Disrupted wall shear stress alters autocrine signaling of brain microvascular endothelial cells at the Blood-Brain Barrier

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BACKGROUND

Flow induced wall shear stress (SS) is known to contribute significantly on the phenotype of microvascular endothelial cells in blood-brain barrier (BBB) formation. Together with the presence of astrocytes, dynamic in vitro models provide more realistic physiological properties of the neurovascular unit[1]. Yet, both direct and indirect relationships have been proposed between brain microvascular damage and abnormal flow patterns caused by upstream stiffened vascular beds[2]. We hypothesize that higher SS rates are responsible of BBB malfunction via autocrine signaling.

METHODS AND RESULTS

In our study, we cultured primary human brain microvascular endothelial cells (HBMVEC) with astrocyte conditioned media (ACM) under different levels of SS on a flow-perfusion bioreactor.
Tight junction formation in our dynamic model was evaluated by immunocytochemical analysis of the tight junction marker Zonula Occludens 1 (ZO-1) (Figure 1).
Expression levels of Claudin 5, P-glycoprotein (Pgp) and Glucose Transporter 1 (GLUT1) were measured by Western Blot. Results showed an up-regulation of the expression in dynamic cultures compared to static cases (Figure 2). Then, conditioned media was collected from HBMVEC cultured under different SS (SSECM), from 0 to 20 dyn/cm^2 and used to culture static co-culture models of the BBB. Functional analysis (dextran permeability) showed that SSECM has a positive effect on the BBB (Figure 3).

