Design and Optimization of a Microfluidic System to Investigate Blood-Brain Barrier Dysfunction in Response to Intrinsic Cues of Alzheimer's Disease

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BACKGROUND

- Alzheimer's Disease (AD) impacts 6.5 million in the US[1] and is incurable despite its well-characterized pathology **(Fig. 1A)**.
- AD studies show junctional weakening[2,3] of the BBB that precedes development of amyloid plaques and tau tangles

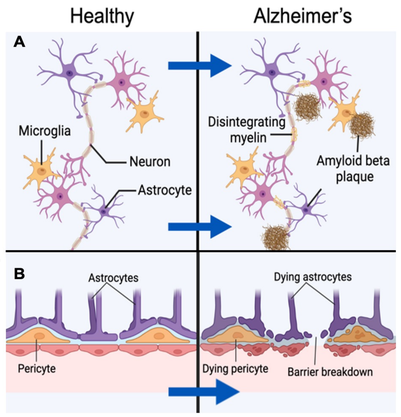


Figure. 1: Contrast in healthy and AD pathologies. A) Myelin disintegration and the ensuing development of Aβ plaques. **B)** BBB astrocyte/pericyte death and barrier breakdown.

- The mechanisms by which BBB progression contributes to AD is unknown because developing a model to control for the many cues of AD is difficult[4]

OBJECTIVES

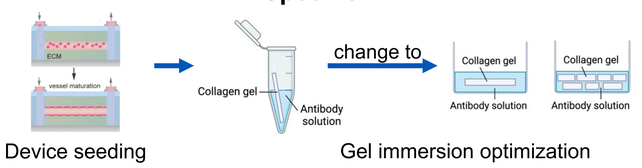
Specific Aim 1: Optimize the current 3D microfluidic system to be representative of AD brain microvessels
- Modify seeding density and alter antibody submersion

Specific Aim 2: Create a process to quantify changes in barrier function during AD progression
- Design a functional assay for Aβ40 and Aβ42 transport

Specific Aim 3: Assess BBB identity and changes in barrier function in response to PSEN M146V and APPswe familial AD mutations

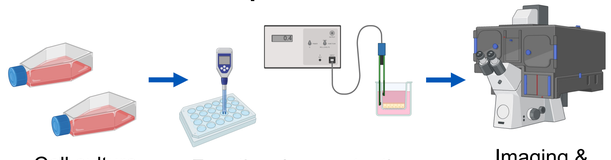
METHODS

Specific Aim 1



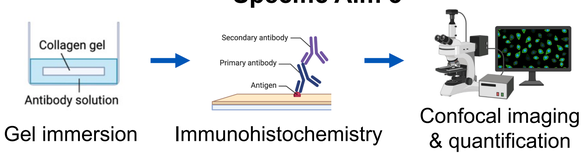
Device seeding — Gel immersion optimization

Specific Aim 2



Cell culture — Functional assay testing — Imaging & analysis

Specific Aim 3



Gel immersion — Immunohistochemistry — Confocal imaging & quantification

RESULTS

A. Foundational Testing

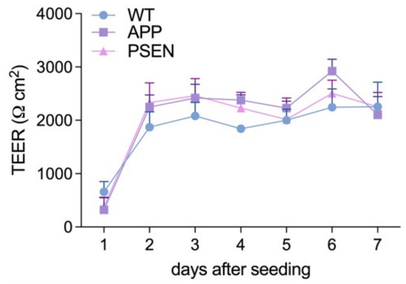


Figure 2. Transendothelial electrical resistance testing (TEER) of all cell lines to confirm endothelial identity and find baselines.

B. Immunohistochemistry

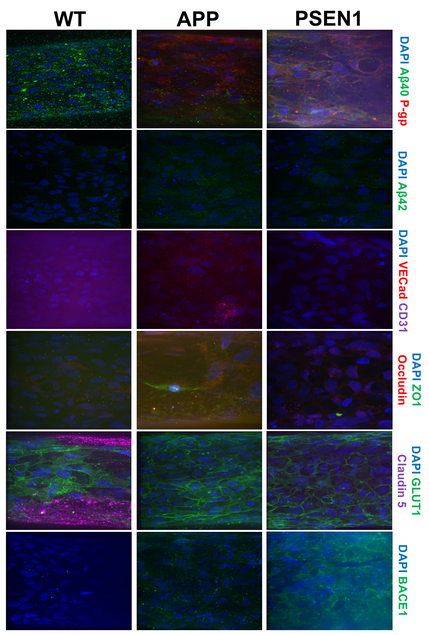


Figure 4. Representative images of maximum intensity z-stack projections of immunohistochemistry for nuclei (DAPI), junctional proteins (occludin, ZO-1), transport proteins (P-gp, GLUT1), endothelial identity markers (VE-cadherin, CD31), and AD-associated biomarkers (Aβ40, Aβ42, BACE1).

