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Vascular Calcification Results in Smooth Muscle Cell Phenotypic Switch

Introduction

The build-up of mineral deposits in the arteries, known as vascular calcification, restricts blood flow and is a frequent complication of cardiac disease. Characterized by the hardening of arteries, the vascular calcification process is like that of bone formation, as the mineral deposition is attributed to osteoblast-like cells found in calcified arteries. The osteoblast-like cells are hypothesized to originate from different sources, including the smooth muscle cells of the artery wall and the nearby endothelial cells via endothelial to mesenchymal transition (EndMT), by undergoing a phenotypic switch [1]. In order to observe this phenotypic change, we have optimized a calcification model of both smooth muscle cells and endothelial cells and characterized the culture for different biomarkers. The cell makers include antigens specific to each cell type as well as markers expressed during the Wnt signaling cascade and BMP signaling, pathways that may be involved in activating calcification [2]. We hypothesize that during calcification, a phenotypic change of vascular smooth muscle cells and endothelial cells occurs, characterized by the upregulation of specific genes involved in Wnt and BMP signaling, such as RUNX2 and BMP2, and downregulation of their cell-type specific markers, such as CD31 and α-smooth muscle actin (αSMA).

Background

Previous studies performed in our lab tested control and calcified vascular smooth muscle cell cultures over time for calcium content and cell markers, using o-cresol-phthalein kit, PCR, and western blots. Within the calcification groups, calcium content was significantly increased and BMP2 and RUNX2 were upregulated, compared to the control groups (Figure 1). αSMA was also downregulated in calcified cells. These results displayed that the smooth muscle cells were undergoing a phenotypic switch. To build upon this conclusion, we sought to perform immunohistochemistry to confirm the results and to begin expanding experiments to endothelial cells.

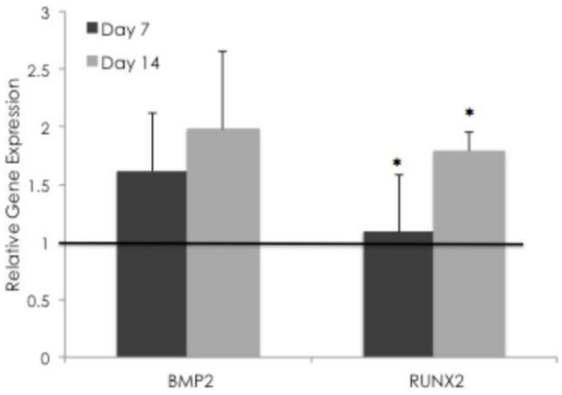


Figure 1. PCR for RunX2 and BMP2: This figure shows the results of the PCR analysis. PCR was run on 7 day and 14 day calcified VSMCs. The housekeeping gene was beta-2-microglobulin (B2MG).

Immunohistochemistry (IHC) is a staining method that utilizes the principle that antibodies will bind to specific antigens on the surface of a tissue to test that tissue for specific cell markers. A primary antibody binds to its specific antigen on the cell surface if present, and a secondary antibody binds to the primary and contains a fluorescent label that can be detected [3]. IHC can be used to observe if calcified smooth muscle cells are exhibiting less muscle cell markers and more related to the Wnt pathway and osteoblasts and if endothelial cells are exhibiting less endothelial and more mesenchymal markers.

The Wnt pathway being tested for is hypothesized to be a key regulator of vascular calcification. The canonical or β-catenin dependent Wnt transduction pathway is a signaling cascade that can regulate transcription and so cell transformation and proliferation. A Wnt ligand binds to Frizzled and LRP receptors in the cell membrane, activating the pathway. This causes the protein disheveled to be recruited. Disheveled inhibits the destruction complex that typically breaks down β-catenin. Because the destruction complex has been disrupted, β-catenin builds up in the cytoplasm and translocates to the nucleus where it affects transcription factors influencing regulation of transcription [4]. Among affected genes are RUNX2 and BMP2, which promote vascular calcification. Using IHC, we can monitor Wnt genes and proteins, such a β-catenin, as well as endothelial and muscle cell markers to see qualitatively the phenotypic changes being undergone by the cells.

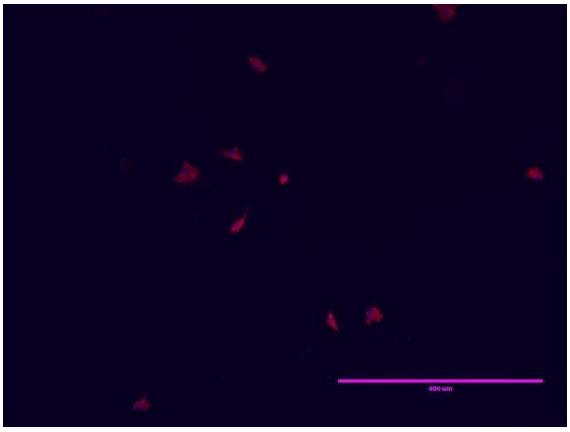
Materials and Methods

Human Vascular Smooth Muscle Cells (VSMCs) and Human Aortic Endothelial Cells (HAECs) were cultured in vitro on 6 well plates. The cells were split into four groups: 7 and 14 day control and 7 and 14 day calcification. The control groups were given normal media (made of Dulbecco's Modified Eagle Medium, 10% FBS, and 1% Pen/Strep). The calcification groups were given media composed of normal media and dibasic sodium phosphate (3 mM). After the incubation period, the cells were analyzed using IHC. Cells were fixed in cold ethanol for 30 minutes, intercellular stains were permeabilized with a triton solution for 10 minutes, and a blocking solution was placed on the cultures for 30 minutes. Plates were incubated with the primary antibody overnight and the secondary antibody for two hours the following day. Primary antibodies included αSMA, axin, β-catenin, CD31, and smooth muscle myosin heavy chain.

Following the antibody incubation period, the cells were stained with DAPI and then observed and imaged under the microscope.

Results and Discussion

The 7-day control and calcified groups did not grow efficiently in the short time frame (Figure 2). The cells were very small and virtually no difference was seen between the control and calcified groups. The fluorescence of each antibody was very inconsistent and subsequently inconclusive. The 14-day control and calcified groups were too confluent and over-grown that individual cells could not be detected, so results for this group were also inconclusive (Figure 3). These problems could have risen due to the seeding density of the cells when plated and concentration of the antibodies used.



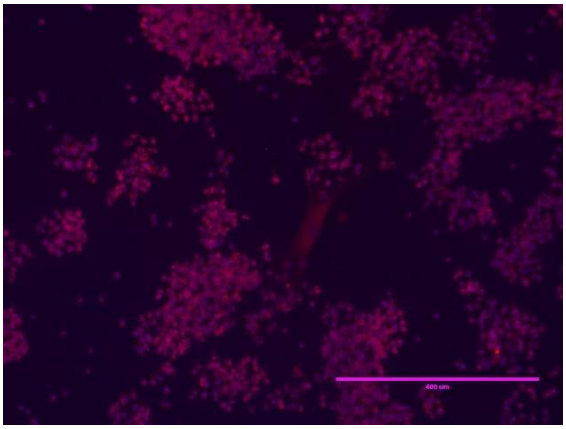


Figure 2. 7 day control HAECs Figure 3. 14 day control VSMCs

Conclusions

Based on the result of our previous studies, vascular smooth muscle cells undergo a phenotypic change during calcification, and endothelial cells also contribute to these changes via EndMT. Immunohistochemistry can contribute supplementary qualitative data to this conclusion. Additional testing will be done in finding an appropriate seeding density and antibody concentration to get viable results from immunohistochemistry. This will allow for better

observation of the phenotypic changes of the cells and a better understanding of how the Wnt pathway modulates calcification. Further experiments will be completed to study the interaction and cross talk between the endothelium and smooth muscle by establishing a co-culture of the cells to create a more accurate model of the vascular wall of arteries and study how the relationship between the two cell types promotes calcification. As our understanding of vascular calcification and its mechanisms increases so does the possibility of developing a target treatment to reduce calcification and improve blood flow in diseased patients.

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The Importance of Voter Education: How Much Are States Expecting by Local Election Officials?

Introduction

Democracies are built on 3 central pillars, participation, representation, and contestation. (Plotke 1997) At the heart of these pillars exists the platform that holds them all up, fair elections. Fair elections allow for citizens to participate in the selection of their representatives and have their vote count equally amongst their peers. In the United States elections are not regulated by the federal government, but by the States. This creates a unique environment for the study of election sciences where each state acts as an individual test subject for many different types of reforms.

Despite this, across the United States we see a distinct lack in a very "obvious" reforms, Voter Education and Voter Outreach. Voter Education only exists as a term in 21 state election codes, 6 states have the term outreach, and only 5 of these states have both voter education and voter outreach mentioned. This highlights an immediate issue, how can we expect citizens to participate in elections if they do not know how to?

In the United States The Voting Rights as of 1965 (VRA), the National Voter Registration Act (NVRA) , and the Help America Vote Act (HAVA), all worked to remove barriers to voting and to raise the ability to modernize the process of voter registration and voting. Only one of these acts, the Help America Vote Act (HAVA), has any guidelines about required information for voters as a form of voter education or outreach. These guidelines are also the only "education" put in place by most U.S states, given the nature of elections in the U.S as well, these guidelines are not very comprehensive and do not create a voter education program.

This leaves us with the question that if there is very little emphasis from the national government, and from state governments, how much are we expecting by local election officials? We see examples from many different localities where despite their state having none or very little information regarding voter education and outreach, where local election officials, schools and students, and local organizations have taken their municipalities voter education into their own hands.

For example in Marion County, Florida, their Supervisor of Elections, Wesley Wilcox, created a program called the "Annual High School Voter Registration Challenge" to encourage voter registration and civic participation across the county high schools. In 2018, the program saw a 479% increase in voter registration from the 2016 election and had participation from all 7 county high schools. Another program started by Conrad Weiser High School in Harrisburg Pennsylvania, exists in a similar framework where a social studies teacher and 4 senior students worked together to improve voter registration amongst the senior class to 92% of seniors being registered to vote. What is also important to note about these two states are that both Florida and Pennsylvania do not have a mention of voter education or of outreach, and we see that these duties have fallen to local officials.

In this project, we ask: How much emphasis do states place on voter education and outreach, and how are these concepts defined, given the absence of federal guidelines? This project seeks to create a comprehensive database of voter education and outreach policies across the United States of America and to decipher whether these types of programs impact voter participation in a positive manner. Currently, research on state voter education and outreach policies is non-existent, which suggests that there are potential information gaps between voters

and local election officials. This research is, therefore, very important and has tangible implications for voter engagement and participation. With this project, I seek to discover how states are involved in ensuring that voters have the opportunity to access elections and cast an informed vote.

Review of the Literature

Researchers of election reform find that voters experience challenges at the polls often due to confusion over complex election processes, such as when and how to register to vote, what constitutes an acceptable voter identification, as well as what to do when they show up to vote in the wrong polling location (Hasen 2005). Such challenges can create significant barriers for voters, as they may not allow them to successfully register to vote (Merivaki 2019), or cast a valid vote (Merivaki and Smith 2018). What is more, the literature on the administration of election documents very robustly that election officials often exercise their discretion when interacting with voters (Hasen 2005; Atkeson et al. 2014; White and Faller 2015). This can impact voters in an uneven manner, thus creating constitutional concerns over the equal protection under the law, or "one person, one vote".

States have been criticized when they implement policies that do not impact, or decrease voter participation, with the most notable example being voter identification laws. In Mississippi, which adopted a strict voter identification law in 2011, state election officials and news pundits claim that because the state invested in informing local election officials and voters on how to use and acquire a voter identification card, implementing voter id has been a "non event", and "voters expressed little, if any, inconvenience at the polls due to the new law."[1]

[1] Sid Salter. June 4, 2014. "Voter ID in election was non-event." The Clarion Ledger. Available at: https://www.clarionledger.com/story/opinion/columnists/2014/06/04/salter-voter-election-non-event/9958567/.

Despite the claims of the state election officials that the success of voter identification law in Mississippi has resulted from efficient dissemination of information, there is very little in the state's election code that outlines the role of election officials in educating voters about elections, including voter identification. The Mississippi election statutes and manuals involve information about the actual day of voting, but there is no information about how to disseminate to potential voters about their ballot structure, acceptable forms of identification or what to do if voters turn out to vote in the wrong polling location. This lack of statutory guidance can lead to discrepancies in how local election officials educate voters, with some localities likely being more engaged than others in order to ensure that voters are informed about voting in forthcoming elections. Given that local election officials are responsible for administering elections across the United States, their ability to interact with voters is directly related to their states' statutory and institutional support.

Without clear statutory expectations about what constitutes voter education and outreach, therefore, local election officials may not consider educating voters about every aspect of elections as part of their job description. If so, then prospective voters are very likely to experience the aforementioned challenges when they attempt to participate in a forthcoming election, have a negative voting experience and a potential aversion to vote in the future (Adona and Gronke 2019).