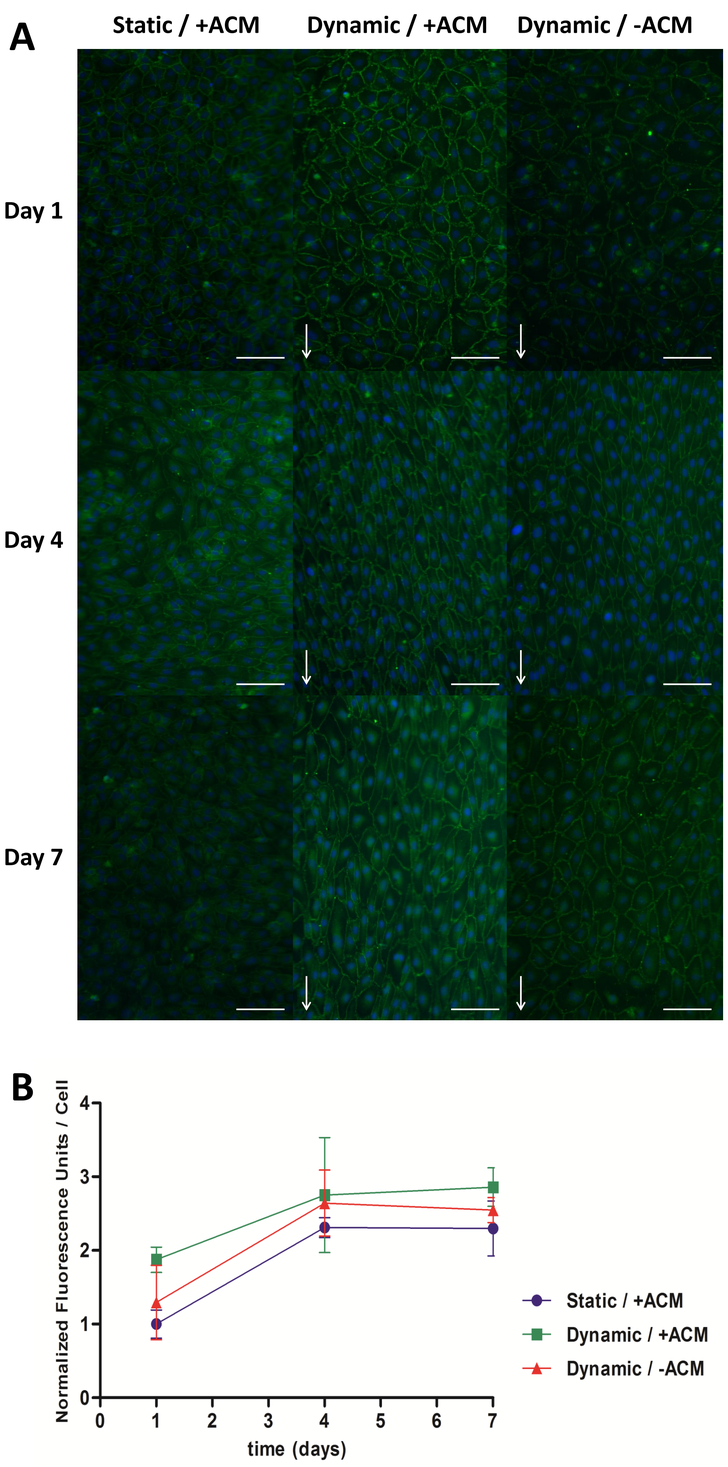

Figure 1. ZO-1 expression of HBMVEC cultured in a flow perfusion bioreactor
Cells were cultured for 1, 4 and 7 days under static and dynamic conditions (5dyn/cm^2) with astrocyte conditioned media (ACM) (C). Then, ZO1 and cell nuclei were labeled to test tight junction formation. (A) Cell morphology is highly affected by shear stress, showing a clear alignment with flow direction (indicated in the figure with arrows). (B) ZO1 expression is higher under dynamic conditions. The combination of shear stress and ACM doubles ZO1 levels at day 1 and remains higher than static culture. There is between 2- and 2.9-fold increase of expression from day 1 to day 4. Steady state is reached after 4 days.
(Scale bar: 10μm) (n=2).

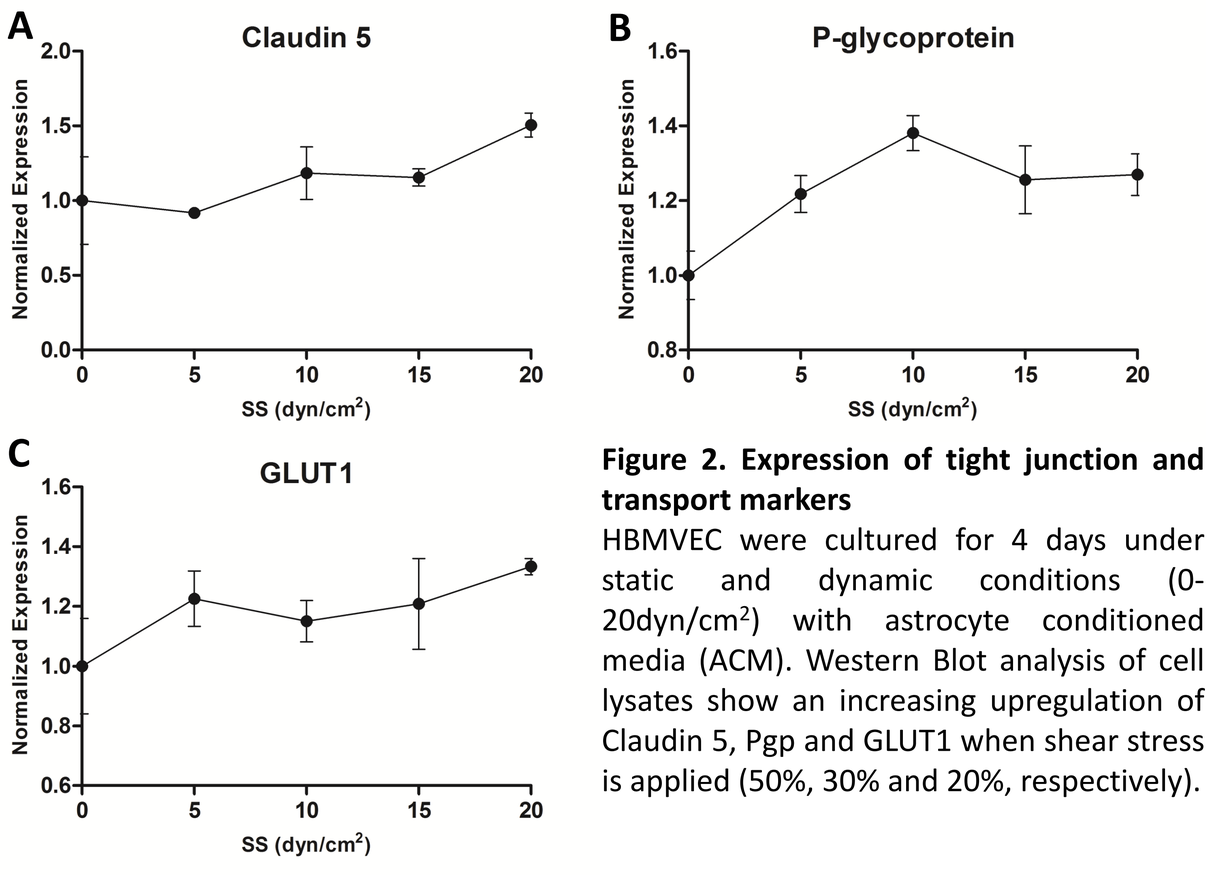

Figure 2. Expression of tight junction and transport markers
HBMVEC were cultured for 4 days under static and dynamic conditions (0-20dyn/cm^2) with astrocyte conditioned media (ACM). Western Blot analysis of cell lysates show an increasing upregulation of Claudin 5, Pgp and GLUT1 when shear stress is applied (50%, 30% and 20%, respectively).

