Development of a pressure-sensitive kinetic blood-brain-barrier Aβ clearance model

Introduction: Alzheimer's disease (AD) is a chronic neurodegenerative disease and the main cause of dementia worldwide. Recently discovered, the glymphatic system is a waste clearance pathway that exchanges the central nervous system (CNS)'s fluids which has been shown to be one of the main facilitators of the clearance of beta-amyloid (Aβ), one of the hallmark pathology of Alzheimer's disease (AD).[1,2] This system carries the soluble Aβ peptide to be discharged through the blood-brain barrier (BBB) by specialized transporters and has been shown to become impaired in ageing, leading to its toxic accumulation in the brain.[3] Furthermore, AD is often implicated with cardiovascular alterations affecting blood pressure and though their treatment has been correlated with reduced incidence of AD and slower cognitive declines in AD patients, their mechanistic relationship remains unclear.[4] Although, Aβ clearance has been mainly characterized *in vivo* in animal models, they also involve the interplay of other cellular and enzymatic clearance mechanisms.[4] Thus, a validated dynamic *in vitro* model would provide a platform to quantify and predict the yet unexplored effects of abnormal blood and cranial pressures in the BBB's clearance of Aβ which are critical to advance our understanding of how one of the most prevalent vascular changes correlated with AD, affects the main clearance gate protecting us from it. **The objective of this proposed research is to build a mathematical model from the kinetic characterization of pressure effects in the transport of Aβ through a microfluidic blood-brain-barrier device to predict the effects of pathologic changes in cerebral perfusion pressure in the clearance of Aβ from the brain.**

Approach: The proposed project will execute the following aims: **(1)** to adapt and validate current microfluidic BBB technology for this project, **(2)** to experimentally characterize the *in vitro* BBB's Aβ kinetic transport as a function of protein load and pressures, and **(3)** to develop a mathematic model of the Aβ efflux across the brain in health and in disease.

Aim 1: Microfluidic models have recently succeeded in recreating and mimicking the selective boundary properties of the BBB; one example includes the NeuroVascular Unit (NVU) model developed at Vanderbilt University by Dr. Wikswo's lab (Fig. 1), involving two distinct chambers representing the blood and the brain sides, separated by a three-dimensional and sequential culture of an immortalized line of the human neurovascular cells building the structure of a complete BBB.[5] Our device will build upon this model to allow for the controlled monitoring of pressure and flow rates while applying its ability to recreate the human BBB's heterogeneity and structural complexity. Firstly, the devise will be equipped with loading port valves, a variable pulsatile pump to mimic vascular perfusion pressure and a syringe pump to maintain an accurate pressure and simulate glymphatic flow on the brain chamber. The boundary's integrity under these dynamic conditions will be validated via transendothelial electrical resistance measurements (TEER) or alternatively via fluorescent microscopy to confirm proper endothelial tight junctions. Thereafter, pH, temperature, viscosity, and salt content on both compartment fluids will be matched to their human physiologic conditions, as protein behavior is sensitive to these factors.

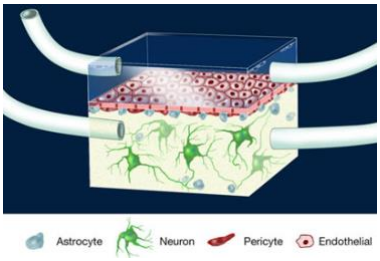

Fig. 1: Wikswo's lab blood-brain-barrier-on-a-chip device.[5]

Aim 2: Commercially-available $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ peptides, will be infused at different concentrations separately on the brain compartment, at different pressures under physiologic mean blood and glymphatic system flow rates to measure the BBB's transport rates at which Aβ leaves the brain compartment. Right after running through the devise the solution will be sampled and

