Development of a Reproducible Endothelialized 3D Hydrogel Channel to Study Cerebral Cavernous Malformation

Prevalence of Cardiovascular Disease Among United States Citizens Living in Low Socioeconomic Status and how it is Systematically Influenced by Diet

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On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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Introduction

Cerebral cavernous malformations (CCMs) are the second most prevalent finding in magnetic resonance images (MRIs) of the brain (Caton & Shenoy, 2024). CCMs are irregularly mulberry-shaped clusters of enlarged blood vessels in the central nervous system, See Figure 1. CCMs can cause inflammation, seizures, headache, hemorrhage, and focal neurological deficits including stroke (National Institute of Neurological Disorders and Stroke, 2024).

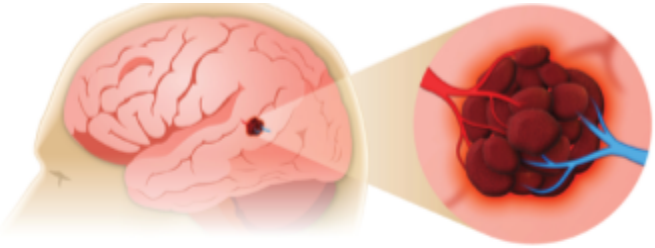

Figure 1, Cerebral Cavernous Malformation (Source: Morrison et. al, 2024)

This cardiovascular disease (CVD) affects the function of endothelial cells by disrupting their ability to form cell-to-cell junctions and increasing the endothelial layer's permeability (Awad & Polster, 2019). Endothelial cells (ECs), see Figure 2, make-up the monolayer lining between the bloodstream and its surrounding tissue, controlling nutrient exchange vital for proper life functioning. However, literature so far has only identified a link between loss-of-function in proteins encoded by three genes and the initial development of CCM (Sahoo et al., 1999). These three genes are the Krev Interaction Trapped-1 (KRIT-1), Malcavernin, and PDCD10. Fischer et. al (2013) hypothesized that CCM functions using Knudson's two-hit mechanism: the first "hit" causing CCM formation is a loss-of-function mutation of one of the three implicated genes, and the second "hit" is unknown.

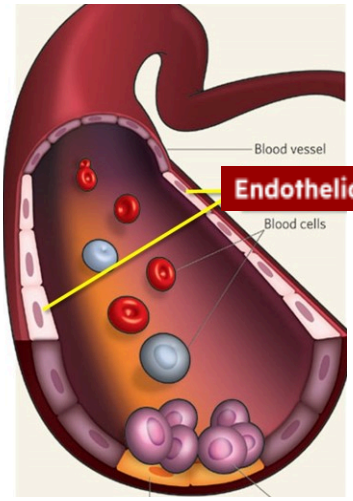

Figure 2, Endothelial Cells, (Source:Cell Applications Inc., 2024)

From blood flow along the wall of the vessel, atherosclerotic lesions have been shown to prevail (Caro et al., 1969). This may also be true for CCM lesions. CCM has previously been

studied in mouse models (in vivo). However, due to the variability between models, it is difficult to study endothelial cellular mechanisms and their response to shear stress in vivo (Maderna et al., 2022). This technical project aims to aid in identifying the role of shear stress in CCM formation in a reproducible model outside of a living organism (in vitro). This in vitro model is a perfusable, endothelial cell-lined, cylindrical channel in a 3D hydrogel that is reproducible and readily manufacturable. Future iterations of this model may introduce a silencing RNA (siRNA) to modify the expression of the gene KRIT-1 in ECs to create CCM-transfected ECs. These transfected cells' permeability measurements can be analyzed compared to our wild-type (non-altered) ECs' permeability to understand the role of shear stress in the causation of CCM lesions. While this model aims to advance our understanding of CCM at the cellular level, CVD extends beyond endothelial dysfunction to encompass broader environmental and lifestyle factors that shape public health outcomes.

The greatest pre-determinant of CVD is an individual's diet (Buttar et al.,2005), operating through multiple pathways that directly impact cardiovascular health. Diets high in saturated and trans fats, such as prepackaged, fast food, and frozen food meals, significantly raise low-density lipoprotein (LDL) "bad" cholesterol levels while decreasing beneficial high-density lipoproteins (HDL) "good" cholesterol (Madell and Nall 2023). This combination leads to atherosclerotic plaque build up, further increasing the risk of heart disease and strokes. While too much cholesterol can be harmful, it is essential for your body to function properly, playing crucial roles in producing substances that help you digest food. These dietary patterns and excessive sodium intake common in processed foods contribute to hypertension, inflammation, and metabolic disruption (Harvard, 2024).