With little information regarding the importance of voter education and its effect on turnout in the United States, looking comparatively at voter education programs in other countries adds validity to the argument that voter education and outreach programs do positively affect voter turnout. In 1994, South Africa had their first national suffrage election, the United States also spent $35 million into South African voter education, $35 million more than was put into U.S voter education at the time. (McGinn 1996) At this time roughly 80% of the population had never voted

and voter rolls were basically nonexistent, and a large portion of the population lived in rural areas or were not very literate. Because of this, the Independent Election Commission (IEC) was formed with the mission to reach all citizens and inform them about the upcoming election and civic participation. The IEC was able to achieve this mission, reaching approximately 91% of the population through media, and a further 2-5% through their programs and programs with political parties. (Moller 1995)

Of new voters, 87% said that without the information they received they would not have been able to vote in the election, stating that the information they received was "easy to understand and useful". With this, the fact that people took personal time to locate voter education information and participate in mock elections, dramas, workshops, and meetings shows voter education and outreach programs were wanted by the populace. (Moller 1995) Further, less than 1% of all ballots were spoiled due to incorrect procedures in filling them out, this also highlights the education received by the citizens was sufficient and successful at its goal of promoting voter literacy. Alvarez and Hall (2006) also found that local officials across the UK engaged in a voter education effort to inform voters about voting by mail in 2001. The authors note that turnout was "almost three times higher than in recent by-elections," suggesting that voter education initiatives can have a tangible impact on participation.

Measuring engagement of local election officials

The terms "voter education" and "voter outreach" were selected for this project to represent the programs implemented by a state to improve voter literacy. This project utilizes a methodologically diverse approach, with the aim to empirically assess the impact of state-level voter education and outreach policies on voter registration and participation. Through collecting

each U.S state's Election Code it was quickly discovered that these terms are very sparse across the country. Not only were these terms not mentioned, but when they were mentioned they were not defined and would require the creation of a typology. Aside from constructing the voter education and outreach typology, the intended methodology is a maximum likelihood regression to systematically assess the impact of voter outreach and education on voter registration and turnout across and within the states. This caused the issue of "What will constitute voter education and voter outreach?" due to this, the typology used here represents the terms as the following:

Term	**Definition**
Voter Education	Any programs required by the State for the enrichment of voter literacy
Voter Outreach	programs required by the state to let their voting age populations know about their Education programs and increase access to these programs

Examples of Voter Education would include the program from Arizona, where the Secretary of State is required to create a candidate guidebook presenting the platform of each candidate in a non-partisan fashion.

Examples of Voter Outreach would include the program from Missouri, where local election officials are required to host public forums about their education programs. Other examples of outreach would also include having information available in every language spoken in an area as we see in California.

With this information, the states were ranked based on the quality of their education and outreach programs.

Voter Education and Outreach: How much do states expect?

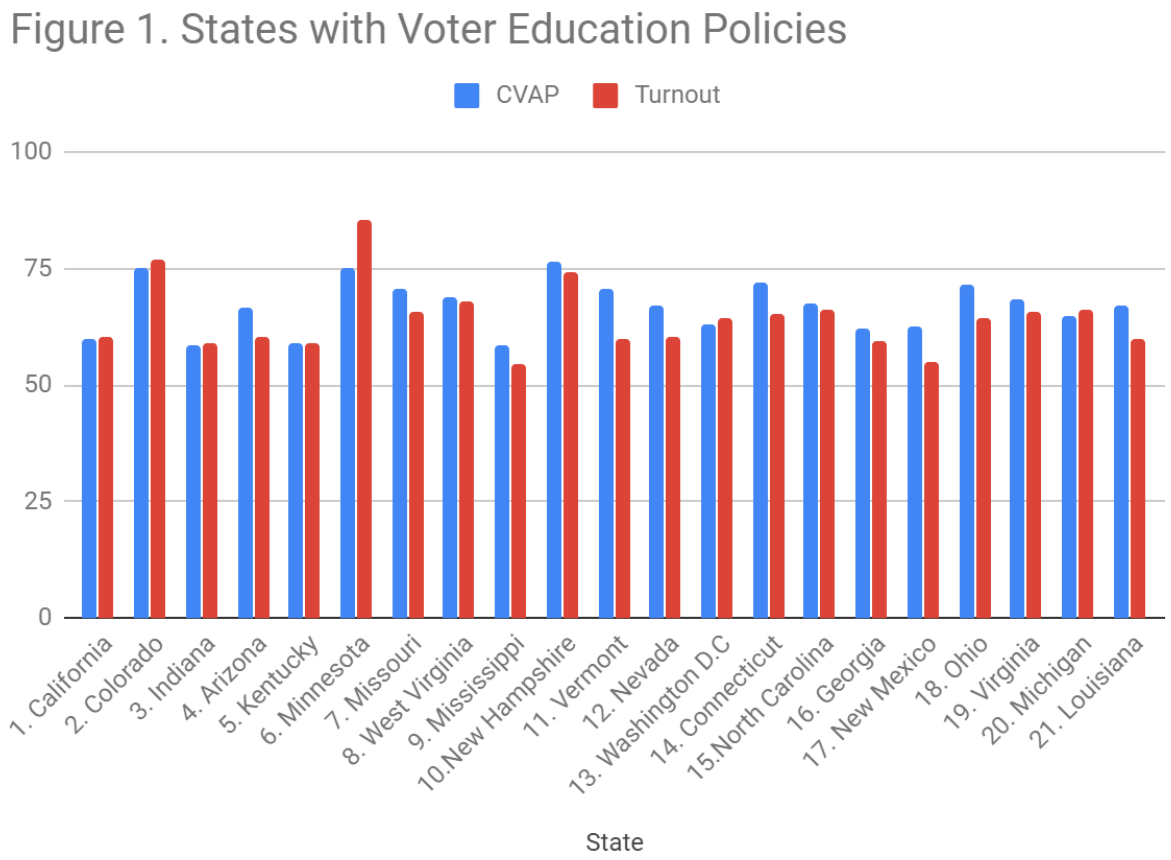

Figure 1 Shows the Citizen Voting Age Population (CVAP) and turnout of each state that has the term "voter education" in their Election Code. These rankings were based on how many people would be positively benefited from the reform, if it was more than the minimum uidelines of HAVA, and how much information was given. States 1-13 all have voter education policies outlined in HAVA and the addition of independent reforms. These states will be referred to as HAVA+ states. States 14-18 only have the term "voter education" and guidelines outlined in HAVA, and nothing else. These states will be referred to as HAVA states. States 19-21 only mention a "voter education fund" and no information about the guidelines of HAVA, how the fund will be allocated, and what constitutes a voter education program it could be used for.

California is the number one state, by election code, in requiring a comprehensive voter education program in each county. To start, the state requires each official to submit their county's voter education plan to the Secretary of State, and the plan must include the following: how media will be used to alert people to the date of the election, about the toll-free voter information hotline, how to vote by mail, community presence, method to identify language minority voters, and all information available on the county website. No other state requires counties to submit this much information to their Secretary of State. California also has sections requiring information about how funds will be used to fulfill the previous requirements as well.

In comparison, the number 2 state, Colorado lags far behind with only two required voter education programs. This program is that all institutions of higher learning that use an online service for students to sign up for classes must also offer the option to register to vote at the beginning of each semester when registering for classes. The second, as Colorado has a unique election reform allowing for ranked choice voting in local elections, requires that any local government that uses ranked choice voting will conduct a voter education and outreach campaign to familiarize citizens with ranked voting and any language required by the VRA.

This difference in requirements highlights the lack of a consistent theme on what voter education should be in a state, and the extent to which the state should inform voters. This information gap becomes even larger as you continue down the list to Virginia which simply states that voter education is the duty of the "registrar" and this person can be compensated for voter education.

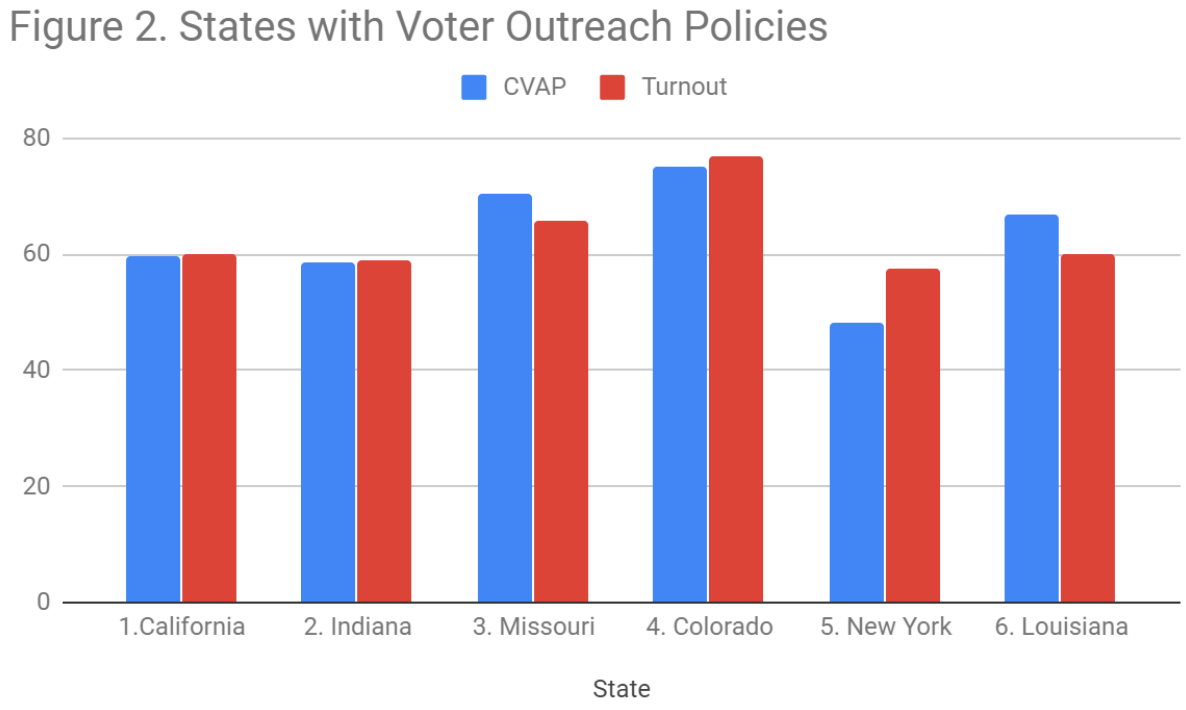

Figure 2 shows states that have the term "voter outreach" in their Election Code. These States are ranked in a similar way as voter education states namely: number of people impacted, and how much information there was about the required outreach program. These states are as well similarly distributed with California leading the charge of comprehensive voter outreach requirements and the number 2 state, in this case Indiana, lagging far behind and each state getting progressively lower.

California for voter outreach has the following requirements: the Secretary of State will create an education and outreach campaign informing voters about the new California Motor Voter Program, an outreach meeting that includes representatives, advocates, and other stakeholders representing each community requiring multiple language ballots and the disabled, at least one bilingual in-person voter education workshop for each minority language falling

under the VRA and for the disabled, a toll-free voter assistance hotline maintained by county officials, at least one public service announcement in the media for english speakers and VRA required languages, a social media strategy, and it all must be posted on the website of the Secretary of State and local election officials.

In comparison, the number 2 state Indiana, requires a voter education/outreach fund, and for the Secretary of State to provide individuals with disabilities the information necessary to Vote, including polling place accommodations and training of election officials, poll workers, and election volunteers on how to properly promote access and participation of disabled peoples in elections.

One thing of note is that 3 of the states with the highest voter turnout in the 2016 election also had voter education and outreach programs. Colorado, which had the highest voter turnout in 2016, (76.89%) was also one of only 6 states with both voter education and outreach programs. Many states also do not highlight whether education and outreach reforms are to be done by the Secretary of State or by a local election official. This lack of information and instructions for local officials is cause for concern.

To further explore what states are doing outside of what was codified into election statutes, a database containing each state's HAVA plan was created. This database contains each individual reform and then says whether each state has a specific reform. After HAVA was passed by the U.S Government each state was required to submit a plan of action on how they would implement HAVA. These plans give us a better look at what all states have planned to do in their states to increase voter education and outreach. Unfortunately just looking over these plans we have no way to guarantee if these reforms occurred in a state or not.

With this new information 40 of the 50 states submitted a plan that would involve education and outreach programs, though as seen in the previous examples, these programs were not created equally. Many states were still quite vague in their plan, and did not address who would hold the power to implement it, be it local election officials or the Secretary of State.

