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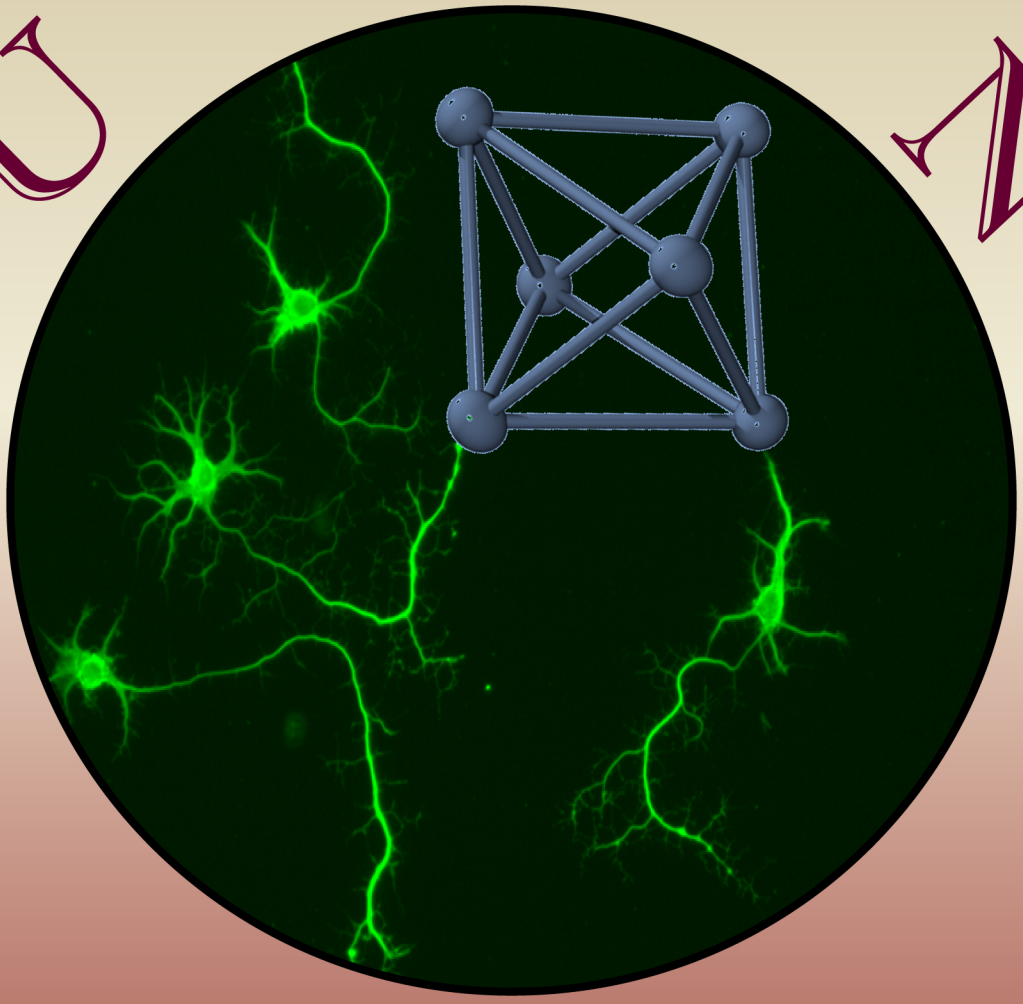

UNIVERSITY OF MIAMI

Undergraduate Research Newsletter

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EDITORS

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Office of Undergraduate Research and Community Outreach

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Foreword

This publication represents the first issue of a new newsletter of the University of Miami (UM). The University of Miami's Undergraduate Research Newsletter (URN) is a collection of original work performed by UM undergraduates that encompasses all areas of academic research. The newsletter is aimed at educating and inspiring undergraduates and students throughout the Miami community to become involved in research. In URN, students will have the opportunity to present results from their research. The newsletter will be circulated to undergraduates at UM and to students in the surrounding high schools and community colleges throughout South Florida in order to inform them about the types of research and research opportunities available at UM.

The contents of this first issue range from the Sciences to the Humanities, and all 16 contributions were reviewed by faculty as well as UM undergraduates, to whom we express our deepest thanks. We hope that as the newsletter gathers momentum, a larger and more complete review committee will be established. The entire review process will be extremely beneficial to both undergraduate contributors and reviewers by introducing them to critical assessment of research work. It is anticipated that the newsletter will be published annually. For this venture to be successful, the support of faculty members is essential. This should be a win-win situation since by encouraging other research groups, a spirit of adventure and commitment to high quality research will naturally occur within the entire academic community.

Lastly, we thank the faculty for allowing their students to contribute their work to URN, and look forward to continued contributions to this newsletter.

Matthew Sacino
Editor and Student Organizer

Burjor Captain
Editor and Faculty Advisor

Rev-erb-mediated Transcriptional Regulation of Interleukin-6 Expression in Macrophages

Veronica Alvarez (class of 2010)

Major: Neuroscience (Psychobiology)
Principal Investigator/Supervisor: Dr. Chris Glass
Department: Cellular and Molecular Medicine, University of California, San Diego
Fellowship/Awards/Recognition: Summer Undergraduate Research Fellowship (2009)
Senior Thesis: No

The Glass Lab aims to determine the transcriptional mechanisms that regulate the development and function of the macrophage, a major regulatory cell of inflammatory processes. The nuclear receptor Rev-erb is a known constitutive transcriptional repressor that plays an important role in circadian rhythm and has been recently shown to be involved in the control of inflammatory processes as well. [3] Preliminary data suggest that Rev-erb binds to an interleukin-6 genomic locus (IL6D), 65kb upstream of the transcriptional start site, possibly implicating its regulation of IL-6 and other inflammatory genes. To further investigate, IL-6 promoter activity was measured through transient luciferase reporter assays after modulation of both Rev-erb and IL6D in macrophages. Results have shown that Rev-erb is likely involved in the regulation of IL-6 expression, however the underlying mechanisms remain unclear.

Chronic inflammation is a state in which the body's inflammatory response remains activated under non-threatening conditions and continues to produce excess pro-inflammatory cytokines that result in the damage of healthy tissues. Chronic inflammation has been shown to play a key role in diseases such as osteoarthritis, arthrosclerosis, diabetes and asthma, as well as others.[2] Regulating the expression of inflammatory genes of the macrophage could potentially attenuate the negative consequences of prolonged inflammation in both a direct and robust manner.

Nuclear receptors such as Rev-erb and PPARγ are important regulators of gene expression and have been considered more selective and potent pharmaceutical targets than more upstream proteins and receptors.[2] Rev-erb is repressor of transcription and plays a role in circadian rhythm and metabolic regulation.[1,4] Preliminary evidence in the macrophage showed an increase in the mRNA levels of the inflammatory gene IL-6 when the Rev-erb gene was knocked out of the cells. Furthermore, a ChipSEQ analysis showed that Rev-erb binds to a distal genomic locus of IL-6, suggesting it regulates IL-6 expression at this site.[5]

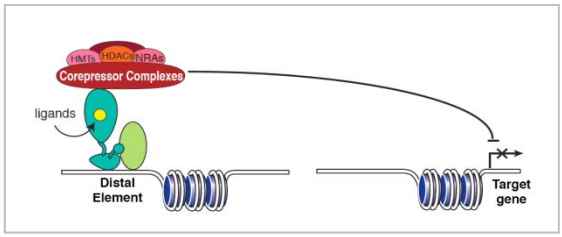

Figure 1: Possible mechanism behind Rev-erb mediated repression or regulation of IL-6 transcription. After activation by its ligand heme, the nuclear receptor Rev-erb may bind to the distal element resulting in the assembly of corepressor complexes and inhibition of transcription of the target gene. [2]

To further elucidate the role of Rev-erb in IL-6 expression, luciferase plasmid reporters with the IL-6 promoter and either a random site (where Rev-erb does not bind) or the distal binding site of IL-6 (IL6D) were transfected into RAW macrophages. Then, cotransfections with either over-expressed Rev-erb, knocked-down Rev-erb, addition of heme or mutations at the distal binding site were done. The expression of luciferase was measured to see how IL-6 promoter activity was affected.

Results show that in the absence of either Rev-erb or IL6D, IL-6 promoter activity is slightly increased compared to control. However, when both are present, the upward trend is normalized suggesting that Rev-erb and IL6D are interacting to repress activation of the IL-6 promoter. When testing the effect of heme with IL6D transfected, results demonstrate a downward trend to also indicate the repression of IL-6 promoter activity through Rev-erb. Our final experiments showed that IL-6 promoter activity was increased when IL6D was mutated,

suggesting that the there are specific regions of the binding site that are essential for the repressive effect of Rev-erb.

Although these results seem likely to indicate that Rev-erb indeed represses IL-6 transcription by binding to IL6D, there are still many questions that need to be answered in order to uncover the mechanism of action. Whether other proteins interact with Rev-erb to carry out this effect, whether this effect is consistent in both the basal and LPS-activated states, and whether Rev-erb acts to repress other inflammatory genes remains unclear at this point. However, if this trend is seen in future experiments, pharmacological therapeutics could be developed to potentially target Rev-erb in order to regulate inflammatory responses and relieve chronic inflammation.

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Pathological Analysis and Health Assessment of Eight Skeletons from Isla San Lucas Penal Colony, Costa Rica

Kristina Astone (Class of 2010)

Major: Psychology (BS) and Anthropology (BS); Biology Minor
Supervisor: Dr. Monica Faraldo
Department: Anthropology
Fellowship/Awards/Recognition: Beyond the Book Scholarship
Senior Thesis: Yes

In this study, I conducted a visual analysis of dental and skeletal pathologies found on eight skeletons excavated from the Isla San Lucas Penal Colony, C.R. in order to assess the overall health of the individuals. I used osteological techniques and bone pathology field manuals to analyze the skeletons. The high frequencies of pathologies displayed on the individuals suggest that they suffered from poor nutrition, excessive mechanical stress, infections, and age-related degeneration.

Physical anthropologists study skeletons and teeth found in the archaeological record in order to gain a further understanding of the lives of the deceased individuals. Pathologies of these bones and teeth are studied in order to assess individual and population health. Teeth contain pathological indicators, such as dental caries, which provide information about these individuals' nutrition. The skull contains indicators of congenital, nutritional, and infectious diseases. Abnormalities in joint articulation and long bone shape suggest nutritional deficits and degeneration from aging or intense physical labor. These physiological indicators provide an immense amount of information that aid anthropologists and historians in understanding the past when written records are not available.[1] Due to a limited written record about the inmates at Isla San Lucas Penal Colony, this research was conducted as part of a multidisciplinary effort to preserve the site and interpret the history of its inhabitants.

