

Research Reports from Undergraduate Students
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Program Director:
Dr. Seth F. Oppenheimer

Edited By:
Katelyn Wright

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Name: Both, Pieter

Faculty Advisor: Donna M. Gordon

Project Title: “Analysis of mitochondrial morphology and genome maintenance in *pcp1* mutants of *Saccharomyces cerevisiae*”

Hypothesis

In the absence of wild type *PCP1*, cells expressing mutant Pcp1p will give rise to abnormal mitochondrial morphology. Given the link between mitochondrial morphology and mtDNA maintenance, we have shown that *pcp1* strains that exhibit the largest degree of mitochondrial fragmentation have lost the largest fraction of their mitochondrial genome, and vice versa, according to qPCR data collected using primers *ACT1*, *COX1*, and *COX3*. This indicates that the relative mtDNA:gDNA copy number (RCN) data obtained by real-time qPCR may be used as a sensitive measure of Pcp1p functionality in these mutants. We predict that RCN data collected using additional primers will be similar to data collected using *ACT1*, *COX1*, and *COX3*.

Summary of Proposed Summer Research

In the first biological replicates of *pcp1* mutants, RCN fluctuated noticeably between data collected using *COX3* and *COX1* as mitochondrial DNA targets. To determine whether these differences reflected preferential loss of certain areas of the mitochondrial genome, additional qPCR trials will be conducted using *ATP6* as a mitochondrial target and *TUB2* as a nuclear target. In addition, RCN between plasmid DNA and gDNA will be determined using the β – lactamase gene as the plasmid target. Plasmid copy number should be relatively equal among all strains.

Summer Research Completed

After viewing a paper discussing the bias in qPCR data obtained from a linear template versus a closed, supercoiled, circular template, it was decided that it would not be productive to use a β – lactamase target in qPCR to determine variation in plasmid copy number among *pcp1* mutants (see PLoS ONE 6(12): e29101. doi:10.1371/journal.pone.0029101). However, all other proposed targets were used in determining mtDNA:gDNA fold changes. These fold changes were calculated as a $\Delta\Delta Ct$. The Ct value of each qPCR reaction is an inversely proportional indicator of the quantity of nucleic acid present at the beginning of the qPCR reaction. A ΔCt is the difference between two Ct values obtained from each primer of a primer pair in a single sample. A $\Delta\Delta Ct$ is the difference in ΔCt among samples. Using the Pfaffl method of calculating the ΔCt , the efficiency of each reaction and the standard deviation of the Ct of each reaction were included in the calculation of the $\Delta\Delta Ct$ (*Nucleic Acids Res* 29(9): 2002–2007). A pair of primers, one nuclear and one mitochondrial, was used in each qPCR experiment to determine fold change. The nuclear target *ACT1* was paired with *COX1*, *COX3*, and *ATP6* in separate qPCR reactions. Each of these reactions was performed three times. Additionally, *ACT1* was paired with two other nuclear targets, *TUB2* and *TIM11*, to estimate the range of $\Delta\Delta Ct$ variation. Because the genomic DNA is identical in all samples analyzed, the $\Delta\Delta Ct$ of samples in a reaction using two nuclear targets is an indicator of precision of the qPCR reaction.

Conclusion:

After calculating the $\Delta\Delta Ct$ of all reactions with an 85% efficiency or higher, it was determined that in the mutants with absent Pcp1p activity, there are consistently lower levels of mitochondrial DNA indicated by *COX3* than *COX1* and *ATP6*. This indicates at least two foreseeable possibilities: 1) If the decrease in mtDNA:gDNA ratios in these mutants is due to degradation of the mitochondrial DNA, this degradation may be site specific to *COX3* as well as other untested target sites, or 2) the mutation may be antagonistic to mtDNA replication, lowering the population of complete mitochondrial genomes in the cell. The results of the gDNA:gDNA fold changes calculated using two nuclear primers indicates that fold changes between about 0.53 and 1.1 are expected from targets with similar copy numbers. Additionally, two of the mutants with reduced enzymatic activity of Pcp1p have similar levels of mtDNA as WT. Both the deletion mutant and the nonfunctional mutant have extremely low mtDNA levels, yet slightly higher than ρ^0 , indicating that there is still some mtDNA present in these mutants. Surprisingly, the deletion mutant consistently has higher levels of mtDNA than the nonfunctional mutant, suggesting the nonfunctional mutation is dominant negative – the adverse impact on the mitochondria of the mutated enzyme is worse than the absence of the enzyme.

Name: Christiansen, Matthew

Faculty Advisor, Department: Dr. Scott Rush, Wildlife, Fisheries and Aquaculture

Project Title: AVIAN HABITAT USE AND POPULATION DYNAMICS WITHIN OPEN PINE FOREST SYSTEMS

Longleaf pine (*Pinus palustris*) ecosystems were once a dominant ecological community from the Atlantic Coastal Plains to the West Gulf Coastal Plains, encompassing an estimated 37 million ha. Today these ecosystems exist as remnants, covering a fraction of their historical range. With the loss of longleaf pine associated ecological communities have also changed, leaving some species imperiled. While longleaf pine has received great attention, other pine systems such as those occurring at the limits of longleaf pine, have received less focus.

Frequent low-intensity fires helped maintain open pine ecosystems by suppressing competition with hardwood understory, promoting a dense herbaceous ground cover, comprised of native grasses. The active suppression of fire by humans has reshaped longleaf pine ecosystems changing their species composition and habitat structure, negatively impacting species long associated with these communities. Bachman's Sparrow (*Peucaea aestivalis*) is one species with declining populations linked with the loss of pine ecosystems. As the distribution of open pine systems has decreased, Bachman's Sparrow is now considered of 'conservation priority' by several wildlife conservation and management organizations (Dunning and Watts 1990).

Many species of birds exhibit area sensitivity by which individuals most commonly occur within larger areas of suitable habitat (Keyel et al. 2012). Bachman's Sparrow has shown area sensitivity, preferring open pine conditions (Pletovich et al. 1998). Given its need for ephemeral, fire-maintained habitats and area sensitivity, Bachman's Sparrow has been selected by the U.S. Fish and Wildlife Service towards strategic conservation planning at the landscape scale (Shelton 2014).

Vegetation composition and forest structure are two main factors that affect avian occupancy and population attributes (demographics). Avian populations can typically be indexed using point count surveys of vocalizing individuals. These methods can be employed to evaluate and compare the composition of avian communities and/or the distribution of species within and among select habitat types. Documenting the presence and absence of community members provides general information on populations but does not address the mechanistic agents behind these changes. To this end, the marking of individuals - through banding - can provide insight into the demographic attributes behind observed change. The benefits and application of bird banding is described at the U.S.G.S. Bird Banding Laboratory's website (<http://www.pwrc.usgs.gov/bbl/homepage/whyband.cfm>). In general, birds are banded to provide information on dispersal and migration, behavior and social structure, longevity, survival and productivity and abundance.

To learn more about a species' habits, dispersal and other life history attributes we need to study the actual species and their populations. Given our limited understanding of the ecological requirements and habitat types that promote the survival and dispersal of most songbird species, including those associated with open pine communities, models alone cannot provide the information obtainable through nationally supported programs. The MAPS program (standing for Monitoring Avian Productivity and Survivorship) was established in 1989 to provide these data. Since its start in 1989 the MAPS program has continued to expand to now over 500 stations operated throughout North America. A MAPS station was established at the Sam D. Hamilton Noxubee National Wildlife Refuge in 2013. This station provides valuable information to the U.S. Fish and Wildlife Service and other groups, helping to determine causes of population change, helping to identify management actions and conservation strategies to reverse population declines. An integral part of the learning process for ecology and field studies is the ability to see and learn about ecological communities and community members. Access to these opportunities is greatly facilitated by the use of non-harmful sampling (using passive techniques such as mist nets).

Through this project, supported by MSU's Shackouls Honors Summer Undergraduate Research Fellowship and the U.S. Fish and Wildlife Service, we focus on identifying key habitat components related to the distribution and demographics of various birds, including Bachman's Sparrow, within the Sam D. Hamilton Noxubee National Wildlife Refuge. Herein, the objectives of this project are two-fold: 1) to collect and evaluate information supporting the MAPS program, assisting the U.S. Fish and Wildlife Service to address site fidelity, habitat use and demographics of avian communities within a pine

ecosystem within the Sam D. Hamilton Noxubee National Wildlife Refuge, and 2) to collect information on the spatial distribution, habitat use and demographics of a pine endemic species, the Bachman's Sparrow.

METHODS

All fieldwork was conducted at the Sam D. Hamilton Noxubee National Wildlife Refuge, an area that spans Lowndes, Oktibbeha, Noxubee and Winston counties, Mississippi. Fieldwork was conducted beginning early May and running through July 2015. Fieldwork incorporated assisting with the existing MAPS station as well as surveying for Bachman's Sparrow (see Cox et al. 2009).

For MAPS, monitoring of the avian community within the site used since 2013, an area off Douglass Bluff Rd. Bird banding took place over one day in each of 10 periods: (1) May 1-10; (2) May 11-20; (3) May 21-30; (4) May 31-June 9; (5) June 10-19; (6) June 20-29; (7) June 30-July 9; (8) July 10-19; (9) July 20-29. During each period 10 12m mist nets were operated within an area encompassing approximately 20 ha, with a targeted density of ~1.5 nets/ha. Nets were opened at sunrise and run for 6 hours, weather permitting, during each session. Primary data collected through the MAPS program includes the species, age, sex, breeding condition (based on presence/absence of anatomical features), wing length (measured with a ruler), date and time captured, mist net where captured (station) and the disposition of each bird (healthy vs. visually unhealthy). Each captured bird was outfitted with a uniquely numbered aluminum bird band, registered through the U.S.G.S. Bird bands have been developed with the species size considered, information referenced through a guidebook (Pyle 1997).

For measuring the distribution and habitat associations of Bachman's Sparrow, we conducted playback surveys to identify this species distribution within specific pine forests throughout the Noxubee refuge. Surveys followed methods described in Watts et al. (1998), with each site was surveyed three times to account for detection probability. Habitat associated with surveys were sampled along transects following methods as described in Haggerty (1998), providing a method to examine the diversity of habitat structure within each study location. Specific habitat metrics included the size of the accessible survey area (measured as radial length across sub-canopy opening, assessed using a laser range finder), sub-canopy height (measured using digital range finders), density of the understory and midstory (defined as number of hits at ≤ 1 m and ≤ 2 m on a pole held at transect), density and size of pine and/or hardwood within, and adjacent to, the focal area, herbaceous density, leaf litter density, tree species composition. For analytical purposes, sub-canopy area was considered as sub-canopy width only as this reflects the smallest radius that could be drawn within each survey area and therefore is a conservative estimate of available area.

Data were analyzed using statistical tools, including occupancy analysis, to link species presence/absence to measured habitat attributes. For occupancy analysis we used the package Unmarked in R version 3.0.2 (R Core Team 2013). Models including detection (p) and occupancy (ψ) were developed using all covariates, and compared using AIC and AIC_c (AIC corrected for small sample size). The top-ranked model was accepted as that with the lowest AIC_c value.

RESULTS

M.A.P.S.

Through the M.A.P.S. program run at Sam. D. Hamilton Noxubee National Wildlife Refuge, May 1–July 29, 2015, we captured 145 individuals. Of these, 94 were new captures, 46 were recaptures (either within year or between years from the same M.A.P.S. location) and five were captured but not banded. Of these species (shown in Table 1). White-eyed Vireo (*Vireo griseus*) was the most common new and recaptured species, followed by Hooded (*Setophaga citrina*) and Kentucky Warbler (*Geothlypis formosa*).

Bachman's Sparrow Surveys

Between May 29 and June 24, 2015 we surveyed 33 locations for Bachman's Sparrows. Surveys were carried out in triplicate with \geq one week between repeat surveys. Through these surveys, Bachman's Sparrow was detected 98 times, most detections occurring at the same survey sites, across repeated surveys. The size of canopy gaps surveyed ranged from 59 – 412 m width (Table 1). Average tree height, density of vegetation (measured in hits per height class), average DBH, canopy height and duff depth are all shown in Table 1.

The naive estimate for Bachman's Sparrow detection (p) was 0.29, with p not statistically significantly related to any covariates considered. The top-scoring model predicting Bachman's

Sparrow occupancy (ψ) at survey sites included the covariates of sub-canopy width and the density of the vegetation within 1 m of the ground (Density Low; Table 1). Bachman's Sparrow occupancy (ψ) related positively with the width of the sub-canopy gap and negatively with the density of vegetation within 1 m of the ground (Table 1, Figs 2–3).

DISCUSSION

Many species of birds exhibit area sensitivity by which individuals most commonly occur within larger areas of suitable habitat (Robbins et al. 1989; Parker et al. 2005; Keyel et al. 2012). Examples of area sensitivity can be drawn from various ecosystems, yet the mechanisms behind these processes are yet not fully understood. Results from our study, like for other species, indicate the occurrence of Bachman's Sparrow within Loblolly pine stands is also positively related to the size of open sub-canopy habitat within the stand.

Current practices to assess the area of a pine stand, such as sub-canopy area, are limited by technologies in GIS and access to expensive surveying equipment. Using range-finders to measure sub-canopy area we were able to show that the presence of Bachman's Sparrow is positively related to the size of sub-canopy openings. To our knowledge, this is the first study to show this association for an open pine species.

Of those habitat metrics measured, presence of Bachman's Sparrow was significantly related to the size of the sub-canopy area as well as the density of the vegetation within one meter of the ground. The negative association between the presence of Bachman's Sparrow and the density of this vegetation indicates that this species is less likely to occur in areas that are not thinned through processes such as prescribed fire. As a species associated with ephemeral habitats (Taillie et al. 2015), continued management using prescribed fire as means to limit the density of herbaceous undergrowth, can serve an effective means supporting conservation of Bachman's Sparrow within the Sam D. Hamilton Noxubee National Wildlife Refuge.

As a species associated with the ephemeral habitats of fire-maintained pine systems, Bachman's Sparrow will abandon sites if not burned within a 3-year cycle (Jones et al. 2013). What's more, in selecting habitats, Bachman's Sparrow can abandon forest patches shortly after fires, returning once regeneration has occurred (Brown 2012). As larger forest patches can provide for a mosaic of fires intensity, increased occurrence of Bachman's Sparrow with larger patch size may reflect this species' use of a variety of burn intensities, facilitating dispersal among sites pre and post-fire activity.

The current study increases our understanding of the processes that affect the presence of Bachman's Sparrow within open pine habitats. However, information on occurrence provides little insight on the demographic processes that contribute to this population. Continued efforts in working with Bachman's Sparrow at the Sam D. Hamilton Noxubee National Wildlife Refuge should expand on occurrence surveys to measure and address specific demographic processes within different habitat types.

ACKNOWLEDGMENTS

This project was supported through the Mississippi State University's Shackouls Honors Summer Undergraduate Research Fellowship Program. Additional support was provided by the Sam D. Hamilton Noxubee National Wildlife Refuge and the Department of Wildlife, Fisheries and Aquaculture of Mississippi State University. We are thankful for this generous and continued support. We are also indebted to S. Veum and Z. Loman who assisted with logistical and analytical aspects of this project.

