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Gaborski NanoBio Materials Lab

Pericytes Contribute Basement Membrane Proteins in a Human Stem Cell-Derived Blood-Brain Barrier Model

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1 Introduction

The blood-brain barrier (BBB) protects the central nervous system by restricting the passage of harmful molecules from the vasculature to the brain. Inflammation can disrupt the BBB and result in development of neurological disorders. A possible link between inflammation and BBB disruption is the upregulated enzymatic digestion of the vascular basement membrane (BM). The BM is composed of networks of extracellular matrix proteins that wrap around blood vessels and provide support for the endothelium. Since the presence of BM strengthens endothelial barrier properties, losing the BM would likely compromise those properties and cause BBB disruption [1]. To study the relationship between inflammation, BM, and breakdown of the BBB, we developed an in vitro model consisting of a microfluidic device featuring an ultrathin and continuously nanoporous silicon nitride membrane. We sought to validate that human induced pluripotent stem cell (iPSC)-derived cells could form a physiological BM on this membrane, which we evaluated by confirming that major BM protein components were produced and that these proteins were concentrated on the abluminal side of the endothelium.

2 Materials & Methods

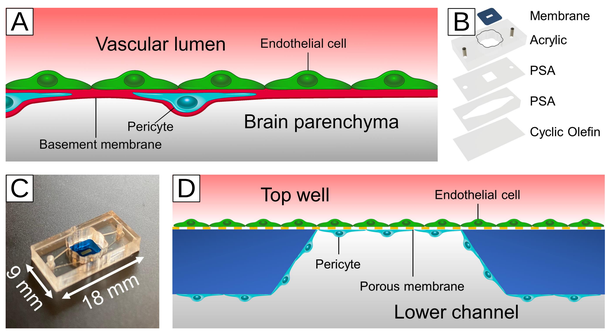

Figure 1. Modeling the BBB with the **micro**physiological system enabled by a **Si**licon-based **M**embrane (µSiM) [2]. **A.** Simplified diagram of the BBB showcasing the relative positions of endothelial cells, pericytes, and the BM. **B.** Exploded view of the µSiM assembly. Components include the optically transparent nanoporous membrane, acrylic housing, pressure sensitive adhesive (PSA) layers, and the bottom cyclic olefin layer. **C.** Dimensions of the µSiM. **D.** Co-culture seeding orientation of human iPSC-derived extended endothelial culture method brain microvascular endothelial cell (EECM-BMEC)-like cells [3] and brain pericyte-like cells (BPLCs) [4] on the µSiM's nanoporous membrane.

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3 Results

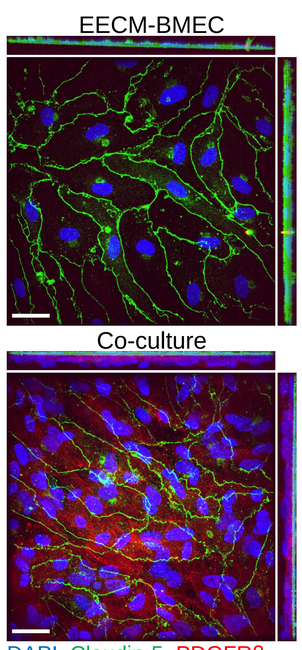

Figure 2. BPLCs are localized below the EECM-BMEC-like cells in co-cultures. Endothelial tight junction protein claudin-5 indicates the position of EECM-BMEC-like cells while PDGFRβ demonstrates the positions of BPLCs. Images were captured at 40x magnification on an Andor Dragonfly Spinning Disc Confocal Microscope with a 0.2 µm step interval. Z-stacks were projected into 2D based on maximum intensity. Scale bar = 50 µm.

