Effect of Shear stress on BBB endothelial cells: A proteomic study by 2-dimensional protein electrophoresis

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ABSTRACT

Human brain microvascular endothelial cells (HBMEC) *in vivo* are continuously exposed to shear stress, a tangential force generated by the flow of blood across their apical surfaces. Shear stress affects endothelial cell structure and function, such as cell orientation with flow direction, distribution of cell fibers, induction/suppression of genes (1-3), production of vasoactive substances and improved cell adhesion (4-6), mitotic arrest of endothelial cells (EC) (7-8)). Shearing forces also induce metabolic changes to counter oxidative stress (7). In this study we focused on the effects of shear stress on EC protein expression at the BBB.

We prepared 2D gels from protein extracts obtained from HBMEC cultured under dynamic (HBMEC were grown intraluminally in a hollow fibers based BBB model and exposed to media flow) or static conditions (HBMEC were grown in flask in absence of flow) for 3 weeks. Protein expression was then compared with a cell suspension. EC were harvested and protein isolated and separated into 4 different fractions (Membrane, Cytoskeletal, Cytosolic and Nuclear).

Cytosolic (73.5%) and nuclear protein (16.4%) constituted almost the total protein content in cell suspension. Cell adhesion induced an incremental expression of cytoskeletal proteins (58.4% of the total proteins) and a significant reduction of the cytosolic fraction. The exposure to flow in addition to cell adhesion (dynamic conditions) caused a further increase in the expression level of cytoskeletal proteins. By contrast, the other protein fractions resulted drastically reduced.

These changes may provide insight in mechanisms leading to pathological conditions such as arteriosclerosis and stroke and could explain the role played by shear stress on BBB endothelial cells.

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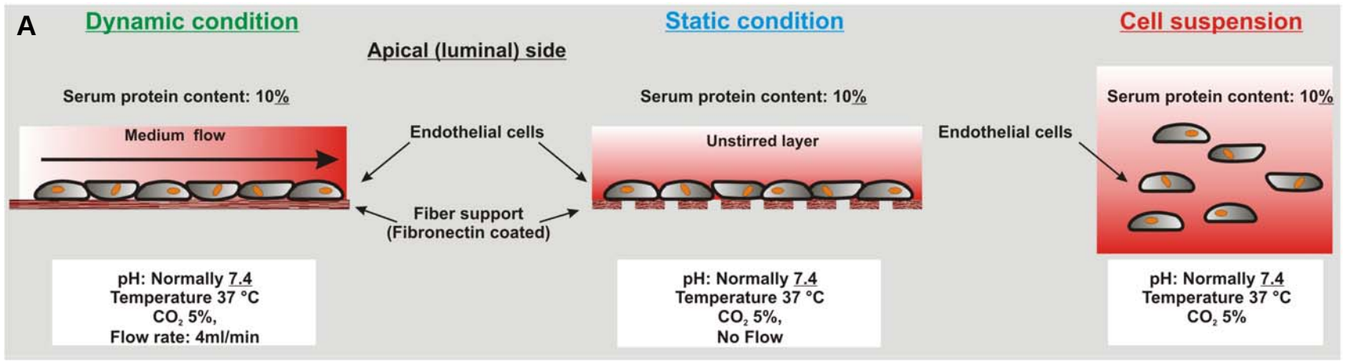
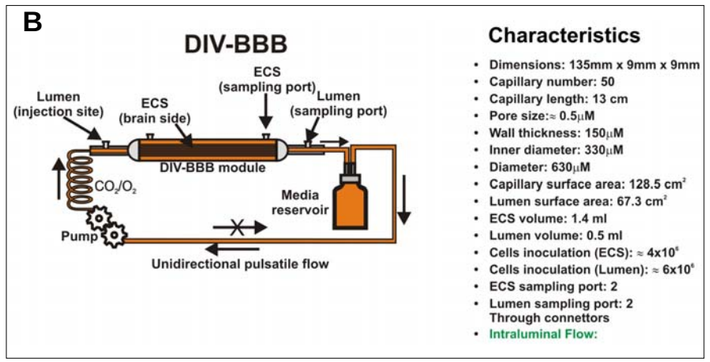
Experimental setup

