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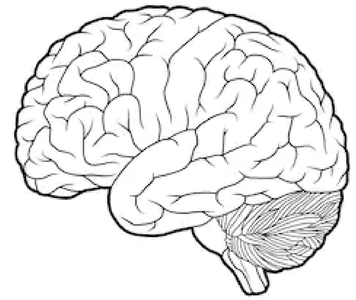


Introduction

The blood brain barrier is composed of specialized endothelial cells that function as a barrier for the central nervous system (CNS).[1] The barrier must be considered in drug development for CNS disorders, and impaired function is associated with neurological conditions such as Alzheimer's disease and stroke.[1]

The purpose of this experiment was to develop an *in vitro* model of the blood brain barrier that incorporated brain endothelial microvascular cells (BMEC) and co-cultures composed of neurons, astrocytes, and macrophages. All cell types were derived from human induced pluripotent stem cells (iPSC) with the BMEC differentiation protocol adapted from Stebbins et al. (2015). The neurons, astrocytes, and macrophages were differentiated separately in accordance to the protocols utilized[3,4,5], and then seeded together when the cultures reached maturity. A 12-well transwell plate format was used for the barrier model; the BMEC cultures were seeded in the transwell inserts and the co-cultures were seeded in the bottom wells. The inserts were coated with collagen and fibronectin, and a Matrigel ECM was used for the bottom wells. A transendothelial electrical resistance (TEER) assay was used as an investigation of barrier function. Barrier function was also evaluated with immunofluorescence by staining for ZO-1 tight junction markers, which are associated with endothelial monolayers. In addition, the model was used to investigate acute neuro-inflammatory responses. Inflammation was induced with the pro-inflammatory mediators poly I:C and LPS, and then barrier integrity was evaluated using the TEER assay. An *in vitro* model of the blood brain barrier could be a valuable tool to observe and assess neuro-inflammatory responses because a human stem cell model is more physiologically relevant.

Methods

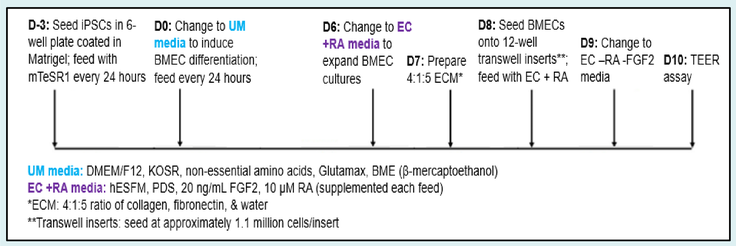


Figure 1. Protocol for differentiating BMEC cultures; adapted from Stebbins et al. (2015)

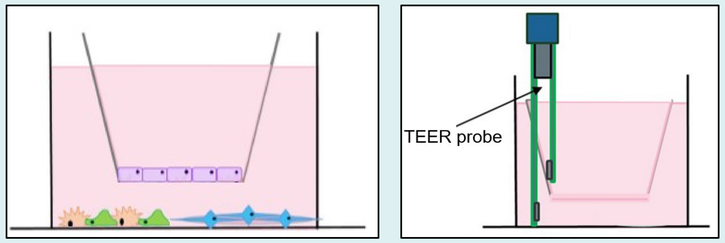
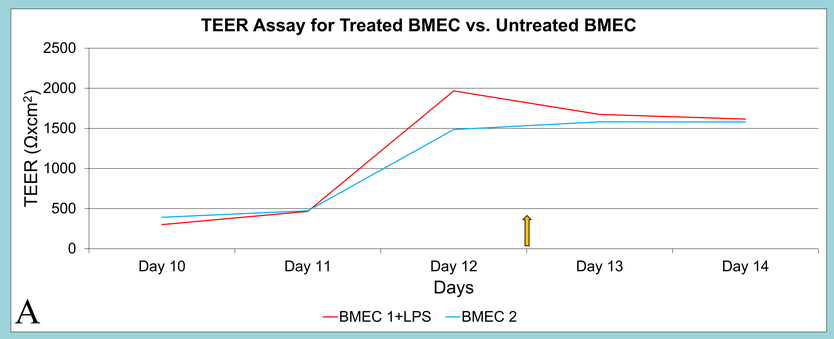


