JOURNAL OF WINCHELL UNDERGRADUATE SYMPOSIUM ABSTRACTS

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Spring 2023

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The abstracts included in this publication describe research presented at the Minnesota Academy of Science's Winchell Undergraduate Research Symposium, held on April 29, 2023, at College of Saint Benedict & Saint John's University in Collegeville, MN

BIOCHEMISTRY

ALTERATIONS IN C:N RATIO TO UNDERSTAND TO UNDERSTAND PLANT METABOLIC PATHWAYS IN RESPONSE TO PRESSURE FROM GOAT GRAZING IN THE INVASIVE SPECIES: *Rhamnus cathartica*

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The high fecundity and germination rates of *Rhamnus cathartica* enable the species to out-compete regional plant native species, causing bare soil conditions and migration of non-plant species to areas of lower *R. cathartica* density. Nitrogen is essential to primary and secondary metabolism, thus minimizing the loss of N to herbivory attack serves to maintain plant performance and fitness. This research sought to explore the relative content ratio of carbon to nitrogen in the invasive species, *R. cathartica*, before and after two sessions of goat grazing to determine if herbivory attack induces nutrient reallocation to metabolic pathways. Dry mass and chlorophyll fluorescence were selected as proxies for carbon and nitrogen assessment respectively. Our results demonstrate *R. cathartica* responds to the first session of goat grazing by investing N in the photosynthetic pathway to compensate for the loss of metabolic tissue. This reinvestment in N mid growing season depletes the plant of resources so that it is unable to effectively respond to another session of herbivory attack late in the growing season. Extending growing season into the fall is an advantage for buckthorn over other native species, but the steeper decline in Chl levels at the end of the growing season suggests that goat grazing lessens that advantage. The lower Chl levels reduce plant efficiency during a time where the plant wants to maximize uptake to prepare for the approaching cold season. With successive grazing, we would expect *R. cathartica* growth in the fall season to decline.

DYSREGULATION OF ARGINASE ISOENZYMES IN FL-HCC: INVESTIGATING THE IMPACT OF NONSPECIFIC ARGINASE-ISOFORM ANTIBODIES ON THE MARKET

Sofia K. Dodge, Allysa C. Steinhaus, James D. Dinger, Riki Mae C. Duevel, and Mong-Lin Yang (Advisor)
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In this project, we investigated the expression of the isoenzymes Arginase 1 (ARG1) and Arginase 2 (ARG2) in fibrolamellar hepatocellular carcinoma (FL-HCC) tissue samples. Previous proteomics data had predicted ARG2 to be up-regulated in FL-HCC without clear indication of dysregulation in ARG1. We utilized western blot analysis to determine protein expression by comparing five FL-HCC patient samples to three normal liver tissue samples. During the analysis, we discovered the non-specificity of several commercially bought ARG2 antibodies. This led to the design and execution of various experiments aimed at troubleshooting and identifying a commercially available ARG2 antibody that is specific for the ARG2 isotype. Once the ARG2 isotype-specific antibody was identified, it was used for western blot analysis. Our data concluded that ARG2 expression is up-regulated in FL-HCC. In addition, our data show that ARG1 expression is sample-specific, pointing to possible stage-specific dysregulation of ARG1 in FL-HCC tumor samples. Overall, our project demonstrates the importance of verifying isoform-specific antibodies while determining the expression of Arginase isoforms in tissue samples. Our findings have important implications for FL-HCC research, as they suggest that targeting ARG2 may be a promising therapeutic strategy. Additionally, our study highlights the need for careful validation of isoform-specific antibodies in cancer research, which can improve the accuracy and reliability of experimental results.

EFFECTS OF GLUCOSE ON BINDING INTERACTIONS OF HUMAN SERUM ALBUMIN WITH POLYSTYRENE NANOPARTICLES

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The interactions of polystyrene nanoparticles with albumin from human serum were studied under several conditions using various spectroscopic techniques. Interactions between polystyrene nanoparticles and albumin form a static protein corona that may affect their biocompatibility. This study aims to further the understanding of protein-polystyrene nanoparticle interactions in physiological environments. The effects of glucose, the most abundant metabolite in blood, were studied to determine how different physiological environments affect protein-nanoparticle interactions. These interactions were studied using nanoparticle titrations and fluorescence spectra to quantify polystyrene nanoparticle quenching of human serum albumin under various conditions. The effects of glucose on protein corona formation can be used to determine how polystyrene nanoparticles will interact in individuals with different blood plasma makeup such as increased blood glucose levels due to diabetes. We found that polystyrene nanoparticles have moderate strength binding of human serum albumin and glucose concentration has a positive relationship with polystyrene nanoparticle binding of human serum albumin.