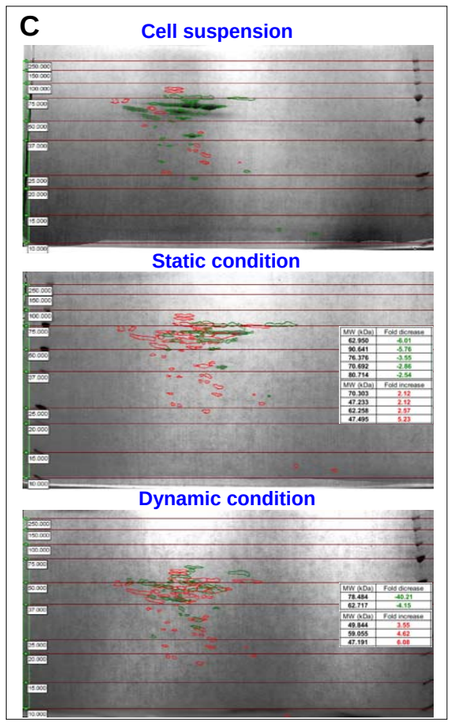


(A) Primary brain endothelial cells were cultured in the DIV-BBB for three weeks. The endothelial cells were exposed to intraluminal flow (4ml/min equivalent to 4 dine/cm2). Parallel culture were establish in flasks under in absence of apical flow. Cell suspension were used as comparison to assess the effect of shear stress and cell adhesion on EC protein expression

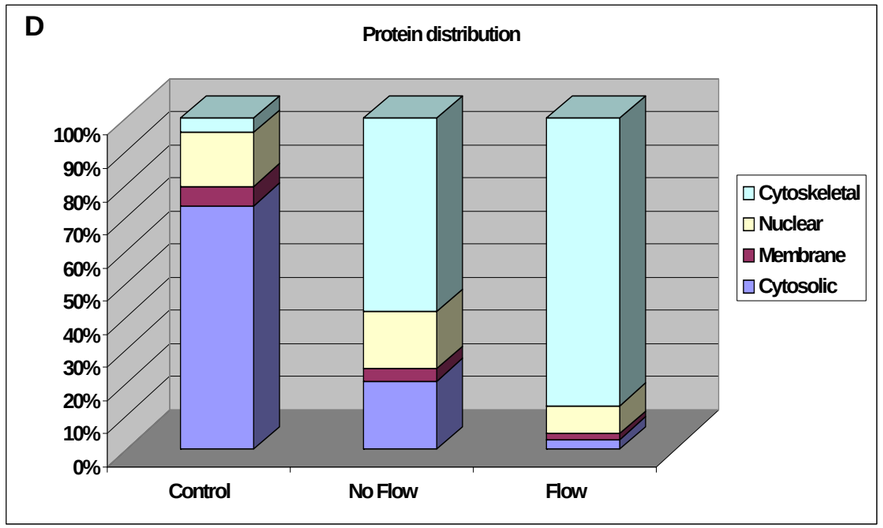
Schematic representation of the DIV-BBB



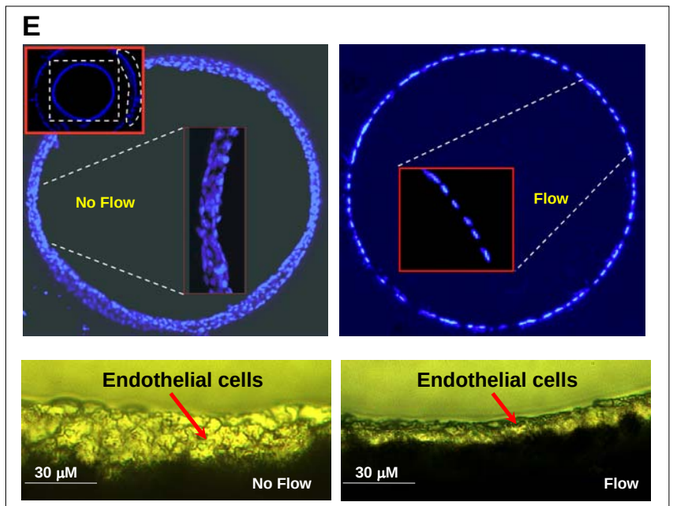
(B) Diagrammatic representation of the DIV-BBB. A bundle of porous polypropylene hollow fibers is suspended in the chamber. Intraluminal flow is generated by a pulsatile pump.



(C) Normalization is performed by Match Ratio Method. In this method the software calculates the ratio of the spot's volume in the base gel, to it's volume in the study gel. The volume of each spot is multiplied by the average of these ratios and then by the area of a single pixel in mm2, if scaling information is present.



(D) Total protein composition (membrane, cytosolic, nuclear and cytoskeletal) observed in EC grown under dynamic, static, and control condition (cell suspension).



(E) EC grown in DIV-BBB under dynamic and static conditions. Note that the exposure to physiological shear stress promoted mitotic arrest by contact and the endothelium assumed the typical monolayer appearance observed *in vivo*. By contrast, in absence of flow EC grew in multilayer fashion.

CONCLUSIONS

Brain vascular endothelial cells *in vivo* are continuously exposed to shear stress, a tangential force generated by the flow of blood across their apical surfaces. Physiologic shear stress is a critical modulator of EC proteins expression level which ultimately affect EC functions and differentiation. Shear stress affects cell orientation with flow direction, distribution of cell fibers, induction/suppression of genes, production of vasoactive substances, mitotic arrest and is also able to induce metabolic changes. These effects take place even in absence of abluminal astrocytes.

References

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