Development and Functional Characterization of a Blood-Brain Barrier Model on a Chip for Predictive Drug Permeability Testing

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Summary

The blood-brain barrier remains a critical challenge in CNS drug development. This work presents the preliminary development and characterization of a microfluidic BBB-on-a-chip model, comparing monoculture and triculture configurations against traditional Transwell systems. Additionally, real time monitoring of fundamental metabolites is being implemented.

Background and Preliminary Results

The blood-brain barrier (BBB) is a highly specialized and selective interface between the bloodstream and the central nervous system (CNS). Formed primarily by brain microvascular endothelial cells, and functionally supported by pericytes and astrocytes, the BBB tightly regulates the passage of molecules into the brain, playing a fundamental role in maintaining neural homeostasis. At the same time, this selectivity represents one of the greatest obstacles in CNS drug development, as the vast majority of candidate compounds fail to cross the barrier in therapeutically relevant concentrations [1, 2 ,3].

Conventional in vitro BBB models, particularly Transwell insert systems, have been widely used to study barrier function and screen drug permeability. However, these static systems lack the fluid shear stress, the three-dimensional cellular architecture, and the paracrine signaling dynamics that characterize the native BBB microenvironment [4]. As a consequence, their predictive value for in vivo drug behavior remains limited.

Organ-on-a-chip technology has emerged as a promising approach to overcome these limitations [5, 6, 7]. By integrating microfluidic flow and compartmentalized cell culture within a miniaturized device, BBB-on-a-chip models can more faithfully recapitulate the physiological conditions of the human BBB. This work presents the preliminary development and functional characterization of such a model.

Two cell culture configurations were established and compared: a monoculture of brain microvascular endothelial cells, and a triculture system incorporating endothelial cells, pericytes, and astrocytes. The identity of each cell population was confirmed through marker characterization, demonstrating the endothelial nature of the monolayer as well as the presence and phenotypic integrity of pericytes and astrocytes in the triculture system. Both configurations were implemented in two formats — conventional Transwell inserts and a microfluidic chip — allowing a direct comparison between static and dynamic culture conditions.

Preliminary functional characterization of the models reveals a trend toward reduced dextran permeability under chip-based and triculture conditions, suggesting enhanced barrier integrity compared to monoculture and static Transwell configurations. These initial findings indicate that the combination of microfluidic flow and multicellular complexity contributes positively to the formation of a tighter and more physiologically relevant barrier. Notably, monolayer stability over time was found to differ markedly between configurations. In both Transwell and chip formats, monoculture barriers showed signs of compromised stability within a short timeframe, limiting the window available for experimentation. In contrast, the triculture configuration demonstrated substantially greater temporal stability, maintaining barrier integrity over longer periods. This is a practically significant finding: greater stability not only extends the experimental window but also allows more information to be extracted from a single chip, improving experimental efficiency and reducing biological variability across replicates.

In parallel, real-time electrochemical sensing of three fundamental metabolites — glucose, glutamine, and lactate — is being implemented. These analytes were selected due to their central roles in cellular energy metabolism and their sensitivity as indicators of cell viability, stress, and phenotypic state [8, 9]. Glucose is the primary energy substrate of brain endothelial cells and a key driver of barrier maintenance; glutamine supports biosynthetic demands and neurotransmitter precursor metabolism in astrocytes; and lactate, as the end product of anaerobic glycolysis, serves as a sensitive proxy for metabolic shifts that may precede or accompany barrier disruption. Continuous monitoring of these metabolites provides a window into the metabolic health of the model that complements permeability-based functional readouts and does not require any intervention or sample sacrifice.

Conclusions

This work presents a preliminary but promising step toward the development of a more physiologically relevant in vitro BBB model. The comparison between monoculture and triculture configurations, across both Transwell and chip formats, highlights the importance of cellular complexity and dynamic microenvironmental conditions in recapitulating native BBB function.

The integration of real-time metabolite sensing adds a critical dimension to this effort. Barrier integrity alone is an incomplete picture of BBB physiology — a monolayer can appear structurally intact while being metabolically compromised, and metabolic disruption often precedes measurable permeability changes. By continuously tracking glucose consumption, glutamine utilization, and lactate production, the model gains the ability to report on its own physiological state in real time, without the need for destructive assays or fixed time points. This multimodal readout strategy — combining structural barrier assessment with metabolic surveillance — is what elevates the BBB-on-a-chip from a permeability screening tool to a genuinely dynamic, information-rich model of BBB biology.

Ultimately, this approach contributes to closing the translational gap between preclinical in vitro models and clinical outcomes, supporting more efficient and biologically grounded CNS drug discovery pipelines.

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