INSERTING A SPECTROSCOPIC PROBE INTO HUMAN MITOCHONDRIAL MALATE DEHYDROGENASE

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Malate dehydrogenase (MDH) is an enzyme that plays a key role in many metabolic processes, reversibly catalyzing the interconversion of (S)-malate and NAD+ to oxaloacetate and NADH. Once oxaloacetate is synthesized, MDH dispatches it to citrate synthase (CS), but it is unclear how this happens. The lack of a spectroscopic probe in MDH makes it difficult to visualize any conformational changes that might happen upon interaction with CS. Our research question concentrates on how incorporating tryptophan (trp) as a fluorescent marker into MDH would reveal interactions between MDH and CS, since fluorescence emissions and λ_{max} shift due to protein-protein binding. Our focus is on determining what mutations in human mitochondrial MDH (hMDH2) would be the most active and fluorescent. Fluorescence and activity data from wgMDH wild type and mutants Y181W, Y323W, and F134W were used to create hMDH2 mutant design criteria. In particular we will present data that shows that distance from the active site, not being in a stable secondary structure, and replacing amino acids of similar size/shape (tyr, phe) lead to the most active wtMDH mutants. Using these data, as well as sequence alignment from human cytoplasmic MDH, which has 4 trp, we designed four hMDH2 trp mutants: tyrosine-136-tryptophan (Y136W), methionine-243-tryptophan (M243W), tyrosine-264-tryptophan (Y264W), and isoleucine-276-tryptophan (I276W). These mutations have been created and sequence verified. Preliminary data on their over-expression will be included. Future work will focus on their purification, activity, fluorescence, and changes in fluorescence upon binding to both citrate as well as citrate synthase.

INVESTIGATING DEK'S INVOLVEMENT IN NEURODEGENERATION IN N2A CELLS

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Alzheimer's Disease (AD) remains one of the most common forms of neurodegeneration. AD results from the loss of neuron function and cell death, which is highly associated with the presence of Tau tangles. Recently the DNA repair protein, DEK, has been associated with AD. Although current literature of DEK and its relationship to neurodegeneration is limited, DEK is suspected to be involved in Tau phosphorylation and DNA double strand break repair, however the mechanism is unknown. Therefore, the goal of this project was to investigate a possible mechanism of DNA repair, regulated by a lipid modification (palmitoylation). To investigate the relationship between DEK and neurodegeneration, we used a cell culture model along with Western blotting and a MTT assay. The results of this project established the presence of DEK in N2a cells. Furthermore, N2a cells were treated with an inhibitor of palmitoylation 2 bromopalmitate (2BP) for 24 hours. This significantly decreased cell viability and reduced total DEK protein levels, suggesting palmitoylation plays a role in protein stability. In addition, treatment with 2BP increased γH2A, a marker of DNA damage, indicating DEK's plausible role in DNA repair malfunctioning and resulted in more DNA damage. Altering DEK's function by changing the structure of DEK to study its effects on double strand repair will help us toward understanding DEK's role in DNA repair, cell death, and ultimately, neurodegeneration.

INVESTIGATING THE EFFECT OF MUTATION F134Y AND F134N ON CATALYTIC ACTIVITY OF MALATE DEHYDROGENASE

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Malate Dehydrogenase (MDH) is an enzyme that is vital for an assortment of metabolic processes such as the tricarboxylic acid (TCA) cycle. It is responsible for making the intermediate oxaloacetate from malate using NAD+ to also form the coenzyme NADH through oxidation/reduction. The purpose of this research is to investigate the effects of MDH mutations F134N and F134Y on the catalytic activity of this enzyme. We hypothesized that these mutations would lower MDH's specific activity due to the changes in overall size and polarity of these amino acid substitutions. To test the hypothesis, the wild type and mutant enzymes were purified using His-tag affinity chromatography. Enzyme activity was monitored with a spectrophotometric assay that follows the disappearance of NADH at 340 nm. The results showed that both mutations lowered enzyme activity by more than 50% compared to the wild type. These findings suggest that the phenylalanine at position 134 might play an important role in flexibility and conformation of the MDH catalytic loop. Future work will focus on studying the effects of the mutations F134Y, F134N and other substitutions at this position on MDH substrate binding and specificity.