Isla San Lucas was a penal colony from 1874-1991 and had the reputation of being the worst prison in Costa Rica. Early in the penal colony's history, it was considered a high security prison where felons, ranging from murderers to petty thieves, were housed. Later, the prison was transformed into a lower security labor camp where inmates were in charge of herding cattle and constructing roads and buildings from large rocks. The only written records that attested to the prison conditions were two books that were written by individuals that escaped from the prison. Each author described the prison as being a horrible place where people were starved to death and murdered; however, archaeologist and scientists

have not found significant forensic evidence to validate their stories.[2]

Eleven skeletons were excavated from a mass grave found in the cemetery on the Isla San Lucas penal colony. The skeletons were associated with artifacts, which led Gabriella Villalobos, the National Museum of Costa Rica's head historian, to the assumption that the skeletons were inmates.[2] Additionally, some of the artifacts displayed dates, for example one button displayed the date 1890, which allowed archaeologists from the National Museum of Costa Rica to date the skeletons. They believed that the skeletons were interred for less than 150 years, indicating the skeletons were archaeologically contemporary. [1, 3] Because the skeletons were in a contemporary context, forensic anthropological applications were used to analyze the skeletal population.[1]

Due to time restrains and the incomplete nature of three skeletons, only eight of the eleven skeletons were included in this pathological analysis. A visual analysis was used in this study because other resources, like x-rays and scanning electron microscopes, were not available. Osteology and Pathology field manuals were used to diagnose abnormalities in bone shape, structure, and texture that are characteristic of certain diseases.[4, 5, 6] Population frequencies were calculated for each pathology (Table 1). A high frequency of dental caries (75%, N=6) and antimortem tooth loss (62.50%, N=5) suggested malnutrition and a diet that mainly consisted of starch found in rice (Figure 1).[7] A high frequency of osteoporosis found on the vertebrae, patella, and phalanges suggested that the inmates endured high levels of physical stress, possibly from labor intensive tasks (Table 1; Figure 2, 3, & 4). There were indications of syphilis and metabolic disease in the skull (pitting), long bones (abnormalities in shape), and pelvis (Figure 5, 6, & 7).[8]

Overall, the health of the eight individuals found in the cemetery on Isla San Lucas penal colony was poor. A pathological analysis of all the skeletons from the cemetery would be necessary in order to examine the overall health of the population from the Isla San Lucas penal colony. Future pathological research should use more advanced techniques and technologies, like bone mass measurements and x-rays, in order to analyze the bones more thoroughly.[6] Comparisons with a contemporaneous mainland population would shed light on the degree to which life in the penal colony was more arduous. This pathological analysis and health assessment of skeletons from Isla San Lucas has provided historians with information about the health of inmates and the living conditions at the Isla San Lucas penal colony.

Figure 1: *Individual 9*: mandible displaying dental caries, attrition, and bone reabsorption (antimortem tooth loss, AMTL).

Figure 2: *Individual 2*: 3rd lumbar displaying osteoarthritis, lipping, and Schmorl's nodes.

Figure 3: *Individual 2*: right patella with enthesophytes and osteoarthritis pits.

Figure 4: *Individual 7*: distal phalanges with tufting and osteoarthritis.

Figure 5: *Individual 7*: skull with lytic lesions indicating the possible early stages of syphilis.

Figure 6: *Individual 7*: right and left tibia displaying a slight bowing of the right tibia and torsion of the left tibia.

Figure 7: *Individual 7*: right and left acetabulum displaying osteoarthritis pitting and over growth and pitting of the pubic symphyses due to possible syphilitic invasion.

Acknowledgement

I wish to thank Jennifer Shook, the photographer, for the magnificent pictures and her professionalism and patience while photographing over 100 skeletal elements. Special thanks to Monica Faraldo for organizing the trip to Costa Rica and inspiring me to study Forensic Anthropology.

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Radiosurgery in the Treatment of Neurofibromatosis Type 2 Related Tumors

Pietro Bortoletto (Class of 2011)

Major: Neurobiology
Principal Investigator/Supervisor: Dr. Aizik Wolf
Dr. Sammie Ross Coy.
Department: Miami Neuroscience Center
Senior Thesis: No

Patients with neurofibromatosis type 2 (NF2) are predisposed to central nervous system (CNS) tumors that grow primarily intracranially in areas where morbidity or complications may arise. Gamma Knife Radiosurgery (GKR) was used to arrest tumor growth while preserving neurological function. By retrospectively reviewing our fourteen year history of treating patients with GKR for NF2, we seek to better define the efficacy of treatment using GKR.

Neurofibromatosis, type 2 (NF2), is characterized by slow-growing, benign tumors that grow along the eighth nerve, which leads from the brain to the inner ear. The tumors, called vestibular schwannomas, can lead to ringing in the ear(s); balance problems; and gradual hearing loss in one or both ears. Gamma Knife Radiosurgery (GKR) was used to arrest tumor growth while preserving neurological function. Radiosurgery is a medical procedure that allows non-invasive treatment of benign and malignant tumors by using gamma radiation to arrest tumor growth. We reviewed a 14 year history of treating patients with GKR for NF2, to better define the efficacy of treatment using GKR.

Neurofibromatosis type 2 is a genetic disorder in which benign tumors grow along the vestibulocochlear nerve. These tumors arise from cells called schwann cells and the resulting tumor is called a vestibular schwannoma as a result of its location and origin. NF2 has an incidence of 1:40,000-50,000 and the first symptoms are usually auditory or vestibular symptoms in Adult patients. Treatment options for patients with NF2 tumors include surgery, radio-surgery, and active surveillance. Many researchers have investigated the best course of treatment for patients with NF2-associated tumors. NF2 tumors pose an interesting challenge in that the decision on how to treat them is rather complex. We sought to better define how effective Gamma Knife Radiosurgery is for the treatment of NF2 related tumors.

From March 1994 to December 2008, 40 patients with neurofibromatosis type 2 were treated using Gamma Knife Radiosurgery at the Miami Neuroscience Center. Radiosurgery uses high doses of radiation to kill cancer cells and shrink tumors, delivered precisely to avoid damaging healthy brain tissue. Gamma knife radiosurgery is able to accurately focus many beams of high-intensity gamma radiation to converge on one or more tumors. Each individual beam is relatively low energy, so the radiation has little effect on intervening brain tissue and is concentrated only at the tumor itself. A head frame is affixed to the patient's head after oral administration of mild sedation and infiltration of the scalp with a buffered local anesthetic agent. The tumor is located and targeted using high-resolution contrast-enhanced magnetic resonance imaging. The images are transferred to the dose-planning computer in the radiosurgery planning location. Computerized

dose planning was performed jointly by the neurosurgeon, radiation oncologist, and medical physicist to determine the maximum radiation dose. Once planning is complete the patient is loaded into the machine and the treatment begins. Treatments can take anywhere from minutes to hours depending on the size, location, and number of lesions to be treated.

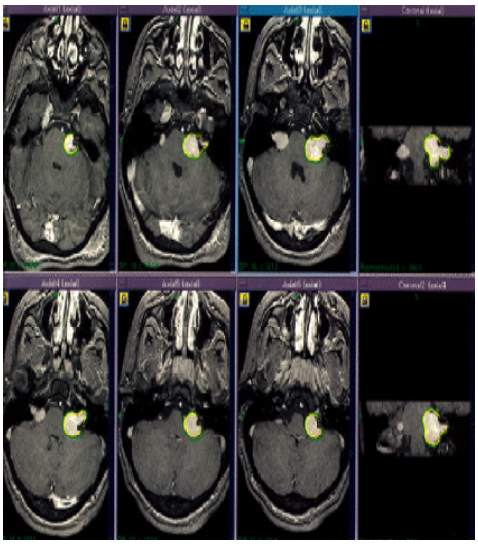

Figure 1: Axial MR images with coronal reconstructions used in radio-surgical dose planning for a left-sided acoustic neuroma.

During the period of March 1994 to December 2008, 153 tumors were treated with GKR. The mean tumor volume at the time of radiosurgery was 4.26cc and the tumors received an average of 11Gy of gamma radiation. Tumor volume is determined via computing software as is the total dose of gamma radiation. The most common indication for treatment was progressive growth on serial imaging studies, although many patients presented with symptoms.

A retrospective analysis was performed on these patients. Review of MRI scans were used to analyze local control of the treated tumors. Local control is defined as the arresting of tumor growth at a specific site. Local control in the treated tumors was 99.35% throughout the course of the follow up period. The median follow-up length was 62 months. . One-hundred and thirty seven lesions were treated with Gamma Knife (Median dose 11.05 Gy; range 10-20 Gy). Only 1 of the treated tumors had to be retreated due to continued growth. However, in addition to arresting tumor growth we also examined the function of the auditory nerve post gamma knife radiosurgery. Useful hearing was preserved in 26 (40%) of 40 patients. This is remarkable in that the tumor grows out on top of the vestibulocochlear nerve, which controls hearing. All patients had been treated on imaging diagnoses of NF2, without histological confirmation of disease. Seventy-two and a half percent of patients were females. The average number of tumors treated was 3.83 (range, 1-15).

Ideally a longer study with more patients would be sought, however NF2 is a rare disorder that is often hard to come by. In addition even longer-term results are desirable to confirm control of growth in acoustic tumors associated with NF2.Radio-surgery is an effective alternative to surgical resection. Radio-surgery has shown the ability to alter the natural course of the tumor by stabilizing the tumor size. As the techniques and indications for radio-surgery become more complex so to will the ability to stabilize the patients and improve their outcome.

The Relationship between Family Home and School Involvement and School Readiness

Johayra Bouza (class of 2010)

Major: Psychology and Religion
Principal Investigator/Supervisor: Dr. Rebecca Shearer
Department: Psychology, School of Arts and Science, University of Miami.
Fellowship/Awards/Recognition : Prime Psychology Program (summer 09)
Senior Thesis: No

The current study examines which type of family involvement, whether home involvement or

school involvement, is a more important contributor to school readiness in Head Start preschoolers. We hypothesize family home involvement, specifically weekly activities, is a more significant contributor for the child's school readiness outcomes. Using the Head Start Family and Child experience Survey (FACES) data set, we conducted multiple regressions to determine relationship between family home involvement, which included weekly activities and monthly outings, and family school involvement. We found that family home involvement (weekly outings) had a stronger relationship with the school readiness outcomes of emergent literacy, emergent numeracy, and social skills.

The conditions related to low socioeconomic status can place children at risk for learning difficulties and low academic achievement. Because family involvement is considered an important aspect in children's education, it can serve as a protective factor to facilitate academic achievement in children of low socioeconomic status (Shumow & Miller, 2001). Studies have found that family home involvement is positively related to receptive language skills, positive behavior, memory, and overall school readiness. (Fanning, 2008; Kephart, 2008; Parker, Boak, Griffin, Ripple, & Peay, 1999). Therefore, it is clear that family involvement has a positive impact on school achievement. What is unclear, however, is whether family home involvement or family school involvement is more important for school readiness. The current study examines which type of family involvement is a more important predictor of school readiness in Head Start preschoolers.

Family home involvement is when family members create learning opportunities at home and in the community by providing a place and materials for homework, visiting the library, and engaging in educational activities (Fantuzzo, 2000). Recent studies have shown family home involvement for low-income preschool children was the most powerful single predictor of school readiness (Fantuzzo, McWayne, Perry, & Childs, 2003). Family school involvement is when a child's family supports the their child in their school setting with activities such as volunteering at school, communicating with teachers, and attending school events(Hill & Taylor, 2004). Head Start children are particularly at risk for deficits in school readiness but the benefits of family involvement in the home and school may help to overcome the added risk Head Start children face.

Figure 1: Head Start Children during Reading Time

The purpose of our study is to examine the relationship between family involvement and school readiness. We predict that family home involvement and family school involvement will be significant predictors of school readiness. Furthermore, we evaluated which type of family involvement is a more significant predictor of school readiness. We hypothesize that family home involvement will be a more significant predictor of school readiness.

Participants included 1,968 Head Start children from a stratified, random sample drawn to be representative of 1,734 Head Start programs nationwide spanning across four geographic regions, urbanicity, and percent minority enrollment (FACES, 1997; U.S. DHHS, 2002).