B. Optimization

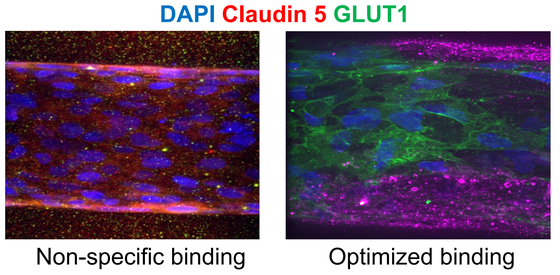


Figure 3. Modification of the gel immersion method during immunostaining replaced non-specific antibody fluorescence outside the microvessel (**A**) with targeted cell-antibody interactions (**B**).

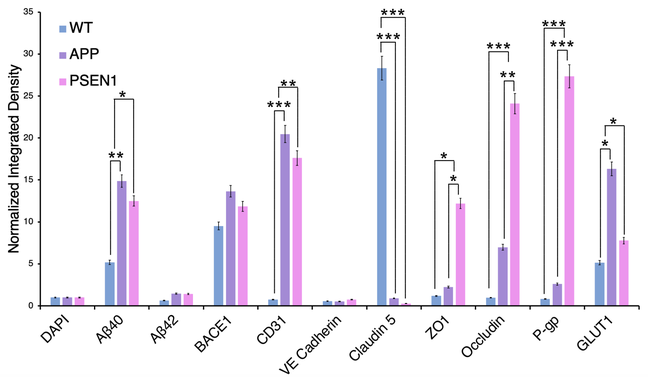


Figure 5. Immunohistochemistry fluorescence for each marker normalized to DAPI nuclear staining. (1-way ANOVA test; *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001)

- Fluorescence normalization of immunohistochemistry staining showed upregulation of CD31, GLUT1, P-gp, Aβ40, ZO-1, and Occludin in AD models compared to the WT control
- Downregulation in AD models was only seen for Claudin 5, with Aβ42 and VE-cadherin signaling appearing to be within error of fluorescence in WT control devices

C. Functional Assay Testing

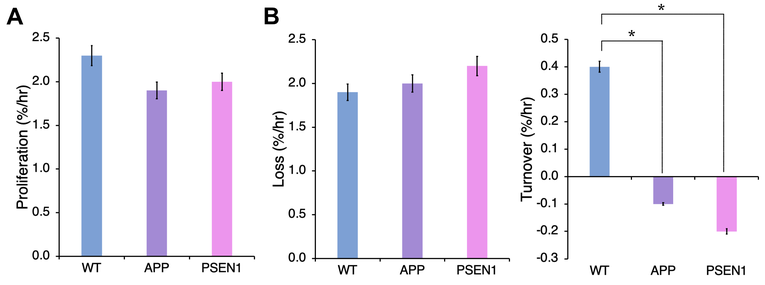


Figure 6. Cell turnover functional testing comparing proliferation (**A**), loss (**B**), and turnover (**C**) rates for all cell lines. (1-way ANOVA test; *p ≤ 0.05)

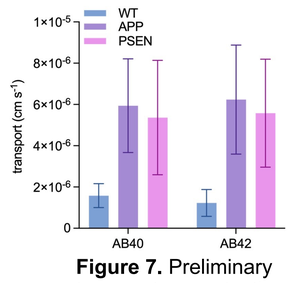
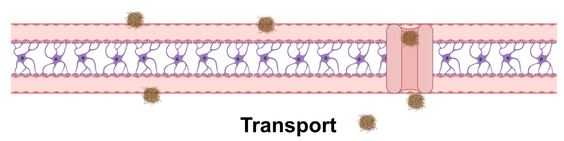


Figure 7. Preliminary transport assay testing.

CONCLUSIONS

- TEER testing established baselines for barrier function, **validating** the microfluidic model for AD studies with PSEN1 and APP mutations
- Modifying the collagen matrix immersion method during immunohistochemistry **optimized staining**
- Both APP and PSEN1 AD familial mutations showed **upregulation** of junctional and transport proteins relative to the WT control devices
- **CD31 upregulation** suggests changes to the endothelial identity of brain microvascular cells in AD
- Cell turnover functional assay testing revealed **reduced cell turnover** in AD
- Transport assays found an **increase in transport of Aβ40 and Aβ42**, prompting further study

FUTURE WORK



- Continue transport assay testing to build a dataset of 9 total replicates, with 3 for each condition
- The receptor for advanced glycation end products (RAGE) is implicated in Aβ transport, conduct transport assay testing with RAGE inhibition and quantify any changes across the 3 models
- Implement extrinsic cues of AD, such as oxidative stresses, into the model and characterize any changes

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