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Table 1. Birds captured through M.A.P.S. activities at the Sam D. Hamilton Noxubee National Wildlife Refuge, May 1–July 29, 2015.

Common name	Status, 2015		
	New	Recapture	Unbanded
Acadian Flycatcher	4		1
Carolina Chickadee	1		
Blue-gray Gnatcatcher	1		
Carolina Wren	3	4	
Eastern Towhee		1	
Eastern Wood-peewee	1		
Hooded Warbler	12	6	1
Indigo Bunting	2	1	
Kentucky Warbler	8	11	1
Northern Cardinal	7	3	1
Pine Warbler	2		
Prothonotary Warbler	2		
Red-eyed Vireo	5		
Summer Tanager	1	1	
Swainson's Thrush	2		
Swainson's Warbler	1		
Tufted Titmouse	4	2	
White-eyed Vireo	27	14	1
Worm-eating Warbler	1		
Wood Thrush	1		
Yellow-breasted Chat	7	3	
Yellow-throated Vireo	1		
Yellow-throated Warbler	1		

Table 2. Vegetation metrics collected from survey points sampled for Bachman’s Sparrow within the Sam D. Hamilton Noxubee National Wildlife Refuge. Metrics defined below table.

Survey Site	Width (m)	Dns_Low	Dns_High	Duff (cm)	Tree_ht (m)	DBH (cm)	Canopy (%)
<i>WPT</i>	265	1.1	2.7	0.63	92.24	25.02	24.25
<i>DLR1</i>	249	0.2	1	0.25	30.5	36.67	25
<i>DLR2</i>	158	1.25	3.35	0.57	30.67	54.59	24.75
<i>DLR3</i>	147	0.1	1.1	0.22	43.89	80.22	28.75
<i>DLR4</i>	238	0.6	3.05	1.69	45.67	47.77	34
<i>WLR1</i>	59	0.9	2.95	0.69	79.8	16.2	15.5
<i>WLR2</i>	67	1	3.1	0.63	82.35	14.43	15.25
<i>WLR3</i>	118	0.75	2.9	0.44	94.8	18.97	21.75
<i>WLR4</i>	273	0.25	2.55	0.88	91.47	16.07	17.75
<i>WLR5</i>	316	0.05	1.35	1	89.23	22.63	23.25
<i>DLR5</i>	176	2.5	3.75	0.81	87.125	16.13	28.25
<i>DLR6</i>	177	0.16	2.3	0.69	81.93	14.2	24.25
<i>LCR1</i>	123	0.95	2.9	1.19	68.47	21.37	19.75
<i>LCR2</i>	152	0.45	3.1	1.03	84.97	26	24.5
<i>SELLR1</i>	198	0.2	2.95	1.19	100.77	19.75	30.5
<i>SELLR2</i>	228	0.15	2.7	0.16	82.2	16	28
<i>SELLR3</i>	129	0.1	3	1.19	97.88	22.7	20.75
<i>SELLR4</i>	232	0.1	0.9	0.06	83.63	21	24
<i>LFR1</i>	148	0.35	3.35	1.25	93.2	18.28	19
<i>LFR2</i>	263	1.3	3.25	0.63	92	21.23	21.75
<i>BHL1</i>	159	2	3.5	0.63	104.5	20.83	21
<i>BHL2</i>	218	0	2.05	0.38	87.33	24.57	28.67
<i>BLR1</i>	190	3.65	4.75	0.44	90.32	25.66	16
<i>BLR2</i>	91	1.2	2.7	0.63	92.64	25.02	28.75
<i>BLR3</i>	96	0.05	2.6	0.34	91.375	15.85	24.5
<i>BLR4</i>	137	0.5	3.45	0.5	93.025	20.05	19
<i>BLR5</i>	412	0.55	2.8	1.5	89.15	19.35	26.25
<i>BLR6</i>	112	0.3	3.3	0.38	91.8	24.33	23
<i>BLR7</i>	113	0.6	3.75	0.5	88.525	13.98	30.75
<i>BLR8</i>	232	2.35	3.35	0.69	92.75	18.4	28
<i>BLR9</i>	246	0.45	3.15	0.69	96.125	16.68	24.5
<i>BLR10</i>	132	0.05	3.3	0.43	97.15	21.98	26.25

Vegetation metrics

Int = Intercept (untransformed naive estimate)

Cnp = Canopy cover (%)

Dns_high = density of vegetation \geq 1m above ground \leq 2m above ground

Dns_low = density of vegetation \leq 1m above ground

Dff = width of duff layer

Wdt = width of sub canopy gap

Table 3. Models predicting Bachman’s Sparrow occupancy and detection within pine stands of the Sam D. Hamilton Noxubee National Wildlife Refuge. Covariates included in models defined below table. Values represent untransformed estimates, blanks indicate covariate not included in model.

Model	ψ(Int)	p(Int)	ψ(Cnp)	ψ(Dns_high)	ψ(Dns_low)	ψ(Dff)	ψ(Wdt)	p(Wdt)	df	logLik	AICc	delta	weight
fm_occ_triples_1	-43.51	0.9103			-24.27		0.40	0.003	6	-50.72	116.7	0	0.577
fm_occ_triples_2	0.3916	-1.126			-17.96	-14.74		0.004	6	-52.01	119.3	2.59	0.158
fm_occ_triples_3	-110.3	-1.165	1.40	-128.2				0.004	6	-52.02	119.3	2.61	0.156
fm_occ_pairs_5	-56.84	-1.94	3.63					0.007	5	-54.62	121.5	4.79	0.053
fm_occ_univ_2	-114	-1.62						0.005	4	-57.18	123.8	7.12	0.016
fm_occ_univ_4	7.618	-1.922	-3.18					0.007	4	-57.33	124.1	7.43	0.014
fm_occ_NULL	7.475	-2.087						0.007	3	-59.08	125	8.32	0.009
fm_occ_univ_1	-2.863	-1.64						0.006	4	-57.98	125.4	8.72	0.007
fm_occ_GLOBAL	-10.42	0.9093	-0.57	-20.91	-38.13	-24.42	0.72	0.003	9	-50.69	127.2	10.53	0.003
fm_occ_univ_3	6.621	-2.086				4.76		0.007	4	-59.08	127.6	10.92	0.002
fm_occ_pairs_1	-5.071	-1.405					0.015	0.005	5	-57.80	127.8	11.15	0.002
fm_occ_pairs_4	5.09	-2.082		5.029		1.20		0.007	5	-59.08	130.4	13.71	0.001
fm_occ_pairs_3	6.67	-2.085			-0.01135	4.79		0.007	5	-59.08	130.4	13.71	0.001
fm_occ_pairs_2	3.388	-2.062		3.335	-0.4598			0.007	5	-59.08	130.4	13.72	0.001

Covariates included in models

Int = Intercept (untransformed naive estimate)

Cnp = Canopy cover (%)

Dns_high = density of vegetation \geq 1m above ground \leq 2m above ground

Dns_low = density of vegetation \leq 1m above ground

Dff = width of duff layer

Wdt = width of sub canopy gap

Fig. 1. Response of Bachman's Sparrow occupancy to width of sub-canopy-gaps within pine stands of the Sam D. Hamilton Noxubee National Wildlife Refuge. Prediction based on 90% credible interval generated using occupancy models.

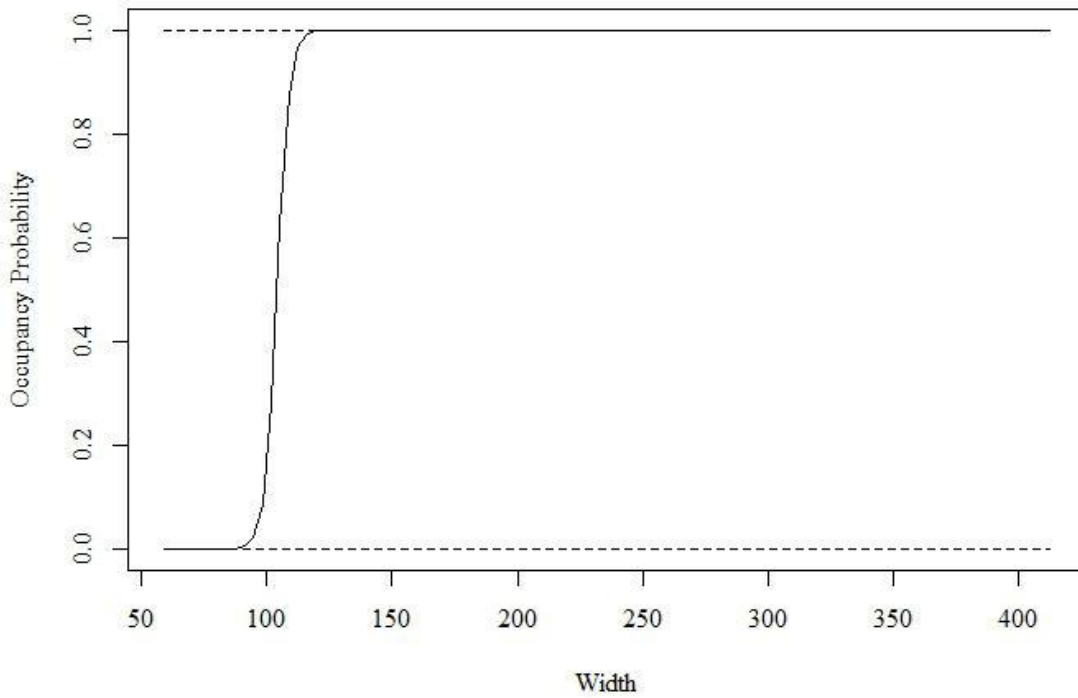
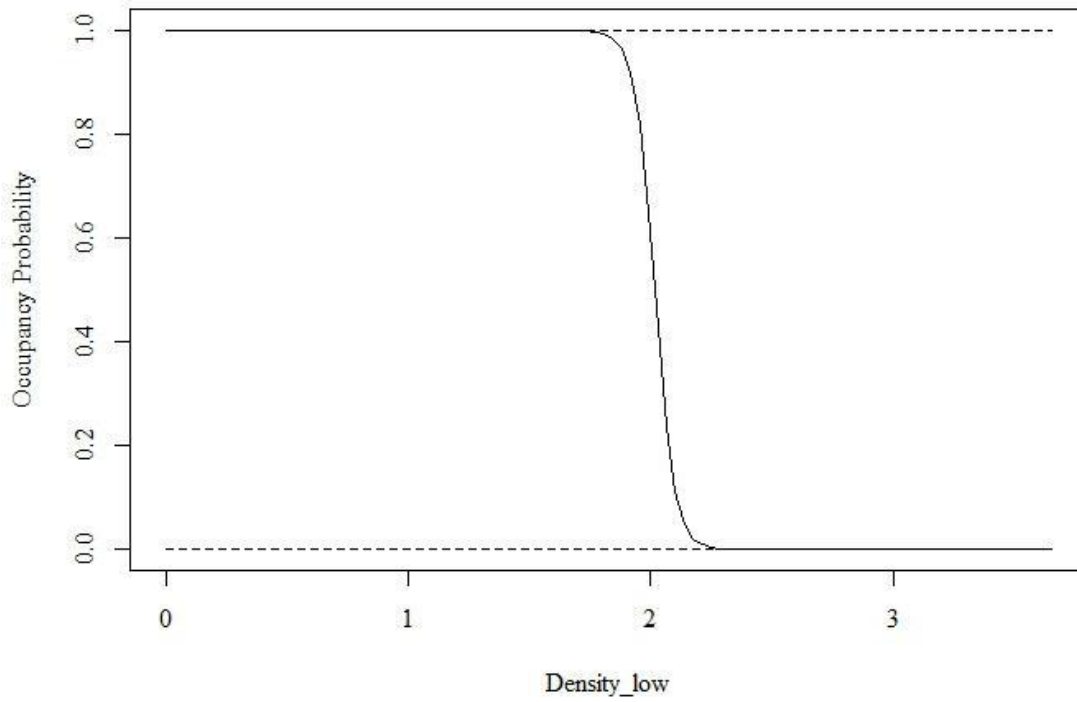


Fig. 2. Response of Bachman's Sparrow occupancy to density of vegetation within 1 m of ground as sampled within pine stands of the Sam D. Hamilton Noxubee National Wildlife Refuge. Prediction based on 90% credible interval generated using occupancy models.



Name: Dunaway, Luke

Major: Biological Engineering

Faculty Advisor: Dr. C. LaShan Simpson

Project Title: A Cell Therapy for Vascular Calcification

Cardiovascular disease is the leading cause of death in the United States. Recently, research has shown that the process in which the arteries calcify is similar to the biomineralization process of bone. Many treatments currently under investigation to treat vascular calcification involve the systemic administration of calcification inhibiting molecules which commonly leads to side effects. Osteoclasts are cells native to bone and are responsible for the breakdown of bone matrix. While there has been some investigation into the use of these cells to demineralize calcified arteries, to our knowledge the use of injectable biomaterials to deliver osteoclasts has never been investigated. Our research group's goal is to develop a method of delivering osteoclast cells to calcified arteries as a method of treating vascular calcification.

After performing an extensive literature search, we have chosen a materials and a protocol that fit our needs. Our group plans to develop oxidized alginate-fibrin microbeads to deliver the osteoclasts to calcified blood vessels. The microbeads will be composed of 7.5% oxidized alginate with fibrin added at a concentration of 0.1%. The microbeads will be cross linked in a calcium chloride/thrombin solution. The microbeads will be evaluated based on their size, degradation rates, and cell compatibility.

These microbeads have shown fast cell release times in past studies. We expect the microbeads to offer high cell viability during injection and to begin releasing the cells within four days. The cells should then begin to degrade the hydroxyapatite matrix while leaving the native elastin matrix intact.

The protocol we are following; however, lacks many details. Thus far we have been refining the protocol to produce higher yields. We are experimenting with different centrifuge speeds and durations as well as different methods of mixing the reactants. As this research continues, we hope to perfect the protocol and begin experimenting with various size microbeads and cell encapsulation methods. This research offers a new method of treating vascular calcification.

Name: Dunn, Christine

Major: Secondary Education, English

Faculty Advisor, Department: Dr. Judith Ridner, History

Project Name: A Shaky Truce: Starkville Civil Rights Struggles, 1960-1980

For this project, Ms. Dunn had several goals. To familiarize herself with the research conducted thus far, she began the summer by listening to and partially transcribing and annotating existing oral history interviews with Starkville residents. As she created these notations, she categorized this information and built a glossary of key terms for use in building the project website.

In transcribing and annotating the interviews, Ms. Dunn was largely self-directed. The project's digital humanities specialist, Ms. Eichmann, suggested the project use the OHMS (Oral Humanities Metadata Synchronizer) to make the interviews accessible to the general public through the website. After familiarizing herself with the information required by the OHMS system, Ms. Dunn began listening to interviews one at a time, sometimes several times through, in order to collect the necessary information including a summary of each "section," "tags" or keywords that could be used to identify each section, and time stamps for the beginning and end of each section. As she made her notes, Ms. Dunn developed a model for others in the project to use as the group continues to conduct interviews and catalogue the information. She included a list of keywords to use as a reference in order to keep the tags consistent and understandable for the general public.

