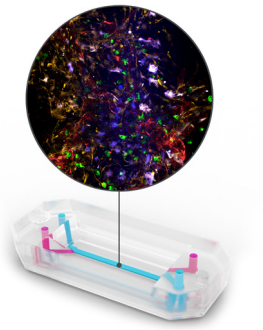
Characterization of the Brain-Chip R1: A First-in-Class, Isogenic Model of the Human Neurovascular Unit



Key Highlights

- **Incorporates five iPSC-derived cell types,** including proprietary BMECs that exhibit a physiologically relevant brain microvascular endothelial–like phenotype
- **Maintains resting-state glia and a tight, stable barrier** throughout a four-day experimental window
- **Features a streamlined, 12-day direct-to-chip workflow** with no pre-plating or expansion steps, enabling robust and reproducible performance
- **Built on the Chip-R1™ Rigid Chip,** which minimizes drug absorption to support reliable BBB transport studies

Abstract

The Brain-Chip R1 is an isogenic, human-relevant Organ-Chip model designed to recapitulate the cellular diversity and functional interactions of the neurovascular unit (NVU). The model integrates five iPSC-derived cell types—neurons, astrocytes, microglia, pericytes, and Emulate's proprietary brain microvascular endothelial cells (BMECs)—within a dynamic, perfused microenvironment that supports physiological barrier formation and cell–cell communication. The 12-day direct-to-chip workflow eliminates pre-plating or expansion steps, producing a consistent, ready-to-use model with a four-day experimental window.

Immunofluorescence (IF) analysis confirms proper differentiation and organization of the brain channel with neurons, astrocytes, microglia, and pericytes exhibiting characteristic morphologies and forming integrated multicellular clusters. qPCR and IF assays further verify that BMECs express key blood-brain barrier (BBB)-associated markers, including GLUT1, P-gp, and the transferrin receptor (TfR1, or CD71), consistent with an endothelial-like phenotype. The model maintains a tight, stable barrier and resting glial state throughout the experimental period, enabling assessment of small-molecule permeability, cytokine secretion, and transporter function. Together, these findings establish the Brain-Chip R1 as a robust, reproducible, and physiologically relevant platform for studying blood–brain barrier transport and neuroinflammatory mechanisms.

Introduction

The NVU plays a critical role in regulating cerebral blood flow and maintaining homeostasis within the central nervous system (CNS)[1,2]. It comprises a diverse network of cell types—brain microvascular endothelial cells, astrocytes, pericytes, microglia, neurons, and an extracellular matrix (ECM)—that together form the structural and functional basis of the blood–brain barrier[2]. The BBB serves as a dynamic, selectively permeable interface separating the bloodstream from the brain microenvironment, tightly regulating the transport of molecules and protecting neural tissue from toxins, pathogens, and immune cell infiltration[1,3].

Despite its biological importance, faithfully modeling the NVU and BBB *in vitro* remains a major challenge. Traditional two-dimensional (2D) monolayer systems are widely used for their accessibility and throughput, but they lack physiological cytoarchitecture, fluid shear, and the multicellular interactions necessary for *in vivo*-like barrier formation[4,5]. Organoid models incorporate more cellular diversity and improved tissue organization, yet they remain limited by batch-to-batch variability, incomplete cellular representation (often lacking microglia), and absence of perfusable vasculature or mechanical cues[6,7]. *In vivo* animal models more closely reproduce BBB physiology, but species-specific differences in transporter expression and endothelial biology, combined with low throughput and ethical constraints, limit their translational relevance for human drug discovery[8,9].

Introduction Cont.

As a result, existing models often fail to recapitulate the selective permeability, transporter functionality, and glial–vascular communication that define the human BBB. More human-relevant, reproducible systems are urgently needed to improve the understanding of NVU physiology and enable predictive assessment of BBB penetrance for therapeutic candidates.