Family home involvement was evaluated by a 22 item parent reported check list of their own frequency of weekly activities (e.g., teaching the child a letter, working on arts and crafts, and talking about television programs) and monthly activities (e.g., family outings such as visiting the library, zoo, or a live show). Family school involvement was measured by 7 item parent report on the frequency of their participation in the child's school (e.g., volunteering in their child's classroom,

attending parent education meetings, or attending teacher conferences).

Figure 2: Head Start Children creating a Mask

School readiness outcomes included emergent literacy, emergent numeracy, and social skills. Emergent literacy was assessed using the Head Start Family and Child Experiences Survey (FACES) literacy sub-scale and the Woodcock Johnson Letter- Word Recognition direct assessment. Emergent numeracy was measured by the Woodcock Johnson Applied Problems-Revised scale direct assessment.

Table 1. *Bivariate Correlations between FACES Family Involvement Variables and Head Start Readiness Outcomes*

	Emergent Literacy		Emergent Numeracy		Social Skills
	FACES Literacy	W-J Letter Word Recognition	W-J Applied Problems	Parent-rated Social Skills	Teacher-rated Social Skills
Child demographic variables					
Age	.38**	.27**	.38**	.11**	.24**
Sex	.10**	.06*	.05*	.12**	.17**
Years in Head Start	.06*	.05	.08**	.02	-.01
Family income (yearly)	.06*	.05	.09**	-.03	-.03
Family involvement variables					
Home involvement (weekly)	.17**	.05	.08**	.11**	.02
Home involvement (outings)	.18**	.06*	.06*	.11**	.06**
Parent-school involvement	.11**	.04	.07**	.07**	.06*

*$p < .05$, **$p < .01$.

Note: W-J = Woodcock-Johnson.

$n = 1,664$, $n = 1,303$, $n = 1,284$, $n = 1,700$, and $n = 1,712$ for FACES Literacy, W-J Letter- Word Recognition, W-J Applied Problems, Parent- rated Social Skills, and Teacher- rated Social Skills, respectively.

Social skills were assessed using teacher- and parent-reports of children's behavior. Teacher rated social skills were measured by a combination of the Personal Maturity Scale (Alexander & Entwisle, 1988) and the Social Skills Rating System (Elliott,Greshman, Freeman, & McCloskey,1988). Parents rated their child's social skills by indicating if certain behaviors, such as making friends easily, trying new things, and showing imagination, are true, somewhat true, or not true of their child.

Table 2. *Relations between Family Involvement Variables and Head Start Readiness Outcomes*

	Emergent Literacy		Emergent Numeracy	Social Skills	
	FACES Literacy	W-J Letter-Word Recognition	W-J Applied Problems	Parent-rated Social skills	Teacher-rated Social skills
Child demographic variables[a]					
Age	.41****	.26****	.33****	.13****	.26****
Sex	.12****	.08**	.06*	.12****	.19****
Years Head Start	-.03	-.01	.01	.00	-.07*
Family income (yearly)	.05*	.05	.07*	-.04	-.01
Family involvement variables					
Home involvement (weekly)	.120****	.03	.07*	.09**	-.01
Home involvement (monthly)	.12****	.04	.00	.06*	.06*
Parent-school involvement	.06*	.02	.06*	.05*	.04
% Variance explained by:					
Overall model R^2	22.0%****	8.1%****	13.0%****	4.8%****	10.0%****

$n = 1,664$, $n = 1,303$, $n = 1,284$, $n = 1,700$, and $n = 1,712$ for FACES Literacy, W-J Letter- Word Recognition, W-J Applied Problems, Parent- rated Social Skills, and Teacher- rated Social Skills, respectively.

[a] Entries are standardized parameter estimates derived in multiple regression. All values reflect the relative contribution of each dimension on the dependent variable (controlling for child age, sex, years in Head Start, family income, and all other family involvement dimensions). Tests assess the deviation of each parameter estimate from zero, where *$p < .05$, **$p < .01$, ***$p < .001$, ***$p < .0001$.

Multiple regression analyses were conducted to examine the relationship between family involvement and children's school readiness. Family involvement variables were used to predict school readiness outcomes, controlling for age, sex, yearly family income, and the amount of years in Head Start. Correlations between variables are provided in Table 1. Table 2 displays the standardized coefficients for the regression models. Family weekly home involvement was a significant predictor of FACES literacy ($b = .12$, $t(1645) = 5.00$, $p < .001$), Woodcock Johnson Applied Problems ($b = .066$ $t(1209) = 2.26$, $p < .05$), and parent-rated social skills ($b = .09$, $t(1645) = 3.23$, $p < .01$). The positive regression coefficients indicate greater family weekly home involvement, which predicts higher scores for children's emergent literacy, numeracy, and social skills as rated by parents. School-based involvement was a significant predictor of FACES literacy ($b = .06$, $t(1645) = 2.49$, $p < .05$), Woodcock Johnson Applied Problems ($b = .06$ $t(1,209) = 2.27$, $p < .05$), and parent-rated social skills ($b = .05$, $t(1645) = 1.91$, $p < .05$).

Family home involvement and family school involvement were found to be important predictors of school readiness for Head Start children. As hypothesized, family home involvement was found to be a more important contributor to school readiness, underscoring the importance of involving families in their child's learning at home. In the future, this research may be used to develop programs to promote

family involvement in their children's learning in the home and support parents' involvement in the school. Additionally, future research may examine the role of family culture and ethnicity with regard to family, home and school involvement. This may lead to the development of culturally and linguistically sensitive interventions to promote home involvement with diverse families.

Disrupting Normal Development of Sea Urchin Embryos with Detergents: A Molecular Approach to a Classic Developmental Biology Experiment

John Dorsey (class of 2010)

Majors: Biology, Anthropology
Principal Investigator/Supervisor: Dr. Athula Wikramanayake
Department: University of Miami Biology Department
Senior Thesis: Yes

Our research centers on the role of the Wnt signaling pathway in regulating axial polarity, germ layer segregation, and gastrulation in the sea urchin embryo. Previous work in the lab has shown that blocking Wnt signaling in the early sea urchin embryo leads to disruption of these processes, and produces embryos that have a distinct phenotype referred to as "animalization". My research focuses on determining if classic embryological experiments that have produced the animalized phenotype are due to disruption of Wnt signaling. In particular, I have revisited a classic experiment where detergent-treatment of sea urchin embryos resulted in animalization, to determine if this effect was caused by disruption of Wnt signaling.

During embryogenesis, blastomeres in the sea urchin embryo divide with an invariant pattern along the primary egg axis, also known as the animal-vegetal axis. At the fourth cleavage division, the four cells at the vegetal pole divide unequally to produce four small cells at the vegetal pole referred to as the micromeres. These micromeres have a critical role in the early patterning of the embryo, and these properties are regulated by Wnt signaling. A classic developmental biology experiment[1] determined that if a certain concentration of a surfactant (detergent) is added at a significant point in the early development of sea urchin embryos the cells will be virtually identical in size and shape at the 4th cleavage division and these embryos lack micromeres and become animalized. Animalized embryos never undergo gastrulation (Figure 3), so they grow until they become too large to function and die (Figure1).The mechanism by which the surfactant causes this is not known, however it is known that SDS (sodium dodecyl sulfate, a common detergent) dissolves cell membranes particularly well.

My mentor's research[2] investigates the Dsh protein (a critical component of the Wnt signaling pathway), which is localized at the vegetal pole of the unfertilized egg and remains at the vegetal pole throughout development (Figure 2). During the 4th cleavage, the micromeres contain the Dsh protein. It is hypothesized that the surfactant delocalizes the Dsh protein and results in the equal cleavage in the classic experiment.

In my research I have successfully duplicated the classic experiment using the surfactant SDS and the sea urchin species Strongylocentrotus purpuratus.

In order to receive the desired effect of the even 4th cleavage, a concentration of 25µg/ml SDS was added to a culture of urchin embryos once they have reached the 4 cell stage. After 30-45 minutes the SDS is removed and the urchins are allowed to develop. The result was a 16 cell stage with all 16 cells of equal size. Approximately 1/3 of these went on to become normal blastulas, whereas another 1/3 became animalized, or lacked any differentiation. Basically an animalized embryo contains cells of all the same size with no specialization.

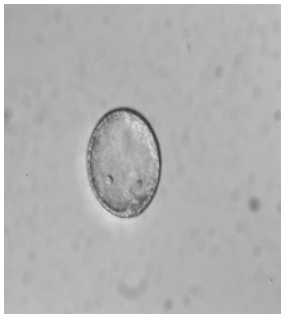

Figure 1: Animalized embryo

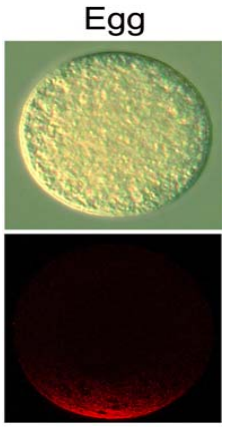

Figure 2: Unfertilized, stained for Dsh embryo

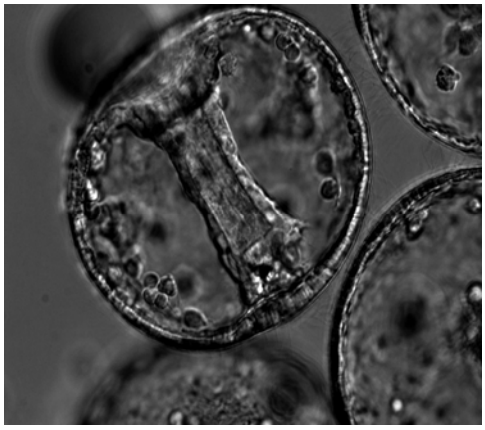

Figure 3: Gastrulation of embryo

It was determined that it might be possible to see the effects of SDS on Dsh in unfertilized eggs. In order to determine if Dsh could be delocalized in the unfertilized egg stage, eggs were subject to SDS for extended periods of time. The result of Dsh antibody staining showed that Dsh remained localized. This is surprising, as it is assumed that the Dsh protein is membrane bound. Due to the fact that SDS is negatively charged, we have attempted to use neutral surfactants to perform the same experiment. These have been successful, but render the eggs so fragile that further investigating them has proven difficult.

In the future it is our intention to continue performing the classic experiment, checking the location of Dsh at every stage after the SDS is added. Also, attempts will be made to 'rescue' the embryos by treating them with SDS, then adding lithium chloride, which is known to activate the Wnt pathway further along, assuming it has been disrupted. In order to determine if Dsh is membrane bound we will be adding lethal doses of SDS or other detergents in an attempt to delocalize the protein. If these attempts fail, the Dsh protein would prove not to be membrane bound, as has been assumed.

Acknowledgement

We wish to credit the photographs to both the author and Jeff Peng.