For the next part of the project in the fall, she worked collaboratively with Dr. Judith Ridner, Humanities Librarian Ms. Hillary Richardson, History Research Librarian Ms. Nickoal Eichmann and graduate students in the history department to help design and construct the project website (<http://starkvillecivilrights.msstate.edu/wordpress/>). In this phase of the project, Ms. Dunn's work in notating and indexing the interviews was instrumental, as she had identified and cataloged key themes that were repeated across multiple interviews. These themes, and key quotes she had recorded from the interviews, helped to build the website's narratives.

Most important, Ms. Dunn also worked independently to create lesson plans for the teacher resources section of the website. Using the themes she had identified from the interviews, she designed lesson plans to adhere to the Mississippi State Standards regarding the study of Mississippi History in the social studies classroom (though the lesson plans could be easily adapted for use across many subject areas). On the website, each of these lesson plans includes links to the interviews that include information about that topic.

Finally, Ms. Dunn also played a critical supporting role at the community forum the project held to launch our website on October 29 at the Hilton Garden Inn. Ms. Dunn assisted in both the planning and staging of this event, which was covered by the *Starkville Daily News*.

Name: Hargrove, Shaquela

Major: Psychology

Faculty Advisor: Kristina B. Hood, Ph. D.

Project Title: Acceptance of Interracial/Interethnic & LGB Couples

The goal of this research is to determine to what extent personal factors such as religion, political conservatism, education level, home location, sexual orientation, gender identity, racial identity and ethnic identity influence support and acceptance of marginalized couples (e.g., interracial/interethnic and lesbian, gay, and bisexual couples.). According to the research, there are some similarities and differences in acceptance, depending on the type of marginalized couple. The literatures shows that most people who accept interracial/interethnic relationships tend to be male (Yancey, 2002; Perry, 2013c), non-White (Perry, 2013c), younger (Yancey, 2002; Perry, 2013c), from the western United States (Perry, 2013c; Yancey, 2002; Johnson, 2005), more politically liberal (Eastwick, 2009; Yancey, 2002; Perry, 2013c; Johnson, 2005), and highly educated (Johnson, 2005).

In contrast, that people who attended multiracial churches, non-Whites, those with a higher education (Perry, 2013d), women, and non-Republicans were more likely to endorse and accept same sex marriages (Perry, 2013b; Horn, 2007). Sex, religiosity, and ethnicity showed significant effects on acceptance: females, those with lower religiosity, and those of Western ethnicity are more accepting of both female and male LGB sexuality (Collier, 2012). In addition, interracial couples were found to be more accepting than intraracial couples of LGB unions (Perry, 2013a). However, there has been little literature on how sexual orientation is related to acceptance of interracial couples, but it has shown to have some correlation with acceptance (Perry, 2013c). Furthermore, few studies have investigated whether there are personal factor similarities of those who are accepting of interracial couples and LGB couples. There are a lot of conflicting conclusions about how certain religious, racial/ethnic group association, couple composition and sexual orientation factors influence acceptance of marginalized relationships, and little research has examined how the acceptance of one group influences the acceptance of another.

Over the summer and during the fall semester, I conducted research on acceptance of marginalized relationship. This research results in the completion of a concept paper on my topic, I am currently working on completing the IRB and set up study in Qualtrics. The proposed study will recruit undergraduate students via the Psychology Research Program via SONA-Systems to complete my study in exchange for course credit. After consent, the participants' will be shown vignettes that display different compositions of interracial/interethnic and LGB couples and asked about how acceptable each couple is to them. I am currently in the process of creating and tailoring all 36 of my vignettes. After participants have completed all of the scale items, including a demographic questionnaire, participants will be given the debriefing form, thanked for their participation, and granted 1 hour of credit. I plan to begin running the study at the start of Spring 2016 and will present my preliminary findings at the Undergraduate Research Symposium Spring 2016.

This research is important because although interracial/interethnic and LGB couples have gained significant support and acceptance over recent years, there are still a great deal of groups or individuals who oppose marginalized couples. Figuring out which factors are associated with opposition could lead to programs tailored for specific groups to educate them on prejudice and the harms caused by discrimination.

Name: Jefferson, Taylor

Major: Psychology

Faculty Advisor, Department: E. Samuel Winer, Psychology

Project Name: Multifaceted Training in Conducting and Disseminating Psychological Science

This research project for Ms. Jefferson had two main goals. The first goal of the project was the development of the necessary skills needed to assume a leadership role in Dr. Winer's research lab. The second goal was to work closely with both Dr. Winer and Jenna Kilgore, a previous and current Honors Summer Undergraduate Research Fellowship winner, to complete publication and presentation submissions using the data collected from a longitudinal study conducted the previous year by Dr. Winer, Ms. Kilgore, and Dr. Michael Nadorff.

Ms. Kilgore first trained Ms. Jefferson on the protocol of the lab, under the supervision of Dr. Winer. Ms. Jefferson was taught every aspect of the protocol so that she could potentially train any new undergraduate research assistants that might join Dr. Winer's lab on the proper protocol for running research participants. Additionally, once she finished her training, Ms. Jefferson began to run research participants. Ms. Jefferson was also able to take a lead role on entering any new data collected in the current study being conducted by Dr. Winer and Dr. Nadorff. Ms. Kilgore and Meredith Pearson, another Honors Summer Undergraduate Research Fellowship recipient, taught Ms. Jefferson how to properly code measures, create codebooks, and check coding in Qualtrics, online programming software package. Based on her progress and mastery of data entry, she was given the leadership role of training new undergraduate research assistants on data entry. She also created a set of codebooks for Dr. Winer and Dr. Nadorff to use for one of their previously conducted longitudinal studies, a large-scale project that was led by Ms. Jefferson.

In addition to her training, Ms. Jefferson was able to aid in the process and completion of a presentation submission. She has conducted multiple literature reviews to date as part of the laboratory, including two separate literature reviews in conjunction with Ms. Pearson and Gage Jordan, a graduate research assistant in Dr. Winer's lab. Additionally, she aided in data cleaning under the supervision of Ms. Kilgore and was also able to help Dr. Winer and Ms. Kilgore run analyses on the longitudinal dataset. Due to her extensive work helping Ms. Kilgore clean and analyze the dataset, Ms. Jefferson earned third authorship on a poster submission that has been accepted to be presented at the upcoming annual ABCT convention in Chicago. ABCT is arguably the preeminent clinical psychology conference in the country, and it is impressive for Ms. Jefferson to have this accomplishment on her curriculum vitae. Ms. Jefferson has also helped in crafting the actual poster for presentation. Thus, her work on all of these projects has greatly contributed to her knowledge of the submission process.

Over the course of her research project, Ms. Jefferson gained immense knowledge about how research is conducted. Being able to work side by side with Ms. Kilgore exposed her to the necessary skills to be an effective leader in Dr. Winer's research laboratory. Additionally, she was able to become more confident in her ability to write literature reviews and conduct searches to find relevant literature to support any findings that may come up in her future data analyses. Her work involving coding and entering data has greatly increased her interest in understanding how certain measures test certain traits and characteristics. Overall, she has gained more confidence in her ability to be an effective and productive researcher and has increased her skillset for future graduate training. Ms. Jefferson has expressed her gratitude for the opportunity the Honors Summer Undergraduate Research Fellowship afforded her to work closely and learn from both Dr. Winer and Ms. Kilgore and has expressed confidence that she is a much stronger researcher thanks to this experience.

Name: Kelly, Sarah

Faculty Advisor: Dr. Sylvia H. Byrd

Project Title: *Effects of cooking camp on self-ethnicity of professional development*

GOAL: Determine how experiential learning through *Fun with Food* cooking camp affects the self-efficacy of professional development in Nutrition majors.

PROGRESS: Since receiving funding to complete this research project, the ninth year of *Fun with Food* camp has been completed. Upon completion of camp, a comprehensive list of counselors from all nine years was compiled. Contact information for these counselors was obtained in order to send the survey once the IRB application has been approved. I have begun filling out the IRB application, but it is contingent upon completion of the literature review, the survey, and drafts of emails that will be sent to participants (counselors). I have worked with my faculty advisor, Dr. Sylvia Byrd, as well as her graduate assistant, Rahel Mathews, to determine key terms and important factors to consider when completing the literature review. I have completed multiple drafts that have been sent to members of our CHANGE (Community Health and Applied Nutrition Group Exchange) Research team for review. We have also worked to draft a survey intended for completion by the participants. Multiple drafts have been reviewed with the goal of adequately answering the research question. The survey will ultimately be delivered via email and administered through Qualtrics. I have also begun to draft the emails that will be sent to participants. There will be a total of four: an initial email containing the survey and three reminders sent to participants who have still failed to complete the survey. The next step is to finalize the literature review, survey, and emails in order to finish the IRB application. Our ultimate goal is to have the survey emailed to participants prior to Thanksgiving so that data collection and statistical analysis can begin in December.

Name: Kilgore, Jenna

Major: Psychology

Faculty Mentor, Department: Dr. E. Samuel Winer, Psychology

Project Title: *Investigating Different Clinical Symptoms of Adults Over Time (2)*

The main purpose of this research project was to continue to expand and analyze the established longitudinal data collection based at Mississippi State University to result in a presentation and/or publication. The goals of this project also included Ms. Kilgore learning and presenting the steps of advanced multi-linear analyses through SAS, SPSS, and R alongside Drs. Winer, Nadorff, fellow undergraduate and doctoral students. Other goals included continuing Ms. Kilgore's work with on an NIMH R-15 grant and training fellow undergraduates for laboratory leadership roles.

This summer Dr. Winer and Ms. Kilgore completed the final wave, wave 4, of data collection, thus spanning this project over a calendar year. Seven hundred six valid-responding participants completed wave 1, 384 valid-responding participants completed wave 2, and 294 valid-responding participants completed wave 3, and 216 valid-responding participants completed wave 4, resulting in a 73% retention rate over from wave 3 to wave 4 and 31% retention rate over the course of the entire year. One hundred thirty valid-responding participants completed all four waves, and 485 valid-responding participants completed at least 2 waves, thus yielding a fruitful longitudinal dataset that allows for the exploration of measures of emotion and psychopathology over time, many of which have never been examined over this timeframe.

A primary focus of the summer consisted of data preparation regarding these four collected waves of data. Ms. Kilgore along with Ms. Pearson and Ms. Jefferson spent hours creating a Qualtrics coding manual and an excel time record, converting Excel documents from Qualtrics to SPSS, and cleaning and organizing the four different waves of data into a single file. To complete the data organization, Ms. Kilgore and Ms. Pearson received training in SPSS longitudinal data organization, which consists of structuring a dataset in one of two ways. Data can be structures such that multiple variables are distinguished via a temporal suffix (i.e., variable.t1, variable.t2) or that instead time is collapsed into single variables and time is its own variable, to be used in a nested model. Multi-linear analysis readings were assigned from the acquired texts; however, it was realized after reading and discussing the initial chapters that Ms. Kilgore would benefit from primarily focusing on data structuring to further her understanding of how data need to be organized to complete these analyses, and from completing initial analyses that would lead to an impactful presentation at a national conference. Moreover, data organization proved to be a very large task that called for a large amount of Ms. Kilgore's time. Ms. Kilgore has also received training in the use of the R statistical software package with regard to subject recruitment.

As an example of the experience Ms. Kilgore gained in data organization and restructuring, Ms. Kilgore helped complete and use an excel timesheet to that contained each MTurk ID and the time and date which the survey was taken, per each wave. The timesheet was compared to an SPSS file and any errors or repeat IDs were edited. Each individual response had to be verified, as several participants incorrectly entered their individuating MTurk IDs, which then had to be manually matched to responses from prior and future waves. After this initial validation, each individual wave was examined again, verified, and organized by at least two undergraduate researchers to ensure reliability and validity of the dataset. Once each wave was verified, all four waves were combined into a single file to be analyzed.

A Qualtrics coding manual was also established to sustain the project past Ms. Kilgore's graduation and includes coding values for each individual measure. Ms. Kilgore led the training of Ms. Taylor Jefferson so that she might assume these duties upon Ms. Kilgore's matriculation. Ms. Kilgore also continued her work with Dr. Winer's NIMH R15 grant. This included taking a leadership role and training other undergraduates as well as running weekly participants. Under the supervision of Dr. Winer, Ms. Kilgore and Ms. Pearson created a training protocol document, consisting of precise instructions on how to systematically train new undergraduates properly for them to adequately run subjects on the R-15 grant. This allows graduate and undergraduate students to ensure protocol fidelity by helping Dr. Winer train them in experimental protocol. This was consistent with a goal of the R-15 grant, which is to contribute to the infrastructure of the investigators' research laboratories as well as the psychology department and university in general.

Ms. Kilgore also took a lead role in training Ms. Jefferson, per Ms. Jefferson's fellowship, on the R-15 protocol, which called for walking her through each task, explaining how to run participants, and playing the role of the participant in order for Ms. Jefferson to practice. Ms. Kilgore also prepared Ms. Jefferson to take over the leadership role in the laboratory upon her graduation. She was also an example to Ms. Jefferson in daily leadership roles, including making sure all materials are prepared for the day, organizing undergraduate schedules for each semester, and aiding graduate students

in other tasks (e.g., helping to arrange subject parking) as needed. Ms. Kilgore along with Mr. Jordan, a doctoral student in the laboratory, worked with both Ms. Pearson and Ms. Jefferson to create the summer and fall running schedules, taking into account each undergraduate's schedule as well as those of the graduate students and professors. This is a large task that consists of producing and iterating schedules of up to ten research assistants in three rooms each of which have specific capabilities that need to be considered during scheduling.

Under Dr. Winer's supervision, Ms. Kilgore also trained Ms. Jefferson and Ms. Pearson on Qualtrics and Amazon.com's Mechanical Turk. Both were shown how to input a survey into the Qualtrics website, share it, and load it to MTurk, as well as how to view and download the data. This training was once again consistent with building the infrastructure of the laboratory. With this training, Ms. Jefferson can continue this research project, and Ms. Pearson is prepared to use this method as a way of conducting her own future research. This experience provided Ms. Kilgore a great opportunity to learn the requirements of being a leader in a research laboratory, including managing, training, and working alongside other undergraduate and graduate students, which will be invaluable to her in her applications for clinical psychology PhD programs and beyond.

The work this summer led to the analyses of the collected data, resulting in two different posters, both accepted to the 2015 Association for Cognitive and Behavioral Therapy conference in Chicago. A graduate student, Mr. Jordan, is the lead author on one poster, while Ms. Kilgore is first author followed by both Ms. Jefferson and Ms. Pearson on the second poster. Ms. Kilgore and Ms. Jefferson took part in the analysis of the Life History of Aggression measure alongside Ms. Salem, a 4th year doctoral student, and Dr. Winer. Ms. Jefferson, as part of her training, was assigned to create the physical poster, and Ms. Kilgore will be presenting at the conference.