To address these challenges, the Brain-Chip R1 was developed as the first isogenic Organ-Chip model of the human NVU. This system integrates five iPSC-derived cell types—neurons, astrocytes, microglia, pericytes, and Emulate's proprietary BMECs—within a perfused microfluidic environment designed to promote physiological barrier formation and cell–cell signaling. Here, we describe the development and characterization of the Brain-Chip R1, including evaluation of BBB marker expression (e.g. GLUT1, P-gp, and TfR1), barrier tightness, and glial functionality over a four-day experimental window. The 12-day direct-to-chip workflow streamlines model establishment by eliminating pre-plating or cell expansion steps, while the Chip-R1™ Rigid Chip provides a simplified Organ-Chip workflow and minimal drug absorption for improved ADME-relevant data. Collectively, these data demonstrate that the Brain-Chip R1 provides a reproducible, resting-state NVU model suitable for studies of BBB transport, permeability, and neuroinflammatory mechanisms.

Goal

To develop and characterize a first-in-class Organ-Chip model of the NVU that emulates human brain and barrier function by incorporating critical cell types in a dynamic microenvironment.

Materials

Instrumentation

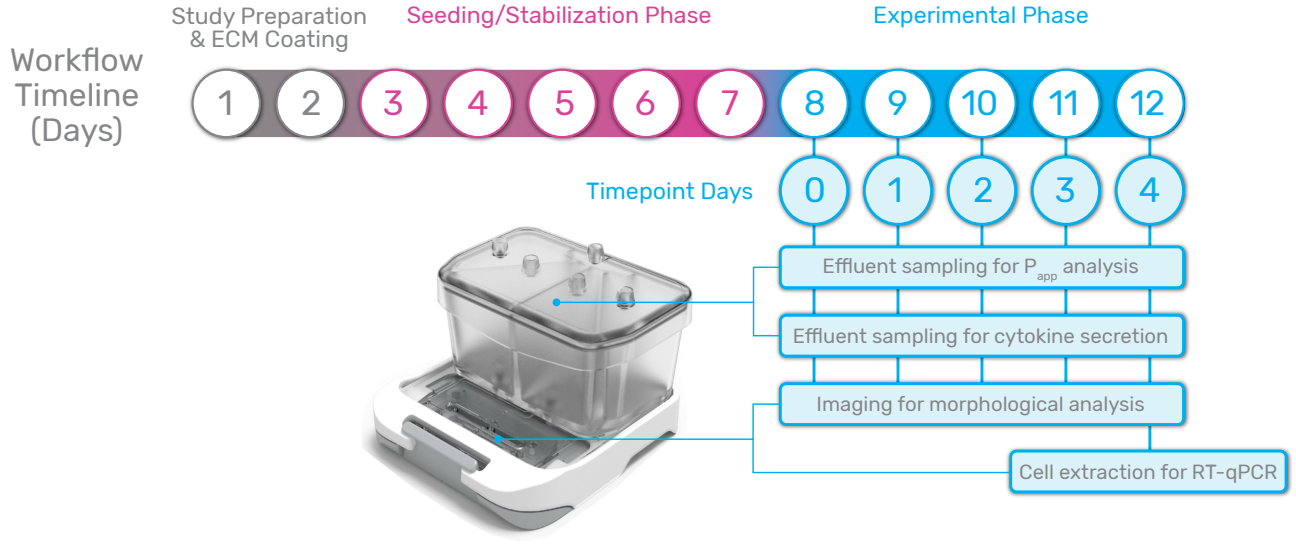
Zoë-CM1® or Zoë-CM2® Culture Module
Orb® Hub Module

Organ-Chip Reagents

Brain-Chip R1 BioKit

- Chip-R1™ Rigid Chips
- Pod-2™ Portable Modules
- Human iPSC-derived cells: iCell® GABANeurons, iCell® Astrocytes 2.0, iCell® Pericytes, iCell® Microglia, Emulate BMECs
- Brain-Chip Brain Channel Media
- Brain-Chip Vascular Channel Media

Experiment Timeline



Experiment Timeline Cont.

A Brain-Chip R1 experiment with a 4-day experimental window can be completed within a 12-day timeline. During the first seven days, the Organ-Chip consumables are prepped for seeding, and then cells are seeded directly onto the chips, connected to flow, and allowed to stabilize before the start of the experimental window. During the experimental phase (Timepoint Days 0-4), daily imaging can be performed to monitor morphology, and effluent can be sampled to analyze barrier integrity and cytokine secretion. At the end of the experimental window, cells can be extracted from the chip for further downstream analysis such as RT-qPCR.

