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3D-printed Perfusion Bioreactor For Brain Vascular Permeability Assessment

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The blood-brain barrier (BBB) plays a crucial role in protecting the central nervous system from viral infections. To recapitulate brain microvasculature more accurately, in vitro platforms must exhibit a supportive 3D architecture to co-culture multiple human cell types in situ under perfusion. Subsequently, we developed a novel 3D-printed microphysiological system (MPS) to recapitulate human BBB for non-invasive, image-based evaluation of antiviral drugs effect on brain endothelium inflammatory responses. Our MPS platform was fabricated using a biocompatible resin-based polymer, which was 3D-printed, followed by the addition of a permeable membrane to create a 3D brain tissue microenvironment with unique geometries. The 3D BBB tissue was engineered from triculture of human cerebral microvascular endothelial cells, mesenchymal stem cells, and glial cells co-cultured on the luminal (blood) compartment of the membrane. To mimic functional BBB, we connected the luminal compartment to an external peristaltic pump driving media from a 3D-printed reservoir. Using this system, we tested FDA-approved therapeutics selected as antivirals for their effect on BBB inflammatory responses when using tumor necrosis factor (TNF-α) to induce inflammation. We monitored BBB permeability with fluorescent-tagged dextran added to the media reservoir. Fluorescent intensity gain in the media on the abluminal (brain) compartment was microscopically recorded to quantify the BBB permeability driven by inflammatory response. We compared this perfusion BBB model with 3D BBB tissue that was statically grown on transwells. Inflammatory response to TNF-α treatment was evaluated both microscopically via immunocytochemistry and by real-time quantitative PCR of total RNA isolated from the 3D BBB tissue constructs. With this system, we discovered a previously unrecognized effect of Vorapaxar (VP), a thrombin receptor antagonist, on the brain microvasculature. While previous studies have found that VP decreases inflammation in coronary endothelium, we report augmentation of TNF-α-driven inflammation in brain microvascular cells in our BBB model. Furthermore, we report that the 3D brain tissue grown within our MPS platform exhibits tighter BBB integrity under perfusion than the statically cultured samples, which we have verified via immunohistochemistry imaging. Our device offers a framework for 3D-printed in vitro platforms, particularly for multiple cell culture models and vascular permeability assays, aiding in the screening of efficient antiviral medical countermeasures against emerging pathogens.