SITE-DIRECTED MUTAGENESIS OF LYSINE 125 IN MALATE DEHYDROGENASE

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Malate dehydrogenase is a multimeric enzyme among living organisms that catalyzes the reverse transformation of malate and oxaloacetate using the reduction of NAD+ to NADH. This reaction plays a role in metabolic pathways including the citric acid cycle, gluconeogenesis, and anaerobic metabolism. The active site of MDH consists of a hydrophobic vacuole containing binding sites for the substrate and nicotinamide ring of the coenzyme NADH. Within the active site there is a loop region containing amino acids 119-137. The charges within the loop region position the substrate in the correct orientation for efficient catalysis. It has been shown that Lys125, within the loop region of MDH, makes essential interactions with NADH and nearby residues that may have been involved in catalysis. Lys125 and Arg124 are in close proximity with each other and with both molecules having a positive charge, they are repelling against each other. We predicted that the position of Lys125 and Arg124 are causing Gly263 to have a less stable hydrogen bond. We hypothesized that if Alanine replaces Lysine at position 125, then Arg124 will have a better position and be more stably bound to Gly263 resulting in a better guide for the substrate to the active site. To investigate our hypothesis, we performed cell lysis, protein purification, SDS-PAGE, and enzymatic assays. We were able to successfully purify and extract our protein. Enzymatic assays determined that our specific activity and turnover numbers were much lower than the wild-type protein, suggesting that our hypothesis was not supported.

SITE-DIRECTED MUTAGENESIS OF SITE D131K IN WATERMELON MALATE DEHYDROGENASE

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Malate dehydrogenase (MDH) catalyzes the conversion of malate to oxaloacetate, and NAD+ to NADH in the citric acid cycle. MDH has a flexible loop region that is important for activity and contains two of the three arginine amino acids required for the active site. Due to the proximity to active sites, we believe that the flexible loop is highly susceptible to mutations. We hypothesized that a mutation at site 131, causing the negatively charged aspartate to become a positively charged lysine, then the activity of MDH will decrease, and there will be a decrease in substrate affinity towards NADH. To investigate this project, we used Nickel purification, Bradford assay, Western blot, and enzymatic assays were used. The experiment showed that the mutation in site 131 of the flexible loop caused a slight decrease in the specific activity of MDH. We observed a specific activity of 25.17μM/Min/μg/mL, and a turnover number of 868 per minute, compared to the wild type specific activity of 29.39 μM/Min/μg/mL and a turnover rate of 1013 per minute. This indicates that the decrease in specific activity is caused by the mutation from a negatively charged aspartate to a positively charged lysine. Our findings suggest that the mutation caused an increase to the proton transfer barrier, therefore requiring more energy to catalyze the reaction from NAD+ to NADH. Future experiments could look at different mutations to see how they affect the kinetics of the enzyme.

SUBSTRATE SPECIFICITY OF GUANYLUREA HYDROLASE: INSIGHTS FROM BIOINFORMATICS AND MUTAGENESIS STUDIES

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Metformin is one of the most often prescribed medications in the world with over 80 million prescriptions per year in the U.S. alone, and its use is expected to increase as the global prevalence of diabetes rises. Guanylurea, metformin's transformation product, is said to be a "dead-end" metabolite that is accumulating in water. Recent studies found that there is an enzyme, guanylurea hydrolase, in Pseudomonas mendocina strain that breaks down guanylurea into guanidine and ammonia. To investigate the catalytic mechanism and substrate specificity of this enzyme, we conducted bioinformatics analyses to identify 13 bacteria that have similar enzymes to guanylurea hydrolase (GUH). Plasmids harboring the genes encoding the guanylurea hydrolase homologs were transformed into E. coli. We purified the enzymes using His-tag affinity chromatography with a Nickell column. Enzyme activity was tested by measuring the amount of ammonia released using the Berthelot reaction. We performed site-directed mutagenesis studies to create enzyme variants and compare their catalytic activity to that of the wild type guanylurea hydrolase. The results showed that most GUH homologs can only degrade guanylurea, indicating that guanylurea hydrolase has a narrow substrate specificity. Preliminary data from mutagenesis experiments indicate that a glutamate at position 211 (E211) determines the enzyme's substrate specificity; changing this amino acid to glutamine (E211Q) allows the enzyme to catalyze the breakdown of biuret, a compound structurally similar to guanylurea. These experiments are key to understanding the breakdown of metformin and its transformation product, guanylurea.

