

3D BLOOD-BRAIN BARRIER MICROVASCULAR NETWORK MODEL INCLUDING HUMAN IPS-DERIVED ENDOTHELIAL CELLS, PERICYTES AND ASTROCYTES

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ABSTRACT

The blood-brain barrier (BBB) is a selective barrier that help to maintain brain homeostasis, however it also creates an obstacle to drug delivery. For years, *in vivo* animal models have been widely used for BBB studies and drug evaluations. Although these techniques are considered the gold standard, 80% of drug candidates that were successful in animal models later failed in clinical trials. For that reason, a cost-effective *in vitro* BBB model that adequately reflects human *in vivo* conditions is required. Here we developed a 3D microfluidic model of the BBB by self-organized vascular network including (iPS)-derived endothelial cells, human brain pericytes, and astrocytes.

KEYWORDS

Blood-Brain Barrier, Microfluidic Model, self-assembled Microvascular network, Organ-on-a-chip, Induced pluripotent stem cells-derived Endothelial Cells, permeability

INTRODUCTION

The blood-brain barrier (BBB) is a complex structure necessary for separating the brain from blood circulation maintaining neural homeostasis and protecting against pathogens.

However, this selective barrier also prevents efficient drug delivery targeting specific brain areas, preventing treatments of neurodegenerative diseases and cancers [1]. Furthermore, traditional and animal models failed to reproduce the complexity of brain barriers, conducting to misleading results in clinical trials.

To overcome those limitations, we developed an innovative 3-dimensional BBB self-organized microvascular network model via vasculogenesis that accurately replicates the anatomical neurovascular organization observed *in vivo*.

METHODS

A PDMS microfluidic model including human induced pluripotent stem cells-derived endothelial cells (iPSC-ECs), human brain pericytes, and astrocytes as self-assembled vascular networks. RT-PCR, vascular permeability and immunocytochemistry assays were performed.

RESULTS

Gene expression of tight junctions (ZO-1, occludin, and claudin-5), extra-cellular matrix proteins (Laminin and Collagen IV), and membrane transporters (PG-P, LAT1, LRP1) was higher in tri-culture condition consistently with quantitative immunocytochemistry analysis indicating BBB-like maturation. Laser confocal microscopy validated microvessel-pericytes/astrocytes contact-interactions. Characterization of microvascular network parameters as vascular diameter, branches length and vascular network area coverage were lower when including pericytes and astrocytes. This revealed that morphological changes were induced by not only the secretion of pro-angiogenic and vasculogenic growth factors but also contact signaling between cells. The BBB model exhibited perfusable and selective microvasculature, showing permeability coefficient comparable to previous models [2], (10 kDa FITC-Dextran: 2.2×10^{-7} cm/s; 40 kDa FITC-Dextran: 8.9×10^{-8} cm/s).

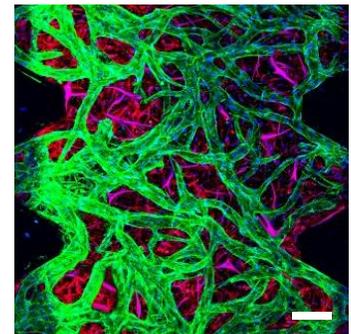


Figure 1. Confocal image of 3D BBB microvascular network model with IPSC-ECs (green), brain pericytes (red) and astrocytes (violet). Bar scale 100 μ m.

DISCUSSIONS

Our BBB microfluidic model has the advantages that all three cell types are seeded simultaneously into a single gel region, producing a perfusable vascular network with permeabilities lower than those of other published models. The contribution of co-culture with pericytes and astrocytes also improved BBB formation and integrity and upregulation of tight junction proteins and membrane transporters by the iPSC endothelial cells, highlighted as potential targets to enhance the penetration of drugs into the brain.

CONCLUSIONS

This robust 3D BBB microvascular model could be potentially applied to patient-specific and neurodegenerative diseases modelling, offering a novel platform to study both drug transport for preclinical screening as well as neurovascular functions within a physiologically-relevant BBB microvasculature.

ACKNOWLEDGEMENTS

Ermenegildo Zegna Founder's scholarship grant, the Joint Ph.D research project (BIOMODE, Compagnia San Paolo), the MITOR research exchange program and support by National Science Foundation are acknowledged.

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