Modeling Cerebral Cavernous Malformation In Vitro

Addressing Racial and Ethnic Discrimination in the Health Care Field Through Training Medical Professionals

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By
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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 malformation (CCM) is a vascular disease that is characterized by the appearance of mulberry-shaped lesions throughout the brain and spinal cord. CCM affects the function of endothelial cells in cerebral blood vessels by increasing their permeability and disrupting their ability to form cell-to-cell junctions (Awad et al., 2019). CCM affects approximately 1 in every 200-250 individuals and can cause inflammation, seizures, headache, hemorrhage, and focal neurological deficits including stroke (National Institute of Neurological Disorders and Stroke, 2023).

There are two forms of CCM: familial CCM, which is genetically inherited, and sporadic CCM, which occurs due to a spontaneous genetic mutation. There are three genes that have been confidently implicated in the development of CCM and they are labeled: KRIT-1, Malcavernin, and PDCD10. Loss or mutation of any one of these three genes can cause CCM, yet the exact mechanisms involved remain unknown.

It has been hypothesized that CCM functions using Knudson's two-hit mechanism: the first “hit” causing CCM formation is a heterozygous, germline, loss-of-function mutation of one of the three implicated genes, and the second “hit” is unknown (Fischer et al., 2013). I hypothesize that the second “hit” is related to endothelial cell exposure to shear stress. It has been proven that hemodynamic shear stress significantly impacts arterial endothelial cell permeability, morphology, and function (Walsh et al., 2001), but there have been no findings linking shear stress to the initiation of CCM.

The majority of CCM cases are due to a loss-of-function mutation in the KRIT-1 gene, located on chromosome 7 (Sahoo et al., 1999). Although CCM can affect people of any race or ethnicity, it has been found that there is an increased frequency of genetically inherited CCM

among people of Mexican descent due to a founder mutation of the KRIT-1 gene (Rigamonti et al, 1988). By researching CCM for my capstone project, I aim to shed light on a disease that predominately affects an ethnic minority group.

In the United States (U.S.), institutionalized racism is embedded in a variety of systems that impact citizen's everyday lives (Braveman et al., 2022). One such system where racial prejudice pervades to this day is health care in the U.S. Studies have shown that racial and ethnic minorities in the U.S. face worse outcomes in various health metrics than white Americans due to a variety of factors (Hill et al., 2023). The relationship between racism and health care is notoriously complex, and is further complicated by environmental, economic, political, and historical influences in the U.S. medical system.

Researchers have recently begun to study how to dismantle the racism embedded in U.S. healthcare and how to reduce health care inequities across racial and ethnic groups. Strategies used to further these goals include implicit bias and communication training for physicians (Ashton et al., 2003) and increasing awareness and accessibility of medical services such as healthcare interpreters (Blay et al., 2018).

Through my technical capstone research, I aim to develop an *in vitro* model of a cerebral blood vessel to study the role of endothelial shear stress in CCM formation. Through my science, technology, and society (STS) research, I aim to analyze how to diminish inequities in U.S. healthcare faced by Mexican Americans using Emily Benfer's health justice framework (Benfer, 2015).

Technical Topic

CCM has previously been studied in mouse models and in human cadavers, but it is exceedingly difficult to study cellular mechanisms and cell response to shear stress in vivo

(Maderna et al., 2022). I aim to develop an in vitro model of a cerebral blood vessel to elucidate the role of endothelial shear stress as the second "hit" in the causation of CCM. Traditional in vitro models culture cells on a two-dimensional (2D) surface, but flow across a 2D surface is vastly different from how blood flow occurs throughout vasculature in the body in three dimensions (3D). To model hemodynamic shear stress on endothelial cells as realistically as possible, I will develop a 3D model of a cerebral blood vessel using a bulk hydrogel as a scaffold.

Hydrogel scaffolds have been widely researched as a basis for manufacturing engineered tissues, organs, and blood vessels in 3D (Lee & Mooney, 2001). Hydrogels are solid, polymeric networks with high water content with highly customizable properties. A hydrogel scaffold can mimic the extracellular matrix (ECM) by providing support for cells, and with the addition of biochemical ligands, such as arginine-glycine-aspartate (RGD), cells can adhere directly to a hydrogel (Bellis, 2011). I plan to use hyaluronic acid (HA) crosslinked with added norbornene groups (known as norHA) as the polymer for my hydrogel. By modifying the weight percentage of the polymer solution, I can tune the mechanical properties of the hydrogel to mimic the natural stiffness of brain tissue, approximately 1000 Pa (Yin et al., 2018). I will make the NorHA solution with added RGD, pour into a custom 3D printed mold around a 22-gauge needle, and crosslink the hydrogel to form a solid using ultraviolet (UV) light.