Model Overview

The Brain-Chip R1 incorporates five human induced pluripotent stem cell (iPSC)–derived cell types representing the key components of the neurovascular unit (NVU): GABAergic neurons, astrocytes, microglia, pericytes, and brain microvascular endothelial cells (BMECs). All cells are derived by direct differentiation from a single donor line and can be seeded directly onto the chip, creating a fully isogenic system that minimizes variability and supports reproducible co-culture of neurovascular cell types.

As shown in **Figure 1**, the model is cultured within the Chip-R1 Rigid Chip, which contains parallel "brain" (top) and "vascular" (bottom) microfluidic channels separated by a thin, porous membrane coated with a tissue-specific extracellular matrix (ECM). The brain channel hosts the iPSC-derived neurons, astrocytes, microglia, and pericytes, while the vascular channel is lined with Emulate's proprietary BMECs. Each channel is maintained using custom, channel-specific media formulations designed to preserve appropriate phenotypes and promote cross-compartment signaling. Because the Chip-R1 consumable is constructed from minimally drug-absorbing materials, the Brain-Chip R1 provides researchers with a reliable platform to assess compound recovery and quantitative assessment of BBB compound permeability (please refer to the Chip-R1 Data Sheet for absorption characterization data).

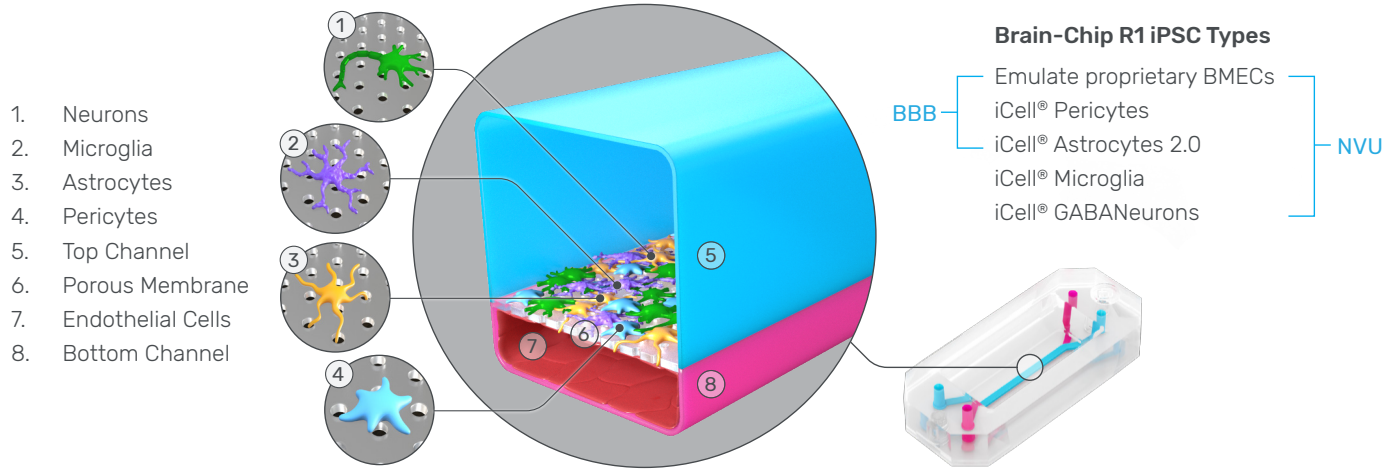


Figure 1: Schematic illustration of the Brain-Chip R1. *The top "brain" channel includes human iPSC-derived neurons, microglia, astrocytes, and pericytes, while the bottom "vascular" channel contains iPSC-derived brain microvascular endothelial cells.*

Results

To verify the composition and organization of the brain channel, immunofluorescence (IF) staining was performed during the experimental window to visualize the four iPSC-derived cell types and assess their morphology and integration within the top channel. The following markers were used to identify each cell population: ionized calcium-binding adaptor molecule 1 (Iba1, green) for microglia, neural/glial antigen 2 (NG2, cyan) for pericytes, glial fibrillary acidic protein (GFAP, red) for astrocytes, and microtubule-associated protein 2 (MAP2, orange) for neurons. Individual channels and a merged composite image illustrate the co-localization and spatial organization of these cell types with physiologically relevant ratios within the brain channel (Figure 2).