Overall, this summer was the most productive yet for Dr. Winer's laboratory and Ms. Kilgore's. Ms. Kilgore was able to learn important leadership skills that will be imperative for graduate school while participating in two longitudinal studies simultaneously. One, the four-time-point longitudinal database, spanning approximately a calendar year, allows for unique answers to longstanding questions about the associative trajectories of person-based and psychopathological constructs.

Name: Kovach, Annie

Faculty Advisor: Raj Prabhu

Departments: Department of Agricultural and Biological Engineering at Mississippi State University, Center for Advanced Vehicular Systems at Mississippi State University, College of Veterinary Medicine at Mississippi State University

Project Title: *Targeted Drug Delivery via Nanoparticles: Novel Treatment for Osteosarcoma*

This summer I was able to continue working on research thanks to the Honors College Summer Fellowship. The research project mentioned is an explorative study of a targeted drug delivery system that we have designed to treat osteosarcoma, or bone cancer. The system is composed of an iron oxide magnetic nanoparticle serving as the base with two proteins attached to the surface: ligand CD80 and VEGF antibody. Together, this system has been hypothesized to be able to target and kill osteosarcoma tumor cells.

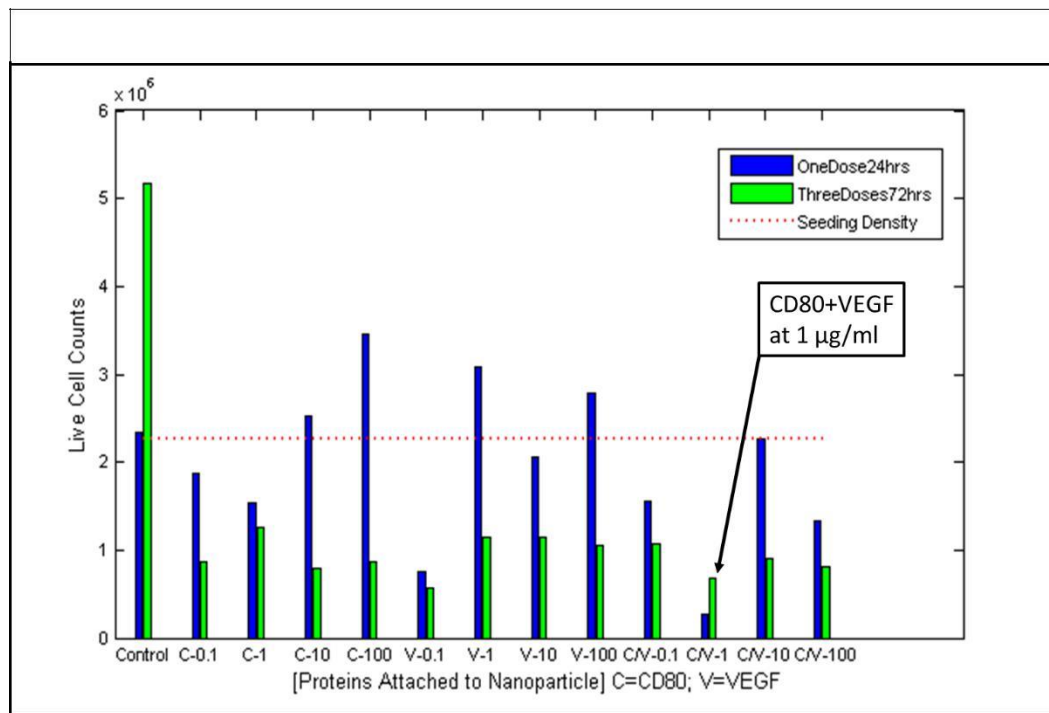
We have chosen Osteosarcoma for several reasons. It is the most prevalent bone cancer in humans. About 400 teens are diagnosed each year in the U.S. alone, making this disease the third most common form of cancer in teens. A big issue with this cancer is that in 15-20% of patients, by the time of diagnosis, the tumor has already metastasized to the lungs and other bones. This being said, the long term survival rate of patients with osteosarcoma metastasized to the lungs is only 30% post-treatment.

The treatment options presently include chemotherapy and tumor excision surgery. Osteosarcoma is a very aggressive cancer which makes chemotherapy often less effective. On top of that, chemotherapy has unwanted side effects that are intensified due to using more to combat this disease in particular. These side effects are mainly caused by the killing of healthy cells along with the cancerous cells. Surgery is the other option in which the tumor is removed. Sometimes there can be residual tumor left behind that will continue to metastasize. Because of this, surgery is often paired with post-surgery chemotherapy. Surrounding healthy tissues can also be damaged during surgery. Neither of these treatments are truly curative so there is a need for a noninvasive treatment that specifically targets cancerous cells, leaving healthy cells unharmed and reducing side effects.

Our proposal includes the previously mentioned delivery system with ligand CD80 and VEGF antibody. The VEGF antibody targets antigens on the tumor cell surface that are involved in tumor growth and metastasis. The antigen is expressed in 63.3% of primary osteosarcoma tumors and in 70% of metastatic osteosarcomas. Ligand CD80 induces apoptosis once it comes into contact with CTLA-4(Cytotoxic T-lymphocyte-associated antigen-4) receptor on the tumor cell surface. The CTLA-4 receptor has been shown to be highly expressed in human osteosarcoma cells. Using the proposed system, we hypothesized that tumor cell proliferation of mouse cells would be decreased significantly.

The process for testing this drug delivery system first involves conjugating our nanoparticles using an OceanNanotech protocol. Flasks are then seeded with cancerous mouse cells and cultured until 80% confluency. Next the well plates are seeded with around 2 million cells per well. Once cells are transferred to the six-well plates, they are allowed to attach for 24 hours. After 24 hours, 10 μ L nanoparticles of varying concentrations and combinations of the two proteins are introduced into six-well plates at concentrations of 0.1 μ g/ml, 1 μ g/ml, 10 μ g/ml, and 100 μ g/ml. The combinations included nanoparticles with only ligand CD80 attached, only VEGF antibody attached, and a combination of the two attached. It is important to note that a control group of non-exposed cells are also evaluated. Three wells are designated for each concentration, each type of nanoparticle, and the control. An additional dose of the corresponding nanoparticles are added at 24 hour intervals to remaining wells to study repeat exposure. Cell counts are performed for each concentration/type after initial exposure at 24 hours and 72 hours. Table 1 shows the results of this experiment; the lower the cell count the more efficient the particular system was at reducing cell proliferation. Note that the system utilizing CD80 and VEGF at one dose for 24 hours and 3 doses for 72 hours performed most effectively.

Table 1. Live Cell Counts at 24hr (1 dose) and 72hr (3 doses).



The experiment verified that using both proteins was more efficient at inducing apoptosis rather than using only one of the proteins. We also found that 1 µg/ml concentration of protein conjugated with the nanoparticles is the optimum parameter as opposed to the 3 other concentrations. For our next round of experiments we will narrow our scope down to 0.1, 1.0, and 10 µg/ml concentrations and focus on the system using both proteins. We will also look at testing on canine cells rather than mouse cells. Canine osteosarcoma occurs much more frequently than human osteosarcoma and it progresses at a faster rate. This makes the canine an ideal model when studying osteosarcoma.

This summer was a significant learning opportunity in the field of researching and doing extensive lab work. I plan on continuing this research and I really appreciate the funding provided by the Bobby and Judy Shackouls Honors College for this fellowship and the other funding I've received from the honors college for attending national conferences. These funding opportunities have opened so many doors, from doing more research at Mississippi State to networking with undergraduates doing research at other universities.

Name: LaCour, Allen

Major: Chemical Engineering

Faculty Advisor, Department: Dr. Dongmao Zhang, Chemistry

Project Title: *Size Effect on Ligand Exchange Reactions with Gold Nanoparticles*

As the result of their unique properties, gold nanoparticles (AuNPs) have applications in many different fields, such as biosensing, solar energy harvesting, and drug delivery. AuNPs are prepared for their given purpose by “functionalizing” them with different ligands, i.e. attaching molecules to their surface. Often, the functionalizing occurs through a ligand exchange reaction with the AuNPs and a sulfur compound, such as a thiol. Characterizing the surface of the AuNPs after this reaction occurs is important for investigating the utility of the AuNPs for a given purpose. In this study, the effect of an incoming ligand’s size on its ability to displace ligands preadsorbed to AuNPs is studied. Four thiols were used as the incoming ligands: sodium hydrosulfide (NaSH), 2-mercaptoethanol (2-ME), L-cysteine (Cys), and Glutathione (GSH). Their molecular weights are 56, 77, 121, and 327, respectively, so they cover a wide range of molecular sizes.

Methodology. AuNPs were first prepared in-house through the citrate-reduction method. 5 solutions of these freshly prepared AuNPs were incubated with adenine, the DNA nucleotide, for approximately 18 hours. Immediately after being incubated the AuNPs begin to spontaneously aggregate (their sedimentation out of the solution. Using absorbance spectroscopy, the amount of adenine that adsorbed onto the AuNPs can be quantified. The AuNPs were then washed thoroughly with water until the supernatant absorbance spectra displayed no activity. Each of the above thiols were then added to a solution of AuNP aggregates, and water was added to the fifth solution as a control. The absorbance of the supernatant was then monitored as a function of time to determine the quantity of adenine displaced from the surface of the aggregated AuNPs. This experiment was then repeated three times.

Results and Discussion. The percentage of adenine displaced from the AuNPs aggregates is shown in Figure 1. The percentage displaced shows a strong dependency on the MW of the incoming thiol. Also, the amount of adenine displaced appears to reach steady state quickly, usually within 10 hours.

The water sample indicates the mechanism by which this displacement occurs. The amount of adenine present in the supernatant of the water sample stays constant over time, which means that no adenine spontaneously desorbs the AuNPs in a water solution. Therefore, the incoming ligand must reach the surface of the AuNP to displace the adenine.

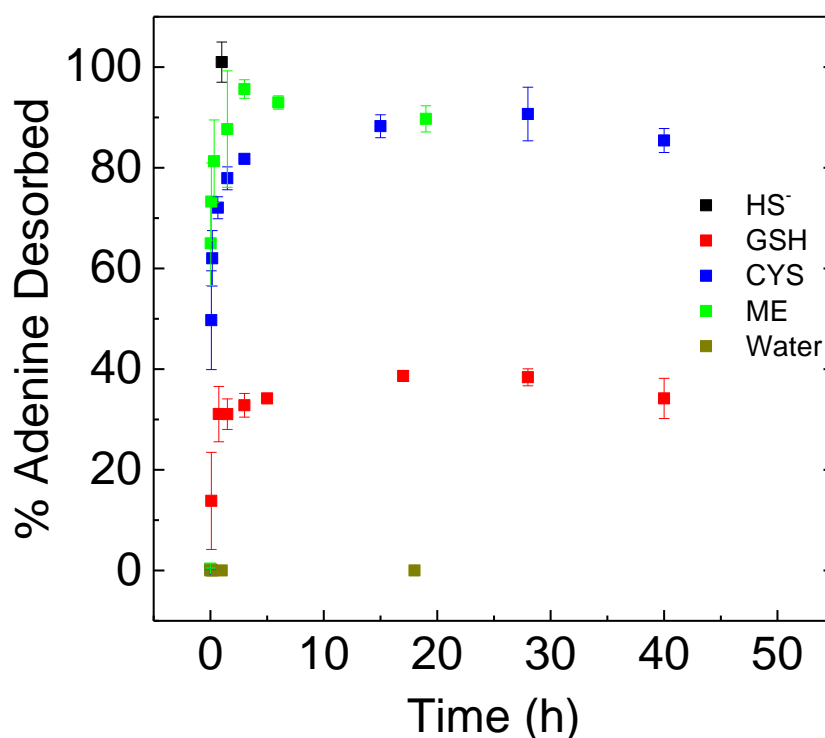


Figure 1. The percentage Adenine displaced of from the aggregated gold nanoparticles with time.

All of the molecules used are thiols, and thiols all bond to AuNPs through a sulfur atom. Therefore it is unlikely that the thiols have different binding affinity to the AuNP surface. This indicates that the size of the molecule is the most likely cause of the differences in desorption percentages.

The size effect probably results from two phenomena. Firstly, adenine is known to form an ordered monolayer upon gold surface. For the adenine to be displaced, the incoming thiol must displace reach the gold surface. Larger thiols can have a more difficult time penetrating the adenine layer in order to reach the surface. Secondly, when the AuNPs aggregate, they form a nanoporous foam structure. Adenine may be trapped inside very small pores, and therefore inaccessible to larger ligands.

Conclusion. The size effect and has large implications for the preparation of functionalization of AuNPs. The work presented here exemplifies the difference the size effect can make in the surface composition of AuNPs.

Name: Langford, Jaslyn

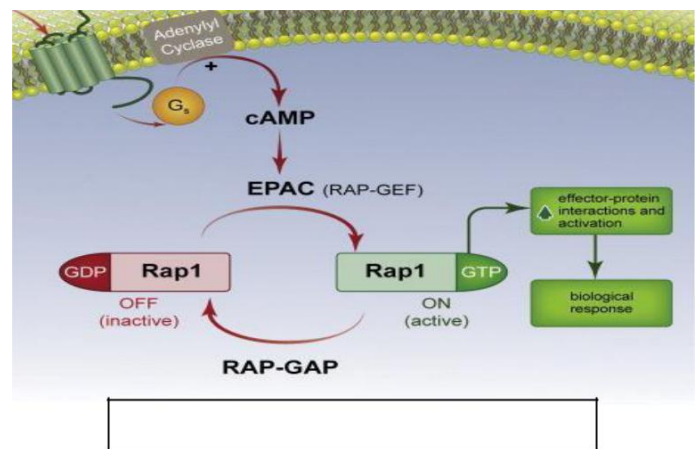
Faculty Advisor, Department: James A. Stewart, Jr., PHD, Department of Biological Sciences, Mississippi State University

Project Title: *ROLE OF RAP1A IN AGE/RAGE-MEDIATED SIGNALING IN TYPE 2 DIABETES MELLITUS*

PROJECT SUMMARY

Increased glucose levels are a defining feature of type 2 diabetes mellitus (T2DM) and considered to be an underlying cause of myocardial dysfunction in the diabetic population. Cardiac fibroblasts are the predominant cell type in the heart and are essential for normal regulation and pathological remodeling of the myocardial extracellular matrix (ECM). Studies have shown that hyperglycemia influences the cardiac fibroblast (CF) phenotype, leading to the increases in collagen I levels, changes ECM modulators, elevated advanced glycated endproducts (AGEs) and expression of AGE receptors (RAGE). In addition, formation and accumulation of AGEs are characteristic features in tissues of type 2 diabetes mellitus patients. Increased AGE/RAGE signaling has been implicated in the potentiation and pathogenesis of cardiovascular diabetic complications⁹. Therefore, changes in the interactions between fibroblasts and the myocardial ECM have been demonstrated to affect heart function by altering ventricular functional¹⁰.

Rap1a, a member of the Ras superfamily of GTPases, is a small monomeric G protein that acts as a molecular switch coupling extracellular events to intracellular signaling (Fig. 1). Through second messengers, such as cyclic AMP (cAMP), calcium and diacylglycerol (DAG). Recent studies have demonstrated a PKA- independent pathway for Epac-Rap1a activation¹¹.



