Studying Neuroinflammation Using a Novel Microfluidic Vascular Brain-On-A-Chip

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Introduction: A key component of neurodegenerative disease pathology is neuroinflammation. However, the role of cerebrovasculature in modulating neuroinflammation is not well-established. We developed a novel 3D microfluidic vascular brain on-a-chip (VBOC) model to study the interaction of endothelial cells (ECs) and astrocytes, specifically in response to medin, an amyloidogenic protein and palmitic acid (PA), the most common free fatty acid in the Western diet, that induce EC inflammation. We herein demonstrate the utility of the developed VBOC in the study of crosstalk between vascular and neuroinflammation that could provide novel insights into the physiological mechanisms linking cerebrovascular disease and neurodegenerative diseases. Additionally, preliminary studies demonstrated the utility of the VBOC to be used with sustained media flow to induce physiologic shear stress on brain microvascular ECs.

Materials and Methods: VBOCs were engineered with a main tissue channel for 3D astrocyte culture (**Fig. 1A**, green), and flanking channels for EC culture and media administration (**Fig. 1A**, red), within a microfluidic device with channel height of 200 µm (**Fig. 1B** shows diffusion through tissue). Human umbilical vein endothelial cell (HUVECs) were cultured in the media channels of the VBOC until monolayer formation (**Fig. 1E**), then human astrocytes, encapsulated in collagen hydrogels, were introduced into the main 3D tissue channel (**Fig. 1D**). Media within the EC channel was changed to ½ astrocyte: ½ EC media to support co-culture, then vehicle, medin (5 µM) or PA (150 µM) were administered for 20 hours (**Fig. 1C**, bottom panel, shows diffusion at 20hrs). Specifically, conditions with and without ECs were compared to vehicle and control, through astrocytic activation, via GFAP staining, and secretion levels of the proinflammatory cytokine IL-8, via ELISA. For media flow experiments, human brain microvascular endothelial cells (HBMVECs) were cultured within collagen-coated media channels until monolayer formation, then flow, at 30 µL/hour, was established for 72 hours. Medin was introduced for an additional 20 hours of flow, and resultant EC monolayer morphologies were compared.

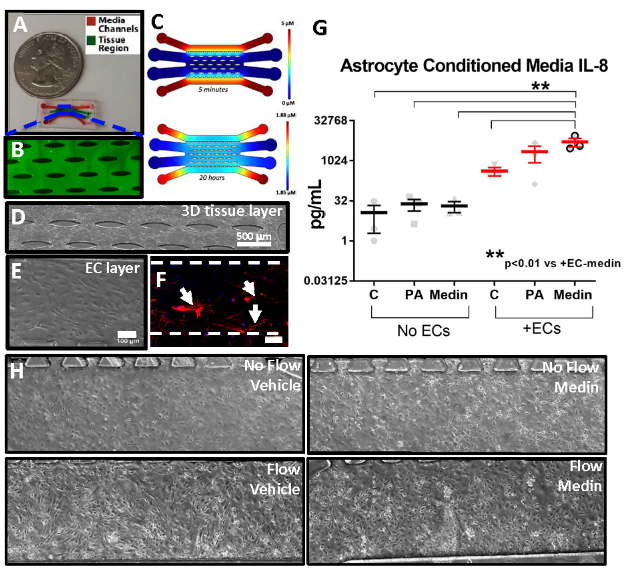

Figure 1. A) Schematic of VBOC. **B,C)** Diffusion across VBOC. **D)** Astrocyte layer in tissue region. **E)** EC layer in media channel. **F)** Astrocytic activation in response to ECs and medin. **G)** IL-8 levels in response to medin, PA and/or ECs. **H)** HBMVEC monolayers under flow and/or medin.

Results and Discussion: Within the VBOC, in the presence of ECs, medin induced astrocytic activation (GFAP staining, red) and astrocytic migration into the EC channel (**Fig. 1F**). Additionally, secretion of IL-8 was increased in the ECs condition, in comparison to no ECs, when exposed to medin, and in the EC condition, in comparison to vehicle control, when exposed to medin (**Fig. 1G**). A similar trend was seen for PA, although not statistically significant. To further the physiological relevance of this model, HBMVECs were cultured in the media channels and exposed to flow. The left panel of **Fig. 1H** compares 72 hours of flow to no flow of HBMVECs, revealing a more confluent monolayer under continuous media flow. The right panel compares flow to no flow, under medin administration, revealing presence of less connected and more rounded ECs as compared to respective vehicle controls, that may suggest altered tight junctions.

Conclusions: The demonstrated VBOC platform enables cell-cell interaction studies to investigate effects of biologic mediators in studying the role of vasculature in neuroinflammation. Administration of medin to ECs resulted in activation of astrocytes, as well as increased secretion of IL-8. Additionally, sustained culture of HBMVECs under continuous flow was demonstrated, with preliminary studies completed on effect of medin on integrity of EC monolayer. This prototype VBOC is a useful model to study molecular mechanisms of vascular and neuroinflammation, with enhanced physiologic relevance by exposure of ECs to flow within 3D environment.

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