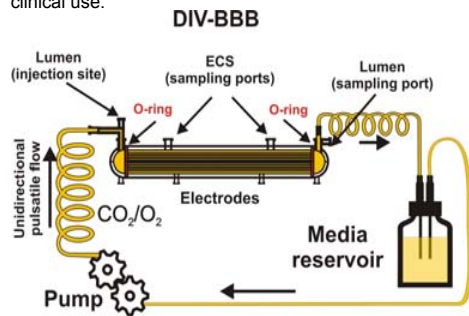


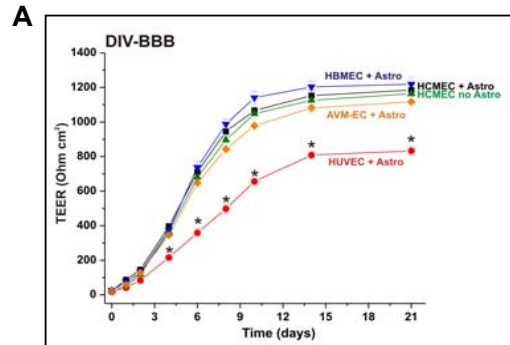
ABSTRACT

In evaluating drugs that enter or are excluded from the brain, novel pharmaceutical strategies are needed. For this reason, we have developed a humanized Dynamic *In Vitro* Blood Brain Barrier model (hDIV-BBB) by culturing a novel immortalized brain vascular endothelial cell line (HCMEC/D3) within a flow-based cell culture system based on hollow fiber technology. In this system, HCMEC/D3 were grown in the lumen of hollow micro porous fibers and exposed to a physiological pulsatile flow. Comparison with well established humanized DIV-BBB models (based on human brain and non brain vascular endothelial cells co-cultured with albuminal astrocytes) demonstrated that HCMEC/D3 cells cultured under flow conditions maintain *in vitro* the physiological permeability barrier properties of the BBB *in situ* even in the absence of albuminal astrocytes. Measurements of glucose metabolism demonstrated that HCMEC/D3 cells retain an aerobic metabolic pathway. Permeability to sucrose and two relevant CNS drugs showed that the HCMEC/D3 cells grown under dynamic conditions closely mimic the physiological permeability properties of the BBB *in situ* (slope = 0.886). Osmotic disruption of the BBB was also successfully achieved. Peak BBB opening in the DIV-BBB lasted from 20 to 30 minutes and was completely reversible. Furthermore, the sequence of flow cessation/reperfusion in the presence of leukocytes led to BBB failure as demonstrated by a biphasic decrease in transendothelial electrical resistance (TEER). Additionally, BBB failure was paralleled by the intraluminal release of pro-inflammatory factors (IL-6 and IL-1 β) and matrix metalloproteinases (MMP-9). Pre-treatment with ibuprofen (0.125 mM) prevented BBB failure by decreasing the inflammatory response following flow cessation/reperfusion. Based on these results, we believe that the use of the HCMEC/D3 would provide a well-characterized and cost-effective surrogate for primary brain vascular endothelial cells in many different BBB applications, from drug delivery studies to screening of novel therapeutic agents prior to clinical use.

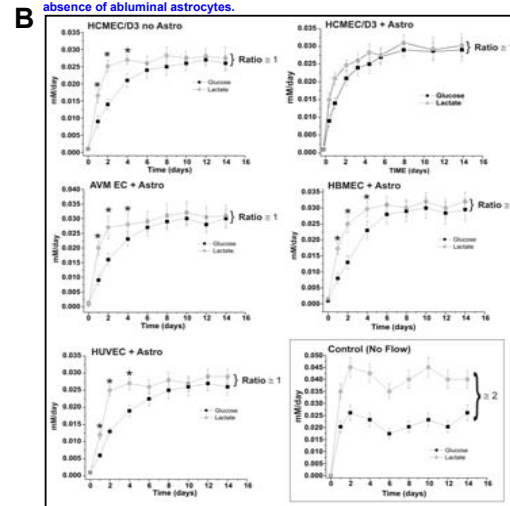


Diagrammatic representation of the DIV-BBB. A bundle of porous polypropylene hollow fibers is suspended in the DIV-BBB chamber. The hollow fibers are in continuity with a medium source through a flow path consisting of gas-permeable silicon tubing. Two three-way stopcocks positioned on either side of the module regulate the access to the luminal compartment.