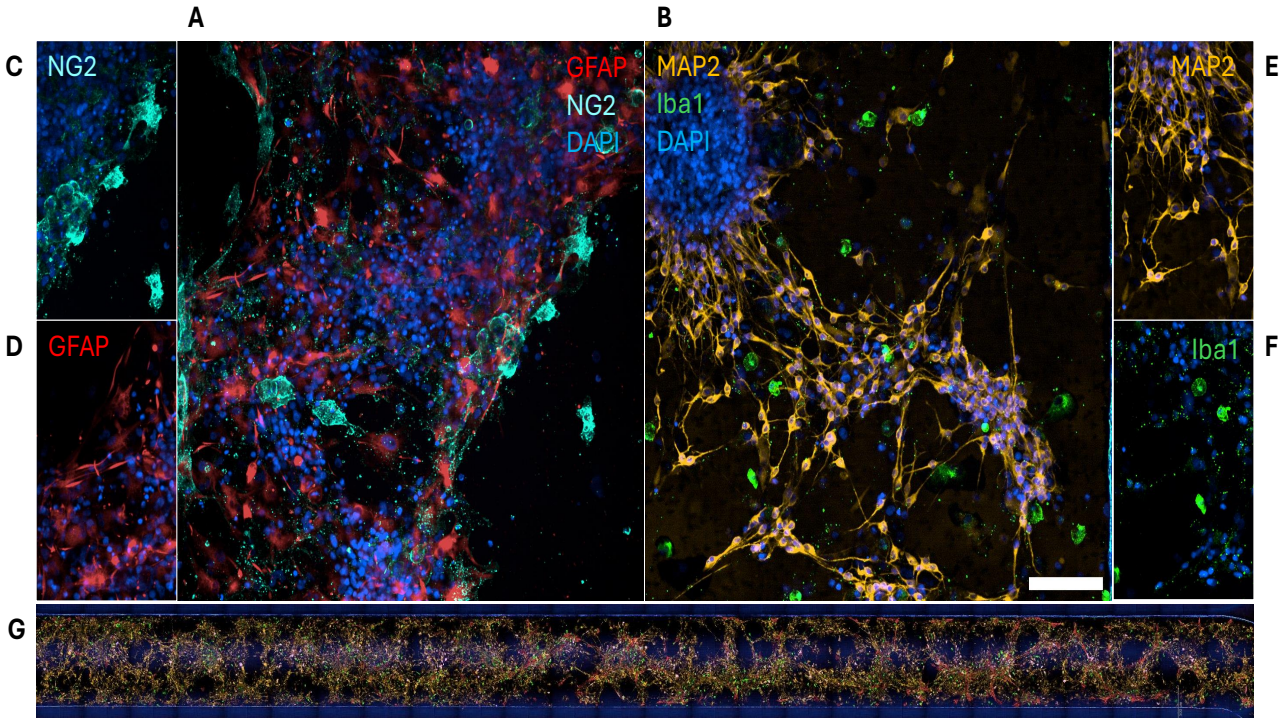


Figure 2: Representative confocal images of the brain channel during the experimental window. *The brain channel was stained for **A)** astrocytes (GFAP, red) and pericytes (NG2, cyan) or for **B)** neurons (MAP2, orange) and microglia (Iba1, green). Images of individual cell types for **C)** pericytes, **D)** astrocytes, **E)** neurons, and **F)** microglia are pictured on the sides. DAPI (blue) is present in all images. **G)** Image of the full channel length demonstrating the formation of a clustered pattern with cell processes extending and forming a network between these clusters. Scale bar equals 100 µm.*

All four populations successfully integrated within the Organ-Chip microenvironment, forming multicellular clusters characteristic of the NVU. Neurons exhibited a bipolar morphology with elongated somas and extended neurites. Astrocytes displayed a stellate morphology with multiple branching projections extending toward neighboring neurons and glia. Microglia appeared in a resting state, characterized by small cell bodies and fine, ramified processes, while pericytes presented an elon-gated morphology with large cell bodies and close association to neuronal and astrocytic structures. Together, these findings confirm that the Brain-Chip R1 supports proper differentiation and organization of key NVU cell types within the top channel.