Despite this clear connection between diet and cardiovascular health, the steady decline in CVD-related mortality has plateaued in the past decade. It remains the leading cause of death in the United States (Tsao et al., 2022). There appear to be regional patterns in CVD mortality, with lower socioeconomic areas of the U.S. consistently having higher rates compared to others (Center for Disease Control, 2022). Limited access to food, housing, transportation, education, healthcare, and medication - along with poor health literacy and risk factor management - contributes to increased CVD prevalence in low-income populations (Minhas et al., 2023). Research performed over thirty years ago and continually expanded upon demonstrated the significant impact of diet in preventing and reversing CVD (Ornish et al., 1990). Yet the most at-risk individuals in the U.S. do not engage in healthy eating habits (Kuźbicka & Rachoń, 2013), trapped in a complex web of socioeconomic barriers that limit their dietary choices.

I aim to develop an in vitro model of a cerebral blood vessel to study the role of endothelial shear stress in CCM formation. From a dietary and systemic perspective, this paper also aims to unpack and investigate the actors currently in place to counter this societal amelioration of CVD predeterminism and mortality in the U.S.

Technical Report

While conventional lab models confine cells to flat 2D surfaces, true anatomy tells a different story - blood vessels are intricate 3D highways where flow dynamics shape cellular behavior. Through the use of 3D biomaterials, such as biocompatible hydrogels, in vitro models that more accurately depict the true environment of tissue microvasculature functions can be constructed.

Hydrogels have emerged as a powerful platform for engineering tissues and organs, owing to their unique polymer networks and high water content (Lee et al., 2023). These versatile materials can be engineered to replicate the extracellular matrix, which regulates cell function, by incorporating cell-adhesion biochemical ligands. Ligands, like arginine-glycine-aspartate (RGD), are essentially hand holds for cells to facilitate cellular attachment and integration (Bellis, 2011). Our approach utilizes a Norbornene-Modified Hyaluronic Acid (NorHA) Ultra Violet (UV) crosslinked hydrogel. Through careful adjustment of norbornene polymer concentration, we can achieve mechanical softness properties matching those of brain tissue, specifically targeting between 600 -1100 Pascals (Pa) for storage modulus and 350 - 600 Pa for loss modulus (Fallenstein et al., 1969). The fabrication process involves combining NorHA with RGD peptides, casting the solution in custom 3D-printed molds, penetrating a 22-gauge needle, and initiating crosslinking via UV exposure to create the final structure. The needle mimics the average diameter of a cerebral blood vessel (<1mm). The UV exposure curing is a form of photocrosslinking that provides an inert and uncontaminated structure for seeded cells to proliferate and form a monolayer in the hydrogel.

Using Newton's law of viscosity, the necessary flow rate to mimic normal arterial shear stress (10 dyne/cm^2) will be determined (Gnasso et al., 2001). A fluid pump will then be used to induce shear stress and introduce flow through the channel in the gel.

Introducing a fluorescein isothiocyanate (FITC)-conjugated dextran dye in the solution to perform a permeability assay will provide the baseline measurement of the hydrogel's perfusability. This fabrication and measurement process will be iterated at least five times. The standard deviation of reproducibility (SDR) will be calculated based on the initial gathered data (Huang & Kacker, 2003). Once the SDR is attained in the iterated measurements we will have demonstrated reproducibility in the manufacturing process we have designed.

Bovine aortic endothelial cells (BAECs) will be cultured on culture-treated plastic in 2D until they reach an 80% confluency, to ensure a sufficient cell count to form a confluent monolayer. Although BAECs are harvested from cow heart cells, they are used because they are easily accessible, maintainable, and cost efficient. A peristaltic pump will be utilized to push wild-type (un-altered) BAECs in cell medium through the channel (see Figure 3), mimicking blood flow and inducing the formation of a confluent monolayer of EC's.

