Perfusion Stabilizes Long-Term Barrier Function of iPSC-derived Brain Endothelial Cells in 3D Hydrogel Channels

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Statement of Purpose

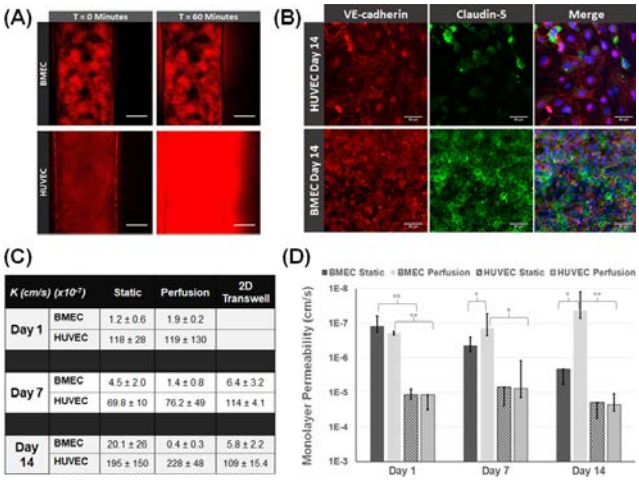
The neurovascular unit (NVU), comprised primarily of brain endothelial cells, smooth muscle cells, and astrocytes, serves as the critical barrier protecting the central nervous system. As such, the NVU is affected by a broad spectrum of neurovascular diseases, as well as environmental toxins and non-neurological pathologies that compromise function. Thus, there is a critical need for more biomimetic *in vitro* tissue models to better understand the complexities of NVU function and provide insight into therapeutic opportunities. In this work, we fabricate a 3D model of brain endothelium using iPSC-derived brain microvascular cells (BMECs) in perfused gelatin hydrogel channels. By demonstrating the ability of perfused BMEC-lined channels to establish and maintain robust barrier functionality long-term, we achieve a foundational step towards creating a clinically relevant NVU model.

Materials & Methods

Porcine gelatin (10% w/v, Sigma) scaffolds crosslinked with 1% (final w/v) microbial transglutaminase (mTG, Modernist Pantry) were cast over 800 µm OD silicon tubing (VWR) supported by an external PDMS (Dow Corning) rig. Once set, the channel formed after removal of tubing was coated with 0.4 mg/ml collagen (Sigma) and 0.1 mg/ml fibronectin (Sigma) and conditioned in complete media overnight before seeding channels with $3x10^6$ cells/ml of either iPSC-BMECs or RFP-expressing HUVECs (Angioproteomie). HUVECs were cultured in DMEM/F12 media supplemented with 5% FBS, 10 mM L-glutamine, 50 µg/ml Ascorbic Acid, 0.75 U/ml Heparin, 15 ng/ml IGF-1, 5 ng/ml VEGF, FGFb, and EGF (Peprotech). iPSC-BMECs were cultured in human Endothelial Serum-Free Medium, supplemented with 1% platelet-poor plasma-derived bovine serum (Alfa Aesar) and 10 µM Y27632 (Tocris). During seeding, media also contained 20 ng/ml FGFb and 10 µM all-trans retinoic acid (Sigma)(*1*). Cell-lined channels were perfused at 100 µl/min unless otherwise noted. Diffusion of AF680 conjugated 3K Da dextran and confocal imaging (LSM 710, Zeiss) weekly. Image datasets were processed with custom Fiji(*2*) and Matlab (Mathworks) scripts to calculate cell barrier permeability coefficients. Constructs were subsequently formalin fixed and analysed for VE-cadherin and Claudin-5 immunofluorescence.

Results & Discussion

iPSC-derived BMECs lining gelatin channel exhibit barrier permeabilities ~10-100 times lower than HUVEC lined channels for all conditions and are consistent with that measured in 2D controls, indicating robust barrier formation in gelatin hydrogels. The barrier strength of non-perfused BMEC-lined channels declined steadily over 14 days, whereas perfused channels maintained barrier integrity. It is likely that perfusion enables barrier stabilization through a combination of benefits including enhanced nutrient/waste exchange, shear-induced mechanical signaling cues(*3*), and reduction of ROS species(*4*), that support long-term cell vitality and function. Immunofluorescence staining of cell-lined channels fixed directly following diffusion analysis revealed characteristic staining patterns for both VE-cadherin and Claudin-5, two junction proteins critical for barrier formation in brain endothelium. Finally, assessment of p-glycoprotein efflux transporter activity with and without inhibition by cyclosporin A showed that BMECs in perfused channels exhibit no significant decline in efflux transporter activity over 14 days; thus, providing additional confirmation of long-term cell functionality.



K (cm/s) (x10⁻⁷)		Static	Perfusion	2D Transwell
Day 1	BMEC	1.2 ± 0.6	1.9 ± 0.2	
	HUVEC	118 ± 28	119 ± 130	
Day 7	BMEC	4.5 ± 2.0	1.4 ± 0.8	6.4 ± 3.2
	HUVEC	69.8 ± 10	76.2 ± 49	114 ± 4.1
Day 14	BMEC	20.1 ± 26	0.4 ± 0.3	5.8 ± 2.2
	HUVEC	195 ± 150	228 ± 48	109 ± 15.4

Figure 1. Perfused BMEC-lined channels exhibit robust barrier function and junctional integrity. (A) 3K Da dextran diffusion across iPSC-derived BMEC and HUVEC lined channels at 0 and 60 minutes. Scale = 500 µm **(B)** Day 14 Immunofluorescence staining of VE-cadherin and Claudin-5 junction proteins. Scale = 50 µm **(C)** Permeability coefficients for cell monolayers calculated from dextran diffusion images. $N \geq 3$ for all samples. **(D)** Graph depicting monolayer permeability versus time for BMEC and HUVEC lined channels maintained under static or continual perfusion. Error = ± 1 SD, (*) indicates $p < 0.01$, (**) indicates $p < 0.0001$ based on a paired Student's t-test.

Conclusions

In this study, we show that iPSC-derived BMECs form confluent monolayer in gelatin hydrogel channels with robust barrier properties. Perfusion under low-shear conditions enables maintenance of barrier integrity for up to two weeks. This work lays the foundation for fabricating a biomimetic in vitro NVU model.

References

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