Discussion and implication for election reforms

This information shows that across the united states there is an information gap between what states are requiring, how they are getting this information out, and who should be distributing it, and that this gap is quite large. The next step is information we can only get from each individual county and it's election official, this will require calling counties and seeing what programs they specifically implement to discuss further how much states are expecting only from local officials. To further this it is important to see if these officials perceptions of voter education and outreach are aligned with their state's expectations. It is also of note that a few states who through earlier discussion should have fantastic turnout rates based purely on education and outreach do not, for example California. California is ranked ahead of all other states, but still does not have as high a turnout rate of many other states even with less programs. This can point to many things, a lack of infrastructure in the state to help local election officials know their duties, asking more of them then they can deliver, lack of resources to properly implement programs and more.

Continuing this project will require an in-depth look at where power is held in each state's electoral system and the individual reforms that separate each state and create distinctions in what each state would require in terms of voter education and outreach.

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Autonomous Vehicle Ability Assessment for Obstacles in Complex Terrains Through Sensor Fusion

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Abstract

Autonomous vehicles in complex terrains require a fusion of data from a variety of sensor technologies such as light detection and ranging sensors (LiDAR), radio detection and ranging sensors (RADAR), and visual imagery technologies to ensure that the vehicle can address possible obstacles distinct from its background. While most testing currently takes place on full-sized vehicles, on a small scale with only a few sensors a mobile platform can be more easily deployed for data collection. Testing this system will give insight to how algorithms may be implemented into full-sized vehicles and what additional sensor information may be required to fully implement off-road autonomy in real-world applications. A basic transport platform will mount numerous sensors of varying type so that it may gain a full field-of-view of its surroundings without visual interference while still protecting the sensing devices inside.

Introduction

As autonomous vehicles become more prolific there is always a need for more development regarding object detection so that collisions may be reduced. While there is currently a large focus on autonomous sensing regarding pathfinding and avoidance in the road setting, overlanding autonomous sensing has been much less developed outside of military applications. These more complex terrains require sensing that will need many more dedicated resources so that effective, safe paths may be taken. Generally, obstacles will be more varied and have locations or dimensions that are not easily identified in autonomous systems built exclusively for road-driving vehicles, such as a having a greater need for positive and negative vertical components to sensing and having no preexisting defined path to center the vehicle. Due to this, in addition to standard LiDAR, RADAR, imaging, and other sensing technologies already in use on autonomous vehicles today more of these technologies will likely need to be employed to cooperatively in new ways to provide the best mapping of obstacles possible in a complex environment. However, implementing these complex sensing systems into a car or utility vehicle can be costly due to both the ownership of the vehicle and the fitting of electronics systems. Instead of going directly to a full-sized implementation this project has aimed to create an intermediate step to allow for initial testing on a smaller scale. This scale of roughly 1:10 brings the advantages of greatly reduced costs and increased manufacturability while not necessarily having to inhibit the use of full-sized sensors meant for standard autonomous vehicles.

Method

The approach to implementing and testing sensor fusion in off-road environments was to follow the path of construction, initial tests, and then final data collection. Construction here is not only a means of making the mobile platform to mount the electronics but also independently testing systems as they are added. The first stage would be making the solid frame for the vehicle so that accurate sizes were available prior to mounting electronics. Electronics would then be decided upon, being the sensors, drive system, and power system. Each of these four sub-systems would all be independently tested and at least be partially implemented prior to being fully incorporated together. Following the initial construction and testing, the completed system is then to be tested

in a simple, controlled environment. This will involve a room with simple geometric obstacles. In this room, extensive tests can be conducted that will help calibrate the sensors and prepare the algorithms required for path planning and mapping. Figure 1.1 shows an example of what the resulting output of a semi-complex map would look like [1]. When the platform is found to be adequately prepared it will then be moved to testing in outdoor environments, the destination of the system. Here is where finalized data collections will occur for any research purposes required.

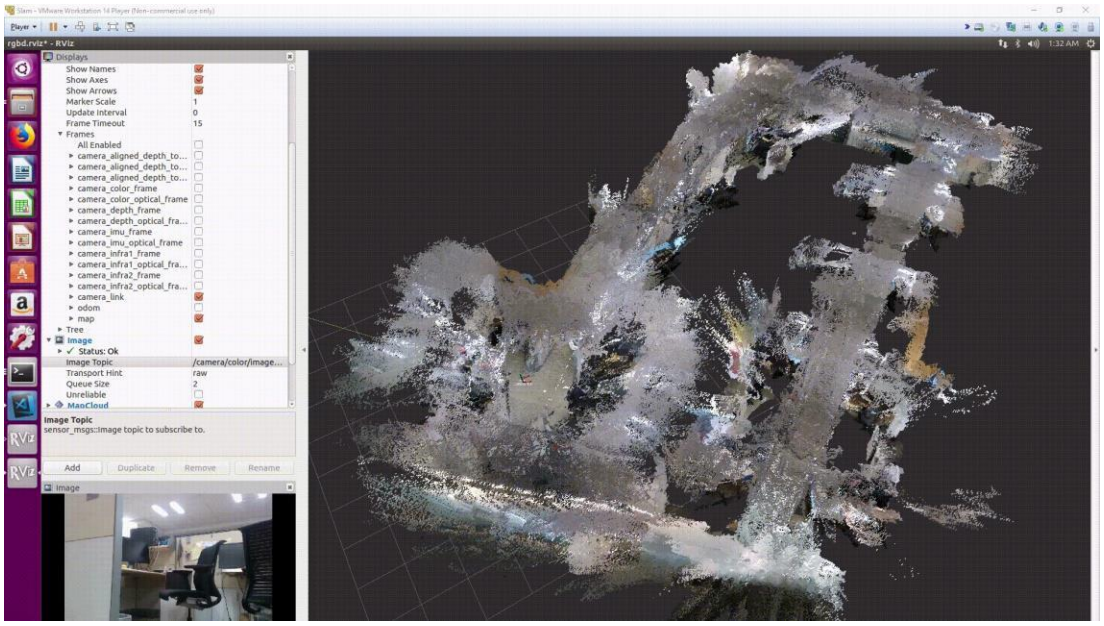

Figure 1.1

Progress

The initial phase of this project was the planning of the frame upon which the platform would be built. The frame would need to be rugged enough to not be damaged in off-road environments but would also need to be easily accessible with an easy mounting system for various sensors. To represent a full-sized vehicle a height of 1 m was required to reach the rough height of a car bumper, which is were most automotive sensors are housed. With these requirements in mind 30 mm aluminum extrusion was decided for the frame. This was due to it being very easy to work with, there being four faces of mounting points per extrusion, and it being sturdy enough to not be damaged in low-speed collisions. From here, a 3D model was constructed to outline a general form of the frame so that size and mounting points of electronics could be decided upon. The exterior cube was to be constructed of 400 mm length aluminum extrusions with 3-point corner connectors and a bottom brace which would provide rigidity. This bottom brace would also assist in mounting electronics, especially the primary embedded computer. An additional platform suspended on four rods above the primary cube would be adjustable in height via shaft collars. These specifications then allowed for electrical system decisions.

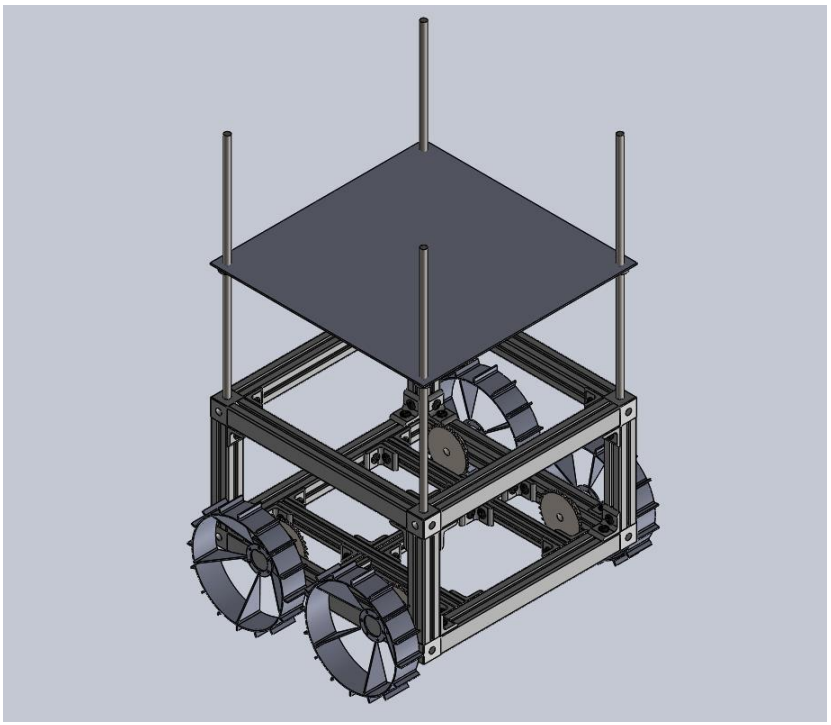

Figure 2.1

The electrical system is primarily comprised of sensing devices and the power system. For sensing devices an Intel® RealSense™ D435i depth camera was chosen for being the only cost-effective depth camera that also had RGB camera capability and an inertial measurement unit (IMU) built-in [2]. This would be the primary visual spectrum sensor. Additionally, a Texas Instruments RADAR was initially planned on being implemented. However, the chosen RADAR was found to be unfit for the system after testing the device. While it was highly effective indoors it became very inconsistent outdoors, with it giving inaccurate readings or shutting off entirely. This along with the device requiring an expensive data capture card to send raw data, required for sensing algorithms, made it no longer feasible in the project. The D435i made up for this in some capacity since IMU tracking paired with visual data could still be implemented together into a lightweight pathing system using simultaneous localization and mapping (SLAM) algorithms. To perform these calculations a Nvidia Jetson TX2 Dev Board [3] was chosen, running off the robotic operating system (ROS) on a 1 Tb solid state drive (SSD) for on-board data storage. The Jetson TX2 embedded computer has the best capabilities by far when it comes to image processing, which is what will be the most computationally intensive in this setting. These devices were then implemented, and initial visualizations of the depth camera were tested.

For the power system the drive train was to be comprised of two D5065 270kv brushless motors [4]. While there was no set requirement for the motors, brushless allowed for high efficiency and reliability along with high torque and high rotational speed. These motors worked together with the ODrive v3.6 motor controller [5]. The system was then tested independently after encoders were aligned to the motors to track position and a Python host program was run to test the motors

in various cases. It was found that the motors could reliably run at 8000 rpm with no load, drawing a maximum of around 1.4 A at 24 V. This acted as a stress test and demonstrated that high power draw would dramatically increase the heating of the external motor housing. This heating would risk melting the 3D-printed enclosure to the motor, so keeping the motors well under this 33.6 W power draw was essential.

Future Work

Currently this project has had interest taken in it by faculty at the Center for Autonomous Vehicle Systems (CAVS) here at Mississippi State University and the next step is to finish this design and improve it in future iterations. Due to the semester beginning developments on this project have come to a sudden halt and additional time will be needed to bring together the independently tested systems involved in this design. Once the electrical system is integrated with the frame a simple SLAM algorithm will need to be implemented and tested with the D435i camera. This can be built upon by more sensors, fusing relevant data as one might in an autonomous car. For now, once the system is complete, localization maps can be made of simple interiors and then move forward until full outdoors mapping is reliable. It has been discussed that this design will then make way for a larger, more robust design, then another iteration after that which will still be much smaller than a car but will be significantly larger than the initial scope developed here. A loose team has already begun to come together to develop the later iterations, though that may be months away.

Conclusion

Autonomous vehicles are difficult, especially when constructing from them from the ground-up. Even on a small scale this has been an immense task of putting together a variety of systems from a variety of fields. Small and scalable robots that simulate the functionality of full-sized autonomous vehicles are one step closer to a more open-sourced and practical way to work with these complex systems. This approach to sensor fusion is an effective divide-and-conquer strategy that will allow for a greater variety of data sets to be taken with much greater ease, which will then pave the way for easier development on fully off- road autonomy.

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An Analysis of the Invasive Green Iguana (*Iguana iguana*) on Little Cayman and Cayman Brac

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Introduction

Invasive species are widely considered to be one of the main threats to the native biodiversity of an ecosystem (Alonso et al. 2001, Kairo 2003). In fact, many biologists and environmentalists claim introduced species have directly impacted the extinction of multiple natives (Gurevitch et al. 2004, Reaser et al. 2007). One invasive species which is causing major conservation concern is the Green Iguana (*Iguana iguana*). Originally native to Central and South America, this species has now been introduced to multiple islands within the Caribbean and Pacific as well as certain regions of mainland North America due primarily to human-mediated dispersals (Villanueva 2016, Krysko et al. 2007, Falcón et al. 2013). In much of their introduced range, the Green Iguana has proven to be extremely successful at establishing invasive colonies. The encouragement of rapid proliferation and the resulting high population densities is due largely to a lack of predators and an abundance of vegetation (Townsend et al. 2003, Smith et al. 2007, Meshaka et al. 2009, Whittaker 2018). Because this species is able to colonize nonnative ecosystems so effectively, the invasion of the Green Iguana has led to concern that their introduction could threaten native iguana species across its introduced range. Many of these endemic populations are critically endangered, including *Iguana delicatissima* of the Lesser Antilles, and the Blue Iguana (*Cyclura lewisi*) and Sister Islands Rock Iguana (*Cyclura nubila caymanensis*) of the Cayman Islands. Therefore, the co-existence of these natives with the Green Iguana has raised alarm that their invasion could further threaten these already endangered animals through potential resource competition and hybridization (Moss et al. 2017, Vuillaume et al. 2015). Evaluating present colonies of Green Iguanas, their modes of introduction, and their ability to successfully colonize an introduced area is paramount in assessing the threat this species poses to native iguana species as well as native ecosystems as a whole.

