Modeling the Neurovascular Unit with Stem Cell Derived Endothelial Cells and Small Molecules Released in the Brain Parenchyma.

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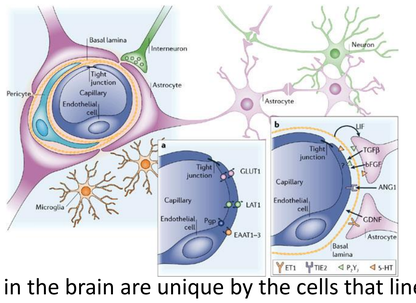
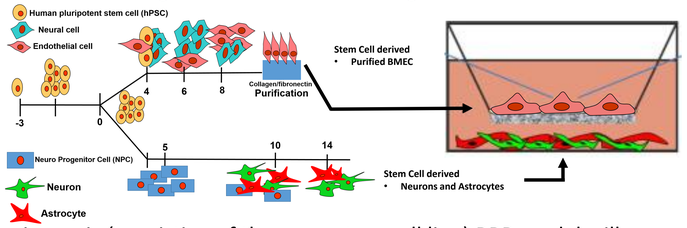
1. Background and Motivation



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.

Isogenic Stem Cell Modeling of the BBB



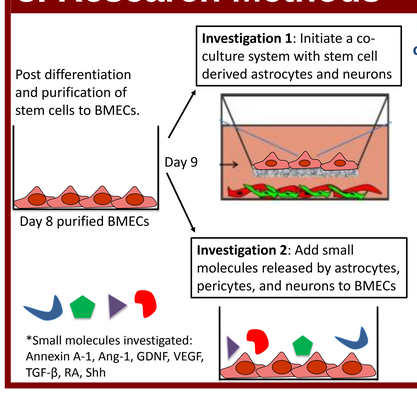
An isogenic (consisting of the same stem cell line) BBB model will eliminate discrepancies in cell origin, while allowing the limitless synthesis of BBB models. These models are then utilized for **drug screening**, and the understanding of **BBB development** and **neurological diseases.**

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 secreted by astrocytes, pericytes and neurons or the initiation of a co-culture system to in vitro systems enhance BBB properties in BMEC populations.

3. Research Methods



Explore the effect of a co-culture system or addition of small molecules on a BMEC population via:

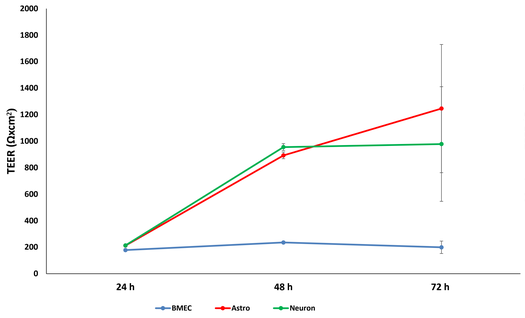
1. **Trans-endothelial resistance (TEER)**: An EVOM volt meter measures the electrical resistance across a layer of BMECs, revealing the tightness of the BBB, in Ω cm^2.
2. **Immuno-chemistry:** Primary anti-bodies, secondary anti-bodies, and fluorescent tags are used to reveal the presence of proteins specific to the BBB. The presence of proteins is qualified via microscopic imaging.

In vivo BMEC populations express unusually high TEER values (~6000 Ω cm^2), and greatly express the tight junctional proteins C5, Occludin, and ZO1.

4. Results

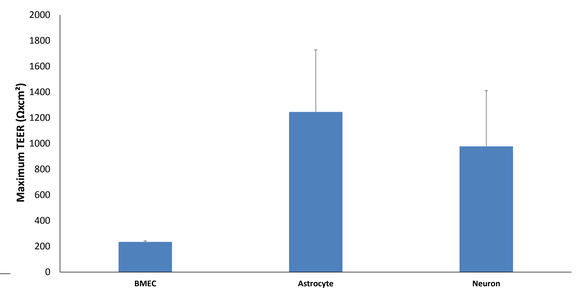
1. TEER of BMECs in a Co-Culture System with Astrocytes and Neurons

A. TEER Values from Various Time Points



Electrical resistance (TEER) of BMECs alone, BMECs in co-culture with astrocytes ,and BMECs in co-culture with neurons was measured 24, 48, and 72 hours after initiation of co-culture.