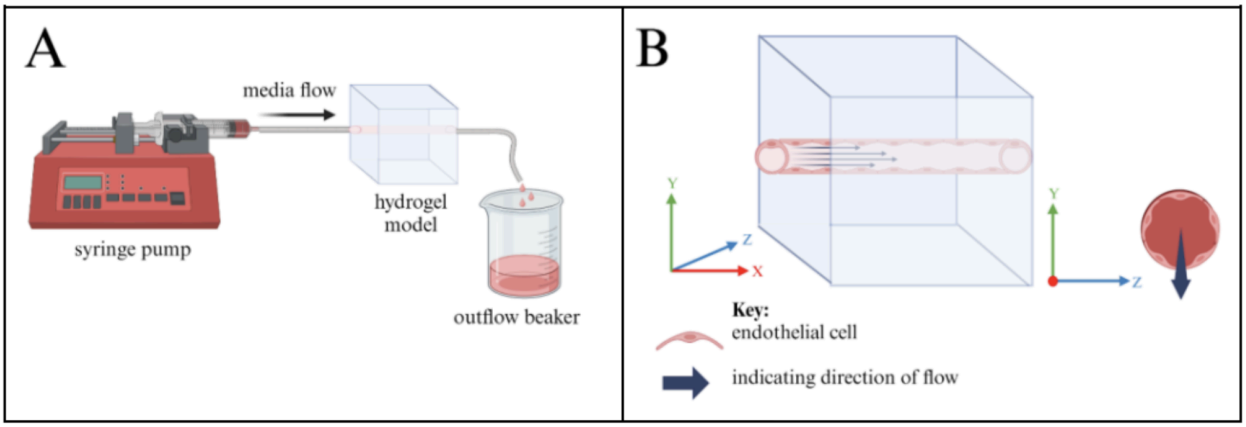

Figure 3.A) Schematic of media flow through hydrogel model. B) Zoom in on the hydrogel model with flow direction. (Source: Created with BioRender based on previous work by Lauren Porter (2023))

To validate that the BAECs have formed an endothelialized vessel, we will measure the distribution of proteins in the vessel using immunofluorescent staining and confocal imaging. We will measure the formation of cell monolayer by staining for the protein f-actin (van Geemen et al., 2014). Additionally, we will quantify the distribution of tight junction proteins between endothelial cells by staining for the protein claudin-1 (Morita et al., 1999). Using immunofluorescence microscopy, a cell proliferation count will be administered to ensure an integral and confluent endothelial layer has been formed 24-48hrs after cell flow is introduced. Once 80% confluency is observed, FITC-dextran dye will be introduced to perform a permeability assay. This process will be iterated at minimum five times and permeability measurements will demonstrate reproducibility through the calculated standard deviation of reproducibility. From here, the degree of permeability in unaltered wild-type EC channels will be compared against the decellularized channels. A lower permeability should be observed due to the presence of an endothelial lining.

Further development and iterations of this in vitro model hold promise to revolutionize our understanding and treatment of CVD as a whole. Beyond providing a platform for testing new drugs and personalized genetic therapies, this reproducible system offers a window into the fundamental cellular behaviors that underlie CVD pathology. Most significantly, by democratizing access to sophisticated cardiovascular research through its cost-effectiveness and reproducibility, this model could help bridge the gap between cutting-edge biomedical research and communities most affected by CVD. At the same time, a parallel challenge emerges. The stark reality that those most vulnerable to CVD often have the least access to these medical innovations. As researchers and clinicians develop increasingly sophisticated tools to study and

treat CVD, we must confront the complex sociotechnical networks that determine who ultimately benefits from these advances.

STS Report: Mapping the Networks of Cardiovascular Health Inequity

Through Bruno Latour's Actor-Network Theory (ANT), we can understand the prevalence of CVD in low-income communities. Not merely as a health disparity but as what Latour terms the "missing masses" - those overlooked nonhuman actors that fundamentally shape health outcomes. These missing masses in our food system include everything from supermarket locations and food prices to transportation infrastructure and agricultural policies. Through ANT's lens, these nonhuman elements emerge not as passive backdrops but as powerful actors, equally responsible for creating and maintaining health disparities as any human decision. Their agency in shaping health outcomes is not incidental but fundamental to understanding how CVD becomes concentrated in low-income communities.