There are very few directly attributed functional roles of Rap1a to ECM remodeling. In preliminary studies, we observed left ventricle (LV) collagen levels were significantly decreased in a Rap1a knockout mouse hearts, and by silencing Rap1a mRNA in diabetic fibroblasts, collagen production and RAGE expression return to that of non-diabetic levels. Both the AGE/RAGE signaling cascade and Rap1a utilize and activate similar signaling pathways involved in cell growth, ECM synthesis and fibroblast differentiation, including ERK1/2 MAP kinases, NF- κ B and JNK. Studies have shown that RAGE activates PKC- ζ signaling pathway propagated downstream by ERK1/2¹². Although there is some evidence of a functional interplay between AGE/RAGE and Rap1a, the exact cause-effect relationship between them remains inconclusive.

The molecular mechanisms governing T2DM-mediated alterations in fibroblasts are currently not well understood. The purpose of this study is to describe the molecular mechanism of Rap1a – AGE/RAGE dependent in remodeling diabetic cardiac fibroblast, leading to the increase of myocardial ECM protein. We hypothesize that **diabetes induces increased AGE/RAGE signaling which will be mediated by active Rap1a GTPase resulting in elevated ECM accumulation in the heart.** The following specific aims will test the hypothesis.

The goal of this study is to define a functional link between Rap1a and AGE/RAGE signaling cascades. We propose that the Rap1a and AGE/RAGE signaling cascades will converge at PKC- ζ to stimulate the downstream mediator ERK. Deactivation of this mechanism by utilizing a loss-of-function approach could lead to reduced fibroblast phenotype changes, decreased collagen accumulation and RAGE down-regulation. The genetically diabetic (db/db) mouse and lean (Db/db) control will be used to determine the mechanistic relationship of the Rap1a and AGE/RAGE cascades. Loss-of-function studies should restore diabetic signaling back to non- diabetic levels.

MATERIALS AND METHODS

Fibroblast isolation and culture - Fibroblasts will be isolated by mincing myocardial tissue and subsequent digestion with collagenase^{13, 14}. Fibroblasts will be maintained in DMEM containing 5% FBS and 10% newborn calf serum. All studies will use cells at P0 for baseline characterization and at P1 for signaling experiments. The purity of the cultures (> 90-95%) will be confirmed by positive staining for the fibroblast-specific marker DDR2 and by negative staining for endothelial cell markers. Hearts from 4 mice used per isolation. Data from 4-5 separate isolations will be collected per group. Currently, we have data from 2 isolations from each group.

Western blot analysis: Lysates from CF cultures and conditioned media will be collected. Western blot analysis will be performed with the following antibodies: CF phenotype markers (α -smooth muscle actin), ECM regulators (AGEs, collagen I, PAI-

1, TIMP-2, MMP-2/9 activation) and signaling proteins (RAGE, PKC- ζ , ERK 1/2, MAP kinases). GAPDH will be used as a loading control. Densitometric analysis performed with NIH image software.

Statistical analyses: Repeated measures analysis of variance (ANOVA) will be used to compare treatment groups for LV physiological measurements. When significant difference ($p < 0.05$) is found, appropriate pairwise comparison methods will be used for inter-group comparison. For all CF experiments, ANOVA with one grouping factor [strain of mouse or treatment] will be used. For signaling studies, power calculations indicate $n = 4-5$ cultures will be required per group to achieve power of at least 95% for testing all pair-wise group comparisons using significance of $p < 0.05$.

RESULTS

Initial observations made by our laboratory demonstrated a possible role for Rap1a in the AGE/RAGE signaling cascade in diabetic fibroblasts (Zhao et al.); however, it was unclear where Rap1a intersected the AGE/RAGE cascade. To identify a link between Rap1a signaling and the AGE/RAGE cascade, we determined whether the downstream effects of AGE/RAGE signaling in diabetes are potentiated by the Rap1a GTPase via a PKA-dependent pathway.

In order to determine the point of overlap between Rap1a and the AGE/RAGE cascade, PKC- ζ phosphorylation was inhibited using PKC- ζ Pseudosubstrate (PS). These studies focused on PKC- ζ as one of the known signaling hubs in the AGE/RAGE cascade (Yu et al. [ENREF 1](#)). Thus, allowing us to define whether Rap1a-mediated AGE/RAGE cascade integration occurs upstream or downstream of PKC- ζ . The data demonstrated that PKC- ζ inhibition returned the elevated signaling proteins in diabetic cells to non-diabetic levels despite Rap1a activation by ISO or EPAC-Ag. Therefore, the Rap1a-mediated alterations in AGE/RAGE signaling were dependent upon PKC- ζ phosphorylation. Using gain-of-function studies, we have defined a novel link between Rap1a and AGE/RAGE signaling cascades, and upon Rap1a activation downstream AGE/RAGE mediators, such as PKC- ζ and ERK1/2, are activated. These mediators increased protein expression of known outcomes in the AGE/RAGE signaling cascade.

A loss-of-function strategy was also used to determine if knocking down Rap1a GTPase expression using siRNA will affect AGE/RAGE signaling in isolated diabetic and non-diabetic cardiac fibroblasts. From our data, Rap1a appears to feed into the AGE/RAGE cascade via PKC- ζ . Therefore, loss-of-function studies should decrease Rap1a-mediated AGE/RAGE signaling cascade outcomes by limiting fibroblast phenotype changes and returning RAGE expression to non-diabetic levels. Rap1a siRNA treatment of diabetic (db/db) and non-diabetic (Het) isolated cardiac fibroblasts significantly decreased Rap1a expression, and Rap1a expression post siRNA treatment ranged from 60-80%. Knocking down Rap1a expression levels in diabetic cells translated into a restoration of signaling molecules, such as PKC- ζ and ERK1/2, back to non-diabetic levels.

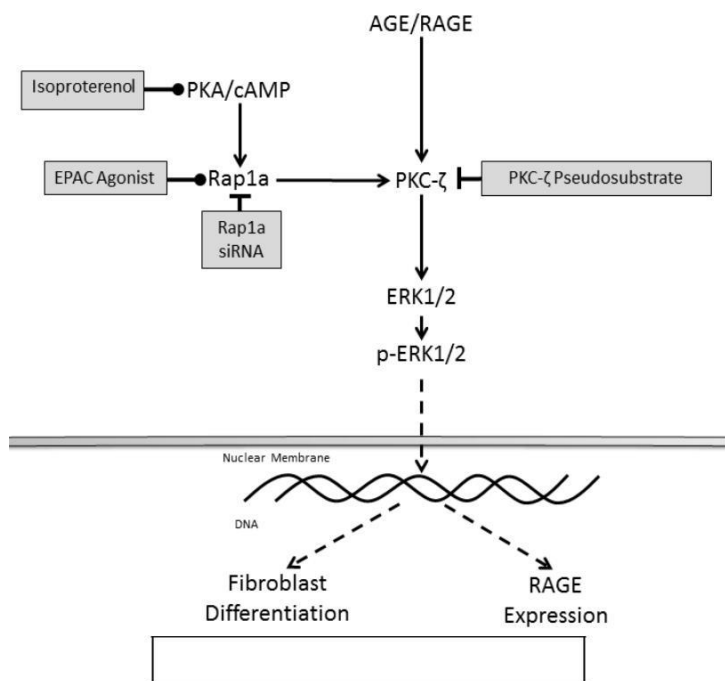


Figure 2. Rap1a Signaling Cascade.

DISCUSSION

Changes in Rap1a expression through either gain-of-function or loss-of function experiments resulted in similar alterations in protein expression in defined AGE/RAGE cascade outcomes, such as α -SMA and RAGE. In addition, AGE/RAGE signal transduction molecules, such as PKC- ζ and ERK1/2, had similar phosphorylation responses upon Rap1a stimulation or Rap1a knockdown experiments. Inhibiting PKC- ζ phosphorylation by administering PKC- ζ Pseudosubstrate in vitro halted downstream progression of the Rap1a-AGE/RAGE signaling cascade. Thus, from data we have determined Rap1a exerts its effects on the AGE/RAGE cascade through crosstalk with PKC- ζ . While further confirmation studies need to be performed, these studies are the first of its kind to provide Rap1a as a unique target for therapeutic strategies aimed at reducing chronic hyperglycemia-mediated ECM production and accumulation in diabetic patients.

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Name: Leonard, Will

Faculty Advisor, Department: Dr. Hugh Medal, Department of Industrial and Systems Engineering

Project Title: A gap analysis of wild land fire response resources in the U.S.

In order to receive research funding for the summer of 2015, I resolved to gather information necessary to conduct a wildland fire response capacity analysis, under the oversight of Dr. Hugh Medal in the Industrial and Systems Engineering Department at Mississippi State University. All data collected for that purpose could later be used by the federal Department of Homeland Security (DHS) to evaluate the vulnerability of the United States to strategically placed wildland fires. In pursuit of that goal, I assisted in gathering and evaluating names for placement in the list of recipients of a survey to evaluate forestry-related agency managers' knowledge about pyro-terrorism, which was to be administered via email. I also began to utilize Geographic Information Systems (GIS) software to prepare for mapping fire crews in states' counties and districts, which could help to determine the locations in the United States most vulnerable to potential pyro-terrorism attacks. In addition, to discover the number of fire crews located in each county or district, I began to contact state forestry commissions' employees to gather the publicly available information related to the same.

To construct a survey, names and contact details of forestry-related government and non-profit organization officials were required. According to one of the professors from the Department of Forestry, at least 1000 names would be advisable to collect a statistically significant amount of data. In pursuit of the minimum goal of 1000 officials, my project partner, Max Moseley, and I divided the 50 states into eastern and western halves, and each of us collected contact details for officials at 25 states' forestry commissions or the equivalent. Notably, despite the fact that public employees' contact information is a public record, several states demonstrated great resistance to revealing email addresses and other specific contact information. Accordingly, more cooperative states are represented in a greater proportion in the final listing of officials' contact information than are less cooperative and non-cooperative states. Also, states with greater populations, along with those of large land area, are more greatly represented, due to their requiring more employees to participate in forestry-related activities. After collecting all forestry agencies' data, additional names were still required. Max and I next considered federal agencies' employees, such as those at the Bureau of Land Management (BLM), DHS, and Federal Emergency Management Agency (FEMA). He and I also looked for officials at the state-level equivalents of the DHS and FEMA. Consideration was also given to additional federal national security agencies, as well as non-profit organizations such as the National Association of State Fire Marshals. Following the gathering of information of all such sources, more than 1200 officials' information had been gathered.

Additional time was spent on learning GIS for use in determining the vulnerability of specific locations in the United States to acts of pyro-terrorism. Specifically, such work could find the difference between the vulnerability of the U.S. to a natural wildfire and that of the U.S. to a strategically-set fire begun by pyro-terrorists. As part of the learning experience, a seminar on basic GIS was taken at the Instructional Media Center conducted by Mississippi State University Libraries. Unfortunately, due to some technical issues and delays in beginning a fiscal year 2016 project after July 1, it was not possible to begin implementing much mapping using GIS. However, under the tutelage of one of Dr. Medal's graduate students and one of the department's post-doctoral associates, Max and I began working with GIS in pursuit of a longer-term goal of conducting spatial analysis of areas' vulnerability to pyro-terrorism.

Furthermore, to ensure that our time was spent as efficiently as possible, Max and I focused on trying to gather information related to the number of fire crews located in each county and district in particular states in various regions across the United States. Such data could assist in determining which areas have the least resources available for combating acts of pyro-terrorism, which would help to ensure that vulnerable areas are not subject to resource rationing that does not provide a maximum benefit to areas affected by pyro-terrorism. In fact, it was determined that a publicly available database of such information for each state is not easily available and does not exist for many states. Accordingly, additional research is required, along with consultation among state and federal officials and Dr. Medal, in order to gather such information, or to determine how to find persons or agencies with easy access to or full knowledge of such information.

Max and I managed to accomplish most of our goals for the summer research project. We gathered contact information for recipients of the survey, to go along with the completed survey assessing officials' knowledge and

perceived vulnerability to pyro-terrorism. Also, although we were not able to use GIS as we had planned, due to the lack of information on fire crews in counties and districts and due to technical issues, we did gain a robust knowledge of GIS, which should prove useful in continued use and mapping in the months ahead. Additionally, the gathering of information related to the number of fire crews in specific areas will hopefully become available or collectable in the near future. Accordingly, the summer research project should be deemed a success for its role in aiding the collection of information to perform an assessment of the vulnerability of the United States to pyro-terrorism attacks.

Name: Lin, Sallie

Faculty Advisor: Dr. Jun Liao

Project Title: Intact porcine heart decellularization with glutaraldehyde

Under the mentorship of Dr. Liao, my interest in tissue regeneration and organ modeling has grown in MSU's Tissue Bioengineering Laboratory. My major focus has been to understand the biomechanical behavior of cardiac and skeletal native and extracellular matrix (ECM) tissue. ECM is a scaffold material important to heart function.

This summer, I worked on understanding the stress relaxation, creep, and failure properties of the porcine mitral and tricuspid valve. The tricuspid valve has been my main focus, and the work is ongoing. If all goes well, I hope to present findings at the Spring Symposium. I have worked in Dr. Liao's lab since freshman year. This summer under the funding of the Presidential Scholarship I was able to refine my skills in the lab. I took charge in tissue dissection in obtaining the mitral and tricuspid valves. With these skills, I was able to help mentor this summer's NSF REU students Lysmarie and Brandon.

In addition to dissection, the tissues were trimmed to certain dimensions and tested on the uniaxial Test Resources, which is programmed to perform the stress relaxation, creep, and failure tests. In mitral valve testing, the lab found that the mitral valve leaflet undergoes minimal creep, a unique feature to these leaflets. I also learned to analyze data from the tests in Excel files to study the trends. My mentee Lysmarie was able to present the mitral valve project at MSU's Summer Symposium, and her poster/abstract "Characterization of the Unique Viscoelastic Properties of the Mitral Valve Anterior Leaflet" was accepted at the 2015 Annual Biomedical Research Conference for Minority Students. I was co-authored with the abstract. Continuing into the Fall semester, I am continuing the tricuspid research and hoping to find comparison and contrasts between the tricuspid valve and mitral valve. Additionally, I am still mentoring new students on the heart dissection and the uniaxial Test Resources machine. In addition to biomechanical testing, I am still aiding in other group projects. Our lab collaborates with other institutions, and I aid in tasks such as refreshing the decellularization solutions that tissue must be washed in to remove cells.

In addition, my graduate student mentor headed a project with the vet school this summer, and I helped out and learned new skills in taking care of research animals. On a shift schedule, I would regularly go in and check on the animals and observe their behavior for any abnormalities. In addition, I learned how to take over tasks at the vet school, such as communicating with faculty and handling animals for scans, when the graduate student mentor was out of town. With the research, I had the opportunity to centrifuge research animal blood samples and use newly acquired aseptic techniques.

