Development of a Human Brain-Chip Model to Study Neuroinflammatory Diseases

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Introduction

Inflammatory processes are involved in a wide variety of diseases and conditions that affect the central nervous system. Recreating these processes faithfully in human based-models has been challenging. Species differences in the function of the brain and blood-brain barrier (BBB) often preclude accurate extrapolation from animal models to human patients. Therefore, it is a great need for human relevant systems that can recreate key aspects of brain physiology and pathophysiology of common diseases. Here, we are developing a human Brain-Chip to model neuroinflammation, a hallmark of many neurodegenerative diseases and changes associated with aging, which enables studies on mechanistic aspects of neural pathology and disease progression.

Brain-Chip Design

To recapitulate key functional aspects of the human Brain-Chip, we seeded human iPS-derived cortical neurons, human primary astrocytes, microglia and pericytes in the neuronal channel (top), and human iPS-derived brain microvascular endothelial cells in the vascular channel (bottom). Coating of the surfaces with *in vivo* relevant extracellular matrix (ECM) emulates the biologically active surfaces cells normally contact and sense in the brain, providing an *in vivo*-relevant microenvironment.

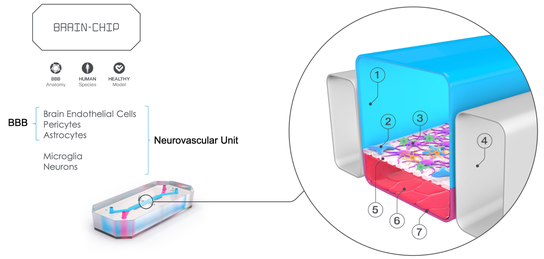

Figure 1: Brain-Chip. 1) Brain Channel, 2&3) Human iPS-derived neuronal cells co-cultured with human primary astrocytes, microglia, and pericytes, 4) Vacuum Channels, 5) Porous Membrane, 6) Human iPS-derived brain microvascular endothelial cells, 7) Vascular Channel.

Reconstructing the Neurovascular Unit

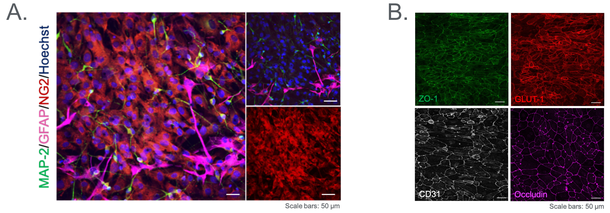

Figure 2: A. Immunocytochemical analysis of iPS-derived neuronal cultures in direct contact with astrocytes and pericytes. Specific markers were used to identify neurons (MAP-2), astrocytes (GFAP), and pericytes (NG2). Blue represents Hoechst-stained nuclei. **B.** Immunocytochemical analysis also confirmed the endothelial monolayer tightness and brain specificity using ZO-1, GLUT-1, CD31, and Occludin markers at day 7 in culture.

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Establishing a Model for Neuroinflammation

To demonstrate the utility of the Brain-Chip for probing the effect of neuroinflammation, we treated the constructed neurovascular unit (brain side) with TNFα, which has been previously reported to mediate neuroinflammation and detected in patients with neurological disorders.

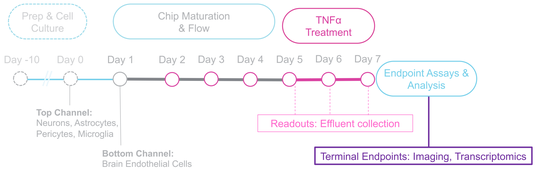

Figure 3: The experimental timeline of the neuroinflammation culture model. On days 0 and 1 we seed cells on the top and bottom channel if the Brain-Chip respectively. The Brain-Chip is then allowed to mature under flow conditions for 4 more days and on day 5, cells on the brain side are treated with TNFα, for 48 hrs. During exposure, effluent is collected, and on the last day are imaged, and cells are lysed for transcriptomic analysis.

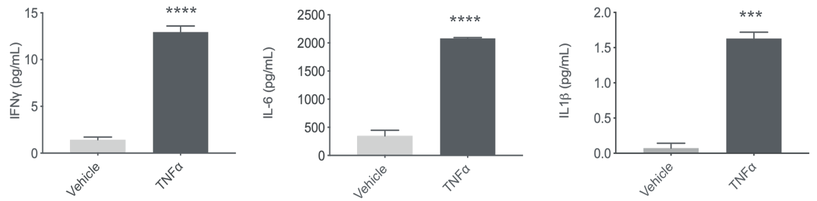

Figure 4: Secretion of pro-inflammatory cytokines. The brain channel of the Brain-Chip was exposed to continuous flow of media containing 100ng/mL TNFα for 48 hrs. The effluent was collected at the 48 h timepoint and cytokines were measured and analysed using MSD human proinflammatory panel. Data are means±SEM, ***p<0.001, ****p<0.0001(n>4 chips).

