Developing a Co-Culture Cell Model of the Blood-Brain Barrier to Investigate Alterations of Barrier Function in Alzheimer's Disease

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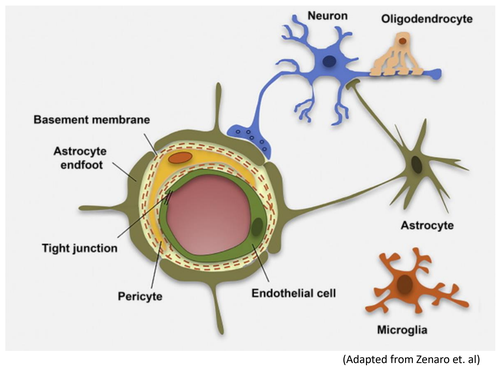
Introduction

- The blood-brain barrier (BBB) is a specialized structure that acts as an interface between the circulation and neural environment, and regulates movement of substances in and out of the brain.
- The barrier is comprised of vascular endothelial cells that work in concert with astrocytes and neurons of the brain.
- Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by chronic brain inflammation and pathological accumulation of amyloid beta peptide.
- Dysfunction of the BBB occurs in AD, but its exact causes and mechanisms remain incompletely understood.
- Presence of amyloid beta peptide in high amounts within the brain may disrupt BBB function, and is thought to be an integral player in AD pathogenesis.



(Adapted from Zenaro et. al)

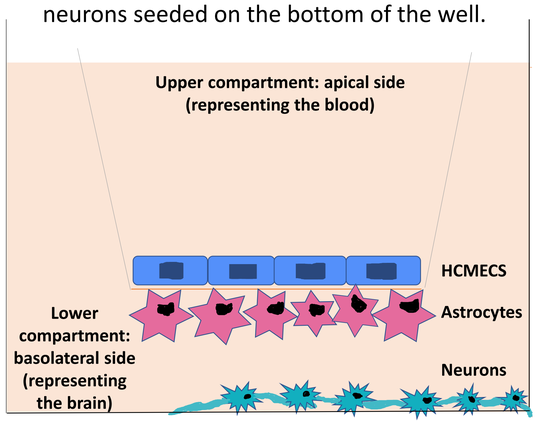
Strategy

Figure 1. Transwell Support System used for construction of this model:

Each well of the multi-well plate contains an insert that divides the well into upper and lower compartments

(Image from Corning Life Sciences)

Figure 2. BBB Model Overview
HCMECs are seeded on the apical (upper) side of the insert, with the astrocytes seeded on the basolateral (lower) side of the insert, and neurons seeded on the bottom of the well.



1.) The bottom of each insert in the lower compartment (the basolateral side) is coated with Poly-L-Lysine solution.

2.) Astrocytes are seeded onto the coated basolateral side of the inserts; they adhere and stabilize in astrocyte medium for 2-3 days.

3.) Once astrocytes are stable, HCMECs are seeded onto the apical side of the inserts (upper compartment); they adhere and stabilize in HCMEC medium for 2-3 days.

4.) Concurrently during steps 1-3, neurons are grown separately on the bottom of the wells in the lower compartment; they grow and stabilize for 4-6 days.

5.) Once all cells are stable, the inserts are combined with the wells so that the different cell types are in close physical contact.

6.) Cells acclimate together in the model for 2-3 days prior to experiments (Figure 2).

Goal

- Development of an *in vitro* co-culture cell model of the BBB to study how cells of the BBB alter their functions and interactions during AD.

Significance of the Model

- This model uses only human cells to closely mimic the physiology of the human BBB:
  - Human brain microvascular endothelial cells (hCMEC/D3 cell line)
  - Human astrocytes (primary cells)
  - Human neurons (MC65 cell line)
- The MC65 neuronal cell line utilized for this model can be induced to express amyloid beta peptides.
- This model will aid in our investigations of barrier alterations during AD by allowing us to study how the BBB functions in the presence of endogenous amyloid beta.

Progress

- Established stock cultures of all three cell types.
- Cultured astrocytes separately on the basolateral side of the inserts (Figure 3).
- Cultured HCMECs separately on the apical side of the inserts.
- Co-cultured HCMECs and astrocytes together on the inserts.
- Cultured neurons separately in the wells (Figure 4).

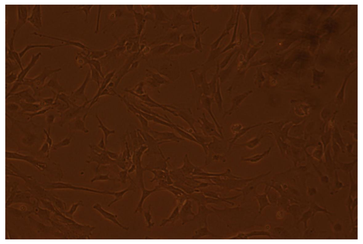


Figure 3. Astrocytes in culture

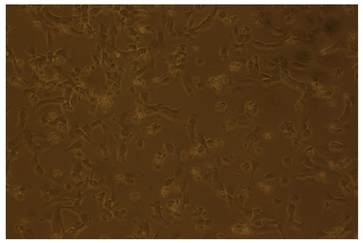


Figure 4. Neurons in culture

Future Directions

- We will assess robustness of the model by evaluating structural and functional barrier properties:
  - Immunofluorescent staining of BBB markers
  - Permeability to tracer molecules
  - Transendothelial electrical resistance measurements

Acknowledgements

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References

1. Zenaro E, et al. (2016). Neurobiology of Disease 107.
2. Stone N, et al. (2019). Frontiers in Cellular Neuroscience 13:230.