Developing a 3D Brain-Chip Interphase Microfluidic Model of TBI-Associated Pathobiology

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INTRODUCTION

Traumatic brain injury (TBI) is one of the leading causes of death and disability among young and productive people and is the most well-established non-genetic risk factor for cognitive impairment, dementia and progression to clinical Alzheimer's disease. However, causes of blood-brain barrier (BBB) deficits observed following TBI are not well understood. A major bottleneck in these important studies is the lack of physiologically relevant human in vitro models. In vitro models provide a highly tunable environment allowing the study of specific molecular and cellular contributions.

To date, few in vitro models have incorporated multiple brain cell types, and thus, are unable to capture interactions that are likely pertinent to the onset and maintenance of pathology following TBIs. To address these critical limitations, we have developed a novel 3D brain-chip interphase microfluidic model using the Mimetas platform.

The 3D cultures allow for the analysis of BBB permeability and changes within the brain compartment. These studies will enable an in-depth examination of the contributing risk factors for post-TBI cognitive impairment pathology in a highly controlled and tunable environment without the confounding effects of a non-human host genome.

Previous models have not examined the role of genetic and non-genetic risk factors that shape pathological changes post-TBI. Future studies will address assessments of ApoE4/4 or BDNF66Val gene variants relative to TBI disease progression and recovery.

MATERIALS & METHODS

Cell Culture and Plating Procedures:
2D and 3D BBB cultures were established with combinations of primary human microvascular endothelial cells (HBMECs), pericytes, astrocytes, neurons, microglia, seeded 2D cultures or in 3D chip cultures employing the Mimetas platform with perfusion to create shear stress in the blood vessel channel.

Sera Administration: LIMBIC CENC cohort

BBB Functional Analysis and BBB/CNS Cell Imaging:
Prior to and 72-hrs after sera exposure, continuous 3D TEER and barrier integrity (BI) tracer assays were performed using FITC-dextran tracer (150 kDa) and imaged on the C10 confocal imager. After functional assays, cultures were fixed and stained to detect changes in tight junction protein (TJP) structure and cell morphology.

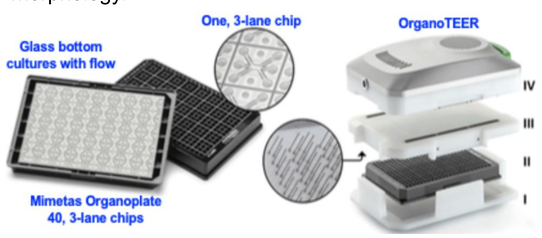

Figure 1: Brain-Chip Cultures using Mimetas Devices
Example of plate and TEER device to study 3D cultures

RESULTS

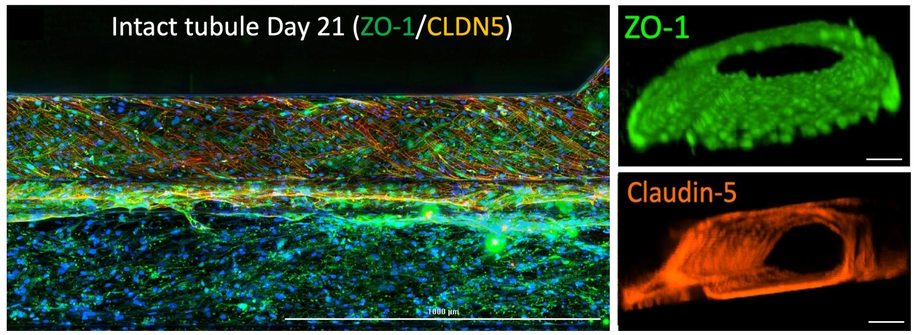

Figure 2: Building a Robust 3D BBB – 3D Brain Capillary Sized Blood Vessel
Mimetas 3-lane 40 or 64-chip plates were seeded with Col-I/Col-IV ECM in the middle channel and left to solidify. HBMECs were seeded onto fibronectin-coated top channels against the ECM for 3 hours. iPSC-derived astrocytes and pericytes were seeded in the bottom channel coated with Matrigel. Finally, media was added and perfusion was established. Images show tubule formation and TJP (ZO-1, Claudin-5) expression after 21 days of culture.