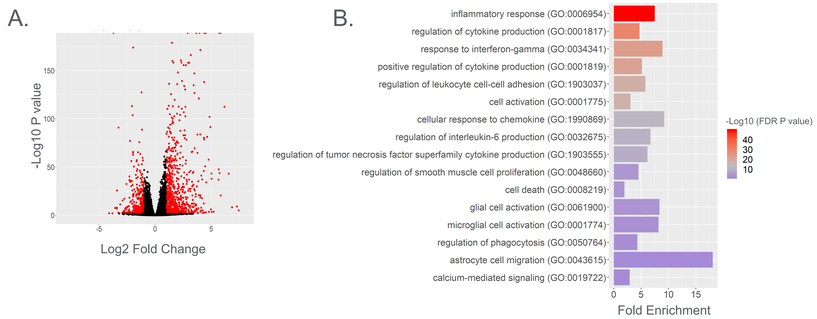

Figure 5: Differential Gene Expression analysis between TNFα exposed Brain-Chips and Healthy Brain-Chips on day 7: **A.** The volcano plot illustrates the number of the differentially expressed (DE) genes (up- and down-regulated) and how they stratify based on their expression changes. Red dots: DE genes significantly up- or down-regulated (adj.p-value < 0.01 and $|\log_2 FoldChange|>1$); back dots: non-DE expressed genes. In total, 1174 genes were found significantly DE in cells of brain parenchyma, 801 up-regulated (in the inflamed Brain-Chips) and 373 down-regulated (in the inflamed Brain-Chips). **B.** Gene Ontology (GO) enrichment analysis based on the 801 up-regulated DE genes between the TNFα Brain-Chips and Healthy Brain Chips. Bar plot presents a subset of the significantly enriched biological processes identified by the enrichment analysis.

Inflammatory Stimulation Effects on Blood-Brain Barrier

To directly measure how BBB permeability changed over time in response to TNFα, we evaluated the transport of 3-kDa Cascade Blue dextran across the BBB. Fluorescent dextran molecule was introduced in the vascular channel and after 24 hrs and 48 hrs of exposure, we measured the amount of fluorescent dextran collected in the Brain-side.

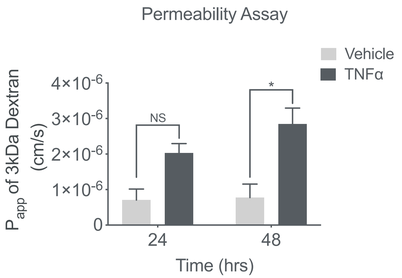

Figure 6. Apparent permeability measured after 24 hrs and 48 hrs of exposure to TNFα (100 ng/mL) introduced on the brain side, transport through the BBB was significantly increased by three times over the control. Data are means±SEM, *p<0.5 (n>4 chips).

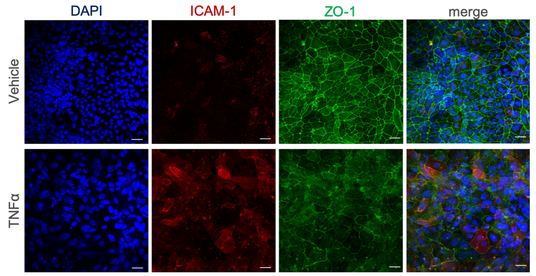

Figure 7: Representative Immunofluorescent staining of endothelial and tight junction markers after 48 hrs of exposure to TNFα on the brain-side showing a decreased expression of tight junction protein, ZO-1, and increased expression of intercellular adhesion molecule-1 (ICAM-1)

Conclusion

The Brain-Chip supports the survival, function, and interaction of iPS-derived cortical neurons, human primary astrocytes, microglia and pericytes as well as BBB integrity for 7 days in culture. Using TNFα we successfully demonstrated a human relevant inflammatory response of key players of the Brain-Chip such as release of major proinflammatory cytokines as well as significant damage of the endothelial tight monolayer resulting in the breakdown of the BBB. This model could enable applications in neurodegenerative diseases including disease mechanism elucidation, target validation, and efficacy testing.

Acknowledgements

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