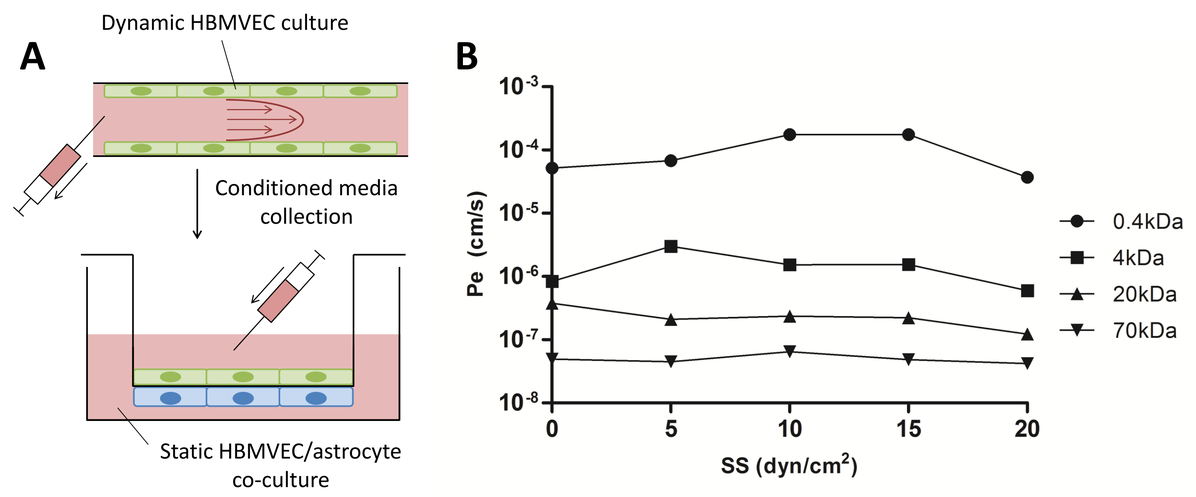

Figure 3. Permeability of in vitro endothelial/astrocyte co-cultures exposed to SS endothelial conditioned media.
(A) HBMVEC were exposed to different levels of steady shear stress for 24h. Conditioned media was collected and used to culture static co-cultures in 24-well plate transwell inserts for 24h. FITC and FITC-labeled dextrans were added in the top chamber of the cultures and permeability was measured. (B) Permeability decreases with increased shear stress in the case of FITC and 4kDa/20kDa dextrans, with its lower permeability at 20dyn/cm^2. 70kDa dextran permeability remains constant. Conditioned media from dynamic cultures has an effect on static co-cultures, suggesting alterations in autocrine signaling of the endothelial environment at the BBB (n=3).

Immunocytochemical analysis, Western Blot analysis and permeability studies support that shear stress induces BBB properties, but no damage is detected in any case. However, endothelial conditioned media from dynamic cultures alter permeability in static cultures, indicating endothelial secretome alterations induced by shear.

CONCLUSIONS & FUTURE WORK

Shear stress promotes Blood-Brain Barrier phenotype at capillary-like rates, but whether high SS rates may damage the neurovascular unit remains unclear. Conditioned media from dynamic cultures alters endothelial autocrine signaling

Future work will be focused on studying the effect of higher shear stress on in vitro BBB models and elucidating the molecular mechanism linking SS, endothelial secretome and BBB expression.

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REFERENCES
[1] Cucullo, L., Hossain, M., Puvenna, V., Marchi, N. & Janigro, D. The role of shear stress in Blood-Brain Barrier endothelial physiology. *BMC Neurosci.* 12, 40 (2011).
[2] Hirata K, Yaginuma T, O'Rourke MF, Kawakami M. Age-related changes in carotid artery flow and pressure pulses: Possible implications for cerebral microvascular disease. *Stroke*, 2006. 37(10):2552-2556.