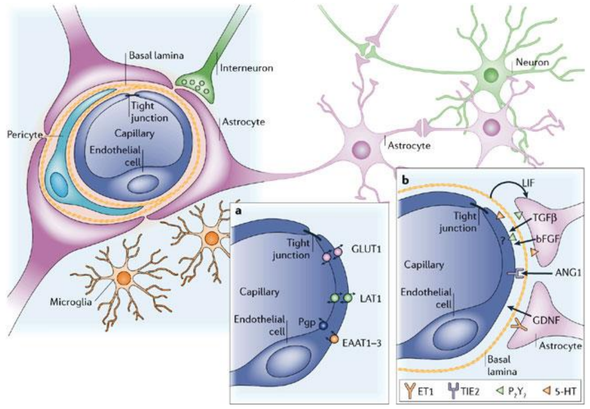
Modeling the Blood Brain Barrier with Stem Cell Derived Endothelial Cells and Astrocytes

Shusaku W. Asai, Scott G. Canfield, Eric V. Shusta, and Sean P. Palecek

Department of Chemical and Biological Engineering, University of Wisconsin-Madison

1. Background and Motivation

Capillaries in the brain are unique by the cells that line it. These cells, called brain microvascular endothelial cells (BMECs), operate in unison with supporting brain cells such as astrocytes, pericytes, and neurons to form the blood brain barrier (BBB).



The BBB's ability to regulate chemical and nutrient transport in and out of the brain via tight junctions and efflux transporters makes it vital for organismal homeostasis.

Literature establishes that small molecules secreted by supporting brain cells maintain *in vivo* BBB barrier properties. Previously, a screening of a portion of the small molecules was performed, and proved to improve BMEC phenotypes in our BBB model.

2. Goal and Hypothesis

Goal: Create an in vitro system that is scalable, reproducible and most similar to in vivo BBB phenotypes.

Hypothesis: The addition of small molecules derived and secreted by Astrocytes enhance our stem cell-derived BBB model.

3. Research Methods

Explore the effect of the addition of small molecules on a BMEC population via:

Investigation 1: Dose cells with small molecules (Days 8-10)

Day: 8 → 9 → 10 → 11

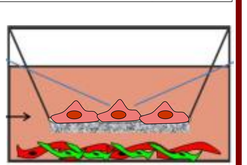
Re-plate cells

Monitor Phenotypes
- Measure TEER
- Fluorescein Permeability
- Immuno-chemistry Analysis

Investigation 2: Dose cells with combinations of GDNF, TGFβ, αGDNF Blocker (Days 8-10)

1. **Trans-endothelial resistance (TEER)**: An EVOM volt meter measures the electrical resistance across a layer of BMECs, revealing the tightness of the BBB, in Ω cm².
2. **Immuno-chemistry:** Primary anti-bodies, secondary anti-bodies, and fluorescent tags are used to reveal the presence of proteins specific to the BBB via microscopic imaging.
3. **Fluorescein Permeability:** A small fluorescent tag in solution is used to measure the permeability and tightness of our BMECs.
4. **Rhodamie123 Accumulation:** The efficiency of the efflux transporter, PGP, is tested via the accumulation of a fluorescent tag.

In vivo BMEC populations express high TEER values (~6000 Ω cm²), and express the tight junctional proteins Claudin 5, Occludin, and ZO1.



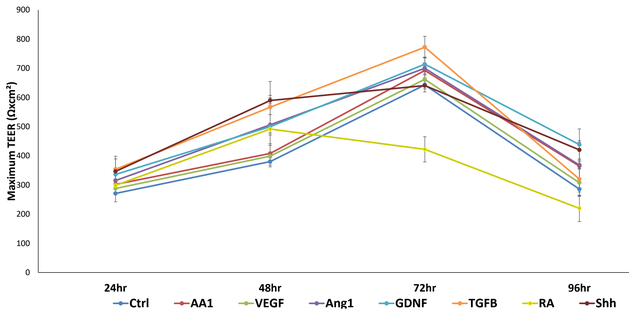
Schematic of current in vivo BBB model, where BMECs lie on the top trans-well.

4. Results

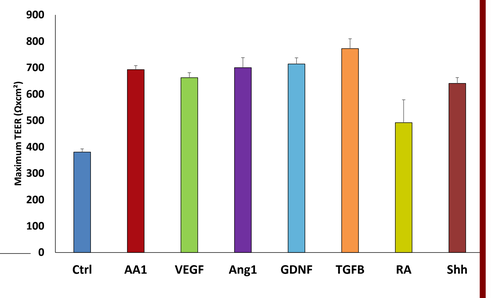
1. TEER of BMECs Treated with Astrocyte Derived Small Molecules

Small Molecules Investigated: AnnexinA1 (**AA1**), Vascular Endothelial Growth Factor (**VEGF**), Retinoic Acid (**RA**), Sonic Hedgehog (**Shh**), Glial Cell-Derived Neurotrophic Factor (**GDNF**), Transforming Growth Factor Beta (**TGFβ**), Angiopoietin 1 (**Ang1**)