Project Significance

Biological invasions are a widespread and significant factor in the loss of biodiversity across many ecosystems (Alonso et al. 2001, Abdelkirm et al. 2005, Vitousek et al. 1997). In fact, the introduction of non-native species to island ecosystems represents the main cause of endemic species extinction for these habitats (Reaser et al. 2007, Blackburn et al. 2004, Sax and Gaines, 2008). Thus, the potential impact the Green Iguana has on endemic species in the Cayman Islands should not be disregarded.

Purpose and Methods

This project sought to further evaluate the invasion status of the Green Iguana in the Cayman Islands by analyzing the relatedness of individuals found on all three islands (i.e. Grand Cayman, Little Cayman, and Cayman Brac). This project was similar in purpose and method to a previous project conducted last year where we evaluated the sibship and parentage of 6 hybrid hatchlings and 14 Green Iguana hatchlings that were captured on Little Cayman in 2016 and 2017, respectively. The purpose of this work was to determine if a single female Green Iguana dammed both of these clutches, which would signify that the number of breeding invasives on the island

had been kept under control. Unfortunately, the results were largely inconclusive due to a lack of genetic variation across the molecular markers used. To attempt in answering this unresolved examination, this project focused on two related questions: (1) Is there an established population of Green Iguanas on either island that is actively breeding; and (2) what efforts can be implemented to reverse an ongoing invasion or prevent establishment from occurring? Analyzing the establishment of Green Iguanas on these islands will allow us to perceive the threat an invasion has on native species, especially endemic iguana species. If we can estimate the number of reproductive females in each of the invasions, we can better evaluate the effectiveness of current control measures.

This project was investigated through a field excursion and the use of molecular methods in the lab. Field work was conducted on the islands of Grand Cayman and Cayman Brac from July 1, 2019 to July 17, 2019 and focused primarily on the impact of the Green Iguana on these island ecosystems. This species has been successfully introduced to both islands; however, the population on Grand Cayman has proliferated most notably with an estimated range between 1.1 and 1.6 million individuals in 2018 (Whittaker, 2018). Molecular methods were conducted in Dr. Mark Welch's Evolutionary and Conservation Genetics lab at Mississippi State University before and after the field excursion. This work included the analysis of Green Iguana samples from the Cayman Islands to verify the relatedness of individuals presumed to have originated from isolated introduction events. Part of my task included increasing the number of molecular markers available to evaluate these individuals because, while Green Iguanas are one of the most notable and widespread iguana species, an extremely small number of species-specific markers have been designed and developed for this species. Therefore, I optimized and screened ~60 markers that were initially developed for the *Cyclura* spp. in *I*guana *iguana* samples. Because these markers captured moderate levels of allelic variation among small populations of rock iguanas (i.e. *Cyclura* spp.), we hypothesized that some variation will exist at these loci in a closely related species (i.e. *Iguana iguana*). After PCR product was generated for these markers, fragment analysis was performed at the Arizona State University DNA Core Laboratory. Allele scoring was performed with PeakScanner software, which allowed for the comparison of allele variation among the different individuals.

Results

Field research was conducted on the islands of Grand Cayman and Cayman Brac. On Grand Cayman, I assisted members of the Blue Iguana Recovery Programme, which is dedicated to the reestablishment of a viable wild population of Blue Iguanas on Grand Cayman, in their daily activities at the breeding facility. This included assisting in basic husbandry tasks as well as administering medication to injured or sick individuals and capturing individuals for health-checks. I also culled invasive Green Iguanas that were captured in the facility. On Cayman Brac, I participated in one of the annual Green Iguana culls, which are organized by the Cayman Islands Department of the Environment. These culling events are generated as an attempt to reduce the overall number of invasive Green Iguanas found on the island. Culls took place between 7 P.M. and 10 P.M., and my tasks included searching through the bush for Green Iguana individuals, noosing animals when spotted, collecting blood and/or tissue samples from captured individuals, and dispatching the individuals once samples was collected. Upon the completion of this cull, we had successfully dispatched 10 Green Iguanas. Nine of these individuals were juveniles (~1-2 years of age), and one was an adult. The blood and tissue samples that were collected from this cull will be utilized in my ongoing project to assess the invasion patterns and genetic variation of Green Iguanas in the Cayman Islands.

Molecular lab work resulted in the screening of 54 markers in 24 individuals; Green Iguana samples were collected from all three islands: Grand Cayman (n=19), Little Cayman (n=1), and Cayman Brac (n=4). PCR product was successfully amplified for 25 of these markers for all DNA samples. I hypothesize that the remaining 29 markers did not amplify because of their inability to bind with fragments in the Green Iguana DNA. This was likely due to the design of these markers, which were developed for species of the *Cyclura* genus (i.e. *C. nubila* and *C. n. caymanensis*). Once PCR product was generated and fragment analysis was conducted, marker evaluation was accomplished through the PeakScanner software. Upon initial assessment, 17 of the 25 microsatellite markers presented more than 2 alleles (Table 1). The ability of these markers to accurately evaluate relatedness among individuals has yet to be determined, but the presentation of multiple alleles allows for a hopeful outcome. Continued analysis of these markers as well

as additional microsatellite markers will be conducted throughout the next year by funding provided through the Biology Student Research Award.

Table 1: Summary of allelic variation among Green Iguana individuals.

Microsatellite Marker	Number of Alleles (N)	Microsatellite Marker	Number of Alleles (N)
1) Z09	3	14) D105	2
2) Z66	2	15) D107	4
3) Z99	4	16) D111	3
4) Z106	3	17) D114	3
5) Z152	3	18) D130	2
6) Z154	2	19) D135	4
7) Z373	3	20) D136	2
8) Z590	3	21) CCSTE-04	4
9) Z609	4	22) CCSTE-05	2
10) Lew115	3	23) CIDK101	4
11) F519	3	24) CIDK113	2
12) C124	2	25) CIDK135	4
13) D102	4		

Future Research

My future project seeks to further evaluate the patterns of nuclear molecular variation and mtDNA haplotype diversity observed in a previous study of invasive Green Iguana populations found on the Caribbean islands of Little Cayman and Cayman Brac. In a previous project, the genotypes of Green Iguana individuals from both Little Cayman and Cayman Brac were analyzed while assessing the sibship and parentage of putative hybrids between the invasive iguana and the endemic Sister Isles Rock Iguana. The analysis revealed a similar lack of nuclear molecular variation across all individuals from both islands. However, there were notable differences in mtDNA haplotype frequencies. This implies a disparity between the spread of nuclear and mitochondrial markers during the colonization of these islands. Given this finding, we hypothesize that individuals colonizing both islands were of similar genetic stock originating from the island of Grand Cayman, where this species has been firmly established, but only a few females have successfully reproduced on Little Cayman and Cayman Brac.
This would simultaneously account for the similarity in nuclear molecular variation and distinct mtDNA haplotype frequencies across the Sister Isles.

To further test the hypothesis that genetically similar individuals populated the Sister Isles but that only a distinct few maternal lines were highly successful, the genetic variation of invasive Green Iguanas found on the island of Grand Cayman will be characterized. If far more molecular variation is uncovered on this island, it would be clear that a limited sample of genetically similar individuals is reaching the Sister Isles. These results would provide the opportunity to assess the effectiveness of current control methods in reducing the number of Green Iguanas arriving and propagating on Little Cayman and Cayman Brac
from Grand Cayman. Continued analysis of the genetic variation of this invasive species in the Cayman Islands will yield a better understanding of how successful introductions and colonization events occur. Further evaluation will also allow us to address potential biosecurity and conservation management planning.

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Nasty Neighbors: DNA Barcoding Mosquito Blood Meals to Formulate Preliminary Data Concerning the Epidemiological Threat of the Invasive Green Iguana (Iguana iguana) to Two Endemic and Endangered Cayman Island Iguana Species

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Background

Mosquito species vary in their host-specificity patterns and ability to transmit pathogens. Prior research shows that viruses normally circulated amongst certain avian hosts can indeed infect mammals and reptiles via generalist mosquito vectors. Reptiles in particular can harbor viruses for extended periods of time, thus allowing overwinter survival despite the absence of mosquitoes. A past study suggests that the Burmese pythons invasive to and prevalent in South Florida play a role in the transmission of local mosquito-borne pathogens [8]. Zoos and captive breeding programs facilitate interspecies disease transmission via mosquitoes, resulting in numerous reported deaths of captive animals, including endangered species, due to high host heterogeneity of zoos and pathogen prevalence [9]. Cosmopolitan feeding habits of mosquitoes affecting diverse taxa raise concerns about the transmission of wildlife diseases and potential zoonotics.

DNA analysis of arthropod blood meals to identify vertebrate hosts has been used as a tool to study the ecology of hematophagous invertebrates in relation to their variety of hosts with the aim of reducing epidemiological risks. Polymerase Chain Reaction (PCR) used to amplify the vertebrate mitochondrial Cytochrome c oxidase subunit 1 (COI) gene present in mixed mosquito blood meals and subsequent sequencing has proven useful in the identification of up to 40 different vertebrate hosts [1]. Thirty-six species of mosquitoes have been identified across the three Cayman Islands (Grand Cayman, Little Cayman, and Cayman Brac) with the most common seven serving as vectors of West Nile virus, Malaria, Dengue, Chikungunya, and various strains of encephalitis [7].

The Cayman Islands additionally host three iguana species of particular interest – two native, endangered species and one invasive species. The Grand Cayman Blue Iguana, *Cyclura lewisi,* is an endangered species endemic to Grand Cayman Island. Though the census population size is currently increasing, it is estimated that fewer than 500 individuals persist in the wild. The majority of this species is restricted to managed protected areas and approximately 175 of these individuals are maintained as part of a captive breeding program [3]. The Sister Isles Rock Iguana (SIRI) *Cyclura nubila caymanensis* is a critically

endangered iguana subspecies endemic to Cayman Brac and Little Cayman. SIRI on both islands continue to experience marked reductions in population size due largely to predation by feral mammals, road traffic, and habitat degradation. It is estimated that fewer than 900 reproductively viable adults persist in the wild [4, 5]. *Cyclura lewisi* suffers from similar anthropogenic/predation influences and continuing range decline. Native to Central and South America, the invasive Green Iguana, *Iguana iguana*, breached the Caribbean as stowaways on barges and through the domestic pet trade. This alien species threatens the native Cayman iguanas with competition for/depletion of resources, possible hybridization [6], and potential introduction of novel mosquito-borne diseases [2].

Investigation of the feeding habits of the Cayman Island mosquitoes could non-invasively illustrate the population dynamics of these three iguana species. If it is found that mosquitoes feed non-discriminately across the three iguana species in question, the issue of cross-transmission of novel disease harbored by Green Iguanas to the two endangered, endemic iguanas arises. This analysis of vector-host specificity thus becomes a conservation effort. We have developed protocol for the investigation of vector-host relationships between the mosquitoes and iguanas prevalent in the Cayman Islands via DNA analysis of vector blood meals.

Our working hypothesis is that mosquitos feed non-discriminately on all vertebrates present on the islands including the three iguana species in question, and may serve as a disease vector among these iguana species. A prediction consistent with this hypothesis is that the ratios of species specific iguana DNA present within blood meals will reflect known population sizes of iguanas across the islands. Further evidence in support of our hypothesis would be indicated by mixed blood meals. Mixed blood meals not only suggest cosmopolitan feeding habits of mosquitoes, but also demonstrate that those feeding habits create opportunities to transmit disease.

