Shear Stress and Co-culture with Astrocytes Determine Brain Microvascular Endothelial Cell Phenotype

S. Salehi Omran[1], SB; F. Garcia-Polite[1,2], MS; P. Melgar-Lesmes[1], PhD; A.A. Garcia-Granada[2], PhD; C.C. O'Brien[1], PhD; E.R. Edelman[1], MD, PhD; M. Balcells[1,2], PhD

[1] Institute for Mechanical Engineering and Science, MIT, Cambridge, MA, USA [2] Universitat Ramon Llull, Barcelona, Spain

Abstract

Introduction: Dementia has classically been identified to be of either vascular or neural origin. These domains overlap and are complementary, thus we consider dementia a disease of the single cerebrovascular unit (CVU). Our objective was to generate a modular platform for co-culture of brain microvascular endothelial cells and astrocytes that also incorporates mechanical stresses, and to then use this model of the CVU microenvironment to study the unit *in vitro*.

Hypothesis: We assessed the hypothesis that endothelial health and blood-brain barrier integrity are modulated by shear stress and co-culture with astrocytes.

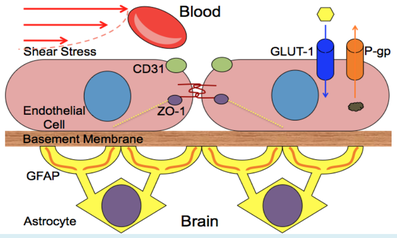
Methods: We lithographed polydimethylsiloxane substrate on Teflon negative molds with subjacent rectangular channels of 0.45 and 2 mm depth for seeding of human brain microvascular cells and astrocytes, respectively, separated by a polytetrafluoroethylene (0.45 µm pore size) membrane under no flow or physiologic flow (6.2 dynes/cm^2) for one week. Immunocytochemical staining for glial fibrillary acidic protein and CD31 was simultaneously visualized by confocal microscopy. Cells from each channel were detached via trypsinization, and expression of transport proteins P-glycoprotein (P-gp) and glucose transporter-1 (GLUT-1), in addition to junction proteins zona occludens-1 (ZO-1) and CD31, was measured by Western Blot.

Results: We stably co-cultured brain microvascular endothelial cells and astrocytes with no chamber leakage or mixing. CD31 staining revealed confluent endothelial monolayer. Expression of ZO-1 by endothelial cells increased in presence of flow and co-culture, by 1.6-fold in combined conditions relative to static monoculture ($p<0.05$). For P-gp, the increase in combined conditions was 5.5-fold ($p<0.05$). GLUT-1 and CD31 levels did not change significantly with co-culture or flow.

Conclusion: Cell biology devoid of microenvironmental cues provides limited insight, especially when considering whole tissues, on the impact of disease. A co-culture system that introduces multiple cells, flow, controlled stress and independent visualization and sampling of each cell domain adds deeper understanding and greater value to *in vitro* biological models and tissue biology.

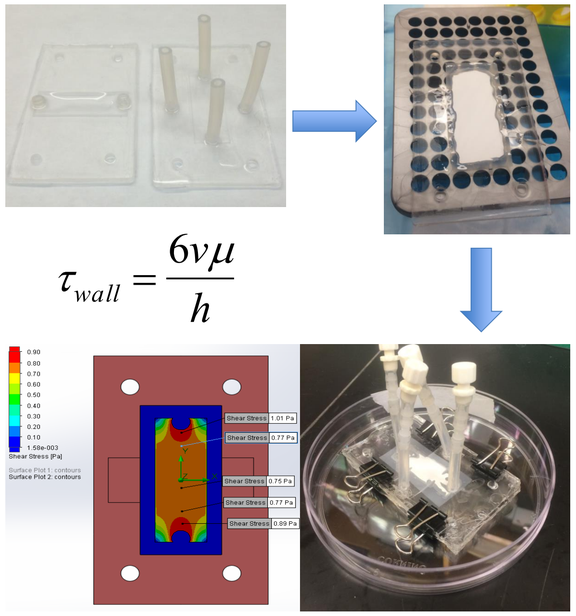
Objective

Produce CVU simulator where cell type, mechanical forces, and chemical environment can be manipulated. Change parameters, e.g. shear stress. Collect useful biological data, such as cell morphology and protein expression.