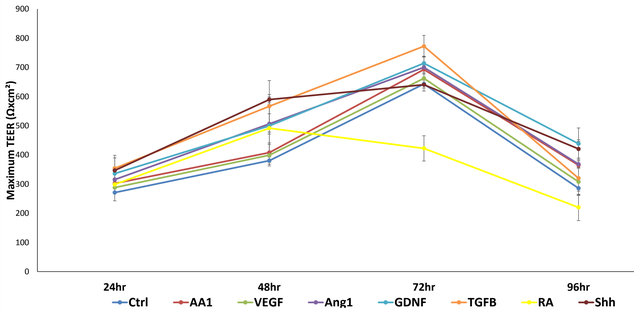
B. Maximum TEER



The maximum electrical resistance (TEER) reached by BMECs alone, BMECs in co-culture with astrocytes ,and BMECs in co-culture with neurons from the same experiment (Figure 1A) was then selected and graphed.

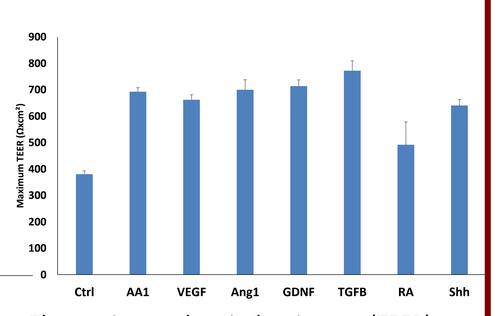
2. TEER of BMECs Treated with Small Molecules from Astrocytes and Neurons

A. TEER Values from Various Time Points



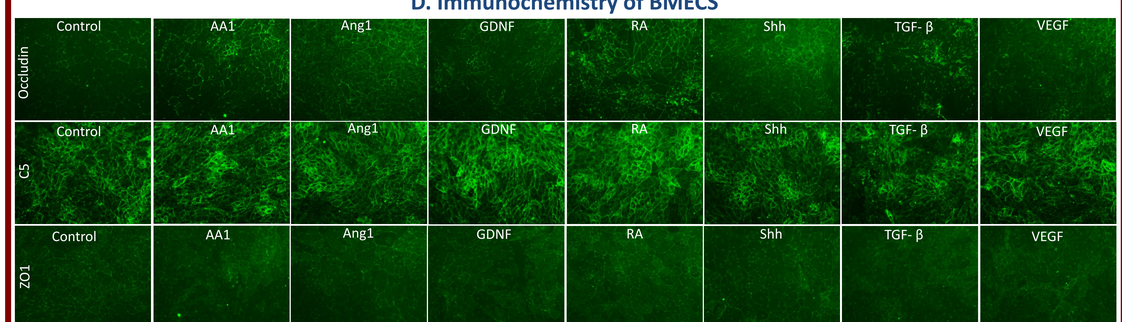
Electrical resistance (TEER) of BMECs alone and BMECs treated with various small molecules secreted by astrocytes, neurons and pericytes was measured 24, 48, 72, and 96 hours after the addition of the small molecules.

B. Maximum TEER



The maximum electrical resistance (TEER) reached by BMECs alone and BMECs treated with various small molecules from the same experiment (Figure 2A) was then selected and graphed.

D. Immunochemistry of BMECS



The presence of Occludin, C5, and ZO1, tight junctional proteins found in the BBB, is qualified via the use of immunochemistry. Images of BMEC populations cultured with and without small molecules released in the brain parenchyma were taken.

5. Conclusions

- The initiation of a co-culture system of BMECs with astrocytes and neurons result in considerably higher BBB properties as indicated by:
  - **Figure 1A** – BMECs in co-culture had higher TEER at 48 hour and 72 hour time points. Both time points had at least a three fold increase in TEER.
  - **Figure 1B** – Maximum TEER values for both BMECs co-cultured with astrocytes and neurons had at least a four fold increase compared to BMECs alone.
- The addition of small molecules released by astrocytes, pericytes, and neurons to BMEC populations increases BBB properties as indicated by:
  - **Figure 2A** – All BMEC populations treated with small molecules had higher TEER values than BMEC control populations without treatment at 48 hours. Five of the 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 2B** - BMECs treated with any small molecules reached higher maximum TEER values than BMECs without treatment.
  - **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.

6. Future Directions

- Inhibit the most effective small molecule pathways in BMEC populations co-cultured with astrocytes, pericytes and neurons. Observe changes in BBB properties of BMECs.
- Observe how blood cells and small molecules secreted by blood cells can affect BBB properties in BMEC populations.
- Observe how shear stress caused by blood flow can affect BBB properties in BMEC populations.

7. Acknowledgements

- Takeda Pharmaceuticals
- National Institute of Health
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8. References

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[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.