RISE RESEARCH INNOVATION SCHOLARSHIP ENTREPRENEURSHIP

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A Microfluidic Device to Study the Neurovascular Niche

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Opportunity

- Neural stem cells (NSCs) possess the abilities to self-renew and differentiate (Fig. 1A)
- The potential of NSCs as neurodegenerative disease therapeutics has motivated scientists to study the determinants of NSC fate
- In 2D cell culture, human NSCs eventually lose their self-renewal and differentiation capability
- In humans, self-renewing NSCs are found in close proximity to brain blood vessels in specialized microenvironments called neurovascular niches (Fig. 1B)
- These microvascular networks (MVNs) are composed of brain endothelial cells (BECs), pericytes (PCs), and astrocytes (ACs), and influence NSC behavior
- **Overarching hypothesis:** Replicating physiological features of the neurovascular niche *in vitro* will enhance human NSC self-renewal
- MVNs can be formed *in vitro*, but these microvessels are not perfused (Fig. 2)

Objectives:
1. Develop perfused brain MVNs *in vitro*
2. Introduce NSCs to MVNs to determine the influence on NSC behavior

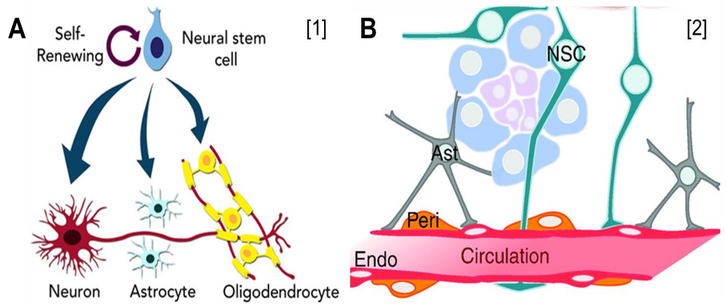

Figure 1. A) NSC can self-renew and differentiate. **B)** NSCs are maintained in neurovascular niches by MVNs composed of BECs, PCs, and ACs.

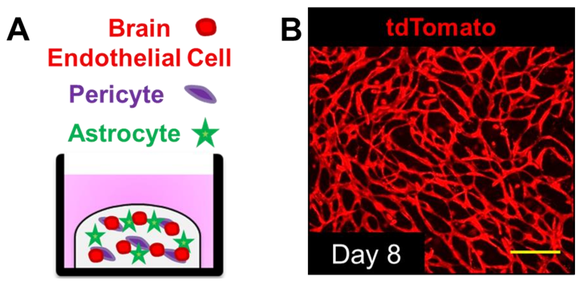

Figure 2. A) Diagram of BECs, PCs, and ACs in a 3D hydrogel. **B)** Non-perfused MVNs (red) formed by Day 8. Scale bar: 200µm.