Methods

To test our hypotheses, we have been developing a protocol utilizing restriction fragment length polymorphisms (RFLPs) between the mitochondrial DNA of iguana species. The known haplotype sequences of the iguana species of interest available through GenBank were utilized to identify informative cut sites for restriction enzymes. Fragment analysis following standard PCR of the NADH-ubiquinone oxidoreductase chain 4 (ND4) gene and restriction enzyme digestion has been tested for accuracy on known blood samples of the species. These samples were increasingly diluted to resemble conditions of mosquito blood meal extractions. This protocol was further tested to determine the maternal lines of putative *Cyclura lewisi* hybrids. A new set of primers were designed to improve clarity of fragment analysis. The capture and preservation of mosquitoes is being performed by the Mosquito Research and Control Unit (MRCU) of Grand Cayman. Once the preliminary assay mentioned above is accomplished, analysis of blood meals can begin. Sterile pipette tips will be used to press deeply on the abdomen of engorged mosquitos to collect blood meals. DNA extraction from the resulting blood meals will be performed using DNeasy 96 Blood and Tissue Kits followed by PCR of the ND4 gene. Samples with clear evidence of iguana DNA will be digested with selected restriction enzymes and then analyzed via gel electrophoresis for determination of specific iguana hosts.

Significance

Given the endangered status of the Blue Iguana and SIRI, the genetic analyses of mosquito blood meals for host specificity may aid conservation efforts by providing a non-invasive measure of population status and by determining the epidemiological threat the invasive Green Iguana poses to its endangered relatives. Prior evidence suggests that mosquitoes feed non-discriminately among vertebrates and could consequently transfer pathogens to vulnerable native species. The concern to public health is valid as zoonotic viruses can be harbored in reptiles even when mosquitoes are absent. The influx and proliferation

of the Green Iguana and the prevalence of mosquitoes on these islands influenced the formation of MRCU – an affiliate of this project. The results of this investigation will help dispel questions regarding the vector-host relationship between mosquitos and iguanas as well as contribute to the conservation of these endangered species.

Conclusion

In order to determine the viability of the protocol we developed, I travelled to Grand Cayman to facilitate the instruction of this protocol at the Mosquito Research and Control Unit (MRCU). During my time working with this department, the director expressed his interest in pursuing the analysis of a wider variety of vertebrate hosts in sampled blood meals. To help determine the potentiality of this analysis, we constructed a list of products and lab machinery that would be required for the developed RFLP protocol within the MRCU lab. We also prepared a list of instructions for the translation of the protocol into the MRCU procedure. While evaluating the capability of the MRCU to run this protocol within their facilities, we determined that another option is also available to analyze all possible vertebrate hosts. This option will be to utilize universal vertebrate primers to amplify DNA from collected blood meals and to ship them off for DNA sequencing from our lab. Because of this decision to utilize universal vertebrate primers instead of the protocol we designed, our project has the potential to expand significantly from simply investigating the epidemiological threat of the green iguana to investigating the indirect host-host interactions of all Cayman Island vertebrates via these mosquito vectors. The MRCU has also been developing new mosquito capturing methods as well as new ways to preserve bloodmeals, which will make them more suitable to survive the shipment from Grand Cayman to our lab at MSU.

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Smooth Muscle Cell Phenotypic Switch in Vascular Calcification

Mary Frances Segars, Dr. LaShan Simpson

Introduction:

Cardiovascular disease is the leading cause of death in the United States, and the state of Mississippi has a higher rate of cardiovascular disease than any other state. Vascular calcification (VC) was once considered an end-stage passive crystallization process but is now known to be a regulated process involving numerous cell types and protein interactions to ultimately cause hardening of the arteries. More recently, research has shown that the process in which the arteries calcify is similar to the biomineralization process of bone. It has been hypothesized that smooth muscle cells native to healthy arteries undergo a genetic switch and become osteoblast-like cells in the presence of high levels of calcium, glucose, and cholesterol. Many researchers have identified this phenomenon but there is still no clear understanding of the mechanism by which this happens.

Characterized by the hardening of arteries, the vascular calcification process is similar to that of bone formation. The osteoblast-like cells responsible for mineral deposition in arteries have been hypothesized to originate from different sources, including the smooth muscle cells, by undergoing a phenotypic switch. In order to observe this phenotypic change, we have optimized a calcification model of smooth muscle cells and characterized the culture for different cell markers. Literature suggests vascular smooth muscle cell calcification may be activated via the Wnt signaling cascade and bone morphogenic protein (BMP) signaling. We hypothesize that during calcification, a phenotypic change of vascular smooth muscle cells occurs, characterized by the upregulation of specific genes involved in Wnt and BMP signaling, such as runX2 and BMP2.

Materials and Methods:

i. Protocol Development

Immunohistochemistry (IHC) is an assay that uses primary and secondary antibodies to evaluate at the cellular markers present to ultimately determine the phenotype of the cells. Before performing our experiment with calcified cells, a functional protocol for IHC was needed. In order to create a protocol, inspiration was taken from many different research papers that used IHC. The new protocol was tested on smooth muscle cells labelled with alpha-smooth muscle actin (aSMA) primary antibody. Because aSMA is a prevalent biomarker for smooth muscle cells, all of the cells should fluoresce.

ii. Cell Culture

Once the protocol was optimized, human vascular smooth muscle cells (VSMCs) were cultured *in vitro* on 6-well tissue culture treated plates. The cells were split into four groups: 7- and 14- day control and 7- and 14- day calcification. The control groups were given normal media (made of Dulbecco's Modified Eagle Medium, 10% FBS, and 1% Penicillin/Streptomycin). The calcification groups were given media composed of normal media and dibasic sodium phosphate (3 mM). By adding dibasic sodium phosphate to the media, calcification will be induced.

iii. Immunohistochemistry (IHC)

After both incubation periods, the cells were rinsed thoroughly with phosphate-buffered saline (PBS) twice. They were then fixed in cold ethanol and blocked with a solution made of bovine serum albumin (BSA), donkey serum, and PBS. Next, the cells were washed with PBS before incubation with the primary antibody overnight. The following day, the primary antibody solution was removed, and the cells were washed with PBS. The secondary antibody was placed on the cells to be incubated for two hours in the dark. After the incubation period, the cells were analyzed under a fluorescent microscope to determine if the biomarkers present on the cells had changed phenotypes once calcified.

Results and Discussion:

After 7 and 14 days, both the control and calcified groups were analyzed using IHC. With the control and calcified group, the cells were IHC stained for aSMA, smooth muscle myosin heavy

chain (MHC), and beta-catenin. aSMA and MHC are both markers for VSMCs. A higher presence of beta-catenin in calcified cells was expected, since beta-catenin is an important protein in the Wnt signaling. Although the calcium content of the cells in the calcium group was significantly increased, there was no difference in the results between the control and calcified groups. All of the markers fluoresced positive. Our results were inconclusive because there should have been a variation between the control and calcified groups.

Conclusions:

Although the results were not as expected, they are still helpful in determining the phenotypic swtich of VSMCs. Additional studies will be performed using IHC to test other markers on the control and calcified VSMCs. In addition to the VSMCs, endothelial cells may also be a source of osteoblast-like cells in vascular calcification via endothelial to mesenchymal transition (EndMT), creating more multipotent cells that can further differentiate[2]. Further studies will be

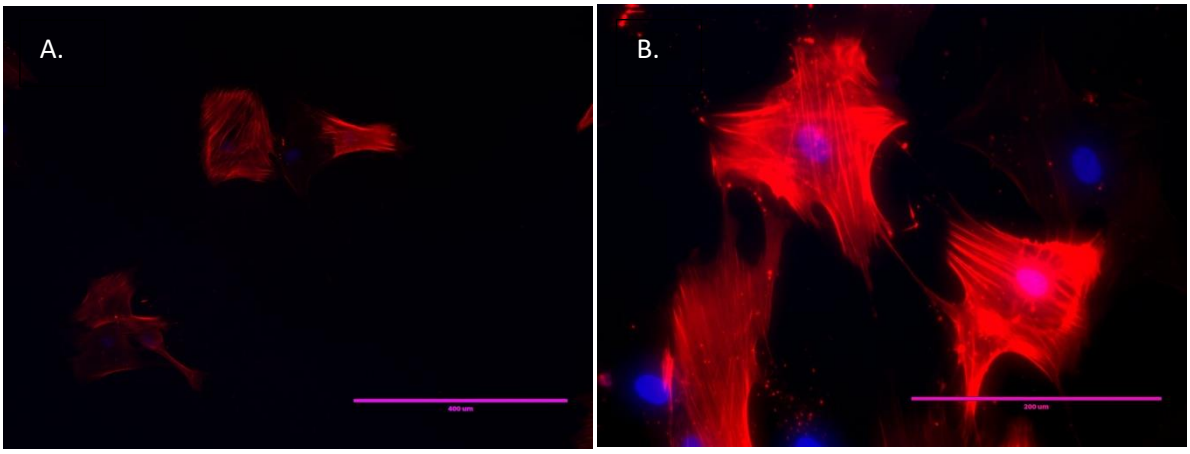

Figure 1. IHC Staining for alpha Smooth Muscle Actin in control group. The red fluorescent color shows the positive staining for aSMA along with DAPI stained nuclei. A. VSMC under 10x.

B. VSMC under 20x

completed to test for cell markers in calcified endothelial cells to observe what role the endothelium plays in calcification.

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The Mechanistic Basis for the Species Difference in the Toxicity of the Insecticide Chlorpyrifos between Juvenile Rats and Mice

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Project Title: *The Mechanistic Basis for the Species Difference in the Toxicity of the Insecticide Chlorpyrifos between Juvenile Rats and Mice*

INTRODUCTION:

One of the most common classes of agricultural insecticide is the organophosphorus pesticides (OPs). There is a growing concern that exposure to OPs induces negative impacts in the brain of developing children. The chemical most commonly linked to these issues is chlorpyrifos (CPF) (Rauh et al, 2012). In order to be toxic, CPF must be metabolized to its active metabolite chlorpyrifos-oxon (CPF-oxon) which then inhibits brain acetylcholinesterase (AChE). This leads to the build-up of acetylcholine in the brain and has negative effects on the nervous system of animals and humans. This occurs at high levels of exposure to CPF. However, most environmental exposures would occur at low levels of CPF that do not inhibit AChE and negative effects on behavior have been observed following exposure to these levels of CPF (Carr et al., 2017). At these levels, CPF inhibits the endocannabinoid metabolizing enzymes fatty acid hydrolase (FAAH) which leads to an accumulation of the endocannabinoids anandamide (AEA) (Carr, 2017).

AEA is a ligand for two receptors, cannabinoid receptor 1 (CB1) and the peroxisome proliferator-activated receptor alpha (PPAR-α). It is not clear which receptor is responsible for the negative effects. In order to test which receptor is involved, “knock-out” mice could be used as a model for the experimentation. “Knock-out” mice have been genetically manipulated which removes one of the two receptors from expression. Most of our studies on developmental CPF exposure have been performed on rats. Therefore, before experimentation could begin, we had to find what levels of CPF exposure in mice resulted in similar levels of inhibition of brain AChE that occurs in the rats. During this work, it became evident that there is a significant species difference in the toxicity of CPF between juvenile mice and juvenile rats with juvenile rats being much more sensitive to CPF that juvenile mice. This toxicity difference in juveniles differs from that observed in adults based on published literature that reported adult mice are more sensitive to CPF exposure than adult rats. Adult mice have a lower LD50 (amount of substance needed to kill 50% of the test population) than adult rats. The basis for this species difference in juvenile rats and mice is not clear.

It is well documented that once metabolized, CPF-oxon also inhibits other enzymes in the blood and liver. These enzymes serve as a protection mechanism. When CPF-oxon binds to these enzymes, it is no longer able to reach the brain and inhibit AChE. It could be is possible that differences in these enzyme levels exist between juveniles of the two species. This project determined the effect of CPF exposure on these enzymes in juvenile rats and mice to determine if there are differences in this non-target protective mechanism. The two main enzymes investigated were carboxylesterase (CES) and cholinesterase (ChE). Both are known to be inhibited following CPF exposure in adult rats and mice.

MATERIALS AND METHODS:

From postnatal day 10 through day 16, CPF was administered daily by oral gavage. Mice pups received either corn oil or 1.0, 2.5, 5.0, or 10 mg/kg CPF, whereas rat pups received either corn oil or 0.5, 0.75, 1.0, 2.5, or 5.0 mg/kg CPF, both at a volume of 0.5 ml/kg. Pups were sacrificed on day 16, 4 hours after the final dosage of CPF, and brain, liver, and blood were collected. The blood was centrifuged to obtain serum and the liver and brain was homogenized in Tris-HCl buffer (pH 7.4).

Brain AChE and serum and liver ChE activity were measured spectrophotometrically with acetylthiocholine (ATCh) as the substrate (1 mM final concentration) and 5,5'-dithiobis (nitrobenzoic acid) (DTNB) as the chromogen. Serum and liver CES activity was measured using 4-nitrophenyl valerate as the substrate (0.5 mM final concentration) and monitoring 4-nitrophenol, one of the hydrolysis products, as previously described (Crow et al., 2007). Protein concentrations were determined using Pierce BCA Protein Assay Kit (Thermo Fisher Scientific).