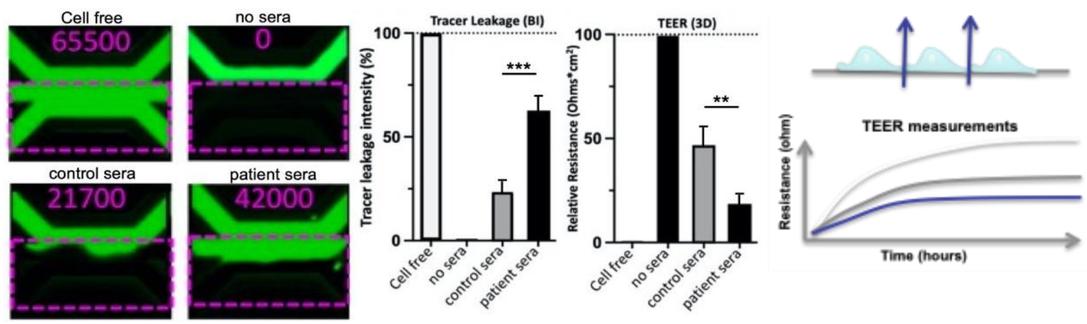

Figure 3: Studying the 3D BBB – Assays to Examine BBB Properties
A representative example is shown of 3D BBB cultures which were exposed to sera from service members and veterans (SMVs) (control or TBI cases) for 72 hours. Shown are the results of BBB leakiness to FITC-labeled tracer (150 kDa) and changes in relative TEER after sera exposure. n=3 technical replicates. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by ordinary one-way ANOVA

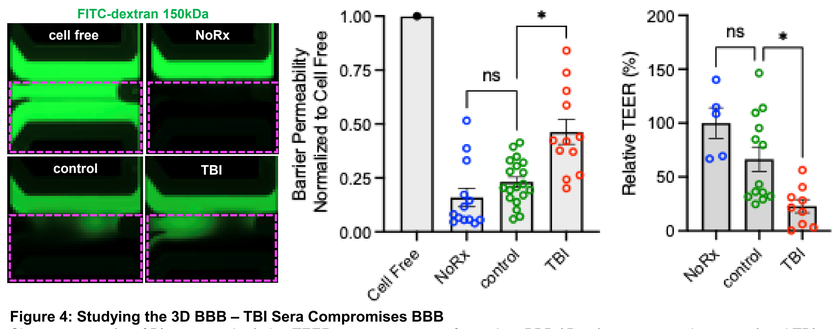

Figure 4: Studying the 3D BBB – TBI Sera Compromises BBB
Shown are results of BI assay and relative TEER measurement performed on BBB 3D cultures exposed to control and TBI sera after 72 hours. Values were normalized to Cell Free (BI assay) or NoRx (TEER measurement). n=12-18 chips. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by ordinary one-way ANOVA

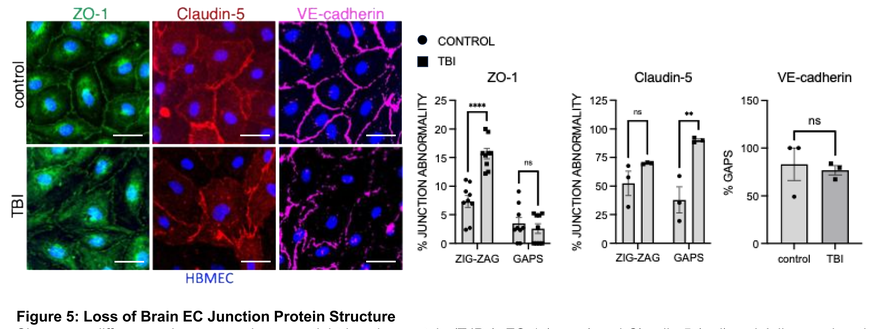

Figure 5: Loss of Brain EC Junction Protein Structure
Shown are differences in structure between tight junction protein (TJPs), ZO-1 (green) and Claudin-5 (red) and Adherens junction protein, VE-cadherin (magenta). n=3-9 chips. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by two-way ANOVA or Welch's t-test.