SYNTHESIZING BETA-LACTONES VIA ENZYMES PRODUCED BY *Nonomuraeacandida* AND *Nocardiaceae*

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The need for new medical drugs is always increasing as society attempts to combat the ever-evolving pathogenic world. Beta-lactones are a class of molecules present in drugs such as Orlistat and Salinosporamide and are useful in medical drugs due to their high reactivity. The idea of making new beta-lactones using enzymes from several bacterial strains is explored in this research. Preliminary experiments done in 2021 suggest that two strains of bacteria (*Nonomuraeacandida* and *Nocardiaceae*) produced a beta-lactone synthase. We performed a follow up study that recombinantly expressed the genes, purified the enzymes via FPLC (fast protein liquid chromatography), and checked the results via SDS PAGE and Bradford Assay. After analyzing multiple growth conditions, we concluded that only one of the bacterial strains, *Nonomuraeacandida* at 16°C and left for 18 hours, had produced a protein. A gel was run to check this result and we observed 3 bands at around 60 kD, which is where a beta-lactone synthase enzyme would be expected. This research demonstrated that it is possible to use a bacterial strain of *Nonomuraeacandida* under certain conditions to produce a beta-lactone synthetase. Future experiments will focus on using enzymes to produce a beta-lactone for medical drugs.

THE ROLE OF PALMITOYLATION IN DNA DAMAGE REPAIR IN OVARIAN CANCER

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Ovarian cancer is one of the most lethal gynecological cancers in women. Often ovarian cancer is associated with errors in our DNA repair mechanisms. Our research focused on ovarian cancer and the relationship with the DNA repair protein DEK. DEK is an oncoprotein that is normally expressed in higher eukaryotes, however, it has been shown to be overexpressed in ovarian cancer tissues. When it is overexpressed, it causes increased cell growth and decreased cell death, classic cancer hallmarks. Furthermore, DEK is thought to be modified by a lipid (palmitoylation) that can regulate its activity. Palmitoylation is the addition of a lipid group to a protein and is known to play a role in cancer, however, the mechanism is unknown. Our research investigated the role of DEK palmitoylation in ovarian cancer. For our research, we worked with a cell culture model, MTT cell viability assays, Western blotting, and colony formation assays. Initially, cell viability assays were performed to determine the effect of the concentration of the chemical inhibitor of palmitoylation 2 bromopalmitate (2BP). We found that the amount of DEK protein decreased with increasing concentrations of 2BP treatments, suggesting palmitoylation plays a role in protein stability. Additionally, we found that 2BP decreases cell viability and colony formations, suggesting changing the structure of DEK affects its function. Through these results, we have a better understanding of the role DEK plays in ovarian cancer, as well as the impact that palmitoylation may have on cancer cells.