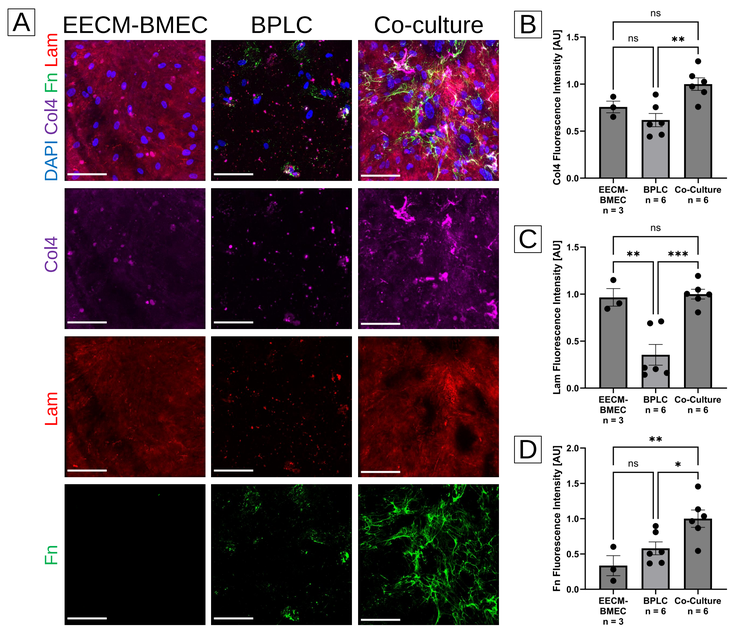

Figure 3. Comparison of BM production in human iPSC-derived monocultures and co-cultures. **A.** Immunofluorescence images of EECM-BMEC-like cell monocultures, BPLC monocultures, and co-cultures featuring both EECM-BMEC-like cells and BPLCs. Extracellular collagen IV (Col4), laminin (Lam), and fibronectin (Fn) were live-labeled prior to fixation. BPLCs tended to fall off the membrane or fail to proliferate in monocultures but appeared to be stabilized by the EECM-BMEC-like cells in co-cultures. Scale bar = 100 µm. Images were captured at 40x magnification on an Andor Dragonfly Spinning Disc Confocal Microscope with a 0.2 µm step interval. Z-stacks were projected into 2D based on maximum intensity. **B.** Barplot of average collagen IV fluorescence intensity. **C.** Barplot of average laminin fluorescence intensity. **D.** Barplot of average fibronectin fluorescence intensity. Fluorescence intensities were normalized to the co-culture condition. Error bars = SEM. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, ns = not significant. Analyzed by one-way ANOVA with Tukey post-hoc test.

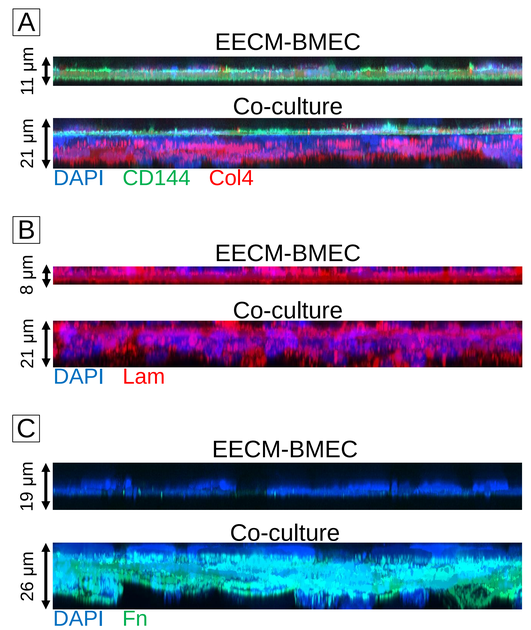

Figure 4. Z-distribution of the BM in EECM-BMEC-like cell monocultures and co-cultures featuring EECM-BMEC-like cells and BPLCs. **A.** X-axis 3D projections obtained from confocal immunofluorescence images of collagen IV (Col4) and endothelial junction protein VE-cadherin (CD144) demonstrate that collagen IV is localized below the endothelium in the co-culture model. **B.** X-axis 3D projections obtained from confocal immunofluorescence images of laminin (Lam). **C.** X-axis 3D projections obtained from confocal immunofluorescence images of fibronectin (Fn). Images were captured at 40x magnification on an Andor Dragonfly Spinning Disc Confocal Microscope with a 0.2 µm step interval. Z-stack thicknesses are indicated to the left of each image.

4 Conclusions

- iPSC-derived pericytes contribute fibronectin and alter the morphology of collagen IV and laminin in our co-culture BBB model.
- iPSC-derived pericytes are stabilized by the presence of endothelial cells.
- The inclusion of iPSC-derived pericytes in our co-culture BBB model causes BM deposition on the abluminal side of the endothelium, thus better mimicking the physiological orientation of the BM.
- With this validated model, we can investigate how pro-inflammatory factors may destabilize the BM and thereby disrupt BBB integrity.

References

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Conflict of Interest Statement:
JLM and TRG are cofounders of SiMPore Inc., which manufactured the µSiM membranes

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