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Fly-ing the Environmental Neurotoxin L-BMAA

Wilfredo J. Escala (class of 2010)

Major: Biology
Principal Investigator/Supervisor: Dr. R. Grace Zhai
Department: Cellular and Molecular Pharmacology, Miller School of Medicine, University of Miami.
Senior Thesis: No

Knowing that it is commonly used in the scientific realm for its ability to generate generations of interest rapidly, as well as its capability of producing surplus amounts of scientific subjects with an already known genome, the Drosophila melanogaster was definitely the most alluring model to jointly study behavior and development. This developmental and behavioral study was centered on the effects of the environmental neurotoxin L-BMAA, its involvement in the continuing onset of neurodegenerative diseases in the pacific islands and its threat as a global toxin. Since the environmental neurotoxin L-

BMAA hypothesis has yet to examine the progressive effects of the neurotoxin, we wish to show in vivo that the environmental neurotoxin L-BMAA truly is a 'slow' toxin that instigates degeneration. In this study we found that L-BMAA exposure causes chronic neurotoxicity, and that Drosophila melanogaster serves as a useful model in dissecting the pathogenesis of Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex (ALS/PDC).

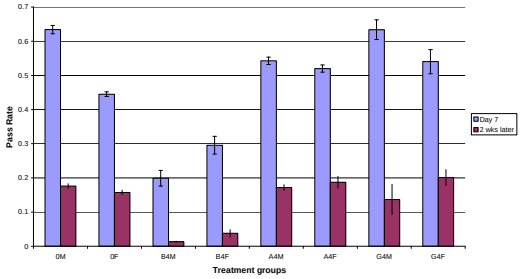

FIGURE 1: Negative geotaxis (climbing) behavior assay at day 7 and day 14, showing the dramatic effect of the neurotoxin L-BMAA compared to the other control groups: Alanine, Glutamic acid and the non amino acid fed flies.

With two schools of thought: one that deems that L-BMAA is indeed a powerful toxin linked with neurodegeneration and one that proclaims that L-BMAA is not an environmental toxin nor that it should be associated with recurring cases of degeneration in the pacific islands, L-BMAA has sparked controversy and a new level of interest in the scientific field of neurobiology and more specifically, neurodegeneration.

Although it has been reported that L-BMAA is a non protein amino acid associated with the Amyotrophic Lateral Sclerosis (ALS) (Rao 2006) digested through the consumption of flying foxes and that it may act as a endogenous neurotoxic reservoir (Murch 2004); it has yet to be shown whether such is the mechanism of the neurotoxin and whether such mechanism truly allows it to functions as a proposed 'slow' toxin. There has also been an absence of an *in vivo* model that can accurately represent the toxicity of L-BMAA without implying sudden and acute toxicity.

To measure the progressive degenerative effects of this neurotoxin, we utilized two assays: Lethality and Climbing. The lethality assay was a method of measuring the survival rate of the *Drosophila melanogaster* and simultaneously replicating the proposed long latency period of the neurodegeneration. After collecting wild type *Canton S* flies, we sorted them into four different groups: L-BMAA (experimental group), Alanine, Glutamic acid and a normal yeast/molasses food source (control groups). After such placements, we measured the survival rate by recording on a daily basis the percentage of flies or *Drosophila* that had died until all the flies in that group had died.

The behavior/climbing assay was a method of studying and quantifying neuromuscular degeneration and the effects on *Drosophila* coordination. It was also an *in vivo* model of verifying whether these effects resembled the behavior of individuals with neurodegenerative diseases such as ALS or Parkinsonism Dementia Complex (PDC). Using the tendency of the *Drosophila melanogaster* to oppose gravity (negative geotaxis), we trapped the collected flies from each group respectively and placed them into a cylindrical structure made from the vertical connection of two vials. Then we would allow the flies 10 seconds to climb 8 centimeters up the vial wall and recorded the number of flies that had crossed the marked 8 cm line. An average of all the flies that passed the 8cm line per their respective group was then calculated and this became the passing rate for each of the amino acid treatments.

It is now clear to us that L-BMAA has a critical toxic role in shortening the lifespan of the fruit flies, and a distinct negative effect in their behavior with a possible dose dependent effect. At this time, we have no conclusive results on the negative effects of L-BMAA on fly nervous system morphology, but it is definitely in the works.

We are also working on expanding the molecular aspect of the project by examining possible mechanisms by which the toxicity of L-BMAA is distributed throughout the nervous system.

Everything considered, it seems that the toxicity of L-BMAA has been underestimated and considering that it can be environmentally bountiful, it should be examined as a strong environmental toxin. This statement is made

considering the fact that the neurotoxin is made by cyanobacteria, which comprises a large percentage of all marine ecosystems.

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Dopamine Could Promote Alzheimer's Disease Through the Catalytic Production of Hydrogen Peroxide

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Major: Chemistry
Principal Investigator/Supervisor: Dr. Rajeev Prabhakar
Department: Chemistry
Fellowships: None
Senior Thesis: No

Alzheimer's disease is a prominent neurodegenerative disorder that has been linked to the aberrant behavior of redox-active metals. This study analyzed the likelihood of dopamine acting as a substrate for the catalytic production of hydrogen peroxide. Computational techniques were employed in order to elucidate the structural and energetic details of the reaction as it proceeds through the proposed catalytic mechanism. The study found every step in the reaction to be exergonic with the exception of the seventh step; it yielded an energy barrier of 14.3 kcal/mol, still within the limit for biologically relevant reactions (15 kcal/mol). The results indicate that the catalytic cycle mechanisms are indeed energetically feasible.

Neurodegenerative disorders have been linked to the overproduction of reactive oxygen species.[1] The most prominent of these is Alzheimer's disease (AD) with an estimated 26 million people suffering from the disease worldwide.[2] One way in which AD physically manifests itself is through the formation of β-amyloid (Aβ) plaques in the brain that have been linked to hydrogen peroxide production.[3] These plaques mainly consist of aggregated Aβ 1-42 peptides, a 42 amino acid polypeptide believed to be responsible for the plaques' neurotoxicity.

A previous study has shown that ascorbate is able to act as a substrate for Aβ-bound Cu (II) to catalytically produce hydrogen peroxide and yielding dityrosine crosslinks representative of Aβ peptide aggregation.[4] This experiment examined the possibility of dopamine acting as the substrate for this reaction.

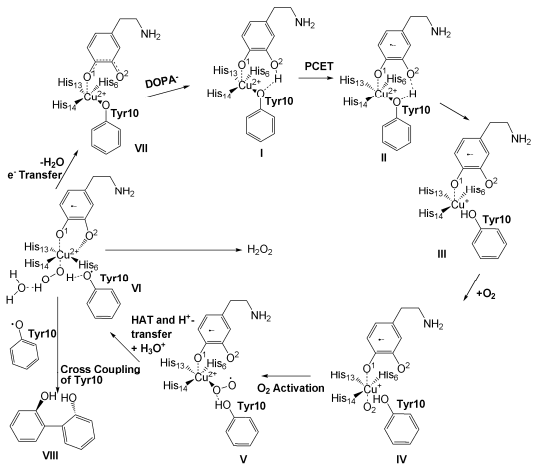

Figure 1. Proposed Catalytic Mechanism.

Computational methods were employed to elucidate structural and energetic details of the reaction that tested the validity of the proposed mechanism (see Figure 1). We've chosen density functional theory and the 6-31G basis set as our model chemistry and used the program Gaussian to make calculations in the gas phase, in solvent, and with an expanded basis set. We used the same model for the catalytic domain of Aβ as Barnham et al: three imines and phenolate anion, representative of three histidines and tyrosine, bound to Cu (II) with the substrate (dopamine in this case) in the axial position. The reaction begins by transfer of a proton from dopamine to phenolate anion and

the donation of an electron from dopamine to Cu (II). These two processes occur through a proton coupled electron transfer mechanism. During the process, dopamine is released by Cu (II) resulting in a 0.4 kcal/mol decrease in energy. The next step involves the release of phenol and binding of dopamine radical anion to reduced copper ion, Cu (I), releasing 2.7 kcal/mol from the system. Introduction of triplet oxygen to the axial position of the copper complex decreased the system's energy by 4.0 kcal/mol. Subsequent binding and activation of oxygen through the donation of an electron from Cu (I) further decreased the system's energy by 0.8 kcal/mol. After the addition of hydronium, hydrogen peroxide and water were formed through a proton and hydrogen atom transfer, resulting in a further drop of 5.4 kcal/mol. The binding of phenolate anion to Cu (II), unlike all previous steps, is an endergonic reaction increasing the system's energy by 14.3 kcal/mol. These results support the conclusion that the reaction mechanisms for the proposed catalytic cycle are energetically feasible.

Similar studies are currently being carried out with catechol, L-dopa, and 6-hydroxydopa using the same catalytic mechanism to reveal other molecules that might be involved in the catalytic production of hydrogen peroxide. Uncovering molecules that may act as substrates for this catalytic mechanism may help shed light on the causes of reactive oxygen species overproduction and Aβ peptide aggregation, two of the main characteristics of AD.

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Reversal of Drug Resistance in Pancreatic Cancer

Heather Miller (Class of 2010)

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Department: Sylvester Cancer Center
Fellowship/Awards/Recognition: American Cancer Society Summer Research Fellowship
Senior Thesis: Yes

Currently there are very few treatment options for patients with advanced pancreatic cancer who progress during or after traditional chemotherapy. One reason cancer progresses in these patients may be due to chemotherapy resistance that pancreatic cells develop through epigenetic changes. The research in our lab focuses on reversing chemotherapy resistance in pancreatic cancer cells by reversing epigenetic mutations with the aim of making chemotherapy more effective. Thus far, our results have been promising and show increased cancer cell death in cells pre-treated with epigenetic inhibitors compared to cells treated with chemotherapy alone.

Pancreatic cancer is the fourth leading cause of cancer related deaths among men and women in the United States, with an estimated 42,470 new cases and 35,240 deaths in 2009.[1] Gemcitabine is the current treatment for patients with advanced pancreatic cancer. However, only a 5.4% partial response rate with a one and five year survival rate of 18% and 5%, respectively, is observed.[1,2] The high fatality of this disease may be attributed to the resistance which pancreatic cancer cells develop to chemotherapy. However, the molecular mechanisms by which pancreatic cancer cells acquire resistance are presently unknown.

One of the mechanisms of resistance may be due to inactivation of pro-apoptotic genes is through epigenetic silencing. Epigenetics refers to DNA modifications without alterations in the DNA sequence. Epigenetic modifications though heritable are reversible, thus making them potential therapeutic targets. DNA methylation is one of the most commonly occurring epigenetic events taking place in the mammalian genome. DNA

methylation is a covalent chemical modification catalyzed by DNA methyltransferase (DNMT) enzymes that catalyze the addition of a methyl (CH3) group to cytosine in the sequence context 5'CG3', also called the CpG dinucleotide. DNA methylation is involved in down-regulation of gene expression and previous studies have shown that many pro-apoptotic genes are epigenetically silenced in pancreatic cancer.[3] Based on this, we hypothesized that methylation of pro-apoptotic genes may contribute to chemotherapy resistance in pancreatic cancer cells. We also hypothesized that by re-activating pro-apoptotic genes it may be possible to enhance the sensitivity of pancreatic cancer cells to chemotherapeutic agents, thereby increasing the efficacy of traditional chemotherapeutic agents.

We examined the role of epigenetically silenced pro-apoptotic genes in two pancreatic cancer cells, MiaPaca-2 and PANC1; however the results shown are from MiaPaca-2 cells only. We focused on the methylation pattern of TMS1, Target of Methylation-mediated Silencing, in MiaPaca-2 cells and MAGE1, a gene which codes for a tumor-rejection antigen in PANC1 cells. We found that the methylation of these genes correlated inversely with gene expression. TMS1 was determined to be fully methylated (Figure 1A), through the use of methylation specific polymerase chain reaction. This correlated with a low level of gene expression (Figure 1B), which was measured by quantitative real time polymerase chain reaction.