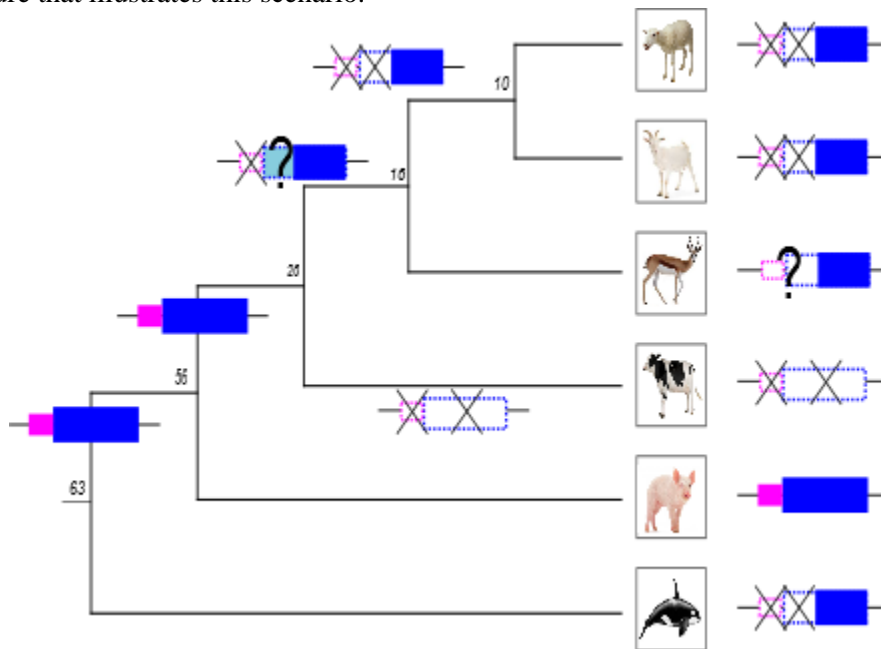
The projects I have worked on are still ongoing: the mitral valve properties, the tricuspid valve properties, and the animal study. Hopefully, the results will be ready by the Spring Symposium. This Fall semester, I did get the opportunity to travel to the 2015 Biomedical Engineering Society Meeting in Tampa, FL, where I helped present "Biomechanical Characterization of Porcine Skeletal Muscle Extracellular Matrix."

Name: Malone, Loggan
Faculty Advisor: Federico Hoffmann

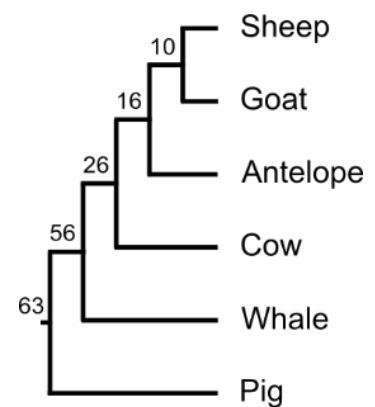
Project Title: Evolution of the Relaxin/Insulin-like gene family in Laurasiatherian mammals

Genes in the Relaxin/Insulin-like gene family encode for peptides that are involved in a variety of physiological functions related to reproduction, including softening of the pubic symphysis during labor and gamete maturation. While continuing my studies on these peptides this summer, I further investigated the progressive erosion of the RLN gene in the lineage leading to bovids. The goal of this research project was to explore how these species lost this gene, how they have managed without its function for millions of years, and still respond to therapeutic treatment with relaxin. Most of the questions we had about the evolution of this gene were answered this summer, but a great deal of my time was spent preparing for an international conference on Relaxin and related peptides in Malaysia, which was held in September.

Taking a closer look at the sequences and Dot plots for the species of interest, including pig, whale, cow, antelope, goat, and sheep, allowed us to depict the evolutionary scenario for the loss of the relaxin gene in bovids. Below is a figure that illustrates this scenario.



Since we determined where RLN disappeared along the lineage, we decided that understanding the phylogenetic relationship among these species would be useful. A phylogenetic tree of these species show divergence dates in millions of years was generated using Timetree.org, which can be seen here. Additionally, we gained insight into how these bovine species can respond to therapeutic treatment with relaxin. The cow, for example, has no identifiable portions of the gene. However, this species has retained all four relaxin receptors, *RXFP1*, *RXFP2*, *RXFP3*, and *RXFP4*, which allows it to successfully respond to the administration of relaxin.



In addition to these new findings, a manuscript is currently being drafted for submission to a peer-reviewed journal. Because most of the research on the Relaxin/Insulin-like gene family for this project has been completed, I now spend my time reviewing the sequences for each of the species whose protein-coding genes I have annotated.

Name: Monroe, Hannah

Faculty Advisor: William Riehm

Project Title: Columbus Decorative Arts Research

My research project this past summer was to observe and provide assistance to the exploration and documentation of antebellum decorative arts in Columbus, Mississippi, by the Classical Institute of the South. Prior to the work in Columbus, I attended training in New Orleans where I was reviewed in material culture literature, was taught how to handle historical artifacts and learned some research methods.

The New Orleans training included one full day of lectures on decorative arts, the general work of the CIS, portraiture, as well as brief histories of the areas they would be visiting over the summer, a tour of the Historic New Orleans Collection and the Williams Residence, and a photography seminar on how to properly set up backdrops and lighting for photographic documentation of artifacts. The second day of training brought us into the field and allowed us to see historical pieces in person not only just in public museum exhibits, but also in the private vaults of the New Orleans Museum of Art and the Cabildo, as well as a viewing of the new *A Louisiana Parlor* exhibit in NOMA prior to its grand opening. Additionally, we received exclusive tours of historical New Orleans houses and were presented with items and spaces to which the general public is not privy.

Once completing the training in New Orleans, I traveled to Natchez, Mississippi, on my own time to meet with the CIS team with the goal of not only building a further relationship with them before the work in Columbus, but also to experience true field-work prior to the start of the research fellowship. We toured Melrose Plantation, and just as with the New Orleans historic houses, were shown areas and artifacts that the average tourist would not see. The team immediately began to educate me on how to determine different types of wood, veneers, and decorative elements involved in furniture construction as well as pointing out the historical accuracy—or inaccuracy—of the antebellum décor shown in the house. After this meeting, I felt more confident in my ability to be of aid in the research occurring in Columbus.

In July, I traveled to Starkville and commuted each day to Columbus, working with the CIS team from around nine o'clock in the morning to around two o'clock in the afternoon, when I would go home and study books that the project director had loaned to me on the subjects of southern material culture and Black Prairie history. The goal of the work by the CIS in Columbus was to collect data on furniture, portraiture, and decorative arts, and the CIS team allowed me to immediately get to work as soon as I arrived the first morning. First, they taught me how enter the information into the item catalogue by allowing me to have control over the excel sheets while they called out information to type in. After letting me catalogue for a couple of hours, the project director moved me from simply typing to being the one handling the item and describing it. I learned how to determine the type of item we were dealing with—for instance credenza and a sideboard are two similar but different items of furniture—and the date they were approximately made to their provenance and their very detailed physical description. Once I mastered inventorying, the team switched me to photography, and for the rest of the first day I just photographed items of silver. The nine days following had similar patterns as we worked in the houses in Columbus under the provenance of the Billings family as well as toured the Tennessee Williams House and Welcome Center and met with the director of the Columbus Convention. By the end of the fellowship, I was able to catalogue artifacts almost independently and instead of being supervised while photographing, I was given free reign.

Since the CIS is a nationally recognized institute, I was honored and excited to be part of their first research endeavor regarding the rich history of Columbus. I hope to work with them again were I ever to have the opportunity. Currently, I am in the process of further study under the direction and personal research of my faculty advisor William Riehm to learn more about decorative arts in the market place. My own goal is to absorb knowledge of the decorative arts in the local region and the heritage of local families and history.

Name: Moseley, Max

Faculty Advisor, Department: Dr. Hugh Medal, Department of Industrial & Systems Engineering

Project Title: *A Gap Analysis of Wildland Fire Response Resources in the United States*

For summer 2015, I worked under the guidance of Dr. Hugh Medal in the Department of Industrial & Systems Engineering. Our project aimed to examine the response capacity of emergency firefighting resources with regard to wildland fires and to conduct an analysis of the preparedness of present emergency responders. In doing this, we hoped to prepare a document for the Department of Homeland Security (DHS) so that they could evaluate the vulnerability of our country to strategically placed wildland fires. As part of this, we also aimed to collect data on the firefighting capacities of each county in certain states from each region of the United States and input that data into a geographic information system (GIS).

Over the summer, I collected names and contact information from state forestry agencies in the eastern half of the United States. The goal of collecting contact information for managers and foresters at the state level was to send out a survey to these leaders to gauge both our country's vulnerability to a wildfire terrorist attack and our ability to combat such an attack if it were to occur. Statistically, in order to ensure an adequate number of responses to the survey, the survey needed to be sent to over 1,500 recipients at the state and federal levels. Once state directories had been exhausted, we turned our direction to federal agencies such as the DHS itself, the Bureau of Land Management (BLM), and the Federal Emergency Management Association (FEMA) to find additional names.

While initially the project planned to use information from several counties to input into a GIS, this aspect of the project was delayed until early October, when we were able to attend an information session that finally instructed how to use ArcGIS, a GIS software. Now armed with a basic understanding of GIS and ArcGIS, my research partner, Will Leonard, and I are receiving additional instructions from Dr. Medal's graduate and postdoctoral students so that we can begin to build our GIS and make further progress in the project.

With the funding from the Honors Undergraduate Research Fellowship, I have been able to help gather our contact list together so that we can begin polling state and federal officials to gain a better understanding of the current preparedness of our country's firefighting resources. I have also been able to learn about ArcGIS, which will be useful when we map out counties' suppression capabilities and perform our spatial analysis. The results of the work I have completed this summer will be eventually included in the report that is sent to the DHS so that they can decide to implement changes to the current system in order to keep our country safe and better protected.

Name: Naas, Kinsey

Faculty Advisor, Department: Dr. Santanu Kundu, Chemical Engineering

Project Title: *Cavitation Rheology of Alginate Gels*

This summer has been a continuation of research from spring 2015. I have been working on cavitation rheology of three different concentrations of alginate gels with different needle sizes.

Background:

Alginate is a polymer extracted from brown seaweed. The extracted alginate ionically crosslinks in contact with calcium ions, resulting in a gel. Polymer gels made from alginate have uses in various fields such as drug delivery, tissue engineering, wound dressing and cell culture¹.

Synthesis:

The alginate gels used for cavitation rheology experiments are prepared in the lab. Initially, alginate powder is mixed in 40 mL of water. Once the alginate powder is fully dissolved, dibasic calcium phosphate is added and the contents are mixed until the dibasic calcium phosphate is fully dissolved. In a separate beaker, GdL (gluconic acid δ -lactone) is dissolved into 40 mL of water; the GdL and water are then poured into the beaker containing the alginate and again the contents are mixed. The GdL provides an acidic environment for crosslinks to develop, resulting in a gel. The mass of calcium salts are varied to obtain three different concentrations of gel.

Technique:

Cavitation rheology is used to characterize the mechanical properties of soft gels. Elastic modulus (Young's modulus) is a characteristic of material to resist deformation (in other words, how strong the gel is). Elastic modulus can be measured using the cavitation rheology technique and such information is useful for designing gels for various applications. Knowing the elastic modulus can ensure that a material has the durability it needs to withstand the deformation it may experience during its lifetime.

Cavitation rheology is performed by inserting a needle, attached to a syringe, into the gel being tested. The syringe is filled with air, and the air compressed at 5 mL/min. As air is compressed in the syringe, the pressure inside the gel increases. Finally, when critical pressure, P_c , is reached a cavity is formed inside the gel (Figure 1). The value of the critical pressure is directly related to elastic modulus and surface tension for Newtonian materials as follows²:

$$P_c = \frac{5}{6}E + \frac{2\gamma}{r}$$

Where, P_c is the critical pressure obtain from experimental data, E is the elastic modulus (Young's modulus), γ is the surface tension of water, and r is the inside radius of the needle.

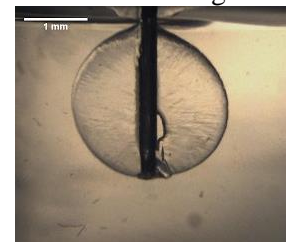


Figure 1: Cavity developed inside an alginate gel.

Accomplishments:

- Synthesized homogeneous alginate gels with uniform mechanical properties.
- Utilized cavitation rheology technique to obtain pressure data for three concentrations of gel with several needle sizes.
- Prepared and presented a poster at the Spring 2015 Shackouls Undergraduate Research Symposium and Summer 2015 Shackouls Undergraduate Research Symposium.

Future plans:

This summer I investigated the physical and chemical properties of alginate gels as described above. This summer's work has gone to better understand the elastic modulus of alginate gels with cavitation rheology. There will be a continuation of this project to fully investigate the elastic modulus of alginate gels. Cavitation rheology data will be fitted in order to characterize the elastic modulus of alginate gels.

¹ Lee KY, Mooney DJ. Alginate: Properties and biomedical applications. *Prog Polym Sci* 2012;37:106-26

² Zimmerlin, J. A. and Crosby, A. J. (2010), Water cavitation of hydrogels. *J. Polym. Sci. B Polym. Phys.*, 48: 1423–1427. doi: 10.1002/polb.21968

Name: Pearson, Meredith

Major: Psychology

Faculty Advisor: Michael R. Nadorff, PhD

Project Title: Moving beyond the cross section

Over the summer, I set out to achieve in two primary goals: to gain experience working on a longitudinal grant-funded experimental research study and to learn how to clean and analyze longitudinal data using an archival data set. To meet my first goal, I aided Drs. Winer and Nadorff with their NIMH R15 grant in order to gain a strong understanding of the project as well as how to run experimental research. This knowledge was purposed to prepare me to serve in a funded position on the grant starting in the fall semester. Without this summer research fellowship, I would not have been prepared to step into my current position of leadership in the lab.

My primary role includes the day-to-day responsibilities of the lab that add to the fidelity of the experiment. This includes ensuring that assessment materials are up to date and organized, creating protocols to streamline different aspects of running the lab, learning procedures for scheduling participants, and other tasks that strengthen the lab's infrastructure. As part of this endeavor, Jenna Kilgore and I created a training protocol for equipping new research assistants. Before the institution of this protocol, Dr. Winer had to individually check out each new assistant, which was very time consuming. Now, we have a standardized method of training so that it is more efficient and allows the option of other senior lab members to aid in the check out process. I was able to utilize this protocol when training three new research assistants at the beginning of the fall semester. Likewise, I assisted Gage Jordan in developing a protocol for parking procedures for the participants in the experiment. With these increased responsibilities, I gained leadership experience that will be invaluable in my future research; few undergraduate research assistants are afforded this opportunity that will set me apart in my graduate school applications.