Next, the vascular channel was examined to confirm formation of a confluent endothelial barrier. IF imaging at the start of the experimental phase (Timepoint Day 0, TPD0) demonstrated a nearly confluent monolayer of BMECs in the vascular channel (**Figure 3**). ZO-1 staining (red) revealed a distinct and continuous signal throughout the vascular channel, confirming the presence of well-defined tight junctions. Additionally, there was continuous signal of Glut1 (green), Glucose transporter type 1, and P-gp (green), P-glycoprotein, implying proper functional state. Regions of the endothelial monolayer were observed to be bridged by the processes of supporting astrocytes extending through the porous membrane from the adjacent brain channel (not pictured), illustrating cross-compartment cellular interaction.

Results Cont.

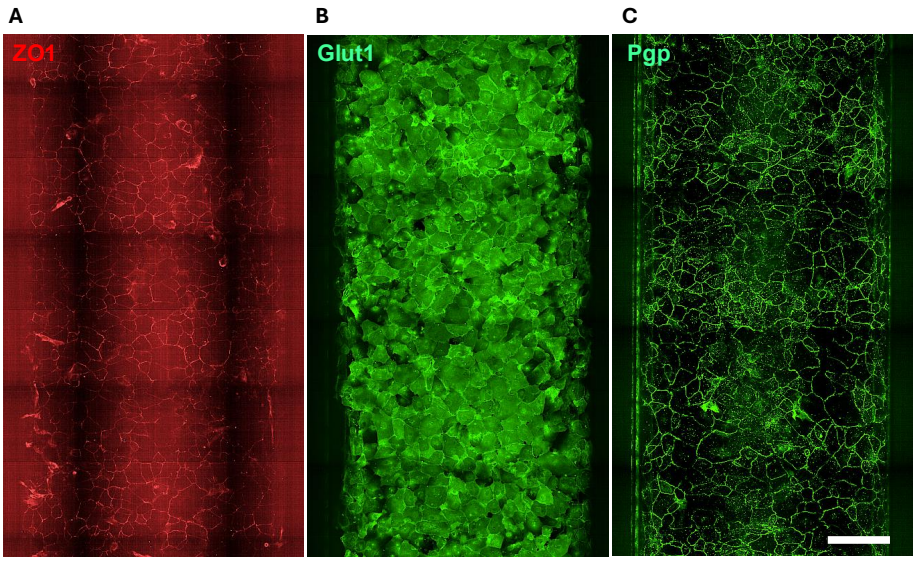


Figure 3: Representative confocal images of the vascular channel at the start of the experimental window (TPD0). *The BMECs in the bottom channel were stained for either* ***A)*** *ZO-1 (red),* ***B)*** *Glut1 (green), or* ***C)*** *P-gp (green). A complete monolayer is observed. Scale bar = 250 µm.*

To further assess the barrier integrity of the BMECs, the apparent permeability (P_{app}) of 3 kDa Cascade Blue Dextran across the endothelial monolayer was measured. As shown in **Figure 4A**, P_{app} values remained below 2 × 10^{-6} cm/s throughout the experimental phase, confirming maintenance of a tight and stable BBB-like barrier throughout the experimental phase.

Finally, a quantitative RT-PCR (RT-qPCR) analysis was performed at the end of the experimental window on Timepoint Day 4 (**Figure 4B**) to further characterize the phenotype of the BMECs. BMECs exhibited expression of canonical tight junction and endothelial markers, including Claudin-5 (CLDN5), VE-Cadherin (VE-CAD), Zona Occludens-1 (ZO-1), and PECAM1, consistent with formation of a mature endothelial monolayer. Additionally, the cells expressed key blood-brain barrier transporters, such as the transferrin receptor (TfR1), Glucose transporter 1 (GLUT1), P-glycoprotein (P-gp), and others, indicating an appropriate BBB phenotype. Importantly, BMECs did not express the epithelial markers EPCAM1, TRPV6, or KRT7, further confirming their endothelial-like phenotype and distinguishing them from epithelialized BMEC models reported elsewhere.