We then determined the cytotoxicity of gemcitabine over the course of 72 hours in each of these cell lines through a cell viability assay and observed increased cell death with

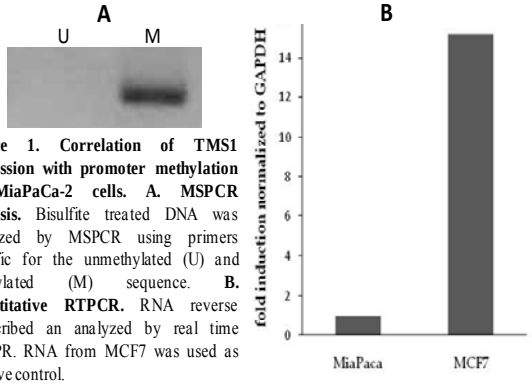

Figure 1. Correlation of TMS1 expression with promoter methylation in MiaPaCa-2 cells. A. MSPCR analysis. Bisulfite treated DNA was analyzed by MSPCR using primers specific for the unmethylated (U) and methylated (M) sequence. **B. Quantitative RTPCR.** RNA reverse transcribed an analyzed by real time RTCPR. RNA from MCF7 was used as positive control.

increasing concentration of the drug. Next, we examined the effect of an epigenetic inhibitor, 5-azacytidine, on gene expression and cell viability over a course of 72 hours. Epigenetic inhibitors, such as 5-azacytidine, can be used to reverse epigenetic changes in DNA. 5-azacytidine is an FDA approved drug, which functions as a demethylase, thus reversing methylation. As with gemcitabine, increasing concentrations of 5-azacytidine resulted in increased cell death. It was also observed that an increasing dose of 5-azacytidine resulted in increased expression of epigenetically silenced genes (Figure 2). Based on the individual drug treatments in each cell line, a combination treatment with 5-azacytidine and gemcitabine was designed. We determined the efficacy of the combination treatment through a cell viability assay. The cells pre-treated with 5-azacytidine showed increased cell death compared to cells treated with gemcitabine alone (Figure 3).

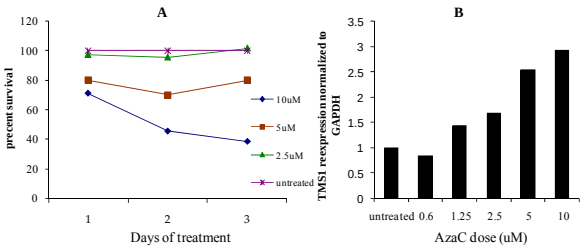

Figure 2. Effect of 5-azacytidine treatment on MiPaca-2 cells. (A) Cell viability after drug treatment, measured by Cell Titer Blue. (B) RT-PCR analysis of TMSI expression after 72 h of 5-azacytidine treatment.

The results of this study suggest a combination treatment of 5-azacytidine and gemcitabine may have a greater efficacy than gemcitabine used alone. The increased sensitivity to chemotherapeutic agents in cells pre-treated with 5-azacytidine is most likely due to the re-activation of epigenetically silenced, pro-apoptotic genes. The results from this study may be applicable to other cancers and may be useful in designing clinical trials for treatment of advanced pancreatic cancer patients who have progressed during or after gemcitabine chemotherapy.

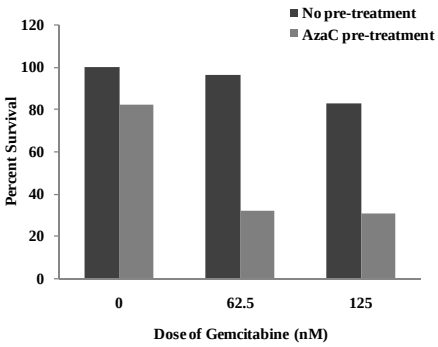

Figure 3. Effect of pre-treatment with 5-azacytidine on sensitivity to gemcitabine in MiaPaca-2 cells. MiaPaca-2 cells were treated with 5 μM 5-azacytidine for 72 h followed by 62.5 and 125 nM gemcitabine treatment.

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Flow Through Beach Sediments

Matthew Phillips (class of 2010)

Major: Marine Biology
Principal Investigator/Supervisor: Dr. Helena Solo-Gabriele
Department: Department of Civil, Architectural, and Environmental Engineering.
Fellowship/Awards/Recognition: Research Experience for Undergraduates (NSF Grant)
Senior Thesis: Yes

The focus of this project is to quantify the vertical transport of bacteria caused by the upward flow of water . A model was built to control and test various rates of flow through a core of sand containing microbes, and to sample the outflow for the microbial indicator organism, enterococci. Preliminary results suggest that the majority of enterococci are entrained and removed from the sediment rapidly at the initiation of flow.

Enterococci are used by the Environmental Protection Agency to test recreational waters for contamination by sewage and fecal matter. It has been shown that these bacteria can also inhabit beach sediments and contaminate water at beaches without a point source of sewage contamination.[1] The process by which microbes are transported from the sediment into the water column has been widely unstudied and quantifying the release of these microbes under varying physical conditions would be useful for implementing predictive measures to ensure public safety in recreational waters.

In order to test and quantify the release of microbes from beach sediments, a microcosm was built (Figure 1) in order to control and monitor environmental parameters such as pressure differentials and flow rate. Hobie Beach was chosen as a sampling site because of its well documented high enterococci concentration in beach sediments and because it is not exposed to a known point source of sewage.[2] Sand cores were taken from the beach at various tidal stages and locations. The sand core was then placed directly into the microcosm for experimentation. Microbe free salt water was then forced up into

the sand core, driven by pressure differentials, and sampled at the top of the core. The water exiting the core was then tested for enterococci. The total enterococci concentration of the sand was also measured before and after the experiment.

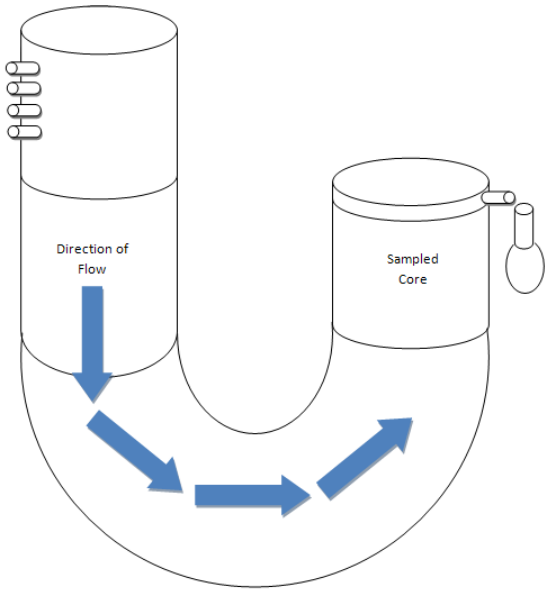

Figure 1: Aseptic water enters the microcosm through the left arm which maintains a constant head difference with the right arm and the sampled core. By varying the head difference, different flow rates and pressure differentials can be created. Water is collected at the top of the right arm, just above the sampled core.

All trials of the experiment thus far have produced similar results. The majority of the enterococci present in the sand sample are entrained rapidly at the initiation of flow. The number of enterococci entrained by water flowing through the sample decreases sharply after the initiation of flow and eventually moves below detectable limits. The slower the flow, the longer it takes for the bacteria levels in the sampled water to go below detectable limits.

The results indicate that the majority of enterococci in the sand are entrained rapidly by the influx of ground water. In vivo, this influx would be driven by the falling tides and the drainage of ground water from the land. Both high tide and flows after rain events have been shown to correspond with high enterococci levels in the water.[2] The study needs to be expanded in order to collect data for a wider range of sand microbe levels and physical parameters. The vertical distribution of microbes in the sediment also needs to be determined in order to fully understand why microbes are being removed at currently observed rates. The deposition of bacteria into the sand also needs to be studied to examine the entire tidal cycle and the possible equilibrium it establishes between the upwards entrainment of bacteria out of the sand and the downward deposition and filtration of bacteria into the sand. In the future, data from these experiments can be utilized in a computer model that will take into account weather, currents, tides and other environmental conditions to predict the levels of enterococci at the study beach and thus enable beach managers to predict times when the bathing waters will be unsafe.

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Chlorine Calorimetry within an Air Tight Environment

Allison Ring (Class of 2010)

Major: Chemistry
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Fellowship/Awards/Recognition: Work study
Senior Thesis: No

A calorimetry device within an air tight glove box system has been designed and constructed, allowing calorimetric research to be conducted with harmful chemicals and air sensitive compounds. Calibration results with Tris-Hydroxymethyl Aminomethane and Phosphorous

Pentachloride have proven the system to be usable and reliable for a reaction with a known enthalpy, so that reliable data can be obtained from it in future experiments.

Calorimetry measures the heat flow and temperature change of a reaction, or the specific heat of a reaction.[1] There are a number of commercial calorimeters on the market, but few can be used with chlorine, a hazardous and corrosive gas. The calorimeter used in this case is composed of glass with Teflon parts. In order to reliably measure enthalpies of reaction with Chlorine, the calorimeter system was first calibrated utilizing Tris-(Hydroxymethyl) Aminomethane (THA), a standard for solution calorimetry. The heat changes associated with electrical input and the introduction of the THA compound were measured in 250 mL of 0.10 M solution of HCl. To measure the chemical changes, glass ampoules were filled with THA and then shattered at designated time intervals. This allowed for comparison between the chemically and the electrically driven changes in temperature. To maintain constant circulation of the HCl, the calorimeter was shaken vertically throughout the experiment. Figure 1 shows a sample graph of the electrical and chemical changes recorded within the system during the experiment.

After a few weeks of conducting the calibration experiments, instrumentation system

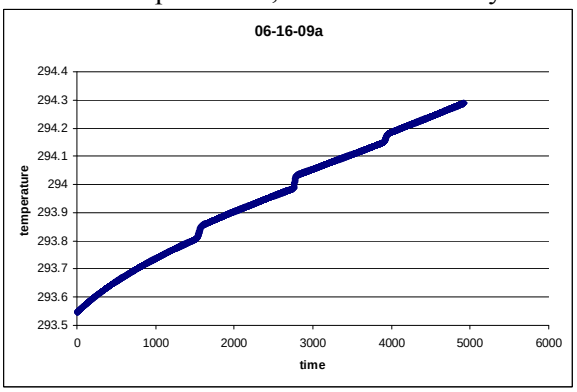

Figure 1: The first jump at around 1700 sec and the last jump at around 4000 sec are both results of electrical input while the jump at around 2800 sec is a result of the chemical reaction of THA with HCl. The jumps depict the temperature change recorded in Kelvin.

were deemed reliable, justifying use of the system for further experimentation.

In order to gather calorimetric data about reactions with toxic chemicals such as chlorine (Cl_2), the calorimetry system needed to be contained within an air tight environment: a glove box. To accurately gather data, the calorimeter must continue to operate in the same manner as before, shaking vertically, while maintaining the safe air tight environment within the glove box. In order to allow this motion through the floor of the glove box, a bellows system was devised. Keeping the motor and the bellows system outside the glove box as shown in Figure 2 is beneficial because valuable working space within the glove box is maximized, and maintenance on the motor and bellows system is easier and more convenient.