Approach

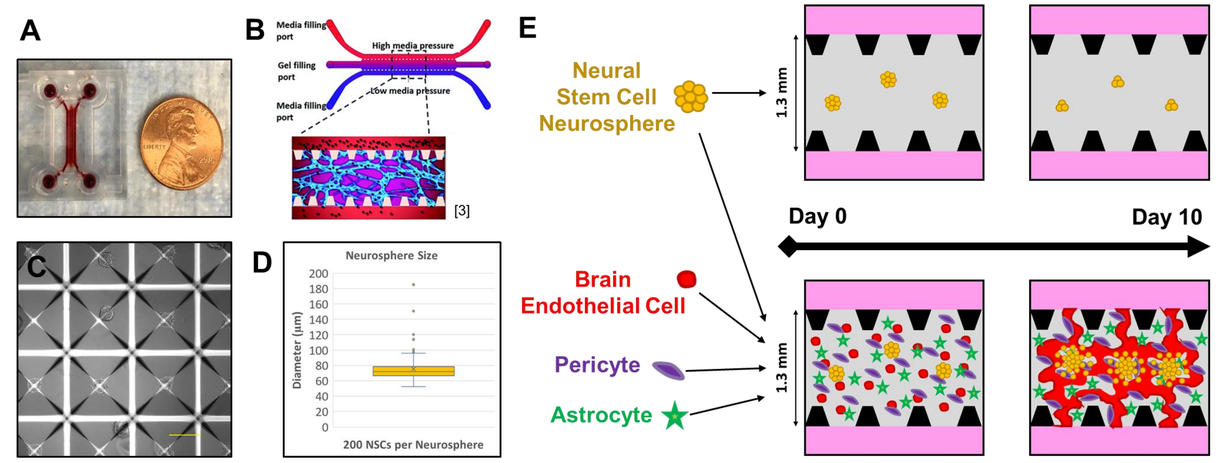

Figure 3. A) AIM Biotech microfluidic device (MFD) **B)** Diagram of MFD creating a hydrostatic pressure gradient to generate perfused MVNs. **C)** Phase image of neurospheres generated for experimentation. **D)** Measured neurosphere diameters. The average diameter was 75.6 µm (n=127). **E)** Experimental design schematic. On Day 0, NSC neurospheres were combined within a fibrin hydrogel with or without BECs (transfected with tdTomato), PCs, and ACs and injected into MFDs. On Day 10, fluorescent microbeads were perfused through MVNs to confirm anastomosis. Next, MFDs were immunostained for NG-2, GFAP, and Sox2 to identify PCs, ACs, and NSCs, respectively. The area of Sox2 fluorescent signal was used as a representation of neurosphere size.

Results

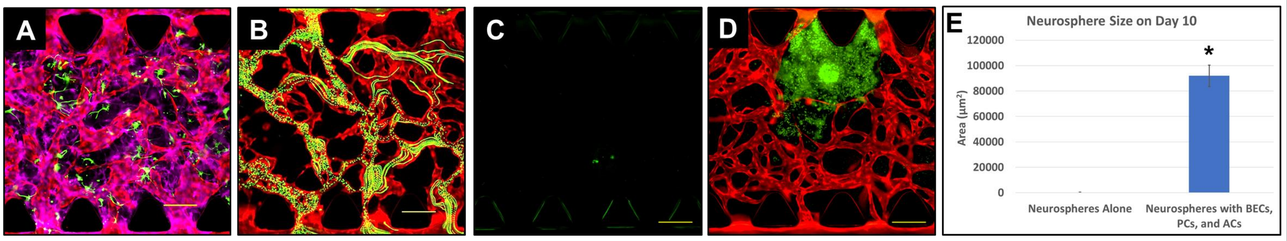

Figure 4. A) MVNs generated from BECs (red, tdTomato), PCs (violet, NG-2), and ACs (green, GFAP) on Day 10. PC coverage of microvessels was observed and AC endfeet extended to make contact with microvessels. **B)** Maximum intensity projection of fluorescent microbeads (green) flowing through the lumen of MVNs. Perfusion and anastomosis was achieved. On Day 10, neurospheres cultured alone **(C)** or with BECs, PCs, and ACs **(D)** were stained for Sox2 (green), a self-renewal marker. **E)** The area of Sox2 fluorescence signal was measured to approximate neurosphere size. Alone (n=64) and with other cell types (n=38), the average area of neurospheres on Day 10 was 242µm^2 and 91968µm^2, respectively. The enhanced area demonstrated that MVNs promoted NSC proliferation. '*' indicates p<0.001. Scale bars: 200µm.

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Impact

- **Impact:** *In vitro* model of the human neurovascular niche with self-renewing NSCs and perfused microvascular networks
- **Beneficiaries:** Neuroscientists studying NSC fate
- **Implementation:** Test NSC-related hypotheses by modifying experimental parameters to observe effect on NSC fate

Modify Parameters: Extracellular Matrix, Cell Type, Luminal Flow → Effect on NSCs: Self-Renewal, Differentiation, Proliferation

- Our MFD model will allow future investigators to test hypotheses relating to the determinants of human NSC fate in an *in vitro* platform
- This model uses human cells and has a high translatability to humans, reducing the need for animal models
- Perfused MVNs allow for NSC pharmacological studies that require drug delivery through microvessels
- Results will increase the therapeutic potential of NSCs as treatments for neurodegenerative diseases

References and Acknowledgements

1) L. Swayne, Frontiers, 2016.
2) L. Otsuki, Neuro. Of Dis., 2017.
3) Y. Li, RSC Adv., 2017

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