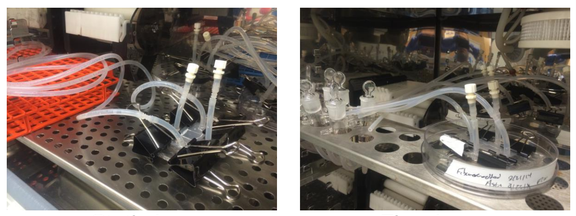


Methods

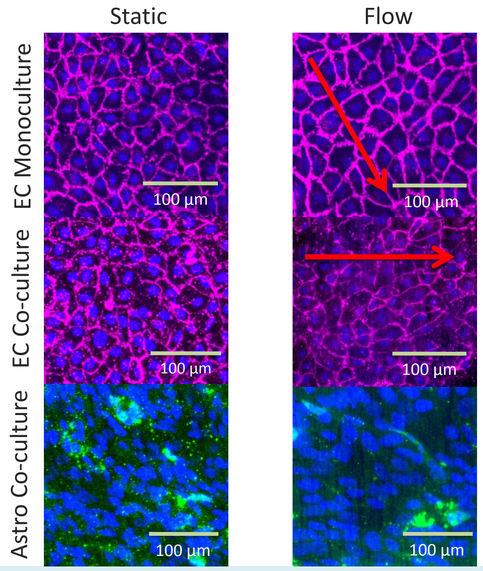
Negative PTFE molds were fabricated by CNC machining. PDMS (20:3 v/v base to elastomer ratio) was cured in molds at 80ºC to produce channels. The molds were bonded together with permeable PTFE membrane (0.4 µm pore size) in between using PDMS and toluene (1:1) at 100ºC. Structure was kept tightly sealed with paper clamps. Membrane was covered (each side separately) with 100 µg/mL fibronectin, then allowed to sit for 2 hours at 37ºC. P6 human brain microvascular endothelial cells were seeded in 0.45 mm channel at high density (10^6 cells/mL) and allowed to sit for 24 hours. P6 human astrocytes or culture medium were seeded in other channel at low density (10^5 cells/mL) and allowed to sit for 24 hours. Device was then either connected to constant flow (29 mL/min, 6.2 dynes/cm^2 calculated) or to static loop of growth medium (EGM-2 with 5% fetal bovine serum). The experiment ended 2 days later and contents fixed for staining, or 6 days later and contents lysed for Western Blot. Immunofluorescence used rabbit CD31 (PECAM) and goat glial fibrillary acidic protein (GFAP) primary antibodies, with donkey secondary antibodies, visualized by laser-scanning confocal microscopy. Western Blot samples were collected from each chamber separately using trypsinization to detach cells prior to cell lysis. 15 µg protein was loaded into each well for SDS-PAGE. Proteins of interest include CD31, ZO-1, GLUT-1, and P-gp.



$$\tau_{wall} = \frac{6v\mu}{h}$$

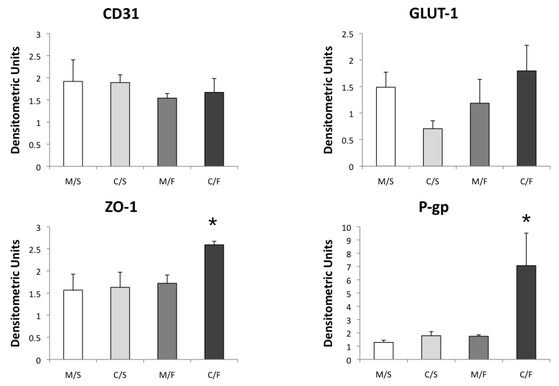


Results



Confocal microscopy on day 4 is seen on left. Blue: DAPI stain, Magenta: CD31 stain, Green: GFAP stain. Red arrow shows direction of flow.

Western Blot results on day 8 are shown below. M: monoculture, C: co-culture, S: static, F: flow.



Discussion

Based on cell counts and staining for CD31 and GFAP, there is no presence of either cell type in other cell's chamber. Device is structurally sound, with no contamination, leakage, or communication bypassing the membrane.

Both endothelial cells and astrocytes produce confluent layers over membrane when seeded at high density, within 96 hours for the endothelial cells and 72 hours for astrocytes. Endothelial cells produce a monolayer by microscopy. Thus, membrane is suitable surface for cell growth.

This configuration produces enough protein for Western Blot analysis, contrary to microfluidic approaches which have not reported such results[1,2]. Combined co-culture and flow increase EC P-gp and ZO-1 expression compared to monoculture of EC in static, in a statistically significant manner. However, GLUT-1 and CD31 expression by EC do not change with presence of astrocytes or flow.

Flow and co-culture appear to push brain endothelial cells towards richer expression of CVU phenotype, consistent with results found by other groups using different techniques for exposing endothelial cells to astrocyte media.[2,3]

Conclusion

We produced an *in vitro* simulator of the cerebrovascular unit. This will be highly invaluable in studying CVU pathology in the presence of dysfunctional endothelium, amyloid beta, carotid arteriosclerosis, among other alterations of cellular, chemical, and mechanical microenvironment of the CVU.

References

1. Booth R, Kim H. Characterization of a microfluidic in vitro model of the blood-brain barrier (µBBB). Lab Chip. 2012 Apr 24;12(10):1784-92.
2. Prabhakarpandian B1, Shen MC, Nichols JB, Mills IR, Sidoryk-Wegrzynowicz M, Aschner M, Pant K. SyM-BBB: a microfluidic Blood Brain Barrier model. Lab Chip. 2013 Mar 21;13(6):1093-101.
3. Cucullo L, Hossain M, Puvenna V, Marchi N, Janigro D. The role of shear stress in Blood-Brain Barrier endothelial physiology. BMC Neurosci. 2011 May 11;12:40.

Acknowledgements

The authors thank the MIT Central Machine Shop for mold fabrication and the Koch Institute for microscopy assistance. SSO thanks Drs. Jeanette Norden and Derek Riebau for sparking his interest in the blood-brain barrier.
SSO is supported by the Sarnoff Cardiovascular Research Foundation.
This work is supported by Programa Estatal de Investigación, Desarrollo e Innovación Orientada a Los Retos de la Sociedad, SAF2013-43302-R.
Contact: sina@alum.mit.edu