Serum Cholinesterase Assay: The serum was initially diluted 1:10 with 50mM Tris-HCl buffer (pH 7.4). For each sample, the diluted serum (10 µL) was then added to 5 wells on a 96 well microplate. Tris buffer (102 µL) was then added into the 5 wells containing the diluted serum. Next, 8 µL of a specific ChE inhibitor (10^{-3}M eserine sulfate) was added into 2 of those 5 wells and these two wells served as a background blank to account for any hydrolysis of the substrate that could occur as a result of things other than the activity of the enzyme. The plate was then pre-incubated for 5 minutes at 37ºC to replicate the mammal internal temperature. After the 5 min pre-incubation time, the plate was placed into a heated plate reader reaction (37ºC) and the reaction was initiated by the addition of 10 µL of a DTNB/ATCh solution (warmed to 37ºC). After the addition of the substrate mixture to the wells, the plate was read at 412nm at 20 sec intervals for 10 min.

Serum Carboxylesterase Assay: The serum was initially diluted 1:50 with 50mM Tris-HCl buffer (pH 7.4) prior to initiation of the assay. For each sample, Tris-HCl buffer (100 µL) was added to 5 wells of a 96 well microplate and to each well, diluted serum (50 µL) was then added. Into 2 wells of those 5 wells, 2 µL of a CES inhibitor (10^{-3}M paraoxon) was added and these two wells served as a background blank. The plate was then pre-incubated for 5 minutes at 37ºC to replicate the mammal internal temperature. After the 5 min pre-incubation time, the plate was placed into a heated plate reader reaction (37ºC) and the reaction was initiated by the addition of 150 µL of a 4-nitrophenal valerate solution (warmed to 37ºC). After the addition of the substrate mixture to the wells, the plate was read at 400nm at 5 sec intervals for 2 min.

BCA Protein Assay: For each sample, 25 µL of the diluted serum was added to 3 wells on a 96 well microplate. A standard curve of several different known concentrations of bovine serum albumin (BSA curve) was prepared for the plate. Using a BCA Protein Assay Kit, 200 µl of Working Reagent was added to each well. The plate was then incubated for 30 min at 37ºC to replicate internal mouse and rat temperature. Following the 30 min, the plate was cooled to room temperature and the absorbance of each well was read at 562nm on a plate reader.

Calculations and Statistics: For the enzyme assays, the absorbance was increased as the product of the reaction forming. The plate reader plotted the absorbance readings as a function of time and calculated the slope of the reaction for each well. For each sample, the average slope of the two blank wells was subtracted from the average slope of the other 3 wells to obtain a corrected slope. For protein assay, the plate reader software compared the absorbance from each sample well to the standard curve to obtain the protein level for each well. For each sample, the average protein concentration was calculated. The corrected slope and the protein concentration was used to calculate the specific activity for ChE and CES for each serum sample. Enzyme specific activities were calculated as nmoles product min^{-1} mg $protein^{-1}$. Analysis was by analysis of variance (ANOVA) using the Mixed procedure (Littell et al., 1996) followed by mean separation by Least Significant Difference. The criterion for significance was set at $p<0.05$.

RESULTS AND CONCLUSION:

Dosages of CPF (1.0 and 2.5 mg/kg) that resulted in significant brain ChE inhibition in juvenile rats which did not cause inhibition in juvenile mice. (Figure 1). Inhibition of mouse brain ChE (14%)

became evident at 5.0 mg/kg, a dosage that inhibited rat brain ChE to a much greater extent (63%). This level of exposure in rats induced a significant reduction in weight gain, a characteristic sign of toxicity. (Carr et al., 2011). However, no similar effect on weight gain was observed in juvenile mice even at 10 mg/kg.

Since liver is considered the primary site of metabolic activation of CPF, differences in liver esterase levels between species could explain the toxicity difference. For ChE, mice had much higher activity than rats (Figure 2). The higher level of ChE in mice could have contributed to the decreased susceptibility. However, based on the percent inhibition, there was not a large difference in effect. For example, at 1.0 mg/kg, inhibition in rats was 66% and in mice was 52% which is close enough to say that liver ChE is not a major key protective enzyme. With respect to liver carboxylesterase (CES) activity, rats has almost twice as much activity than mice (Figure 3). However, even with this higher level activity, inhibition of CES enzymes reached over 95% at 1.0 mg/kg in rats while inhibition in mice was only 58% at the same dosage. If this enzyme was playing a significant role, rats should be more resistant to CPF since they have twice as much activity.

Figure 1. Brain AChE

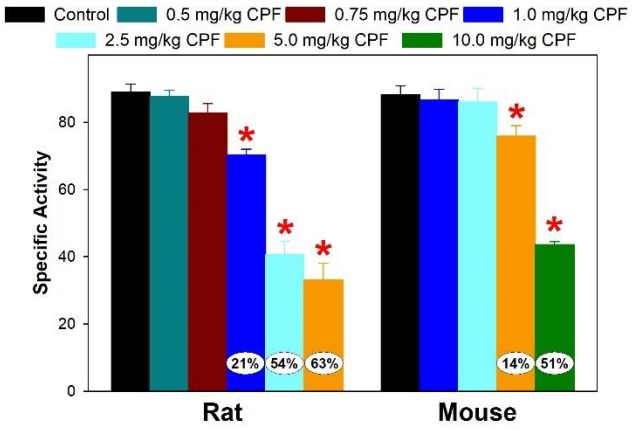


Figure 2. Liver ChE

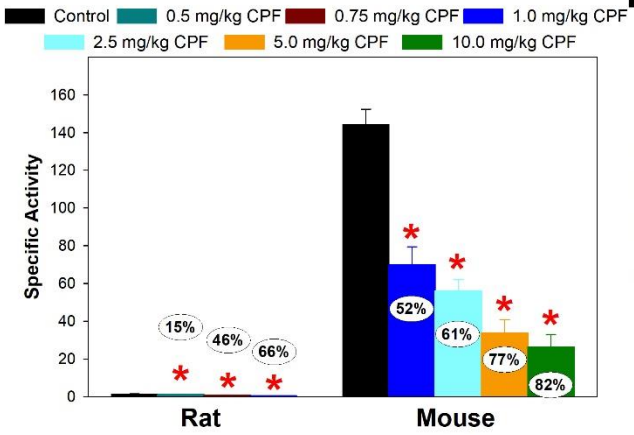
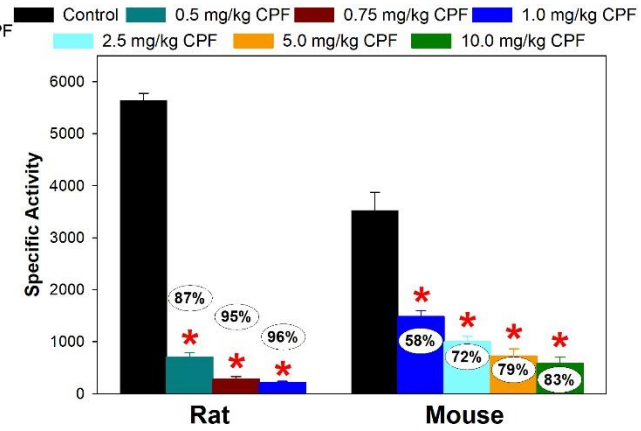


Figure 3. Liver CES



The level of serum ChE activity was greater in control juvenile mice than in juvenile rats (Figure 4). While this suggest that this enzyme could provide some protection during CPF exposure, there was a similar percent inhibition level in both juvenile rats and juvenile mice. For example, at the same dosage of 1.0 mg/kg, inhibition was 53% in juvenile rats and 42% inhibition in juvenile mice. This pattern is similar to the pattern of ChE inhibition in the liver. Serum CES activity was eight fold higher in juvenile mice than in juvenile rats (Figure 5). At 1.0 mg/kg CPF, serum CES activity in rats was virtually eliminated (~95% inhibition). The same dosage only resulted in 24% inhibition in mice suggesting that a significant level of CES protective enzymes remained in the serum of mice. It took raising the dosage to 10 mg/kg in mice to reach similar levels of inhibition that was observed in rats.

Due to the large amount of CES enzymes in the blood in mice, CPF does not reach the brain except for at higher dosages, while the low levels of CES enzyme activity in rat blood allow more CPF to reach the brain at lower exposure levels, causing inhibition of brain AChE at lower exposure levels.

Figure 4. Serum ChE

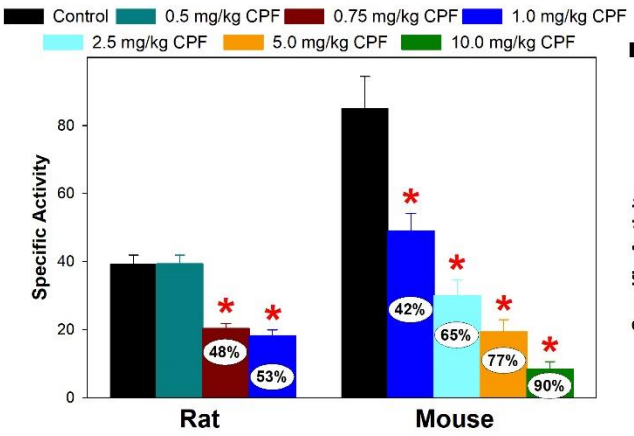
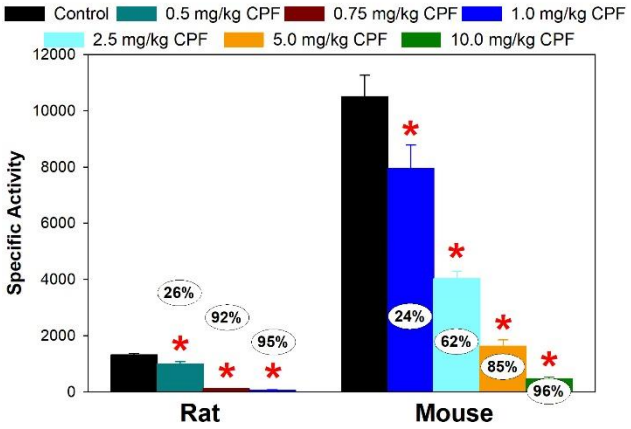


Figure 5. Serum CES



Our results suggest that serum CES plays an important role in the susceptibility of juvenile mice and rats to CPF. The higher level of CES in mice serum, as compared to rat serum, delivers more protection from the active metabolite of CPF to reaching the brain. The basis for the differences of CES between juvenile rats and mice is unclear. It is well known that adult rodents have higher levels of CES in their blood, which is an important factor in why juveniles are more susceptible than adults to OPs such as CPF. It is possible that mice may simply develop faster than rats and reach adult levels of blood CES activity much earlier than do rats. However, blood CES levels is only one parameter that plays a role in protecting against OP toxicity and other factors could play a role in the observed species differences. These include: 1) Differences in CYP450 activation and detoxication of CPF between species; 2) Difference levels of other protective enzymes (PON1); and 3) Different affinities of the protective enzymes or target enzymes for CPF-oxon (the known inhibitor of CPF).

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Grass Roots Organizing and Colorblind Ideology in Association with the Maintenance of White Womanhood

By Emily Tingle

Introduction:

Although women in the South have been portrayed as playing a background role as caretakers of the home, women have always played a large role in shaping politics in the south often under the guise of maintaining white womanhood and the racial order. Women have been able to expand their sphere of domesticity into the public realm by engaging in politics through the issue of education. During the Jim Crow era, white women were political actors instilling policies such as censoring textbooks, endorsing candidates, writing newspaper columns; all to maintain white supremacy (McRae). However, post-Jim Crow and the Civil Rights Movement racism has been rebranded across the country and has become less explicit and more subtle. This new form of rebranded racism in the 21st Century has been coined "colorblind ideology" by sociologist Eduardo Bonilla-Silva. This new racial structure focuses on the "covert nature of racial discourses and practices", more people avoiding "racial terminology", and the "invisibility" of structures that are used to reproduce racial inequality (Bonilla-Silva, 18). Today, many white women in the South have taken surprisingly progressive measures by advocating more equal funding for public schools while criticizing the move for privatization and charter schools. However, preliminary observations have suggested that while these women are taking some progressive steps to achieve some racial equality through more funding in public schools, they also engage in some forms of colorblind ideology through the refusal to address race as an issue and the racial inequalities that currently exist within public schools. By not addressing issues like tracking that help extend racial inequality, these women

are being complacent in addressing race even though they are advocating more progressive measures. Another issue is that these women have significantly more power and the ability to make change than their Jim Crow counterparts. Many of them are engaged in grassroots organizations ranging from official campaign organizations to political action committees which involves engagement through social media, fundraisers, and donations to political candidates' campaigns. This level of sophistication that involves political power through campaign contributions and more engagement in the 21st Century through social media gives these women more political power than the white women in the 1950s and 1960s.

