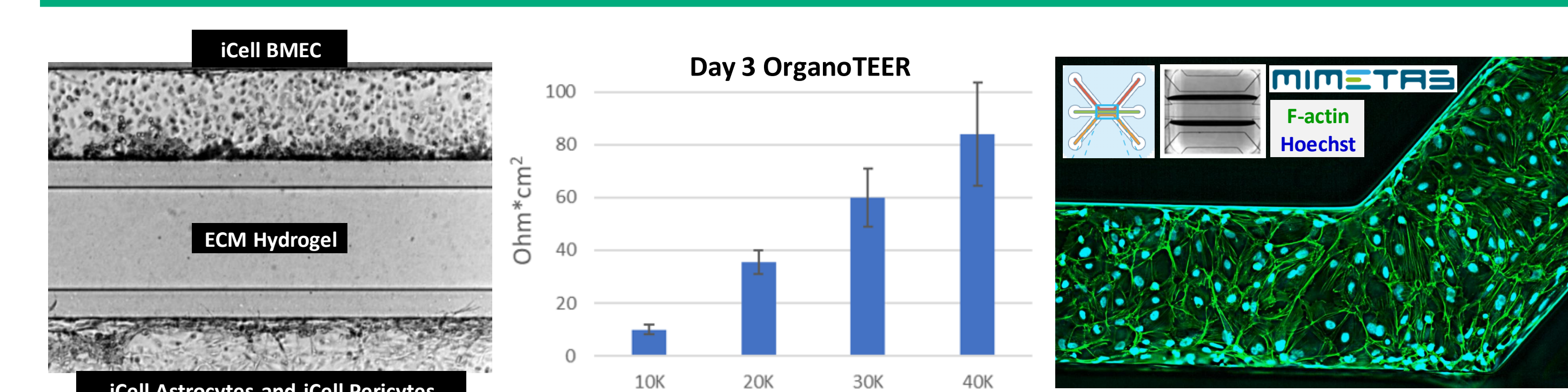


3D Cell Culture of Human iPSC-derived BBB Model

Another approach to examine cell-to-cell interactions at the BBB is to culture cells in 3D as self-assembling multi-cellular spheroids. The precise number of total cells, defined ratios of each cell types, media formulation, ultra low attachment (ULA) plate type, etc. are important variables that require optimization to achieve functional barrier properties and permeability. While this application is still ongoing, we have successfully cultured tri-culture spheroids at a ratio of 2:1:1 for >1-2 weeks in various ULA plates and can immunostain the cells to understand the relative expression and localization of important BBB markers throughout the 3D structure.

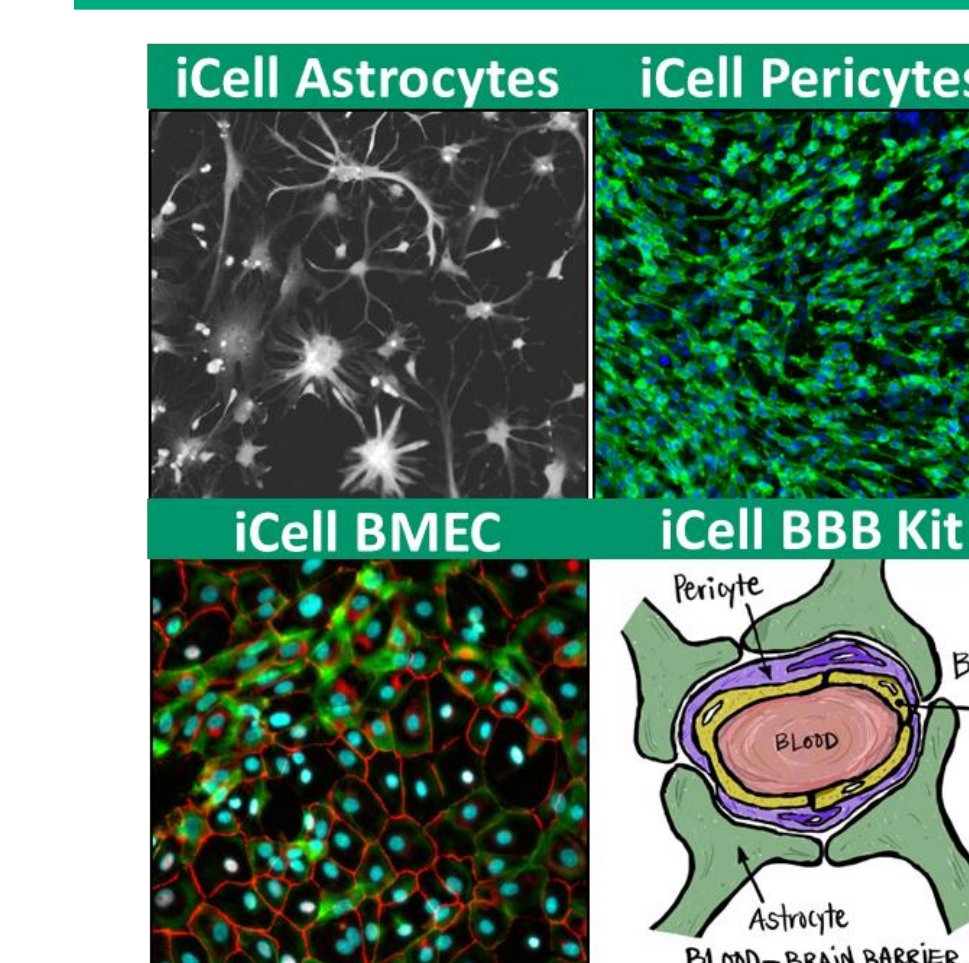


Organ-on-a-Chip with Human iPSC-derived BBB Model



Expanding beyond 2D culture or Transwell inserts to any microphysiological system is of high importance, but it is not a seamless transition. Here, the iCell BBB Kit is used with an OrganoPlate 3-lane 40 microfluidic device (MIMETA). Increasing cell densities of iCell BMEC result in increasing TEER values recorded on the OrganoTEER system.

Summary of Human iPSC-derived BBB Model



FUJIFILM CDI provides a solution to model the human BBB utilizing human iPSC-derived cell types. Here we have presented detailed characterization of iCell® Astrocytes, iCell® Pericytes, and iCell® Brain Microvascular 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 robust and reproducible TEER values in different assay formats. Additionally, barrier integrity assays and compound permeability can be assessed with the complete iCell BBB Kit. Future work includes expanded pharmacological validation of drug permeability and transporter activity, refinement of 3D spheroid assays, as well as expansion into other "brain-on-a-chip" platforms.