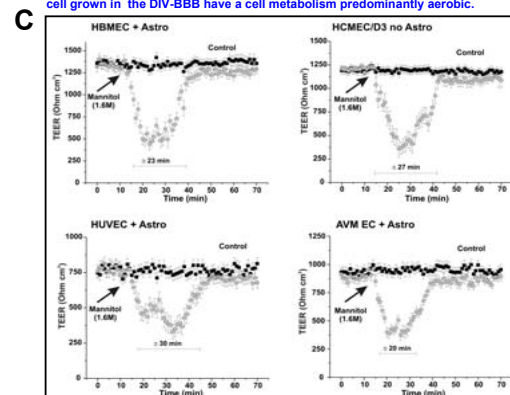
This work was supported by Alternative Research Development Foundation (ARDF) and Philip Morris USA and Philip Morris International external research awards to Luca Cucullo and by NIH-2R01 HL51614, NIH-R01 NS43284 NIH-R01 NS38195, NIH-R41 NS 054348-01A1 and Philip Morris USA and Philip Morris International external research awards to Damir Janigro.



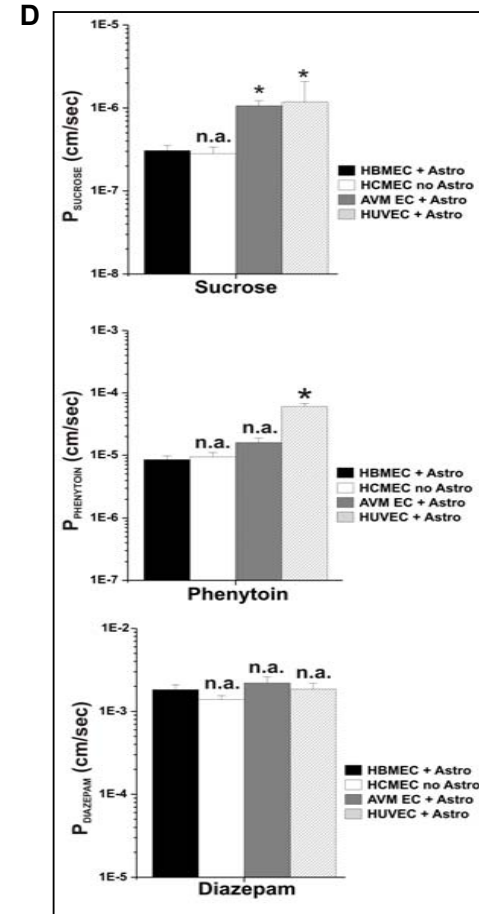
(A) Under dynamic conditions, endothelial cells shown a robust differentiation resulting in the development of stringent barrier. No difference was observed between HCMEC/D3 alone and HCMEC/D3 co-cultured with albuminal astrocytes. This data confirms that this cell line is capable of developing a tight barrier even in absence of albuminal astrocytes.



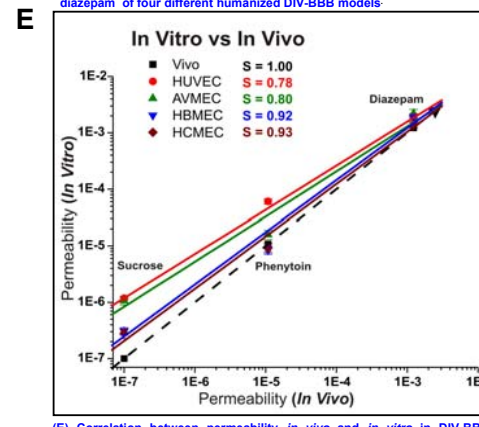
(B) Regardless of the type of vascular endothelium used, the sharp metabolic increase in glucose consumption is paralleled by a similar increase in lactate production but only under dynamic culture condition. This suggests that in the cell grown in the DIV-BBB have a cell metabolism predominantly aerobic.