Today's white women have considerable power and influence but fail to take fully progressive measures to instill equality in schools. While they may have the best intentions regarding the push to fully fund public schools and instilling a public backlash on charter schools and privatization practices, the lack of attention they give to measures that could help institute full equality in public schools is problematic and their complacency leads to the subtle maintenance of white supremacy.

Although this project closely examines grass-root organizations, this project is about far more than this kind of organizing around the topic of education. Instead, this study focuses on how modern-day racism continues to shape politics today. By not considering racial inequality in schools and instead embracing silence on the topic of race, these organizations likely perpetuate racial inequality and maintain the current racial structure. As many Americans have strong beliefs of America working as a meritocracy through schools providing a basis for an even playing field, unequal schools for minorities keep them at a disadvantage while many Americans claim they should be able to overcome more unfortunate circumstances despite their lack of

opportunities and systematically being discriminated against (Johnson, 10-11). The result has been an increase in the racial wealth gap between Blacks and whites despite the belief that America has become more equal and racism is a problem of the past (Johnson, 1). Instead, racism has simply been rebranded in a subtler form that even exists in progressive political organizing through colorblind racism. This study, thus, elaborates on existing scholarship about new forms of racism—and how racial ideology and social structures mutually reinforce one another.

Questions:

1. To what extent do white women confront the roles that race and racism play in the educational opportunities and outcomes of youth in the South?

Literature Review:

This study draws a great deal from the research of Elizabeth Gillespie McRae and her work *Mothers of Massive Resistance: White Women and the Politics of White Supremacy*. McRae highlights how southern women took an active role in preserving segregation specifically through grassroots efforts and often featured at the center advocating for policies involving education. Women became political actors in the public sphere by viewing schooling as an extension of the home (McRae, 14). The focus on motherhood changed the political language to minimize the focus on racial identity and instead focus on gender identity (McRae, 14). However, segregationists instilled the idea that "good" womanhood meant performing whiteness and used that ideology to continue varying degrees of racial segregation (McRae, 14). To maintain the white supremacist order of society, white women focused on school board decisions, local political campaigns, college scholarships given for essays on maintaining racial segregation,

rewriting textbooks that upheld white supremacy, and supporting de facto segregation (McRae, 18).

The research conducted in this study would provide a continuation of McRae's analysis of what role white women play in upholding the racial order through political activity even if they consider themselves to be more progressive and supporters of more equal schooling.

The second piece of literature that this study draws on is *Racism without Racists: Colorblind Racism and the Persistence of Racial Inequality in America* by Eduardo Bonilla-Silva. Bonilla-Silva identifies "colorblind ideology" as a new form of racism that has emerged to replace the more explicit Jim Crow era mainstream racism ideology (Bonilla-Silva, 51). In the realm of education, there has not been a broad institutional pattern, but racism still exists through patterns of white flight and tracking that guarantee advantages to whites (Bonilla-Silva, 51). One of the frames central to colorblind racism is the minimization of racism (Bonilla-Silva, 57). Minimization of racism "suggests discrimination is no longer a central factor in affecting minorities' life chances" (Bonilla-Silva, 51). This is generally used to excuse many racially motivated actions by whites and institutions through the removal of de jure segregation (Bonilla-Silva, 57).

This study will demonstrate the use of colorblind ideology through studying how white women respond to whether they believe racism exists today and affects the structure of education. In addition, preliminary observations have shown the use of minimizing racism by suggesting it is a subject that should no longer be discussed and is not relevant to their policy agenda.

Despite the Best Intentions by Amanda E. Lewis and John B. Diamond shows how even in many schools that are "diverse" and "progressive" may aid in helping continue the pattern of

racial inequality (Diamond & Lewis). These schools continue to be racialized due to problems like tracking and opportunity hoarding which closely resemble segregation and continue the pattern of separating students of color from white students (Diamond & Lewis). Continuing in the ideology of colorblindness, "basic classes" become the new term for talking about black students, while "honors classes" become the new term for talking about white students (Diamond & Lewis). While many people believe that equal education is getting better, studies show that "while legalized segregation of schooling ended more than 50 years ago, many schools and districts in the United States remain highly segregated" (Diamond & Lewis, 84-85). Parents are also involved in instituting this inequality. According to Diamond and Lewis's theory of schools as a marketplace, parents' economic capital, along with social, cultural, and symbolic capital plays a huge part in the educational advantages for themselves and their children (Diamond & Lewis, 53). Schools as a marketplace for educational services provide a highly established example of how the patterns of both race and class interrelate where parents with economic gains establish patterns of race which eventually perpetuate the patterns of class. Due to patterns of race and class in American society, "white skin represents a form of symbolic capital which brings with it better treatment and more educational opportunities" (Diamond & Lewis, 53). Those educational opportunities will eventually lead to privileged children being more likely to succeed in higher-paying jobs in the workforce and continuing the pattern of class. This also causes the continuation of the "racial achievement gap" which is used to later justify the continuing patterns of race and class in America. These problems show that “race does not necessarily matter less than it did in the past. It just matters differently.” (Diamond & Lewis, 84).

This work reflects the goal of the study in showing how racism has simply been rebranded in the 21st Century and through issues like tracking and cultural capital lead to segregation and

structural inequality within schools. This also reflects on how the current class stratification is reinforced and recycled due to minorities being placed at a disadvantage. The study would add to this piece on how white women rationalize tracking and handling culture capital in order to secure their own children's advantages in schools.

The final work that this study draws upon is Heather Beth Johnson's *The American Dream and the Power of Wealth: Choosing Schools and Inheriting Inequality in the Land of Opportunity Structural* showing how many Americans across all classes use the idea of the "American Dream" and education as "the Great Equalizer" in order to excuse structural inequality that is made possible partially by the construction of race (Johnson, 14). The contradiction between the ideology of "you can do anything if you work hard enough" and the admittance of intergenerational wealth being a factor alongside the inherent inequality among schools is perplexing, but exists in order to stabilize the proponent ideologies of some races are better than others and that structural inequality exists not based on structures but on individual people's bad choices (Johnson, 28-29). This ideology of meritocracy often defeats efforts in order to reduce inequality (Johnson, 29). The ideology of "we deserve our inheritance" maintains structural inequality and affords some people vastly different opportunities than others even in the same career field. This is caused not by lack of education, but by lack of resources caused by intergenerational wealth (Johnson).

This becomes problematic since education is often viewed as "the Great Equalizer" when it is a racialized system that also discriminates based on class (Johnson). Although most people believe in "the American Dream" that if one works hard enough one can accomplish anything, most people simultaneously also believe that schools are inherently unequal and that your education helps lead to your success (Johnson, 169). The way people talk about schools has

become racialized even by using seemingly race-neutral language. Many parents consider the "good" schools to be "whiter" and often schools with more funding due to intergenerational wealth influencing the class system (Johnson).

This piece will reflect the questions in my study when I ask why these organizations are interested in promoting public education and in looking at how they talk about "good schools" versus "bad schools". I expect that there will be at some point in the interview a participant explaining the American Dream or reasoning for meritocracy. I also expect there to be the use of seemingly race-neutral language throughout all the interviews.

Methods and Procedures:

Following IRB approval, participants who meet the study's inclusion criteria (being a member of/ preferably on the board of an education political action committee or campaign or other relevant organization) were contacted through a snowball sampling technique starting with contacts that I had in various organizations. These potential participants were presented with a recruitment script via email. Participants in this phase of the study are only from Mississippi in order to only engage in differences in women in Mississippi's participation in education policy in the 1950s-1970s and now. Interviews lasted 60-110 minutes, depending on how much the participant has to say. As this is part of an ongoing study, this cycle of the project currently consists of 3 fully analyzed interviews, with other interviews ongoing. Interviews were recorded with a digital audio recorder. In order to establish rapport and build trust, the interview began with more small talk questions about their children, community, or summer plans. Interviews consisted of questions about their perspectives on how their organizations are organized, what led them to becoming involved in public education activism, what policy agendas they support,

and whether demographic characteristics like race and gender play a role in maintaining inequality within schools.

Upon the completion of the interviews, all interviews were transcribed using transcription equipment and coded using MAXQDA to analyze the results. My identity as a white woman will have some impact on this project. As a white woman interviewing other white women, trust may be easier to establish, and the participants will probably be more candid in their conversations. However, I do not believe that my identity as a white woman will lead to less scrutiny in the analysis of the interviews due to my background from a mixed-race family and growing up in a majority-minority school. Although I identify primarily as a white woman, I am less likely to gloss over or excuse subtle forms of racism due to my experiences. I also believe that it is important for white women to be interviewed by other white women who understand their part in being socialized within a structure of racism, but at the same time does not excuse their complacency or actions that replicate the structure.

Results:

All of the women in this study strongly emphasized their belief in fully funding public schools in Mississippi and ending the growing movement of privatization in public education such as the introduction of charter schools and vouchers. The women in this study were very deliberate in their statements about how they believed every child should have equal opportunity to succeed regardless of their demographic background, however, all of the women became flustered once the topic of tracking was introduced. For instance, while advocating that every child should have a more equal experience in public education and critiquing white flight to a nearby suburban school, one advocate instantly became agitated upon the issue of tracking:

> **Interviewer:** Some people say that tracking, dividing students into basic, honors, or AP classes, divides children by race in schools. Do you agree or disagree?
> **Anne:** …Um…I have mixed emotions on that because I can certainly see where it does um but my children are in a very diverse district where they're in the minority. They are in advanced classes, AP classes, so to me, that's important for them and I may be biased there because my children are-I want my children to have every opportunity to be in the same situation as the kids in [nearby suburban school]. And I probably don't notice as much in [city in Northeast MS], we don't have as many white families in [city in Northeast MS], but even the honors classes and the AP classes are still pretty darn diverse. But um I can see that argument I can see that but I feel like you do- when you take the flip side of that like here in [city in Northeast MS], a lot of times because we're 70% free or reduced-priced meals and our scores have dropped, they've gone up and down these schools have so we're like B scores now so we're not that bad. In the past, the school district zone put so much emphasis on bringing the lower tier up that they're not going to keep the high achieving students where they need to be. I think it can go both ways where resources are trying to build the bottom tier up, the next tier up, the special needs students up, all pockets where we struggle. Sometimes not enough is done to keep the high achieving students where they need to be. So it goes both ways.

Upon the mentioning of potential racism within her own children's schools, Anne immediately clams up and begins defending some of the privileges her children receive by stating how her white children go to a majority-minority school and that around 70% of the children in the school come from impoverished backgrounds. To Anne, the privileges her children receive they deserve since she's compensating by sending them to a more racially and economically diverse school.

Similarly a successful businesswoman, Christy, who left her old job to work full time for a public education organization, made it clear throughout the interview that she fully recognized the role one's parents' income plays in the education they receive as well as being one of the most vigorous advocates for fully funding all public schools. Unlike Anne, Christy's children attend a very privileged, mostly white suburban public school in the same district as Anne and Christy's children are not enrolled in AP or Honors classes. However, when asked the same question, Christy's response also did not advocate taking away tracked classes:

Interviewer: some people say that tracking, which is dividing students into basic, honors, and ap classes, divides children by race in schools. What are your thoughts on that? I know it's a very divisive issue.
Christy: No, I think it is. I go back to every child deserves to have just because this group gets to do this intensive study to prepare them for certain tests that could get them a leg up. Why are we not doing it for everybody? You know? I-I don't
Interviewer: Are you describing that you believe that there should be the same level of rigor for every child or that every child should have that same potential opportunity?
Christy: Potential opportunity. Not every child's going to ultimately react well to it. And that's when we go into if we had plenty of money and were able to give specialized education to everybody, we could do that. I guess what I'm saying is I want everybody to have a chance to be tested for um to go into these classes or to go into these classes. I don't want to say "oh well you can't do that we're just going to go on and see" I guess but I guess we already are doing that okay so let me back up. Based on map testing, you are identified with a certain academic with uh certain academic standings and you can get into advanced classes so yes not everybody can do advanced classes, so I guess sometimes there has to be something like that. But as far as just everyday learning, I think everybody should have the same opportunities and get the same rigor and really just focus on good quality education not the "oh well we got to teach to the test" or "oh we don't have time to do that" or "we don't have computers so we can't do that" everybody should have a rigorous schedule within reason and then if you can go above you go into advanced, if you need more help you go into a tier program, but the basics should be everybody gets the same rigor no matter what school district you're in and that would go back to providing computers. That Delta school does not have computers for every child. You can't provide the same if they don't have the same.

It's obvious that Christy wants to stick to the script of saying, "well everyone should be equal", but she diverts from that by saying that there should still be AP classes, just that everyone should have an opportunity to take an AP class which avoided the question. However, when asked about race in tracked classes, Christy responds by saying it's mostly just due to a child's family's economic status:

Interviewer: And this is another question but in some of the better public schools' um they've noticed that it's mostly white children in the AP and Honors classes.
Christy: Mmhmm I can see that.

Interviewer: Why do you think that is?