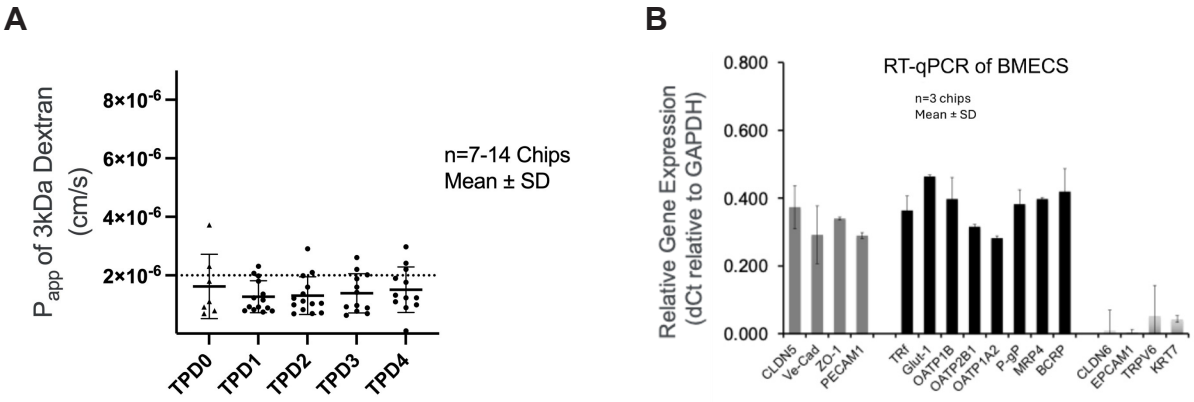


Figure 4: Characterization of BMECs. A) *Assessment of apparent permeability (P_{app}) with 3 kDa Cascade Blue Dextran. Data are expressed as mean ± SD. N = 7-14 chips per Timepoint Day (TPD).* ***B)*** *RT-qPCR analysis of gene expression of BMECs for various tight junction, transporter, and epithelial markers. Data are expressed as mean ± SD. N = 3 chips.*

Results Cont.

The Brain-Chip R1 is designed to maintain a physiologically resting, non-reactive glial environment throughout the experimental phase (Timepoint Days 0-4), while retaining the capacity to mount an appropriate response when stimulated. To evaluate this, cytokine secretion and IF imaging were used to assess inflammatory state and glial activation across the four-day experimental window.

Cytokine profiling confirmed that the model starts in a resting state at the beginning of the experiment (TPD0), with low baseline levels of IFN-γ, IL-1β, IL-6, and TNF-α measured in the top channel effluent (Figure 5A). When the model was subsequently stimulated with IL-1β (via addition to the top channel inlet) to simulate a neuroinflammatory event, a robust increase in secretion of all four cytokines was measured (Figure 5B). This response demonstrates that the glial populations remain quiescent under baseline conditions but are functionally responsive to inflammatory cues, reflecting physiological behavior of the human NVU.