A. TEER Values from Various Time Points

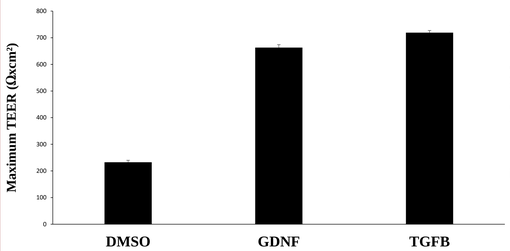


B. Maximum TEER

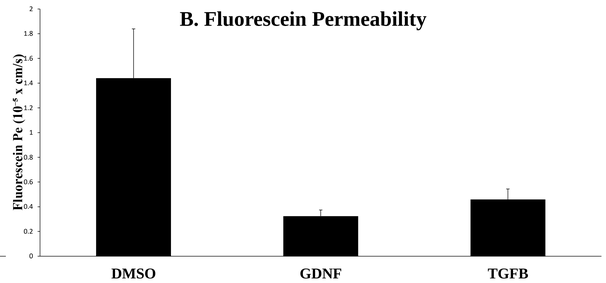


2. Phenotype Monitoring of BMECs Treated with GDNF and TGFβ on Days 6 - 10

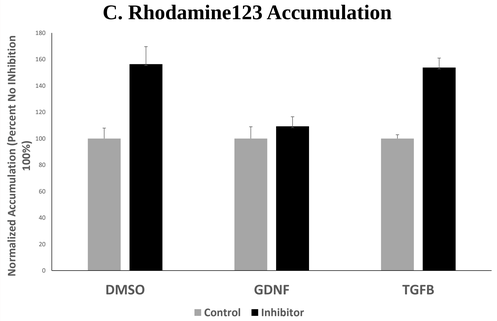
A. Maximum TEER



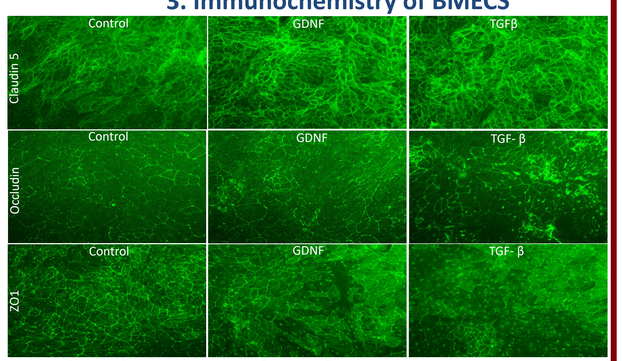
B. Fluorescein Permeability



C. Rhodamine123 Accumulation

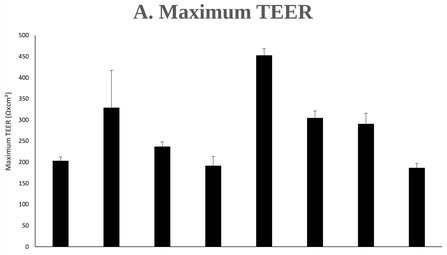


3. Immunochemistry of BMECS



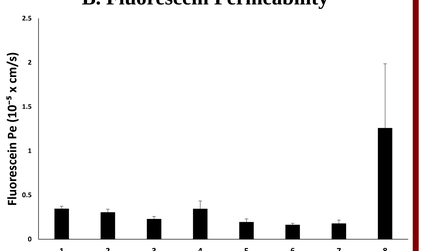
4. Phenotype Monitoring of BMECs Treated with GDNF and TGFβ on Days 8 - 10

A. Maximum TEER



Groups:
1 - Control
2 – Monoculture (+GDNF)
3 – Monoculture (+Blocking)
4 – Monoculture (+GDNF, +Blocking)
5 – Co-culture
6 – Co-culture (+ Blocking)
7 - Conditioned Media
8 – Conditioned Media (+ Blocking)

B. Fluorescein Permeability



5. Conclusions

- The addition of small molecules released by astrocytes to BMEC populations increases BBB properties as indicated by:
  - **Figure 1A** – All BMEC populations treated with small molecules had higher TEER values than BMEC control populations without treatment. Five treatments had higher TEER values at 72 hours, and six treatments had higher TEER values at 96 hours compared to BMEC populations without treatment.
  - **Figure 1B** - BMECs treated with any small molecules reached higher maximum TEER than controls.
  - **Immunochemistry-** The addition of various small molecules on BMECs indicated higher expression of the tight junctional proteins Occludin, C5, and ZO1 as indicated by the brighter cell junctions in images.
- The addition of the astrocyte derived small molecules, GDNF and TGFβ enhances BBB properties as indicated by:
  - **Figure 2 A,B,C –** These figures serve as verification for figure 1, shown by the increase in TEER and decrease in permeability .
- The inhibition of the astrocyte derived small molecules, GDNF and TGFβ diminishes BBB properties as indicated by:
  - **Figure 4 A,B –** BMEC populations that were subjected to a GDNF inhibition/blocking showed lower TEER and higher permeability in certain groups compared to their control counterparts.

6. Future Directions

- Continue inhibition and blocking experiments to further verify preliminary results.
- Observe how blood cells and small molecules secreted by blood cells can affect BBB properties in BMEC populations.

7. Acknowledgements

- Takeda Pharmaceuticals
- National Institute of Health
- Palacek and Shusta Laboratories

8. References

[1] Abbott, N. Joan, Ronnback, Lars, Hansson, Elisabeth. "Astrocyte-endothelial interactions at the blood-brain barrier." *Nature Reviews Neuroscience 7, 41-53 (* January 2006). 10.1038/nrn1824

[2] Lippmann, E. S. et al, Derivation of blood-brain barrier endothelial cells from human pluripotent stem cells. *Nature Biotechnology* 2012, *30* (8), 783-791.

[3] Alvarez, J. I., Katayama, T., & Prat, A. (2013). Glial influence on the Blood Brain Barrier. *Glia*, *61*(12), 1939–1958. http://doi.org/10.1002/glia.22575

[4]Almutairi, M.M.A et al, Factors controlling permeability of the blood-brain barrier. *Cellular and Molecular Life Sciences 2015, 73 (1), 57-77.*