To incorporate cells into my model, I will culture bovine aortic endothelial cells (BAECs) on culture-treated plastic in 2D until they reach the desired density to create a monolayer across the model vessel. I then will passage the BAECs, load them into a syringe, and inject them into the vessel. I will rotate the vessel 360° for two hours at a rate of 8 rotations per minute, allowing for BAECs to adhere to the walls of the vessel. I will then perfuse the channel with cell medium

using a peristaltic pump and tubing. Using Newton's law of viscosity, I can calculate the necessary flow rate needed to mimic normal arterial shear stress (10 dyne/cm^2) (Gnasso et al., 2001).

To validate that the BAECs have formed an endothelialized vessel, I will measure the distribution of proteins in the vessel using immunofluorescent staining and confocal imaging. I will measure the formation of cell cytoskeleton formation by staining for the protein f-actin (van Geemen et al., 2014) and I will measure the distribution of tight junction proteins between endothelial cells by staining for the protein claudin-1 (Morita et al., 1999).

Once I have established a 3D, perfusable, in vitro model of an endothelialized cerebral blood vessel, I will genetically model CCM by using silencing ribonucleic acid (siRNA) to knock down the expression of KRIT-1 in BAECs. I will then use western blotting and sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to compare the concentrations of KRIT-1 proteins in transfected and wild type BAECs.

When I have confirmed that transfected cells do not express KRIT-1, I can seed them into the hydrogel model under identical conditions to wild-type BAECs. I will compare the permeability of transfected cells with shear stress exposure, transfected cells with no shear stress exposure, wild type cells with shear stress exposure, and wild type cells with no shear stress exposure using a fluorescein isothiocyanate (FITC)-conjugated dextran permeability assay (Pauty et al., 2017). By creating a 3D, perfusable artificial blood vessel that is lined with a monolayer of endothelial cells, I can study the role of endothelial shear stress in the onset of CCM *in vitro,* reducing reliance on animal or human testing.

STS Topic

In the United States, there are significant disparities in healthcare across racial and ethnic groups, with racial and ethnic minorities consistently faring worse than their white counterparts across a variety of metrics of health (Hill et al., 2023). One study found 24% of Latinx Americans self-reported their health as "poor" or "fair" in a survey compared to only 14% of white Americans (Baumgartner et al., 2021). In addition, Latinx Americans have the highest uninsured rates of any racial or ethnic group in the United States (Ramos, 2022).

Research on racial and ethnic health inequities has increased dramatically in the past 3 years. A search of the phrase "health inequity" in the database Web of Science returns 19,633 results, with 56.955% of results being published from 2020 to 2023. To study how to combat these inequities, I will use Emily Benfer's health justice framework (Benfer, 2015). This framework has three main arguments: (I) there are a variety of social, economic, and environmental determinants that significantly affect an individual's health, (II) there is an inextricable link between the U.S. legal system and U.S. citizens' health, and (III) healthcare workers have a responsibility to work towards achieving health justice to improve the overall health of U.S. citizens.

First, Benfer (2015) asserts that an individual's health is a result of a combination of environmental, economic, and political factors, not just one's genetics. Most prominently, minorities and people living in low-income communities face the most severe negative health effects. Second, Benfer discusses the use of legal regulations that both intentionally and unintentionally negatively impact minority health. One example of an unintentional legal measure negatively affecting an individual's health is nuisance ordinances. Nuisance ordinances are a type of third-party policing law that typically call for the legal eviction of tenants who call emergency services (such as 911) over three times per month. Benfer cites a study that found that

approximately 33% of nuisance citations were generated by domestic violence (Desmond & Valdez, 2012). Domestic violence is obviously detrimental to an individual's health, and by penalizing citizens for calling 911 with threat of eviction, the U.S. legal system prevents individuals' from accessing necessary resources for health treatment. An example of an intentional legal measure negatively affecting an individual's health is U.S. eugenic sterilization laws that allowed for the legal compulsory sterilization of individuals deemed "unfit" for reproduction (Lira & Stern, 2014). These laws did not specify entire racial groups for sterilization, but rather authorized medical professionals in hospitals and mental institutions to sterilize patients classified as "feebleminded" or with conditions "thought likely to be transmitted to descendants" (Lira & Stern, 2014).