Christy: Okay so it goes back to that initial map testing that gets you into that 7th or 8th grade advanced English or Math class. Okay so if you aren't doing well on the map test, okay so that goes back to what's going on at home. Were you read to as a child, do you have a computer at home, do you have internet access at home, do you have two parents at home? Do you have one parent that is helping you with homework? You know even

> an upper-class white woman may not help her child with homework. So, it goes- there are lots of different examples of what might be going on with that child with the map test to start them on that track. And it most often is probably a minority, but like I said I know plenty of white parents who don't sit down and do homework with their children or read to their children. Well all of that is proven that will help give that child a leg up on standardized testing. So yes, your assessment is true I would think that more white children who are of a higher economic level are probably in classes. You can also look at the amount it costs to be in an AP class and take the test.

Although Christy openly admits that there are more white children in AP classes, she defends tracked classes by saying it's a parent's responsibility on how their child performs in assessments and compensating her argument by saying that some white parents also do not perform that responsibility. However, like Anne, but for different reasons, Christy does not advocate for getting rid of higher tracked classes but suggests just making sure every child is tested for them.

Discussion:

The women in this cycle of the study do have a grasp of how racial inequality is perpetuated through white flight and the economic cycle of poverty. They even are progressive, in the sense that they are advocating for public schools to receive better funding and be put on an equal playing field. However, they fail to see how their own privilege perpetuates racial inequality within schools. While advocating for equal treatment for all children, they are unwilling to give up the racial and cultural capital privileges their own children receive. The study's participants engage with the ideology that there is an unequal playing field in education, but that within schools that playing field is removed and a meritocratic system works. This has led to the belief that honors and AP classes are needed even if they hinder racial inequality since, within schools, there is a stronger belief that all children are on an equal playing field.

While these women have successfully combated structural inequality in the field of education through their advocacy for equal funding and anti-privatization, they have failed to fix their blind spots when it comes to seeing the privilege they maintain within schools.

The potential impact of these women's racial blind spots is that while the racial gap in education may decrease if these women succeed in their goals, there may also be further use of colorblind ideology regarding tracking if all public schools are equal. This could lead to many white advocates failing to critique their privileges through their use of cultural and social capital. With economic inequality between schools removed, colorblind ideology may change to argue more against parenting styles and IQ scores than focusing on how much of an impact a child's race or socioeconomic status may play in them not receiving equal opportunities. This study reflects the obvious need for white women to think critically about how their children's' successes were aided by their own privileges and intervention to better provide an equal playing field within schools.

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Name: LeAnn Ward
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Faculty Mentor, Department: LaShan Simpson, Ag. & Biological Engineering

Title: Development of A 3D Vascular Model Using Porcine Arteries

Introduction:

Vascular calcification is a condition associated with cardiovascular and kidney disease. Cardiovascular disease is the leading cause of death for both men and women in the United States. Main blood vessels in the abdomen, the aorta, carotid, and iliofemoral arteries are especially affected by cardiovascular disease. Calcification is seen in the vascular smooth muscle cells (VSMC) of the artery, and it can appear when cells responsible for building and maintaining VSMC change into osteoblast-like cells (Donghyun, 2011). Osteoblasts are the cells responsible for building bone. When the status quo of the arteries is disrupted by conditions like cardiovascular disease, kidney disease or diabetes, mineralization of the smooth muscles begins to occur. Many factors seems to affect the initiation and increase of mineral deposition in the arteries, such as heightened calcium and phosphate levels, bone morphogenic proteins 2 and 4, arterial stiffening associated with aging, and transformation growth factor-β1 (Donghyun, 2011).

Some research is currently focused on studying these elements in order to prevent or possibly reverse the process of vascular calcification. A solution that would inhibit and rid of the mineralization of the arteries could save or prolong the life of those suffering from the condition. However, calcification is an active process in the body with many moving parts. A sufficient *in vitro* model is needed for in-depth studies of this process. The goal of this project is to create a table top model that can be used for calcifying human cells. Two protocols were tested on porcine renal arteries for the purpose of decellularizing the samples. If it can be shown that all the porcine cellular material and DNA were removed with only the non-living tissue matrix remaining, then future work can be done to begin seeding human vascular smooth muscle cells onto the tissue.

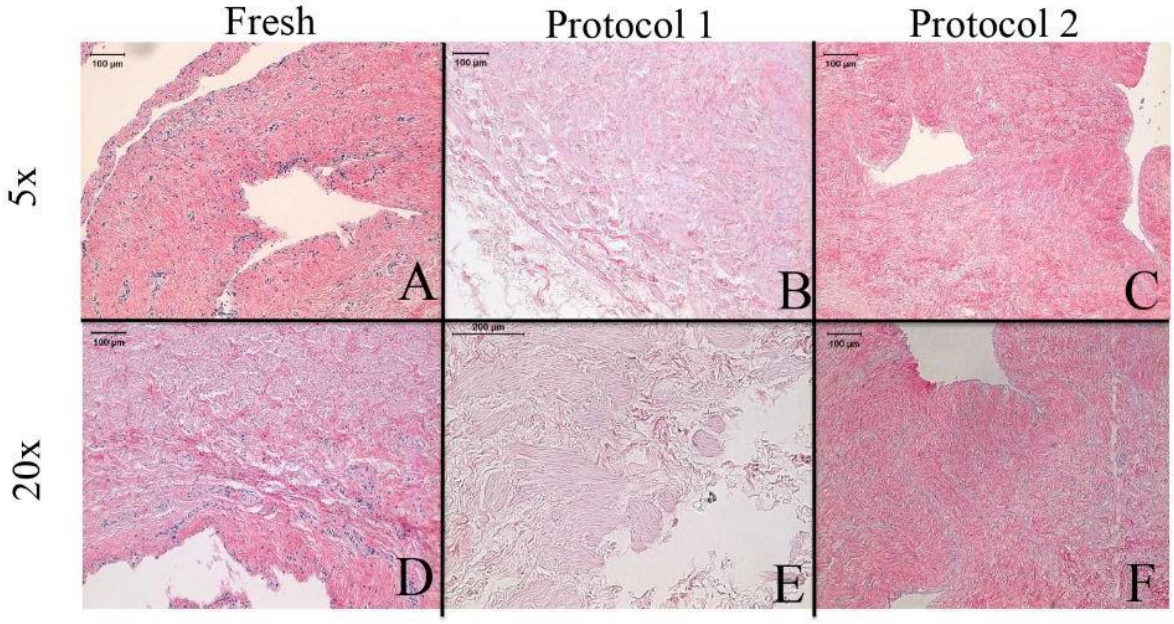
Materials & Methods:

Fresh porcine renal arteries were cut into similarly sized 1-inch pieces. They were separated into three groups: fresh samples, Protocol 1 samples, and Protocol 2 samples. The fresh samples were stored in 1X phosphate buffer solution (PBS) at 5-10 degrees Celsius. Protocol 1 and Protocol 2 were each decellularized over a 6-day period under continuous shaking using two different methods.

Pieces of all three samples were made into slides to be examined. A hematoxylin and eosin (H&E) stain was done to examine the tissues of the decellularized artery. Masson's Trichrome Blue stain was also performed on all of the samples to analyze the connective tissues. Both stains are capable of identifying the presence of a cell nucleus.

The rest of the samples were gathered, and DNA analysis was performed using a GloMaxTM-Multi Jr Method for DNA Quantitation using Hoechst33258.

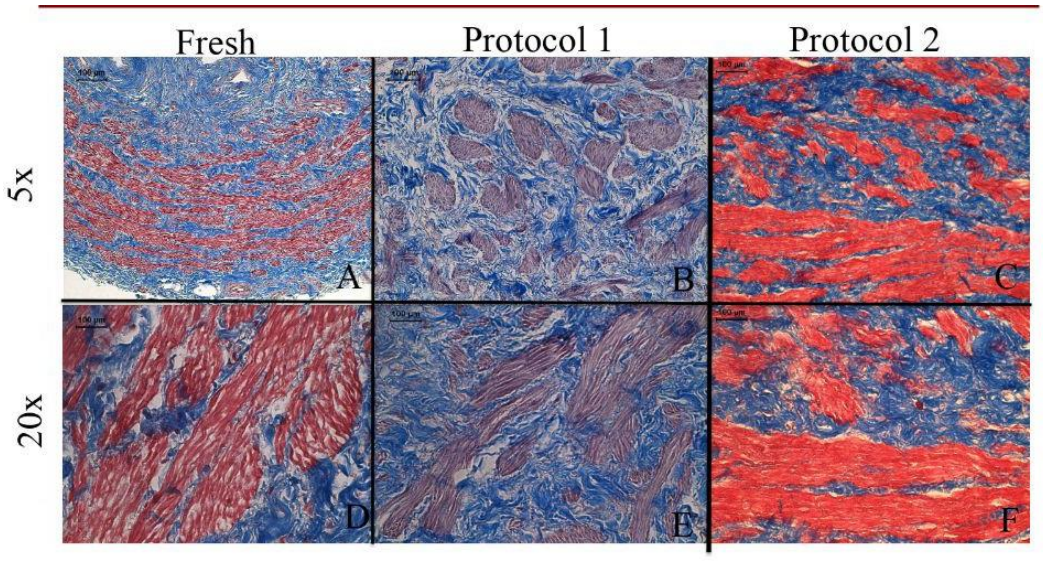
Results:



A: fresh porcine artery 5x, B: protocol 1 decell 5x ,C: protocol 2 5x ,D: fresh porcine artery 20x ,E: protocol 1 decell 20x ,F: protocol 2 decell 20x

Figure 1: All 3 Samples Underneath the Microscope with H&E Stain

In an H&E stain, as shown in *Figure 1*, the presence of a nucleus and nucleic acids that would contain DNA would be indicated by a dark blue-purple color (Fischer, 2008). Other materials made up of proteins like the cytoplasm of the cell or the extracellular matrix would be stained pink (Fischer, 2008). It can be seen clearly in *Figure 1* that only the fresh samples that did not undergo decellularization protocols contained any genetic materials.



A: fresh porcine artery 5x, B: protocol 1 decell 5x ,C: protocol 2 5x ,D: fresh porcine artery 20x ,E: protocol 1 decell 20x ,F: protocol 2 decell 20x

Figure 2: All Three Samples Underneath microscope with Masson;s Trichrome Blue

For a Masson's Trichrome Blue stain, all nuclei present will be stained black and collagen tissue will be stained blue. The red indicates the background of the image. It can be seen in *Figure 2*, that nuclei can only be seen in the fresh sample. This is a secondary confirmation that the cell materials were destroyed and washed away by Protocol 1 and Protocol 2.

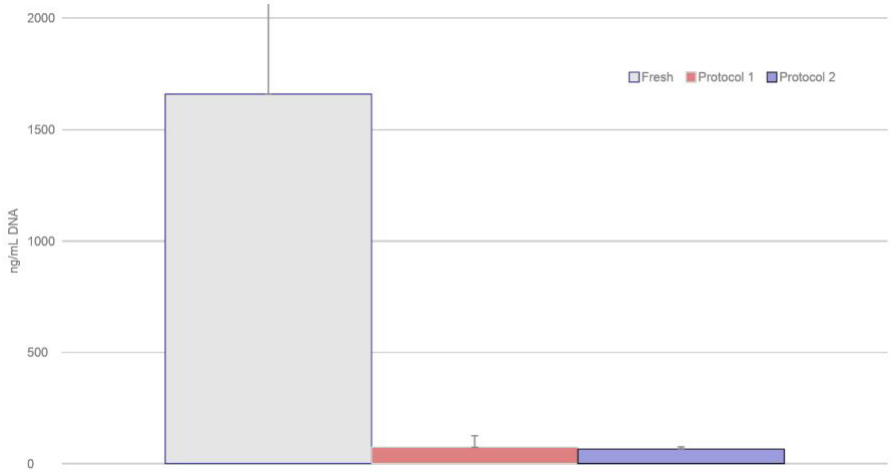


Figure 3: DNA analysis results of the three samples

The presence of porcine DNA was measured for each of the samples. The results of the analysis revealed that the fresh sample had a significant amount of DNA remaining. This was the expected results. Both Protocol 1 and Protocol 2 had little to no DNA material left.

Conclusions:

Both stains and the DNA analysis confirmed the success of this experiment. It was seen in both *Figure 1* and *Figure 2* that Protocol 1 had an oddly dull colorization compared to the other two samples. These protocols will be performed again on porcine carotid arteries instead of renal arteries to further confirm the protocols' success and to investigate the apparent dullness of Protocol 1.

Future work with human VSMC and calcification is planned. Further testing must be applied in order to test the mechanical strengths of the samples. If the samples continue to have successful results, work will be done to seed cells onto the arteries to be tested for their feasibility as 3D vascular models.

An accurate 3D model would have great use in the study of vascular calcification and its causes because it could better simulate the conditions of a diseased artery in humans.

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