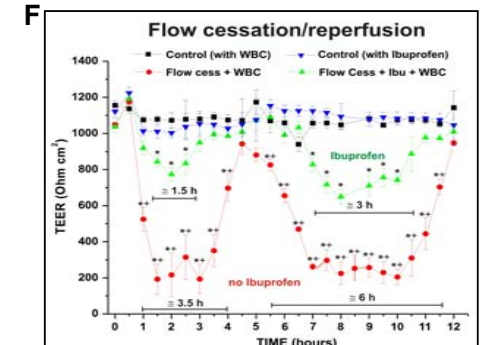
(C) Hyperosmolar opening of the BBB in DIV models. The figures show real time TEER changes induced by intraluminal perfusion (30 sec) with hyperosmolar mannitol (1.6M). Note that the duration of peak BBB opening varied from 20 to 30 minutes following mannitol perfusion and was completely reversible.



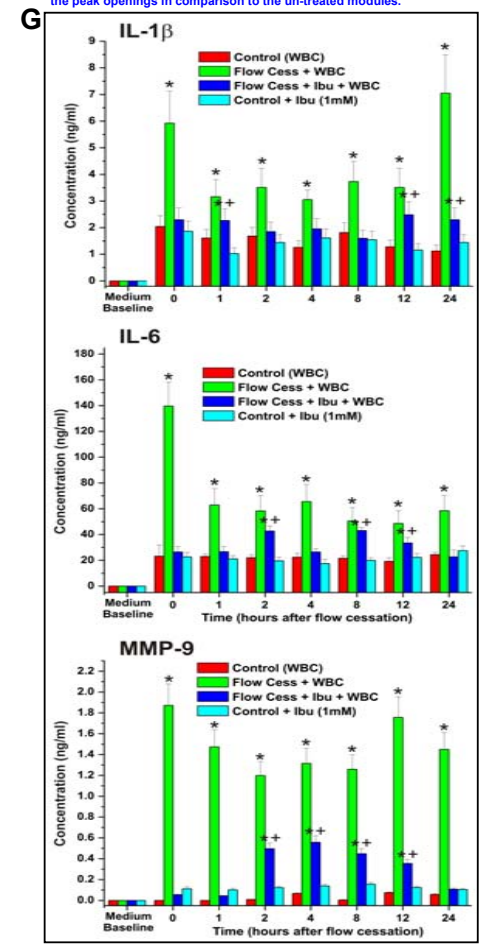
(D) Side by side permeability comparison to sucrose, phenytoin and diazepam of four different humanized DIV-BBB models



(E) Correlation between permeability *in vivo* and *in vitro* in DIV-BBB system. The dashed line indicates the idealized relationship if the data *in vivo* were identical to *in vitro*. Note that permeability obtained in the DIV-BBB established with the use of HBMEC (S = 0.92) and HCMEC/D3 (S = 0.93) endothelial cells accurately reflects the *in vivo* scenario.



(F) Functional assessment of the HCMEC/D3 *in vitro*-based BBB in an experiment of flow cessation reperfusion with WBC. Flow cessation/reperfusion caused a biphasic opening of the BBB. Pre-treatment with ibuprofen partially prevented BBB failure and shortened the duration of the peak openings in comparison to the un-treated modules.



(G) Measurement of pro-inflammatory cytokines IL-1 β , IL-6 and matrix metalloproteinase 9. Levels of IL-1 β , IL-6, and MMP-9 were measured in medium samples in all experimental conditions. Pre-treatment with ibuprofen significantly reduced the release of IL-1 β , IL-6, and MMP-9.