A study of over 17,000 forms recommending sterilization of patients between 1920 and 1945 in California showed that Latinx patients were disproportionately subject to eugenic sterilization, particularly those of Mexican descent (Novak et al., 2018). The study found Latinx men were at 23% greater risk of sterilization than non-Latinx men and Latinx women were at 59% greater risk of sterilization than non-Latinx women. The study argues that the reason for this disproportionately high rate of sterilization among Latinx people was due to racial biases against them. The study also asserts that during this time period, Mexican American women were stereotyped as "hyper-fertile," poor mothers, criminally inclined, and sexually deviant.

The final argument of the health justice framework is that all people should have a fair opportunity to attain their full health potential. The health justice framework establishes a set of four tenets for establishing health justice in the United States: (I) the U.S. government must develop and enforce legislation to protect citizens from known causes of negative health effects, (II) the U.S. government must amend or repeal laws that are known to adversely affect health

and must examine all potential health risks on the population as a result of enacting a new law, (III) U.S. society must make a conscious effort to end discrimination and racial biases, and (IV) the U.S. government must listen to and invest in communities that have been directly negatively affected by health inequities (Benfer, 2015).

Research Question and Methods

I aim to focus specifically on tenets III and IV of the health justice framework (Benfer, 2015) to ask the question: how can we train health care professionals to reduce racial and ethnic discrimination faced by Mexican Americans in the medical field in the U.S.?

I plan to answer this question by completing a literature review of published strategies for reducing racism in the medical field and using thematic coding to analyze common ideas throughout these strategies. I will specifically target studies focusing on Mexican American individuals in the United States.

In addition, I will conduct interviews with both healthcare professionals at the University of Virginia (UVA) Medical Center and Mexican Americans in the Charlottesville area who have received treatment through UVA Health. I will connect with the organization Sin Barreras to establish connections with local Mexican American individuals who have received medical care from UVA to be interviewed (*Sin Barreras*, 2023). Example questions for Mexican American patients include, "How was your experience communicating with physicians and other medical professionals at the UVA hospital?" and "Have you ever utilized UVA Medical Center's interpretation and translation services? What was your experience like?"

I will connect with healthcare professionals working at UVA Medical Center including physicians, nurses, hospital administration, social workers, and other employees in the healthcare industry by contacting the Chief Diversity and Community Engagement Officer for UVA health

(UVA Health, 2023). Example questions for medical professionals include, “what training did you receive towards addressing implicit bias and racial discrimination in medical school? What about in your training at the UVA hospital specifically?” and “How have you interacted with UVA hospital’s translation and interpretation services?”

By interviewing both patients and medical professionals, I hope to gain a more well-rounded perspective on what problems exist in racial and ethnic discrimination training in medical care. By specifically interviewing Mexican American patients, I hope to follow the fourth component of the health justice framework: we must listen to and invest in communities that have been directly negatively affected by health inequities (Benfer, 2015).

Conclusion

Through my technical capstone research, I aim to develop a 3D, perfusable, *in vitro* model of a cerebral blood vessel to study CCM formation. By developing an *in vitro* model, I aim to study the mechanisms involved with the onset of CCM without testing on animals or humans. Currently, treatment options for CCM are limited. Patients with CCM can manage specific symptoms with medication or have lesions surgically removed, but surgeries are not always successful (Mouchtouris et al., 2015). Understanding the cause of CCM is a step towards developing comprehensive treatments with higher success rates.

Through my STS research, I aim to investigate how to best train health care professionals to reduce racial and ethnic discrimination faced by Mexican Americans in the medical field. By conducting a literature review and interviewing medical professionals and Mexican American patients in the Charlottesville area, I aim to form a well-rounded approach towards decreasing racism in the medical field. I hope to create a more inclusive, accessible medical field for all patients, especially Mexican Americans suffering from CCM.

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