The food system particularly exemplifies what Latour terms "obligatory passage points" - unavoidable channels through which actions must flow. With 31.6% of low-income households experiencing food insecurity and 19 million Americans living in food deserts (Meng 2021), ultra-processed foods become literal obligatory passage points, forcing certain dietary choices through their accessibility and affordability. When these foods comprise 58% of U.S. caloric intake (Harvard, 2024), it's not just about individual choice - it's about the network of agricultural subsidies, food distribution systems, and pricing structures that make these foods the most accessible option in low-income areas.

Technical mediation, a key concept in Latour's framework, explains how these nonhuman actors actively shape health outcomes rather than serving as passive barriers. For instance, when CDC data shows hypertension mortality rates ranging from 6.1 per 100,000 in Wyoming to 18.0

in Mississippi (Center for Disease Control 2022), this disparity emerges through what Latour calls "programs of action" - embedded scripts that guide human behavior through material constraints. A food desert isn't simply an absence of grocery stores; it's an active network of zoning laws, property values, and transportation systems working together to limit food choices.

Following ANT's emphasis on the agency of nonhuman actors, we see how these networks create what Lovasi et al. (2009) describe as "built environments" that disproportionately burden disadvantaged communities. For instance, in areas with limited access to fresh food, the presence of fast-food outlets becomes what Latour calls a "delegate" - a nonhuman actor enforcing certain behaviors through its very presence and accessibility. The estimated annual medical cost of obesity leading to cardiac-related health risks ($173 billion) represents the cumulative effect of these delegates systematically shaping dietary choices in low-income communities.

By acknowledging these interconnections through ANT's lens, we can better position research to address both the biological mechanisms of CVD and the complex sociotechnical networks that determine its prevalence. This theoretical framework demonstrates how health disparities are not simply social problems awaiting social solutions, but emerge from the complex interplay of material, technical, and social actors that must all be considered in any attempt at intervention.

Research Question and Methods

Recognizing that cardiovascular health disparities emerge from complex sociotechnical networks rather than isolated social factors, my research aims to map the critical intersections of food access, policy, and health outcomes across Virginia's diverse counties. Through systematic

comparison of counties across Virginia, I will trace how local, state, and federal policies interact to create disparate health outcomes. This state-level analysis provides a manageable yet comprehensive scope for understanding how ANT's concepts manifest in real-world health outcomes.

The methodology employs ANT as both a theoretical framework and analytical tool to trace the complex web of relationships between policies, institutions, and health statistics. Through systematic literature review, I will map how federal and state policies interact with county-based food environments across Virginia. By correlating CDC cardiovascular mortality data with USDA food insecurity mapping at the county level, I will identify how these policy networks materially affect the prevalence of health risks. The analysis will extend to examining county-level food assistance programs, local zoning laws affecting food retail distribution, and school lunch programs and their nutrition standards.

Geographic analysis will focus on comparing urban and rural county health outcomes, while economic structure review will examine county-by-county food pricing disparities and transportation infrastructure's impact on food access. Special attention will be paid to local agricultural subsidies and food retail distribution patterns, following ANT's emphasis on how nonhuman actors mediate social outcomes. Through this methodological approach, I aim to move beyond simply documenting disparities to understanding how networks of policies, practices, and infrastructure actively maintain them, particularly focusing on how ultra-processed foods become dominant actors in low-income food networks through pricing policies and marketing strategies.

Conclusion

This research bridges two critical aspects of CVD: the development of a reproducible in vitro CCM model and the investigation of socioeconomic networks that perpetuate CVD prevalence in low-income communities. The technical deliverable—a perfusable, endothelialized 3D hydrogel channel—will provide researchers with an accessible, cost-effective platform to study CCM formation and test potential treatments. This model's reproducibility and affordability could democratize cardiovascular research, making it more accessible to a broader range of institutions serving diverse communities.

The STS deliverable—a comprehensive analysis of how actor-networks influence cardiovascular health disparities—will illuminate the complex systems that maintain inequitable health outcomes. By mapping these networks through Latour's ANT framework, we can identify key leverage points where policy interventions could most effectively improve food access and cardiovascular health in low-income communities. Together, these deliverables work synergistically. As we develop more accessible research tools and better understand systemic barriers, we move closer to addressing both the biological mechanisms of CVD and the socioeconomic factors that determine its prevalence.

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