To meet my second goal I worked closely with Drs. Winer and Nadorff to complete a longitudinal data analysis project using archival data in the lab. As part of this project, Jenna Kilgore and I intended to clean the data, conduct longitudinal analyses using MLM, and write up a manuscript with Dr. Winer for publication. Drs. Winer and Nadorff were to lead a statistical group over the summer that would cover longitudinal data analyses using several different statistical packages (R, SPSS, and SAS). However, the cleaning process took significantly longer than was first anticipated. We had to recode hundreds of thousands of observations by hand before any analyses could be conducted; the whole cleaning process took approximately 100 hours to complete. As part of this process, I also learned how to utilize Amazon.com's Mechanical Turk and Qualtrics online survey software to collect longitudinal data. Because this process took so long, we were unable to go through the statistical textbooks in much depth. These texts will be an excellent resource for my future research both at Mississippi State and elsewhere.

Despite this setback, I was able to complete a statistical analysis and abstract for a different data set. Dr. Nadorff obtained these data from the Sleep and Human Health Institute. Together with Dr. Barry Krakow, Natalia D. McIver, Victor Ulibarri, Dr. Winer, and Dr. Nadorff, I drafted and submitted an abstract to the Association for Behavioral and Cognitive Therapies (ABCT) conference's special interest group (SIG), Child & Adolescent Anxiety. Additionally, I completed a literature review for Affies4Kids (<http://affies4kids.com/>), a PBS-affiliated company that aims to improve preschoolers' self-esteem through self-affirming statements and engaging media.

The knowledge and experience I gained this summer during the honors research fellowship is invaluable to me. It gave me a depth and breadth of knowledge that I otherwise would not have and for which I am immensely grateful. I now feel more prepared for future research, graduate school studies, and leadership opportunities within the lab.

Name: Price, Cody

Faculty Advisor: Dr. Nicholas C. Fitzkee

Project Title: *Characterization of novel elastin-like proteins*

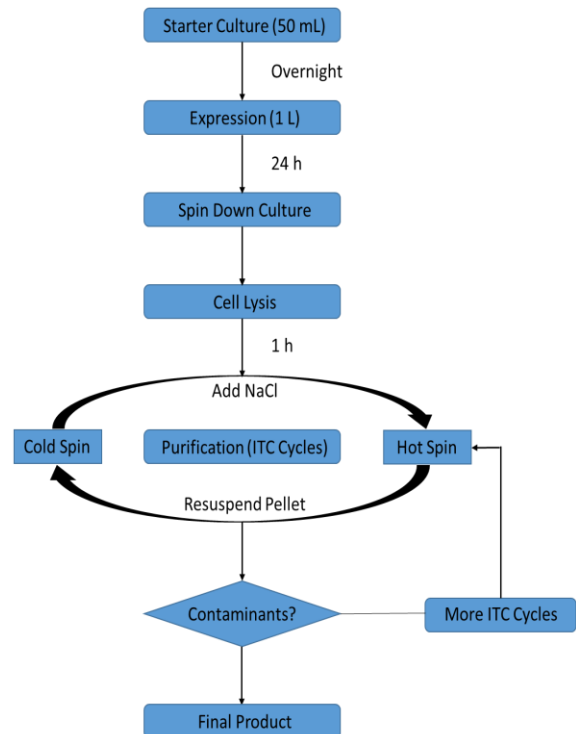
This summer we developed a detailed procedure to purify Elastin Like Proteins (ELP), and label ELP with MTSL (a paramagnetic spin label).

1. For ELP purification instead of using traditional methods of a chromatography column to separate the proteins, we utilized ELPs' physical property of reverse aggregation. Above the ELPs transition temperature it aggregates, below this temperature it dissolves in solution. Through a series of hot and cold centrifugation steps above and below the transition temperature we were able to purify our protein. A summary diagram of the ELP Purification procedure is to the provided.

2. During MTSL labeling we faced a large problem: we could not determine ELP concentration using the traditional UV-Vis method. Without our ELP concentration we could not conduct accurate NMR studies. Since our protein only had one Cysteine site, protein concentration equals sulfhydryl concentration in solution. Ellman's reagent quantitatively determines how many sulfhydryl groups are in solution. Using Ellman's reagent we were able to indirectly determine the concentration of our protein.

My work this summer allowed Yue Zhang, a PhD student in the Fitzkee laboratory to conduct further and more detailed NMR studies. He is using this data to write a paper on ELP Modeling that will soon be submitted for publication. Elastin Like Proteins share several common factors with intrinsically disordered proteins (IDPs). ELP may serve as a model protein to study IDPs.

ELP Purification



Name: Rands, Gabrielle

Faculty Advisors, Department: Dr. Robin Fontenot, Dr. John Thomason, Department of Clinical Sciences, College of Veterinary Medicine, Mississippi State University

Project Title: *The Effect of Glucosamine on Equine and Canine Platelet Aggregation and Thromboxane Synthesis*

Introduction

Canine and equine athletes are commonly prescribed glucosamine in order to treat or prevent osteoarthritis, a potentially painful condition that leads to the degeneration of the cartilage, joint lining, ligaments and underlying bone. Extensive research has proven glucosamine to be an effective form of treatment and prevention of osteoarthritis³. Unfortunately, glucosamine was observed to inhibit platelet function in vivo in guinea pigs⁴. Inhibition of platelet function leads to problems with the formation of a platelet plug in hemostasis and thrombosis. Patients could potentially have issues in surgery, due to a lowered amount of platelet function. Investigation of the effect of glucosamine on canine and equine platelets could aid in determining the risk of a surgery. Problems with bleeding are essential to know prior to surgery, which is why patients are suggested to avoid taking non-steroidal anti-inflammatory drugs (NSAIDs). These compounds have been proven to reduce platelet function and therefore lead to problems in surgery. By researching the effect of glucosamine on platelet function, veterinarians can adequately prepare by advising against the administration of glucosamine prior to surgery.

Platelets are derived from megakaryocytes located in the bone marrow of animals¹. They aid in the prevention of blood loss by forming a platelet plug, which seals the wound. The formation of a platelet plug involves a process called platelet aggregation, otherwise known as the adherence of platelets to one another. In vitro tests use this process to determine platelet function by using agonists to instigate aggregation. Platelet function can also be analyzed through thromboxane B₂ EIA assays. In order to determine canine and equine platelet function in this project, turbidometric platelet aggregation and thromboxane B₂ EIA assays were used. Ultimately, glucosamine was hypothesized have an effect on both canine and equine platelet function because of how the previous study in vivo with guinea pigs.

Methods

Sample Collection and Preparation

In this study, eight horses and eight dogs had blood samples withdrawn from the jugular vein via *ppendorf*. The blood was drawn into a light blue top tube with sodium citrate. Samples were immediately placed into the centrifuge and spun down accordingly. Once completed, the platelet rich plasma (PRP) was removed from each sample and the tube was returned to the centrifuge and spun down again. The second spinning of the centrifuge is necessary in order to retrieve the platelet poor plasma (PPP). The PRP and PPP were placed into their respective clean storage tubes. Each horse/dog had his own PRP and PPP containers.

Turbidometric Platelet Aggregometry

The PRP was left to incubate at room temperature for five minutes in its respective concentration of glucosamine (0ul, .2ul, 2 ul, or 20 ul) before it was placed into a glass cuvette with a stir bar. The PPP was placed into the glass cuvette without any addition of glucosamine or a stir bar. Both cuvettes were incubated for one minute in the warming chambers, and then placed into the aggregometer for the test. Once the aggregometer was set up for the test (test information entered, calibrated), the agonist was added into each of the PRP cuvettes. In the adenine diphosphate (ADP) agonist tests, exactly 2.5 microliters of ADP were used for both dogs and horses. While in the collagen series of tests, 1.25 microliters of collagen were used to determine platelet function in both animals. The ADP tests ran for six minutes and the collagen tests ran for eight minutes. Once each of the tests was complete, the used PRP cuvettes were removed and the process was repeated with new PRP cuvettes. Exactly three cuvettes were used for each concentration of glucosamine in each test.

Thromboxane B₂ EIA

Once again, the PRP incubated for five minutes at its certain concentration of glucosamine. Then it was placed into an *ppendorf* tube and frozen until ready to use with a thromboxane B₂ EIA kit. When ready to run the assay, the incubated samples were thawed using body heat until room temperature. The EIA buffer and wash buffer solutions were prepared according to the Cayman Chemical Thromboxane B₂ (TXB₂) EIA Kit booklet. Then, these solutions were used in order to establish the TXB₂ EIA Standard, the TXB₂ AchE Tracer, and TXB₂ EIA Antiserum. The plate was opened and each well was filled as described in the booklet. Once every well was completed, the plate was covered with a clear plastic film then placed into a box and refrigerated overnight at 4° C. After the plate was incubated in the refrigerator, the plate was developed using Ellman's Reagent, the wash buffer, and the TXB₂ AchE Tracer. The plate was covered with

the plastic film again and then refrigerated for 90 minutes. Finally, the plate was read using a plate reader. The data was collected and analyzed to determine the effect of glucosamine on thromboxane B₂ in canine and equine platelets.

Results

Statistical analysis of the data is pending; however, the raw data suggests that glucosamine did not have a significant effect on either species. Below is an example of the raw data from a canine platelet aggregation test involving ADP as the agonist.

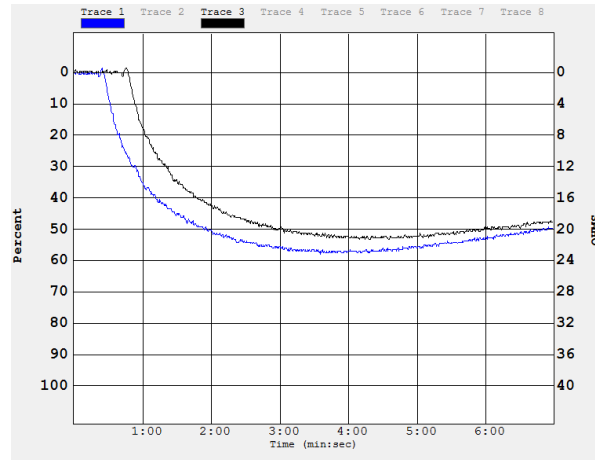


Figure 1

The data consists of a graph (Figure 1), which shows the percentage of light that was allowed to pass through the sample of PRP over the duration of the test. From this graph, certain numbers were recorded to determine platelet function such as the amplitude, slope, lag time, and area under the curve. This data was compiled into an Excel spreadsheet where a preliminary analysis of a box and whisker plot suggested there was not a significant effect on platelet function on either species. In Figure 2, the box and whisker plot demonstrates an example of how the data from all of the tests from one animal was summarized.

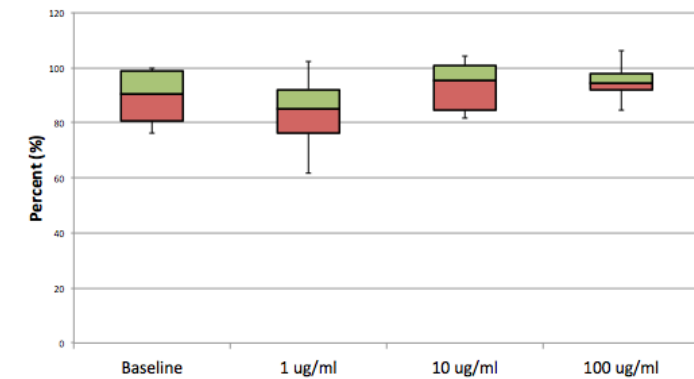


Figure 2

References

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- ² Cayman Chemical Company. Thromboxane B₂ EIA Kit. 2014.
- ³ Down, S. "Equine Osteoarthritis: Management Overview, Options And Treatments". *Veterinary Times*. 2009.
- ⁴ Lu-Suguro, J.F., Hua, J., Sakamoto, K., and Nagaoka, I. "Inhibitory action of glucosamine on platelet activation in guinea pigs". *Inflamm. Res*. 2005.

Name: Sanderson, Audrey
Faculty Advisor: Dr. Kathleen Alley

Project Title: SSILTT Findings

My Role in Research

Over the course of the summer, I helped Dr. Alley prepare for the SSILTT training sessions. Prior to the summer training sessions, I attended the follow-up meetings from last year's group to begin to understand the scope of the program and the positive impact it has on teachers and their instruction. This past summer, I transcribed several hours of interviews and recordings from the SSILTT meetings. Not only did this assist Dr. Alley in her research, but also allowed me to analyze which teaching strategies worked best according to the participants, and those strategies that did not work as well. Transcribing was the primary role I had in the research this summer, although I also began to learn how to code the transcriptions for different purposes. The qualitative data I recorded was in the process of being sent to a professional to be quantitatively analyzed.

Preliminary Findings

Multiple methods and instruments have been used to collect data to examine the impact SSILTT participation has on teachers' ability to create lessons/units in accordance with Mississippi College and Career Readiness Standards, increasing rigor through building strong content knowledge, developing critical skills, and strategically using technology and digital media. Currently, both quantitative and qualitative methods are being used to evaluate SSILTT 2014 and 2015, and data is in the process of being analyzed. Below I share preliminary findings from this analysis.

The following quotes provide preliminary evidence of the positive result of SSILTT on local teachers. You can tell by what they have said that they are more confident and have a larger pool of resources to use when planning their instruction.

Quotes from Focus Group Discussions

"I now have an instructional 'kit' to use to engage my students. With limited resources and time, it is now easy for me to use strategies and skills learned during SSILTT to help my students."

"I now have a variety of teaching techniques that have been introduced and proven to be successful in my own class. I feel more confident and in control of my classroom. I have many learning or 'aha' moments with my students as we learn together."

"I have learned to allow students to take charge and learn more from each other in small groups. They are engaged with the new strategies I am using, and I rarely have behavior issues."

"My students are more confident in their ability now that they have used so many literacy strategies. They have the foundation to write paragraphs and essays using multiple sources. It wasn't easy but it was a lesson that will follow them always."

Instructional Strategy Use and Familiarity, Pre/Post Test Results

This questionnaire contained a list of 77 instructional strategies, rated by professional development participants as yes/no first to indicate their familiarity with the strategy, followed by the frequency of their use of said strategy (never, rarely, sometimes, often). The questionnaire was given three times (first day, prior to intervention; fall follow up meeting, 9 weeks after the conclusion of the intervention). SPSS was used to analyze participants' familiarity with strategies before and after the institute, as well as changes in participants' self-reported use of said strategies before and after the institute's conclusion. Preliminary findings indicate:

- There was a significant difference in participant familiarity with strategies before the training ($M=0.69$, $SD=0.16$) and after ($M=0.82$, $SD=0.11$); $t(15) = -2.83$, $p=0.013$.
- Paired-samples t-test results comparing participant self-reported use of strategies before the training ($M=2.36$, $SD=0.50$) and after ($M=2.91$, $SD=0.51$) were also significant; $t(15)=-2.746$, $p=0.015$.

It is obvious through not only qualitative data, but also concrete quantitative data, that teachers implementation of effective teaching strategies increased due to SSILTT.