After the initial calibration of the calorimeter system it is important to calibrate it with a chlorine based reaction.[2]

$$PCl_3 + Cl_2 \rightarrow PCl_5$$

The calibration with this reaction is more closely related to our research, therefore a better test reaction. This reaction's computed value for ΔH is -374.9 kJ/mol.[1] Since it is a known quantity, we can assess our experimentally determined accuracy.

Knowing the calorimetric data is important to supplement research projects. The ultimate goal is to measure the enthalpy of

Figure 2: The glove box with the moveable mechanisms and bellows system below. The calorimeter holding vessel and calorimeter is pictured attached to the metal rod that extends through the floor of the cast iron glove box and connects to the bellows system.

Chlorine addition to dual platinum core compounds. This project is done in collaboration with Tim Cook and Dr. Daniel Nocera at Massachusetts Institute of Technology. This data is needed to determine the amount of energy released chlorine is bonded to the platinum compound. These and related compounds form the basis for solar energy storage and conversion. These calorimetric studies are essential in order to quantitatively assess the amount of converted light energy.[3] Additional use of this system is planned to map the strength of the metal-chlorine bond for a wider range of compounds of interest for both solar energy conversion and catalysis. None of this will be possible until the construction and calibration of a calorimetry system capable of dealing with the toxic, hazardous, and corrosive chlorine gas is complete.

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Hedgehog Signaling Regulates Cell Adhesion Molecule (CAM)-Mediated Neuronal Morphogenesis

Matthew Sacino (class of 2011)

Major: Neurobiology
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Department: Neurological Surgery, Lois Pope Life Center, Miller School of Medicine, University of Miami.
Fellowship/Awards/Recognition: Lois Pope Fellowship
Senior Thesis: No

Our research revolves around neuro-regeneration after injury. Focusing on the Hedgehog Signaling pathway we have investigated the role of the Smoothened (Smo) protein in regulating neuronal growth and differentiation. Embryonic and adult rat hippocampal neurons were treated with Cyclopamine (pharmacological inhibitor of Smo) and Sonic Hedgehog fragments (activator of Smo) and grown on different substrates. Results have shown that neurons treated with Sonic Hedgehog fragment have shown increased neurite length and branching.

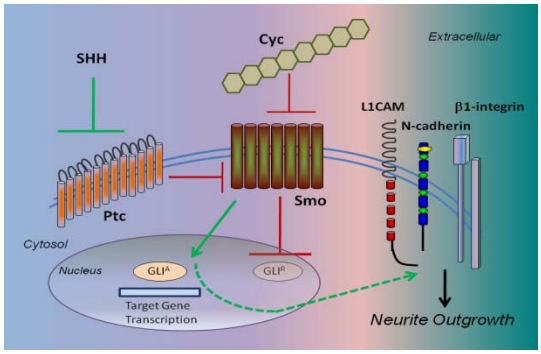

Figure 1: Signal Transduction of Hedgehog Signaling Pathway.

The G protein-coupled seven-transmembrane receptor Smoothened (Smo) is activated by Sonic hedgehog (Shh) signal transduction and pharmacologically inhibited by the small molecule Cyclopamine (Cyc), see Figure 1.[1] Previous data from our lab has led to the hypothesis that Smo regulates axon and dendrite length and branching. To test this hypothesis we determined the effects of cyclopamine and an active Shh fragment on neuronal morphogenesis. Embryonic day 18 rat hippocampal neurons were grown on laminin (Figure 2), which activates β1-integrin receptors.[2] Neurons were cultured in five conditions: untreated, DMSO (vehicle control), cyclopamine (5μM and 10μM), and active Shh fragment C25II (3 μg/mL).

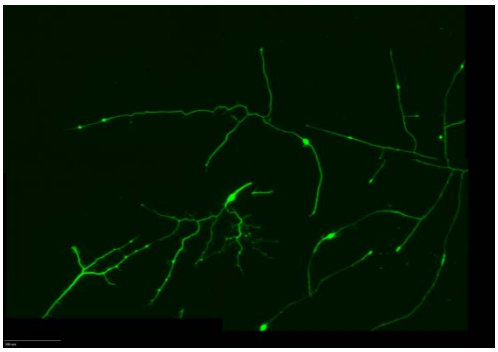

Figure 2: Embryonic Day 18 rat hippocampal neuron grown on laminin substrate. Neurons treated with Shh C25II fragment showed a marked increase in morphogenesis while neurons treated with Cyclopamine exhibited a decrease in morphogenesis.

The second part of this experiment involved analyzing the neuronal gene expression of Hedgehog signaling components to determine whether the genes are expressed in the embryonic rat hippocampal neurons used for the neuronal culture. Reverse-Transcriptase Polymerase Chain Reaction was run on adult rat hippocampal and cerebellar granule neurons as well as and postnatal day 8 (P8) rat cerebellar granule neurons. Gene expression was tested for the components of the signaling pathway, including: Sonic Hedgehog (Shh), Indian Hedgehog (Ihh), Desert Hedgehog (Dhh), Patched 1 and 2, Smoothened (Smo), and the 18s ribosomal subunit.[2]

Results taken from L1, N-cadherin, and laminin substrates have shown that activation of Shh has increased the outgrowth of hippocampal neuritis in culture. Cyclopamine, through inhibition of the Smoothened protein, has decreased neurite outgrowth in culture. Furthermore, Rt-PCR analysis has shown that many Shh regulating genes including that of Smoothened Protein are expressed in hippocampal neurons. These results support our overall hypothesis and goal for neuronal re-growth after injury. Future directions for our study include further experimentation within the Hedgehog pathway directing gene expression and corresponding in-vivo studies.

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An In Vitro Study Comparing Aneurysm Clotting Times of Flow Diverter Devices Using a Milk-Rennet System

Jessica Schmidtman (class of 2010)

Major: Biomedical Engineering
Principal Investigator/Supervisor: Dr. Chander Sadasivan, Dr. Baruch B. Lieber.
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Fellowship/Awards/Recognition: Honors Summer Research Fellowship (summer 2009)
Senior Thesis: No

Our research involves flow diverter induced thrombosis as a method for cerebral aneurysm treatment. Whereas each diverter tested has distinct flow kinetics, and consequently, different clotting times. We used a milk-rennet system to form a milk clot in a sidewall aneurysm model implanted with different diverters as previous research suggests that the milk clot forms in locations similar to in vivo thrombus. The progression of the growing clot was imaged via 3D rotational angiography (3DRA), and its volume was calculated. Results suggest that the time taken from initiation to complete filling of the aneurysm is inversely related to the device pore density (PD). Such a milk-rennet system can be used to compare aneurysm occlusion efficiency of different flow diverter devices with a bench-top setup.

A cerebral aneurysm is a weak or thin spot on a blood vessel in the brain that bulges out and fills with blood. If the aneurysm bursts, blood will leak out into the brain leading to a hemorrhagic stroke. Flow diverters are being

evaluated as a treatment method for cerebral aneurysms as it is known the device slows the blood flow within the aneurysm inducing an intraaneurysmal thrombus.[1] Rennet added to milk induces proteolysis of the kappa casein protein facilitating coagulation of the milk.[2] This milk-rennet system has been used to simulate in vivo thrombosis previously, however those studies have focused on heart valves or arterial stenoses and not on cerebral aneurysms.[3, 4] We used such a milk-rennet system to study flow diverter induced coagulation in a silicon sidewall aneurysm model (Figure 1).

Prior to beginning each experimental trial the milk was activated with 0.1 % by volume of 6 M $CaCl_2$ and subjected to a coagulation test to insure reproducible results. Next, the device was deployed across the neck of the aneurysm, and the model was inserted into our flow set-up (Figure 2). Milk flowed at a steady rate through the model (Reynolds number 300). The temperature of the milk was held constant at 37^0C with a hot water bath. A 5% by volume solution of rennet was continuously infused (0.6 ml/min) upstream of the aneurysm. Three trials of varying time periods were run for each device in an attempt to capture 50, 75, and 100% clot formation. Upon completion of each trial, the system was flushed with water, a contrast agent was infused through the aneurysm, and the clot was imaged via angiography. 3D reconstruction was then used to calculate the clot volume and produce the image in Figure 3.

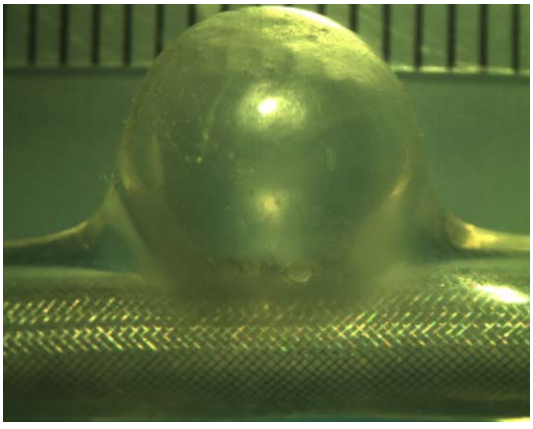

Figure 1: Silicon model with a diverter device deployed across the neck of the aneurysm. Scale markings are 1 mm.

Results show that the milk clot consistently begins forming at the distal part of the aneurysm dome and is completely formed in 20, 8, and 12 minutes for devices A, C, and F, respectively.We tested the clotting times of three different devices (A, C, and F) having PDs of 6, 15, and 25 pores/mm^2 respectively.

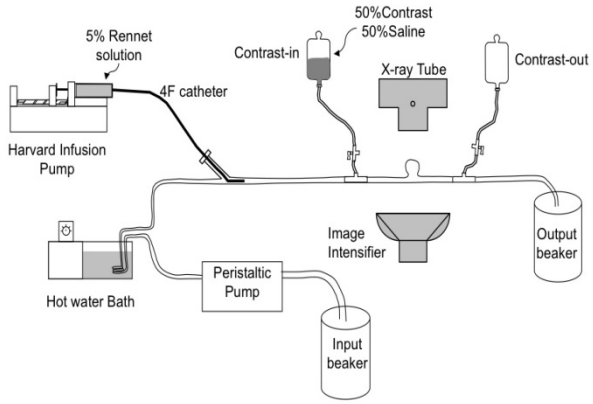

Figure 2: Set-up for flow experiments.

The control case without diverter implantation was 3% full after 25 minutes. The data was fitted to a linear model and trend line equations were used to calculate the time to 100% aneurysm occlusion (Figure 4). The clotting times were found to be approximately 10, 6, and 4 minutes for the low, medium, and high PD devices, respectively.

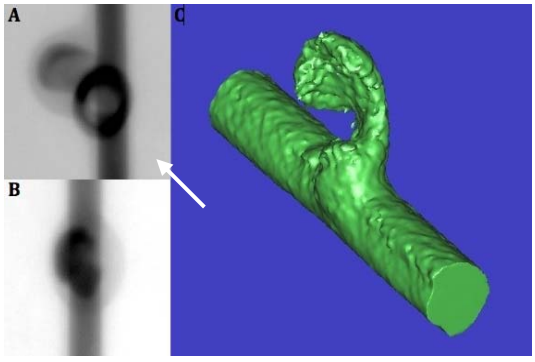

Figure 3: A) Lateral view and B) Anteroposterior view of milk clot imaged after an 18-minute trial with device A.

The dark circle (arrow) on the lateral view is from the Plexiglas casing holding the aneurysm model. Note the contrast within the aneurysm appears dark whereas the milk clot does not provide any attenuation. C) 3D reconstruction of device A (18 min) in which the contrast is a negative representation of the milk clot.