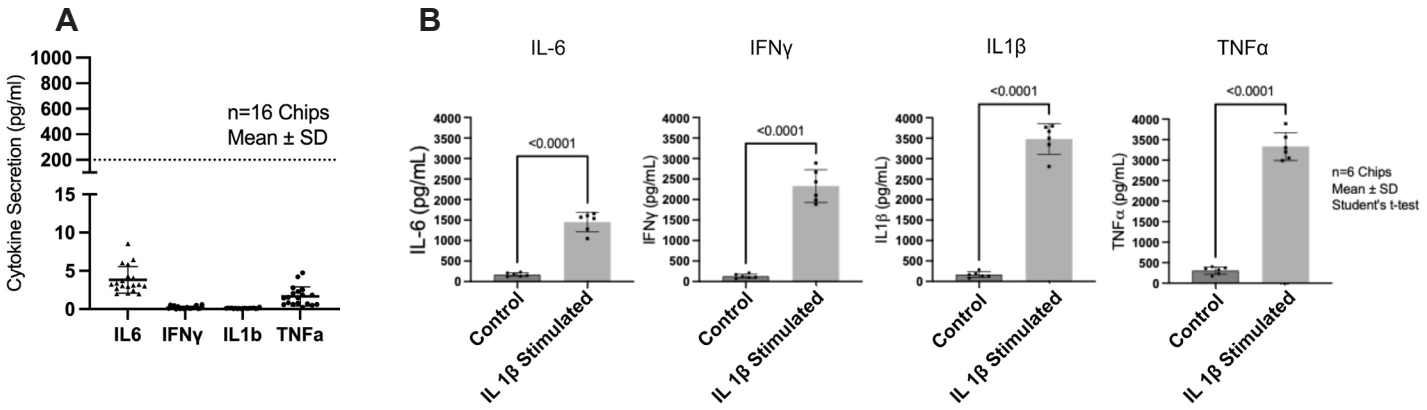


Figure 5: Characterization of the resting and stimulated inflammatory states of Brain-Chip R1. A) *Low cytokine levels of IFN-γ, IL-1β, IL-6, and TNF-α were measured from brain channel effluent at the beginning of the experimental phase. Data are mean ± SD. N = 16 chips.* ***B)*** *Stimulating the model with IL-1β induces a spike in all four cytokine levels, emulating a neuroinflammatory event. Data are mean ± SD. N = 6 chips.*

To further validate the maintenance of the resting phenotype, glial cells were compared at Timepoint Day 0 under two media conditions using IF imaging (**Figure 6**). When cultured in a commercially available triculture medium, glial cells exhibited protoplasmic morphology indicating signs of activation, including hypertrophy and retraction of fine processes. In contrast, cells maintained with Emulate's custom brain channel medium retained a resting morphology, characterized by small, ramified microglia and fibrous astrocytes with extended projections containing mitochondria. These observations highlight the importance of optimized media formulations in supporting stable, physiologically relevant glial states on chip.

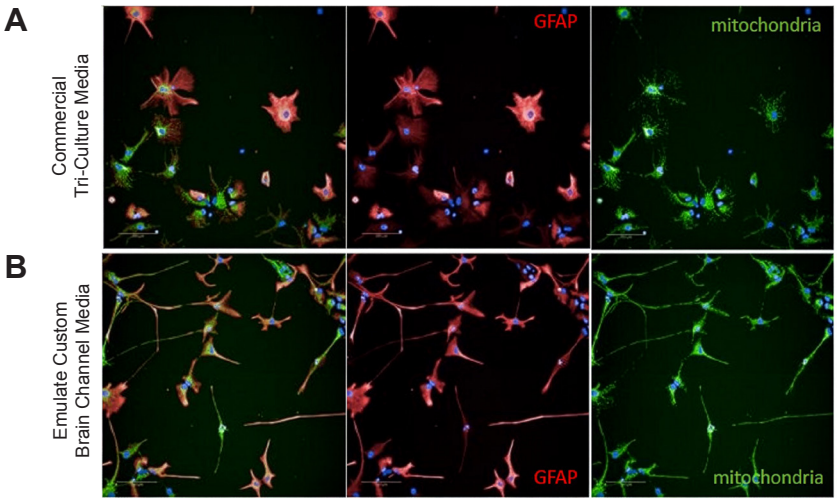


Figure 6: Effect of culture media on resting state of glial cells. A) *Astrocytes stained for GFAP (red) and mitochondria (green), cultured in a commercially available tri-culture media.* ***B)*** *Astrocytes stained for GFAP (red) and mitochondria (green), cultured in Emulate's custom brain channel media.*

Conclusion

We successfully developed the first five-cell isogenic iPSC Organ-Chip model of the human neurovascular unit (NVU), establishing a new benchmark for *in vitro* brain research. The Brain-Chip R1 integrates five iPSC-derived cell types within a dynamic microenvironment to recapitulate key structural and functional features of the NVU. Characterization confirmed that Emulate's proprietary BMECs exhibit a brain microvascular endothelial–like phenotype with expression of key BBB transporters, while co-cultured glial populations remain in a resting, non-reactive state. The model forms a tight, stable barrier that supports a four-day experimental window with robust reproducibility.

Together, these findings establish the Brain-Chip R1 as a physiologically relevant and experimentally consistent platform for assessing BBB transport, glial functionality, and neuroinflammatory responses. By enabling researchers to interrogate human-specific mechanisms of brain physiology and disease, the Brain-Chip R1 represents a critical advance toward more predictive and translational approaches in CNS drug discovery.

References

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