Aβ measured by ELISA. After multiple trials, a differential mass balance on the two compartments will be used to calculate two diffusive rate constants, one for the Aβ going from the brain to the blood side, and a reverse rate to account for potential Aβ reuptake into the brain compartment, allowing for the overall rate of Aβ transport to be calculated for different Aβ concentrations and pressures. To validate the physiologic operation of both of the known Aβ transporters, LRP1 and RAGE, in the cells, their commercially-available natural ligands will be similarly run as a control, comparing their transport rates to their physiologic literature values.[6]

Aim 3: Using the calculated Aβ transport rates, statistics on R will be used to confirm the data's power and a mathematic model on MATLAB will be built to describe the Aβ efflux as a function of Aβ concentration and the pressures across both chambers, thus, quantitatively correlating the relationship between Aβ transport at different healthy and pathologic peptide burdens in the human brain, and under physiologic and abnormal pressures on both sides of the BBB. The developed model will be validated testing its predictions on the quantitative change in Aβ accumulation in the microfluidic device after sudden or chronic pressure changes likely to be produced by stroke, traumatic brain injuries, and cardiovascular diseases. This will produce a predictive computational model of the decrease in BBB-mediated Aβ clearance with ageing and disease.

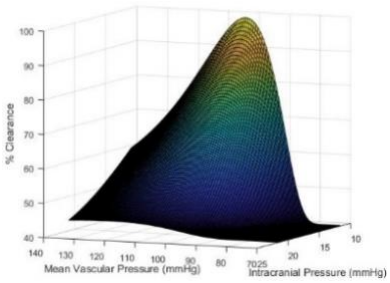

Fig. 2: Possible effects of pressure in Aβ clearance for a fixed Aβ load.

<u>Broader Impact:</u> The development of a mathematical model focused on cranial and blood pressure will allow for the estimation of Aβ accumulation in the brain due to reduced BBB clearance and predict the increased risk to acquire AD after people develop hypertension, hypotension, stroke, or traumatic brain injuries, when it is much easier to take preventative and therapeutic measures than trying to arrest an evolved AD later on. Furthermore, by considering Aβ concentrations, this adaptable predictive model will be able to incorporate future Aβ biomarkers' data for personalized medicine diagnostics using the patient's own biometrics. Finally, the improved microfluidic device will serve as a platform for characterizing the effects of abnormal blood pressures in the arterial pulse-driven glymphatic system, thus, describing its synergistic dysfunction in disease, and for studies describing the pharmacodynamics of brain-penetrant drugs in altered vascular perfusion or cranial pressure conditions.

Studying the link of the discussed highly prevalent clinical conditions has important repercussions in everyone's everyday lifestyle decisions. To generate awareness, I will use my continued participation at the Society of Hispanic Professional Engineer's national conferences and the blog I will manage during grad school to present and break down the significance of my findings to the general public, inspiring them to pursue similar projects in STEM.

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[1] Nedergaard, Maiken. "Garbage truck of the brain." *Science* 340.6140 (2013): 1529-1530.

[2] Tarasoff-Conway, Jenna M., et al. "Clearance systems in the brain - implications for Alzheimer disease." *Nature Reviews Neurology* 11.8 (2015): 457-470.

[3] Kress, Benjamin T., et al. "Impairment of paravascular clearance pathways in the aging brain." Annals of neurology 76.6 (2014): 845-861.

[4] Hamel, Edith, et al. "Neurovascular and cognitive failure in Alzheimer's disease: benefits of cardiovascular therapy." *Cellular and molecular neurobiology* 36.2 (2016): 219-232

[5] Brown, Jacquelyn A., et al. "Recreating blood-brain barrier physiology and structure on chip: A novel neurovascular microfluidic bioreactor." *Biomicrofluidics* 9.5 (2015): 054124.

[6] Deane, R., et al. "Clearance of amyloid-β peptide across the blood-brain barrier: implication for therapies in Alzheimer's disease". *CNS & Neurological Disorders-Drug Targets* 8.1 (2009): 16-30