These results support the hypothesis that the PD of the diverter device inversely correlates to its clotting time as the increased PD better facilitates flow stasis within the aneurysm. Future directions for our study include experimentation and research into a link between the milk clotting times and in vivo thrombus formation time.

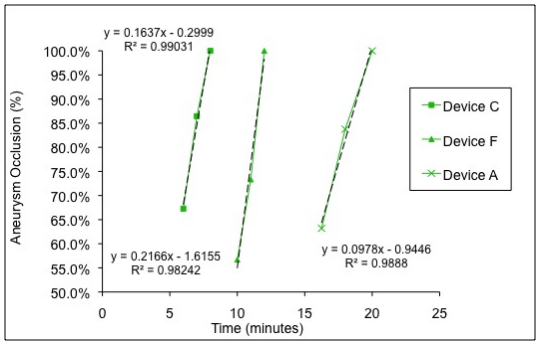

Figure 4: Graph depicting percentage aneurysm occlusion versus time to occlusion for each of the devices tested. The trend line equations shown were used to calculate the respective clotting times.

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Surrogate Genetics: Heterospecific Complementation of Viral Recombinases

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Department: Biochemistry and Molecular Biology
Fellowship/Awards/Recognition: None
Senior Thesis: No

It is unknown if viral recombinases are host specific or, if properly expressed and localized, are capable of catalyzing recombination in other hosts. Specifically, we are testing if an animal viral recombinase supports high efficiency bacterial "Recombineering". To test this, a plasmid expressing a Human Herpesvirus recombinase gene is being constructed and then placed into E. coli in order to see if the Herpes recombinase catalyzes single-strand DNA Recombineering as measured by a fluorescent reporter target gene. Results from this study will be used to optimize Recombineering for making genetic disease models and gene therapy.

DNA viruses employ a highly conserved mechanism for homologous genetic recombination. Some systems of these DNA viruses have been adapted to perform high efficiency genome modification in bacteria: recombineering. Recently, the Red recombination system of the bacteriophage lambda has been found to employ similar enzymatic and mechanistic properties as Human Herpesvirus Type I (HHV-I).[1] As the mechanisms are highly conserved, we hypothesize that viral recombinases co-evolved with their hosts to maximize recombination efficiency. This would likely make recombinase gene expression and targeting host specific. To test this, the HHV-I recombinase genes will be reconstituted in Escherichia coli in order to compare it to the lambda Red system. As recombination is an efficient means of changing sequences, many viruses of many organisms have been found to carry recombinase genes,[2] but each virus is rather specific to its host. Thus

far, attempts to reconstitute different bacteriophage recombinases in E. coli host cells have resulted in low recombineering efficiency.[3] To increase the efficiency in mammalian cells, a pRARE plasmid[4] has been inserted into E. coli to overcome the codon bias in the bacterial cell by providing the cell with human t-RNA genes. To place the HHV-I recombinase genes into E. coli, the UL29/ICP8 genes were cloned into a vector, pSIM6, through polymerase chain reaction. The pSIM6 plasmid was previously engineered to express human genes, ampicllin resistance, and encode a recombination reporter gene, the green fluorescent protein. These vectors must be placed into the E. coli cells containing the pRARE plasmid using electroporation. Immediately after electroporation, the cells will be placed onto a plate with a medium to support growth. The medium will also contain ampicillin to rid the sample of any cells that did not effectively recombineer to contain the new genes. After the cells have recovered and grown into colonies, the colonies will be counted and examined for ICP8 expression using the Western Blot method. Once ICP8 expression is confirmed, a test for recombination efficiency will be performed using a synthetic oligonucleotide (a small DNA sequence) substrate that, if recombineered into the cell, will change the fluorescent color of the green fluorescent protein. From this, the recombination frequency and efficiency can be determined. These data will help determine if recombination is indeed host specific. Thus far, no sufficient data has been collected. If the resulting data supports that recombination is heterospecific, then recombination can be applied very broadly. The method can then be employed in many different types of cells. Effectively, DNA sequences from specific organisms can be placed into different organisms to modify their genomes through viral recombination. The laboratory would like to use recombination to create genetic disease models and to develop gene therapy. Currently, a graduate student is working on the reciprocal experiment in human stem cells to hopefully be used as a tool to fight diseases, specifically Progeria. Recombination using DNA viruses has many opportunities and can be applied to a broad spectrum of biomedical problems. If data is found that supports heterospecificity in recombineering, the method will be applied to gene therapies and disease models. If not, more data will support that it is indeed host specific and new approaches for overcoming this barrier will have to be created and approached.

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Content and Frequency of Prayer of Patients with HIV/AIDS in Relation to Health Locus of Control, Depression, and Perceived Stress

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Senior Thesis: No

The purpose of this project was to relate the content and frequency of prayer of HIV/AIDS patients to factors such as health locus of control (HLOC), depression, and perceived stress, which have been associated with the progression of HIV/AIDS. Data was obtained at a single time point from questionnaires which

HIV/AIDS patients completed as part of a larger longitudinal study. As hypothesized, patients with a "chance" HLOC pray for their health (i.e. positive correlation). It was also found that patients with an "other people" HLOC do not pray for their own well-being (i.e. negative correlation), and finally, patients who are less stressed pray for non-specific things (i.e. negative correlation).

It has been demonstrated that the progression of HIV/AIDS can be predicted by psychological factors.[1] Research has shown that some HIV/AIDS patients use prayer as a "self-care strategy for managing [their] symptoms".[2] However, little research has been done to relate prayer to other factors such as HLOC, depression, and perceived stress, which have been found to be associated with the progression of the illness. The aim of the present study was to determine how the frequency and content of prayer of HIV/AIDS patients is related to HLOC, perceived stress, and depression.

There were 134 participants, 90 men and 44 women. They were all HIV+ patients in the mid-range of illness (CD4 between 150-500 cells/mml^3), and they were not drug-dependent. Participants came to the University of Miami's Positive Survivors Center every 6 months as part of a large longitudinal study. Informed consent forms were given to participants to read and sign at each visit. During each visit, participants filled out questionnaires, they were interviewed and had blood drawn. The data used for this project was taken from a single time point; the time point at which patients were asked more extensive questions on what they prayed for. Participants were compensated with $75 at the end of each visit. The prayer data was collected via 2 open-ended questions on the questionnaire which asked, "How often do you pray?" (Scored by frequencies) and "What do you pray for?" (Scored by content analysis). Other instruments used in the questionnaire were the Beck Depression Inventory, the Perceived Stress Scale, and the Health Locus of Control.

Data analysis revealed 2 broad categories: what people pray for and for whom they pray. Within each category, groupings were formed when over 10% of the sample prayed for something specific. Having a "chance" HLOC was positively correlated with praying for personal mental/physical health. There was a negative correlation between "other people" HLOC and praying for personal well-being. Finally, there was a negative correlation between praying for non-specific things and perceived stress.

Table 1:

Percentages of WHAT HIV/AIDS Patients Pray For		Percentages of WHO HIV/AIDS Patients Pray For	
What HIV/AIDS patients pray for	**Percentage**	**Who HIV/AIDS patients pray for**	**Percentage**
One's health (physical and/or mental)	42.1%	Self	69.2%
Well-being, happiness, being a better person	17.3%	Significant others, family, relatives, loved ones, friends	29.3%
Non-specific	16.5%	The living, everyone, all creations	15.0%
Strength (physical and/or mental), courage, ability to cope	15.8%		
Guidance, decision-making, direction, right thinking	14.3%		
Give thanks	12.8%		

Table 2:

Correlations of WHAT HIV/AIDS Patients Pray For and HLOC, BDI, PSS, and Prayer Frequencies

WHAT HIV/AIDS patients pray for	HLOC-Internal	HLOC-Chance	HLOC-Doctor	HLOC-Other people	BDI	Perceived Stress Scale	Frequency of Prayer
Health (physical and/or mental)	-.087 .320	.187* .031	-.040 .645	-.051 .562	-.007 .932	.039 .654	.107 .222
Well-being, happiness, being a better person	-.078 .373	-.080 .357	-.110 .207	-.181* .037	.000 .999	.045 .605	.071 .421
Non-specific	-.004 .967	.089 .307	.068 .440	-.033 .705	-.066 .451	-.189* .030	-.039 .662
Strength (physical and/or mental), courage, ability to cope	-.049 .578	-.020 .822	.059 .497	.116 .186	-.084 .336	-.046 .602	-.041 .639
Guidance, decision-making, direction, right thinking	.038 .666	-.140 .109	.023 .792	-.082 .352	.000 .992	-.048 .586	-.040 .647
Give thanks	.078 .370	.066 .451	.092 .292	-.016 .857	.101 .249	.125 .151	.111 .207

Key: Correlation Significance * p < .05

As hypothesized, having a "chance" HLOC was positively correlated with praying for personal mental/physical health. Therefore, it can be implied that HIV/AIDS patients who believe their health is due to chance, would find comfort in praying for their health, since they do not perceive themselves to be in control of such. In addition, the negative correlation between "other people" HLOC and praying for personal well-being may be best explained by the idea that if HIV/AIDS patients believe their health is controlled by others, they may not necessarily

find comfort in praying for their own well-being. One possible interpretation for the finding of the negative correlation between praying for non-specific things and perceived stress is that patients who are not stressed out are not praying for anything in specific.

Future studies where health locus of control is assessed should use the modified health locus of control scale where "God" is used as a category. In addition, future studies should focus on identifying coping strategies other than prayer that may be more suitable for HIV/AIDS patients with an internal HLOC. In addition, future studies with a longitudinal design should examine which comes first: perceived stress or prayer.

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Insights into a Possible Mechanism of Amino Acid Oxidation to Reduce the Presence of Reactive Oxygen Species Characteristic of those Found in Alzheimer's Disease

Woody Taves (class of 2009)

Major: Chemistry
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Senior Thesis: No

Using quantum mechanical modeling we studied the possible mechanism for the oxidation of sulfur-containing amino acids, methionine (Met) and cysteine (Cys), catalyzed by amyloid beta peptides bound with redox-active metals: Cu^{2+}, Zn^{2+}, and Fe^{3+}. Two mechanisms were studied: (1) stepwise and (2) concerted. Our calculations for two distinct stepwise pathways showed prohibitively high barriers of 27.3 and 35.1 kcal/mol, and were thus ruled out. The concerted mechanism, however, had a biologically feasible barrier of 14.3 kcal/mol. The substitutions of Cu^{2+} by Zn^{2+} and Fe^{3+} increased the barrier to 19.6 and 16.9 kcal/mol. The substitution of Met by Cys resulting in barrier of 15.8 kcal/mol for the Cu^{2+}-Aβ complex.[1]

In the United States alone Alzheimer's Disease affects one in eight persons over the age of 65 with a new case developing every 70 seconds. The indirect and direct costs of care to these persons are totaled to over $148 billion annually.[2] Alzheimer's disease was discovered by Alois Alzheimer in 1906 and is characterized by two abnormal structures present in the brain known as plaques and tangles. Pertinent to this work are the plaques. Plaques are aggregates of amyloid beta (Aβ) protein fragments that are 40-42 amino acids long that are located in between nerve cells and are thought to disrupt normal cell activities.[3] One of the most important of these amino acids is the single Methionine (Met) residue located at position 35 in the following primary structure of the Aβ peptide shown below:

"$DAEFR_5HDSGY_{10}EVHHQ_{15}KLVFF_{20}AEDVG_{25}SNKGA_{30}IILGLM_{35}VGGVV_{40}IA_{42}$"[1]

This Met has been reported to play a critical role in the Aβ-peptide aggregation and the oxidative stress mechanism. This work pertains more to the oxidative stress mechanism. In the oxidative stress mechanism theory Aβ-peptide in conjunction with redox active metal ions (Cu^{2+}, Zn^{2+}, and $Fe^{3+)}$ and oxidants initiates protein oxidation, lipid peroxidation, and uncontrolled production of reactive oxygen species (ROS). This Met has been shown to give oxidant and, through Nishino and Nishida's Electronspray mass-spectrometry (ESI-Mass) experiments using bound Cu^{2+} catalyst and H_2O_2 also to give, antioxidant properties to the Aβ-

peptide. As an oxidant it serves as an electron donor and subsequently aids in the generation of ROS. As an antioxidant it will be oxidized to form Met(O) by utilizing a ROS such as H_2O_2 via the metal catalyst and thus impeding the mentioned pathway of ROS in addition to using ROS as the substrate. The reaction is shown below:

"Met + H_2O_2 → Met(O) + H_2O"[1]

A mimic of the Cu^{2+} catalyst that was more biologically favorable was made using the quantum mechanical modeling program, Gaussian, at the Computational Chemistry Lab. Virtual models were designed to represent the reactant complex. Optimization calculations were performed by the computer and the energies of our complexes were monitored. Vibrational and solvent effect calculations were performed throughout the reaction pathway.