Figure 2. Diagram of co-cultures in transwell plate format and TEER assay procedure; adapted from Stone et al. (2019)

TEER Assay Analysis



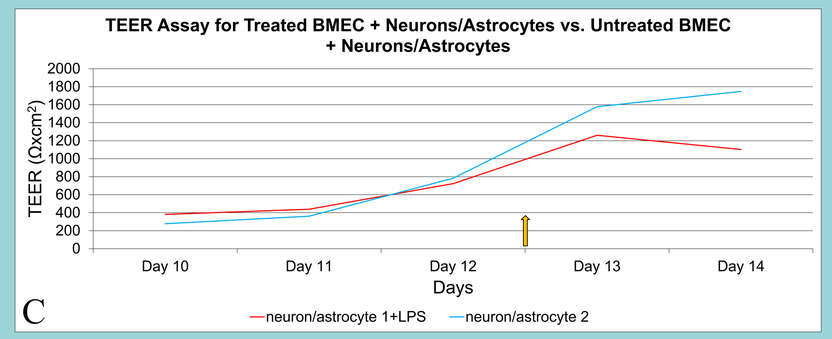
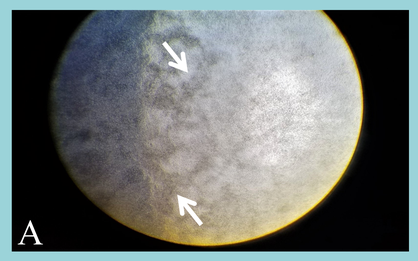
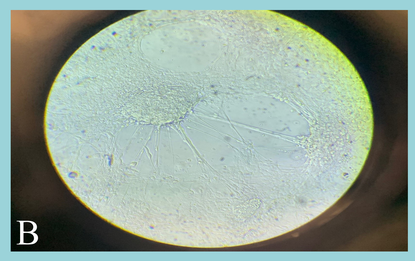


Figure 3. TEER assay analysis for treated and untreated cultures; TEER measurements were taken at 24-hour timepoints starting at Day 10, cultures were treated with LPS on Day 12 (indicated by arrow)
A. BMEC cultures in transwell inserts; no cultures in bottom wells
B. BMEC cultures in transwell inserts; neuron cultures in bottom wells
C. BMEC cultures in transwell inserts; neuron and astrocyte co-cultures in bottom wells

Cell Morphology





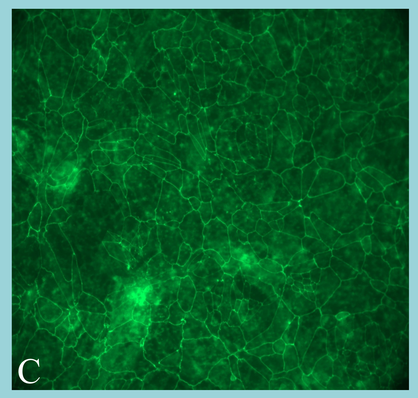


Figure 4.
A. Untreated BMEC Day 12 100x; arrows indicate cobblestone pattern associated with monolayers

B. iPSC-derived neurons 200x

C. Untreated BMEC Day 14 widefield 20x; stained with ZO-1 tight junction marker

Conclusions and Future Work

- Data analyzed from the TEER assay (Fig. 3) suggest that treatment with the inflammatory mediator LPS affected barrier integrity in comparison to the untreated cultures.
- BMEC cultures grown on a collagen-fibronectin ECM in the transwell inserts exhibited ZO-1 tight junction proteins in a "cobblestone" pattern (Fig. 4C), which is typical of endothelial monolayers.
- Optimization of the *in vitro* blood brain barrier model is ongoing with the future goal of utilizing additional cell types in the model that are also implicated in the blood brain barrier, as well as using other iPSC lines.
- Future projects may incorporate RT-PCR and confocal imaging to further investigate acute neuro-inflammation in the blood brain barrier model.

References

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Acknowledgements

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