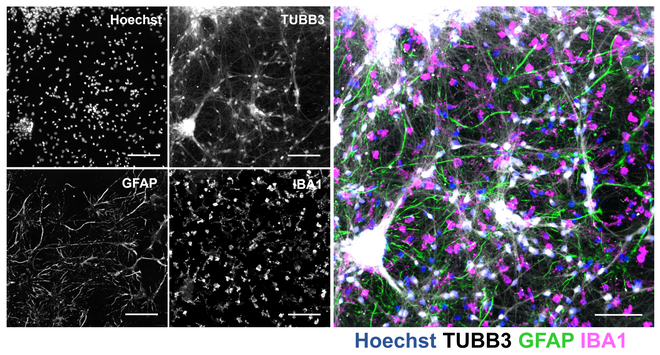

Figure 6: Studying CNS Cellular Networks
Shown are representative immunofluorescence images of CNS tri-cultures composed of iPSC-derived neurons (TUBB3, white), astrocytes (GFAP, green), microglia (IBA1, magenta) and nuclei (Hoechst, blue).

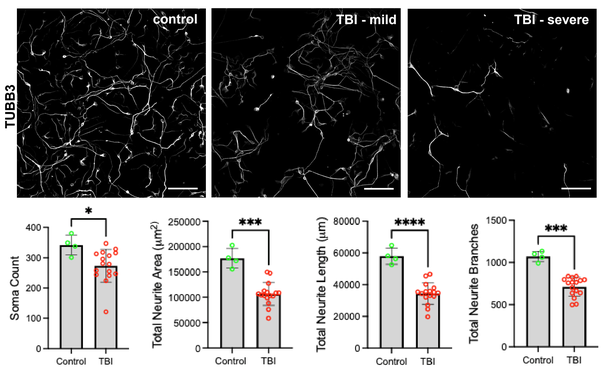

Figure 7: Studying neurons in CNS Cellular Networks
Low-density tri-cultures were exposed to TBI sera from SMVs for 3 days. Shown are the effects on neurons (soma) and neurites (area, length and branches). n=3-15 cultures. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by Welch's t-test for normally distributed data or Mann-Whitney nonparametric t-test

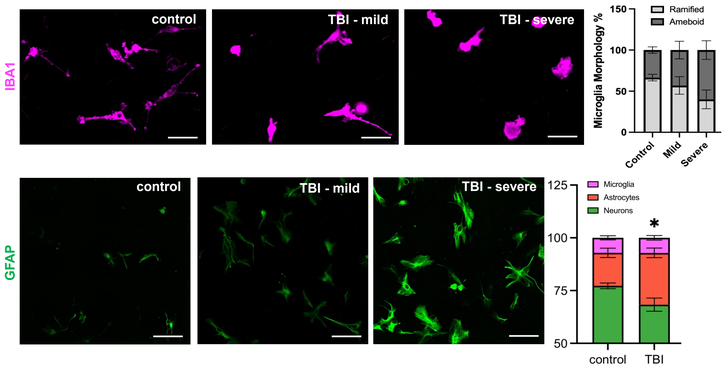

Figure 8: Studying Microglia and Astrocytes in CNS Cellular Networks
Some low-density tri-cultures were exposed to sera from TBI SMVs for 3 days. Shown are changes in cell morphology of microglia (IBA1, magenta) and astrocytes (GFAP, green) and changes in the ratio between cell types: neurons, astrocytes, and microglia. n=3-5 cultures. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by Welch's t-test for normally distributed data.

SUMMARY

- We have successfully developed complex 2D and 3D models for studying the BBB and NVU in relation to TBI-associated pathology.
- Through BI and TEER assays, we show that TBI sera significantly affect the integrity of the BBB.
- TBI sera-induced BBB deficits are likely the result of an increased number of gaps and zig-zags of junction proteins between brain ECs.
- Looking at CNS cells, TBI sera appears to lead to a decrease in neuronal soma count and neurite area, length and branching.
- Morphological changes indicating activation of microglia and astrocytes driving neuroinflammation, are seen with TBI sera exposure.
- Future directions will include looking at the role of genetic and non-genetic risk factors that shape pathological changes post-TBI.

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