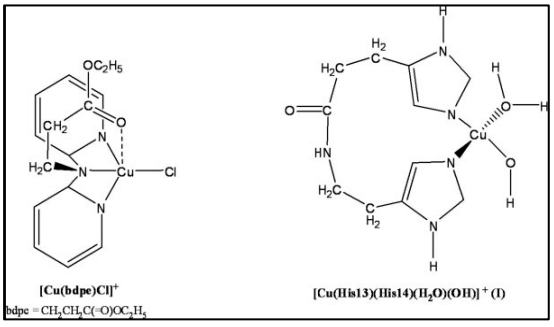

Figure 1: The structure on the left is the original catalyst studied under ESI-Mass and the structure on the right is the biologically appropriate mimic used.

Our results showed our two distinct stepwise mechanisms to be thermodynamically unfavorable due to having activation energy barriers lying outside the biologically relevant limit of 15 kcal/mol. Our concerted, one-step, reaction pathway had a favorable activation energy barrier of 14.3 kcal/mol. Afterward the substitutions with other metals Zn^{2+} and Fe^{3+} showed higher barriers of 19.6 and 16.9 kcal/mol respectfully and were less favorable, as was the substation of Met with cysteine (Cys) increasing the barrier by 15.8 kcal/mol. Data is shown in the charts and graphs below.

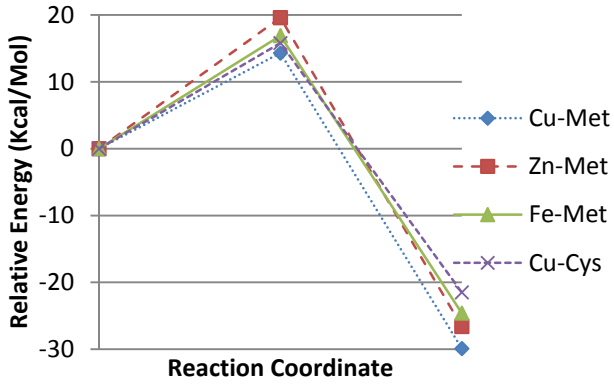

Figure 2: Potential Energy Diagram showing the energy differences of the concerted mechanism with different metals and amino acids.

	Relative Energies			
Complex:	Cu-Met	Zn-Met	Fe-Met	Cu-Cys
Reactants	0 kcal/mol	0 kcal/mol	0 kcal/mol	0 kcal/mol
Transition States	14.3 kcal/mol	19.6 kcal/mol	16.9 kcal/mol	15.8 kcal/mol
Products	-29.9 kcal/mol	-26.6 kcal/mol	-24.6 kcal/mol	-21.5 kcal/mol

Chart 1: Relative energies of the concerted mechanism with different substrates.

The results explicitly indicate that of all the metal complexes, the Cu^{2+} -Aβ complex is the most efficient catalyst for the oxidation of Met to Met(O), These results will provide a deeper insight into the oxidation of the amino acid methionine and pave the way to develop more efficient catalysts for the generation of Met(O) which posses antioxidant properties that could be used to reduce the amount of ROS in patients suffering with neurological disease such as Alzheimer's.

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Diagnosis: Heart Attack--Looking Deeper into Cardiac Troponin I

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Principal Investigator/Supervisor: Dr. Roger M. Leblanc
Department: Chemistry/Nanoscience
Fellowship/Awards/Recognition: Foote Fellow
Senior Thesis: No

Acute myocardial infarction (AMI), also known as a heart attack affects about 1.2 million Americans annually. A final diagnosis of this condition requires a cardiac Troponin I (cTnI) test. The protein in the troponin-tropomyosin complex of the myocardium, is released in measurable quantities 3-6 hours after a heart attack, but due to the limited knowledge of cTnI's nature, the various assays available are highly inconsistent. In order to develop more accurate and rapid assays, we hypothesized that an image of cardiac troponin I can be obtained through atomic force microscopy (AFM). The AFM image of cardiac troponin I obtained gives insight into the molecule's structure, something that must be taken into account in the development of new assays.

Every year about half of the 1.2 million Americans that experience a heart attack die, making coronary heart disease the nation's single leading cause of death.[1] Atherosclerosis, the build-up of fatty deposits (plaque) inside the artery walls, is the cause of approximately 90% of acute myocardial infarctions (AMI). Rupture of the plaque lining arteries often triggers coronary thrombosis, which involves platelet activation and clotting. If a clot blocks an artery for several minutes, oxygen debt causes premature tissue death of heart muscle.[2] Upon a patient's entrance to the emergency room, complaints of severe chest pain illuminate the possibility of heart attack. To confirm an AMI, doctors look for elevated levels of cardiac troponin I (cTnI) in the blood by using one of various assays on the market. Cardiac troponin I, a regulatory protein in the troponin-tropomyosin complex of the myocardium (Figure 1), functions normally in heart muscle contraction, but after a heart attack certain proteases degrade cTnI causing its release into the bloodstream[3]. The level of cTnI in a heart attack patient can jump from the healthy value of 0.06 ng/mL to 100-1300 ng/mL and remain elevated for 6-10 days, significantly longer than other markers used (Figure 2).[4,5] Some problems with the test, however, include the 3-6 hour delay between the attack and its appearance in the blood and the little consistency among the available cTnI assays due to lack of knowledge about cTnI's structure and physiochemical properties. In order to investigate the protein further, we turned to AFM, a very precise microscope that works by using a cantilever tip that contacts the surface to be imaged (Figure 3). The surface emits an ionic repulsive force that bends the tip of the cantilever upwards. A laser spot is reflected off of the cantilever and onto a split photo detector, where the amount of bending is measured. The amount of bending is then used to calculate the force, which if kept constant while scanning the tip across the surface, records the surface topography of the molecule.[6] The sample of 0.7 mg/mL cardiac troponin I (MW 24,000 g) in a phosphate buffered saline solution was obtained from MP Biomedicals and placed on the AFM slide using the drop method. Individual proteins in an aggregated state were seen in the AFM image. Figure 4 contains these images. Since very little work has been done with cardiac troponin I thus far, it is difficult to conclude much about the molecule's nature. We believe, however, that the information about troponin I's structure obtained in this study and future studies can be applied to the development of faster and more accurate assays. These assays will speed up the diagnosis of acute myocardial infarction, allowing for life-saving procedures to occur earlier in the treatment process. This results in saving lives, which is the ultimate goal of this study. Understanding of the physiochemical properties of cardiac troponin I, a significant biomarker of AMI, is most definitely a step in accomplishing this goal.

Acknowledgement

Thanks Jhony Orbulescu, the University of Miami College of Arts and Science Women and Minorities Research Program, and the University of Miami Honors Program for funding and support.

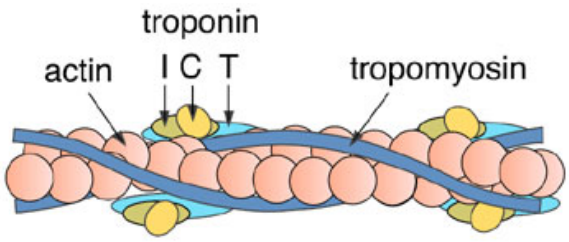

Figure. 1 The troponin-tropomyosin complex bound to actin in the myofibril[7].

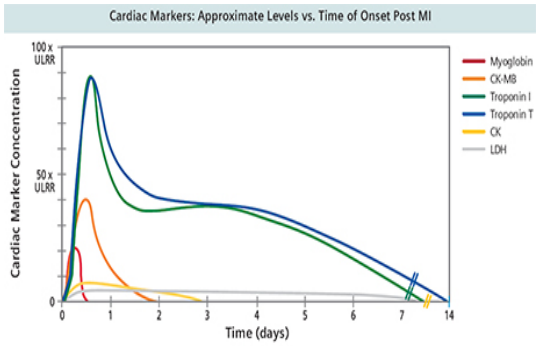

Figure 2: Approximate time span of cardiac markers[5].

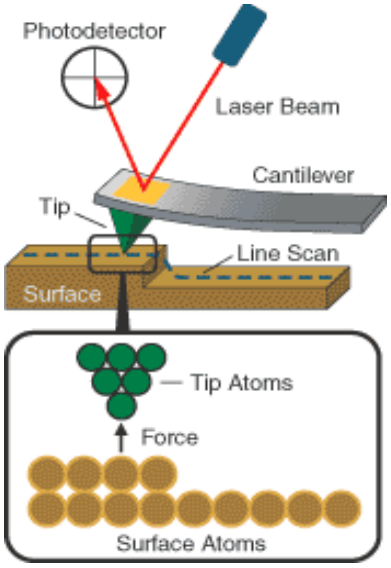

Figure 3: Diagram explaining how the atomic force microscope produces an image of a molecule's topography[6].

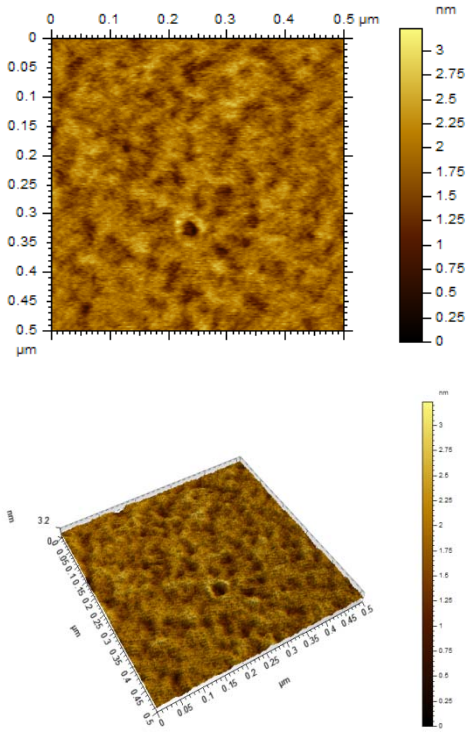

Figure 4: Atomic Force Microscopy image of cTnI obtained by Jhony Orbulescu using AC mode, drop casting technique.

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