What I Have Learned

The number one thing that I learned through this experience is how effective intensive professional development can be for teachers. It was amazing to hear their personal stories about how SSILTT helped them become better students and also motivated their students to learn through the new strategies and activities they had discovered. It was also interesting to hear firsthand accounts of what actually works in the classroom. As an elementary education major, I am constantly learning about new pedagogical methods of teaching—some of which may work better in the classroom than others. By listening to the personal accounts of the SSILTT teachers, I was often able to connect what I have learned in my courses to what the teachers said was effective in their classrooms. The SSILTT program also taught me how to conduct research and all that goes along with it—IHL training, transcribing, coding, editing, publishing—and not to mention the SSILTT curriculum itself. Having the opportunity to shadow Dr. Alley through this research process has proved to be invaluable to me because I have learned more than I thought possible in a short span of two months. Ideally, I would love to be a graduate assistant next year while obtaining my Masters degree in Curriculum and Instruction, and I believe this Research Symposium has prepared me with a solid foundation from which to understand how to conduct research. As a future teacher, this Symposium and the SSILTT program has taught me that professional development can prove to be exponentially valuable to you as a teacher because it allows you to explore new pedagogical methods and instructional strategies that will motivate not only you, but your students as well.

Name: Smith, Destini

Major: Psychology

Faculty Advisor: Kristina B. Hood, Ph. D.

Project Title: *Condom use and sex education*

2015 Summer Undergraduate Research Fellowship Follow up

People of color are at an increased risk of contracting a sexually transmitted infection (STI) or human immunodeficiency virus (HIV) (Center for Disease Control and Prevention [CDC], 2014). Although people of color participate in protective behaviors that appear to decrease their risk of STIs and HIV, cultural and societal barriers make them more susceptible to infection (CDC, 2014). The current study sought to determine if condom use self-efficacy and sex education could predict perceived STI and HIV risk. Multiple hierarchical linear regressions found that condom use self-efficacy and an increase in topics covered in sex education predicted an increased perceived risk. However, condom use self-efficacy was found to be a stronger predictor of perceived STI and HIV risk. Also, receiving sex education from parents significantly predicted perceived STI and HIV risk, such that sex education from mothers was related to a decreased perceived risk and sex education from fathers was related to an increased perceived risk. The interaction between condom use self-efficacy and sex education was not significant. The results can be implemented in communities without access to HIV/STI prevention programs to train people of color on using condoms so that they can have confidence in protecting themselves.

Participants were recruited through Amazon Mechanical Turk (MTURK), and each individual received \$1.00 for completing the survey. The money from the fellowship was used to pay the participants and to pay MTURK for their services. Over 300 adults have participated in this study to date. I will be defending this undergraduate honors thesis November 5, 2015. Also, I have presented my research at the Southeastern Society of Social Psychologists Conference in Winston-Salem, NC, this past October. After my defense, I plan to submit a procedural modification to test college students. Then, once I have collected data from college students, I want to compare their response to the responses that I received through MTURK. I intend to present my findings at the 2016 Spring Undergraduate Research Symposium.

Project Title: 3 D Printed Microreactors

Abstract

Micro-reactors are devices in which chemical reactions takes place within micro-channels. Micro-reactors technology is very attractive because it decreases experimental cost by using a small amount of reagents, increases reaction control by providing accurate control of reaction time, temperature and pressure. The purpose of this research is to design and to fabricate micro-reactors using 3D printing technologies. Micro-reactors with internal flow channels are designed using computer-aided design (CAD) software. Using a Cube Pro Duo 3D printer, these micro-reactors with incorporated internal flow channels have been fabricated. The micro-reactors fabricated using the cube Pro duo 3D printer is made with polymers such as PLA (polylactic acid) and ABS (Acrylonitrile butadiene styrene). Due to the low chemical resistivity of these two polymers, various polymers with better chemical resistivity such as PP (Polypropylene), PC (Polycarbonate) and PETT (poly(ethylene-co-trimethylene terephthalate)) are being investigated. A new printer is being assembled to process the above mentioned polymers or any other nonproprietary polymers.

Material and Method

Microfluidic reactors for the synthesis reaction will be designed with 3D CAD software (Ivent Cubify). The design will be made by incorporating microchannel networks of varying dimensions and geometry in small devices. After designing the reactor using CA, a polymer will be chosen based on its properties. In order for the synthesis reaction to occur properly, a flexible, tough and chemically inert polymer must be used. Additionally, the polymer must be compatible to 3 D printing technology. PLA (polylactic acid) and ABS (Acrylonitrile butadiene styrene) are the most used polymers in 3 D printing. Nevertheless, different polymers, such as polypropylene, may be a better alternative due to its unreactive nature. By using a rheometer, the rheological properties and melting temperature of this polymer will be compared to that of PLA and ABS to check if it is compatible for 3 D printing.

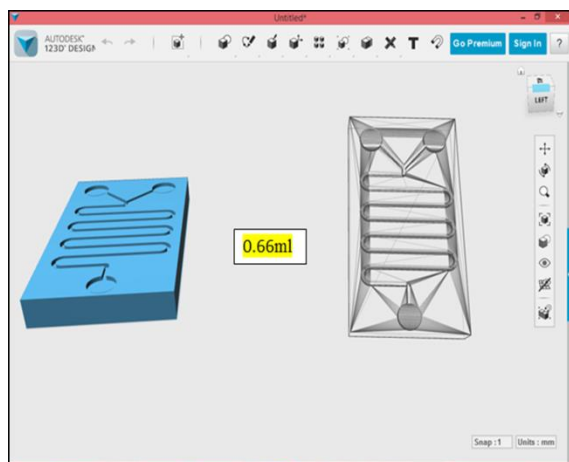
Designed Prototype

- Microreactors are designed using a 3D CAD software package (Invent Cubify and AutoDesk 123D).
- The shape of these microreactors is chosen for short design and print time with the robustness required for a flow system.
- Two types of reactor designs; close and open designs.

Open Reactor Designs

Flow channels are designed on a flat surface and covered with acrylic glass.

Prototype 1



Prototype 2

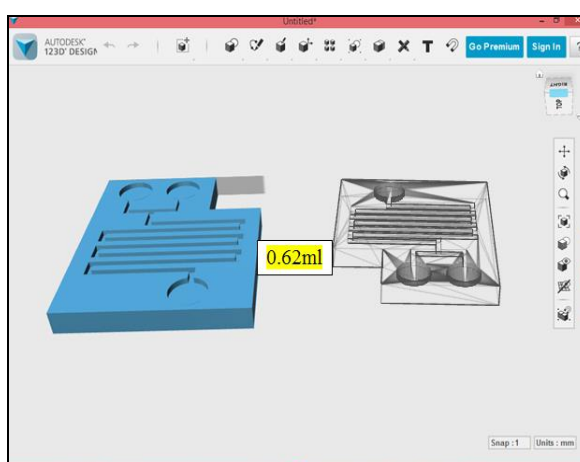


Figure 1: Open Reactor design

Table 1: Characteristics of Open Design Microreactors

Characteristics	Prototype 1	Prototype 2
Dimensions (mm)	50*60*6	50*90*10
Channel dimensions (mm)	1.5*1.5	1.5*1.5
Theoretical volume (ml)	0.66	0.62
Printing time (min)	125	93
Polymer mass (g)	28.7	17.77

Fabrication Procedure

The 3 D printed microreactors are covered using an acrylic top as shown below.

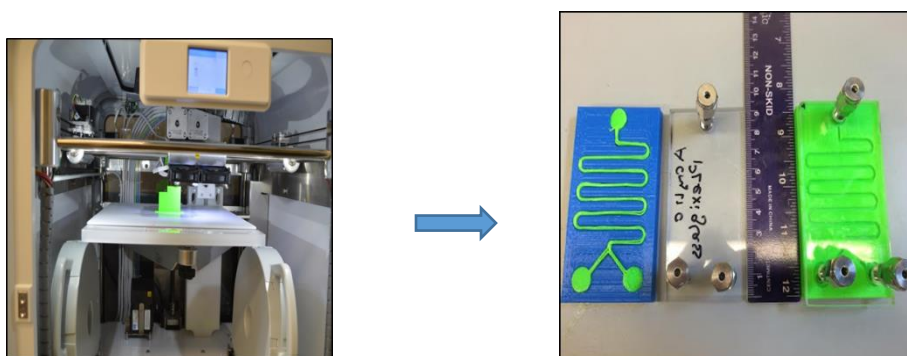
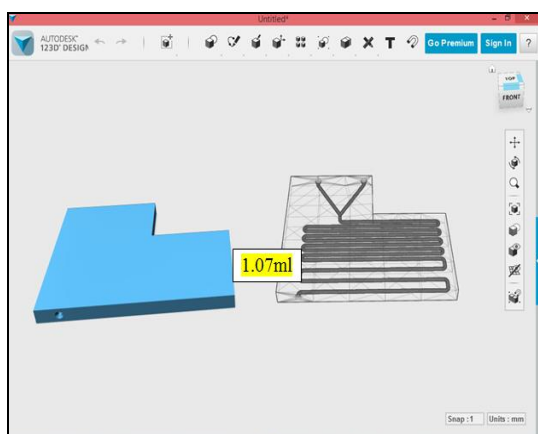


Figure 2: Open Micro reactor Fabrication Procedure

Close Reactor Design

Prototype 1



Prototype 2

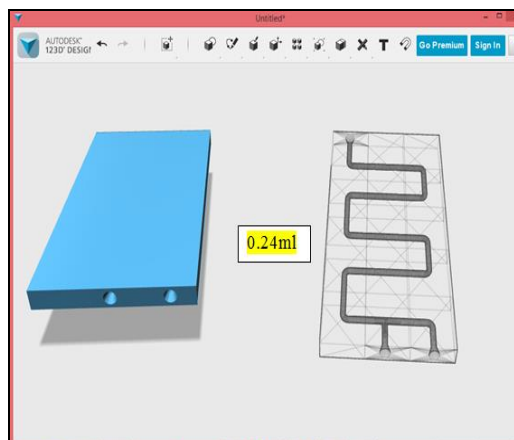


Figure 3: Close Reactor Design

Table 2: Characteristics of Close Design Microreactors

Characteristics	Prototype 1	Prototype 2
Dimensions (mm)	35*75*5	67*74*5.5
Channel dimensions (mm)	1.5	1.5
Theoretical volume (ml)	0.24	1.07
Printing time (min)	76	153
Polymer mass (g)	16.2	35.9

Rheology

The variation in viscosity due to change in shear rate in thermoplastics such as ABS & PLA is crucial in the understanding of the extrusion process in 3 D printers. When deciding an appropriate 3 D printer extruder temperature for a particular polymer, the polymer’s viscosity data should be considered. Moreover, these viscosity data are made more relevant when presented over a range of shear rate. The following rheological procedure was used.

- Determine the viscosity at which polymers are extruded by the 3 D printer extruder.
- Rheological investigations for PLA and ABS were conducted to obtain the viscosity values at the typical extrusion temperatures

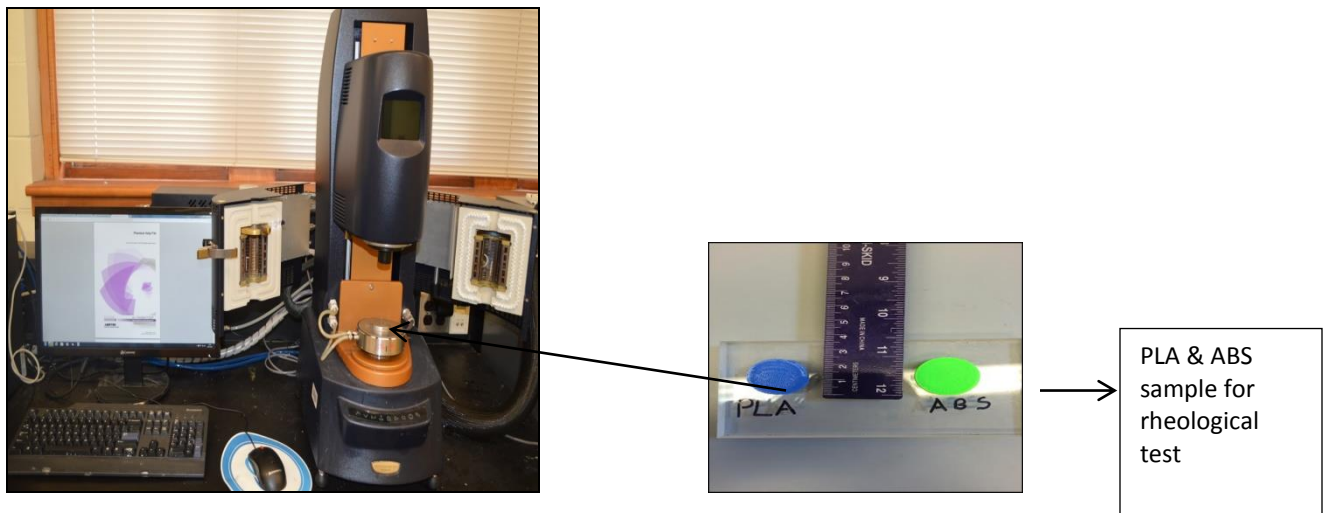


Figure 4: Rheological Analysis of PLA & ABS

Using the rheometer a viscosity curve for PLA and ABS was determined. This viscosity curve will serve as calibration for future materials. To determine the print conditions, a rheological analysis will be conducted on the polymer and the point of intersection of its viscosity curve and that of either PLA or ABS will be the set printing conditions for that polymer.

Rheology Results

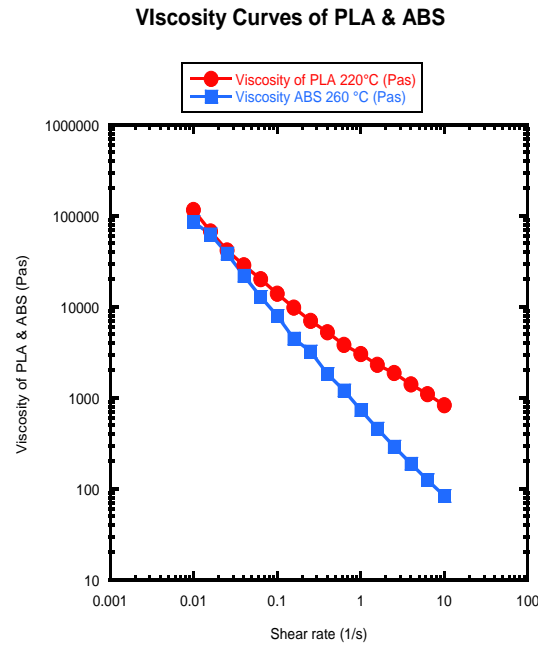


Figure : Rheology of PLA and ABS

As shown on the figure above the viscosity of PLA at 220°C and ABS at 260°C are very close over a shear rate range (0.0159 -0.0398 (1/s)). Nevertheless, at the lower end of shear rate range there is a visible deviation in viscosity between ABS and PLA. From this data it can be deduced that the cube Pro Duo 3 D printer operates at the following parameters.

Conclusion and Future work

A Bukobot 3 D printer has been assembled which provided the possibility of printing with different polymers such as PP (Polypropylene), PC (Polycarbonate) and PETT (poly(ethylene-co-trimethylene terephthalate)). Open and closed microreactor prototypes will be fabricated with PP and PETT. My next research goals will be using an SLA 3 D printer to fabricate a fully functional microfluidic device with microchannels less than a 100 microns.