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New In Vitro Model to Study Multicellular and Flow Control of Blood-Brain Barrier

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Abstract

Brain endothelial cells of the blood-brain barrier (BBB) have been shown to be regulated by supportive cells, such as pericytes and astrocytes, and shear stress exposure. However, studies investigating the impact of pericytes and astrocytes on brain endothelial cell function have identified both beneficial and detrimental results. Additionally, most studies investigating the relationship between shear stress and brain endothelial cell function lack physiological relevance via the use of sub-physiological shear stress magnitudes and/or via the absence of pericytes and astrocytes. In this study, we developed a millifluidic device compatible with standard transwell inserts to investigate BBB function. In contrast to standard polydimethylsiloxane (PDMS) microfluidic devices, this model allows for easy, reproducible shear stress exposure without common limitations of PDMS devices such as inadequate nutrient diffusion and air bubble formation. In no-flow conditions, we first used the device to examine the impact of primary human pericytes and astrocytes on human brain microvascular endothelial cell (HBMEC) barrier integrity. We found that astrocytes, pericytes, and a 1:1 ratio of both cell types increased HBMEC barrier integrity via reduced permeability to 40 kDa fluorescent dextran, which was associated with increased expression of tight junction protein, claudin-5. Interestingly, we also observed a significantly lower permeability to 3 kDa dextran in HBMEC-pericyte co-cultures compared to HBMEC-astrocyte and HBMEC-astrocyte-pericyte co-cultures. Based on these findings, we hypothesize pericytes may be providing increased barrier support to the BBB model compared to astrocytes although they both function as permeability reducers. After using the device to generate 24-hour flow at 12 dynes/cm2, we observed that shear stress exposure significantly reduced dextran permeability in HBMEC monolayers, but not in tri-culture models consisting of HBMECs, pericytes, and astrocytes. These results indicate that co-cultures may demonstrate a more pronounced impact on overall BBB permeability than flow exposure. In both cases, flow exposure was interestingly associated with reduced expression of both claudin-5 and occludin. However, ZO-1 expression, and localization at cell-cell junctions increased in the tri-culture but exhibited no apparent change in the HBMEC monolayer. Under flow conditions, we also observed alignment of HBMECs in the tri-culture while no such phenomenon was observed in HBMEC monolayers, indicating supportive cells and flow are both essential to observe brain endothelial cell alignment in vitro. Collectively, these results support the necessity of physiologically relevant, multicellular BBB models when investigating brain endothelial cell function in relation to the BBB. Additionally, our findings provide clues on the role of shear stress and supportive cells within the BBB, a critical step to elucidating the physiology of the neurovascular unit.

This is the full abstract presented at the Experimental Biology meeting and is only available in HTML format. There are no additional versions or additional content available for this abstract.