THE ROLE OF SARS-COV-2 ORF8 PROTEIN ARKS MOTIF ON NOVOBIOCIN BINDING

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The discovery of the SARS-CoV-2 virus during the COVID-19 pandemic required scientists to develop medical solutions to reduce viral spread and symptoms, prompting novel therapeutic drug methods to be developed. This experimental project focused on targeting the unique accessory protein, Open Reading Frame 8 (ORF8) in SARS-CoV-2 through studying its interactions with novobiocin. ORF8 helps evade immune system checks, is involved in inflammatory responses from the cytokine storm, and most importantly, was proposed to act as a histone mimic at the histone-H3 ARKS motif that causes post-translational changes in chromatin, further worsening these problems. Previous experimental work from our lab has shown that novobiocin [Kd = 54.5 ± 3.14 μM] and three other computationally verified ligands bind to ORF8. To probe the role of Arg in the histone-H3 ARKS motif, specific mutation was done in position 52 from Arg to Met, Glu and Leu respectively, resulting in drastic intermolecular force changes that affect novobiocin's ability to bind to the ORF8 pocket. In silico analyses for the mutagenic ORF8 found the variants still docked successfully to ORF8 according to Swissdock. Primers for the ORF8 R52 mutants were then designed, and mutagenic plasmids were sequence verified. The mutant ORF8 proteins were overexpressed, purified, and Kd values for binding to novobiocin were determined via intrinsic fluorescence spectroscopy. These data will help further understand the role of SARS-CoV-2 ORF8 protein ARKS motif and how its interactions affect novobiocin binding, potentially benefiting future studies attempting to repurpose novobiocin for treatment of the virus.

ZEAXANTHIN IN THE ROLE OF PHOTO-PROTECTION

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Zeaxanthin is a pigment made by plants in the presence of light. Previous studies conducted on moss support the notion that zeaxanthin has photo-protective properties against intense sunlight, as well as extreme cold. Moss are particularly resilient in stressful conditions and act as good models for studying the benefits of zeaxanthin. We examined the correlation of thermal dissipation via light energy within two moss genera, *Dicranum* and *Calliergon*. The variable measured is the efficiency of photosynthesis (Fv/Fm) of the recovering moss after receiving hydration. Moss were pre-treated with light or dark exposure and then desiccated, in order to "preload" some with zeaxanthin in comparison to no zeaxanthin. Then moss were exposed to light treatments in the desiccated state, followed by hydration and monitoring recovery of Fv/Fm. Furthermore, pigment analysis of each group was done to determine differences in the level of zeaxanthin formed between the two species in each subgroup. The results showed that moss with zeaxanthin recovered slightly more quickly from desiccation and contained higher zeaxanthin levels in the chemical screening. The results suggest that prior presence of zeaxanthin poses some benefit in desiccation recovery. Such findings can play a critical role in how plants can work to adapt themselves in the changing climate.

CELLULAR AND MOLECULAR BIOLOGY

BIOINFORMATIC AND GROWTH CURVE ANALYSIS OF THE POORLY CHARACTERIZED YEAST GENE YLR407W

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Yeast (*Saccharomyces cerevisiae)* is a model organism and is used in the study of cell systems due to how similar it is to other eukaryotes. The aim of this project was to identify functions of the poorly characterized yeast gene YLR407W. Bioinformatics was performed to understand more about this gene. For example, it helped pinpoint the nucleus as a probable location. We conducted an experiment to determine whether altering the pH of media, an external factor, would influence the growth of the deletion mutant YLR407Wdel compared to that of the Wild Type (WT) strain. Cells were grown in Yeast Peptone Dextrose media at different pH levels, and OD 600 readings were taken hourly over a 24-hour period. Growth curves showed early promising results since for some pH conditions the deletion mutant grew better than the WT. This result suggested that YLR407Wdel potentially had a growth advantage which could indicate involvement of YLR407W in growth suppression. Further experimentation should be done to confirm if the gene is involved in growth suppression at different pH levels and if so, determine the underlying molecular mechanisms. Additionally, future study could involve work focusing more specifically on the nucleus of yeast cells such as exploring DNA methylation. This research could play a role in characterizing the function of the yeast gene YLR407W.

DEVELOPMENT OF IMPLANTATION METHODS FOR HELA AND HEK293 CELLS ON THE CHORIOALLANTOIC MEMBRANE

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The chorioallantoic membrane (CAM) of a chicken embryo is a vascularized membrane that plays a vital role in gas exchange, calcium transport, and nutrient uptake. It can be cultured ex ovo as an immunodeficient cancer and tissue engineering platform for researchers. This project aims at developing cell implantation methods for the ex ovo CAM platform. Our project began with the testing of two simple implantation methods described in previous literature using filter paper discs and plastic rings. Next, we compared the growth of HeLa and HEK293 cells cultured ex vivo with variable media volume and cell seeding volume to identify key considerations for the development of implantation methods depending on the identity of cell types in the experiment. We found that the ideal ratio of cell seeding volume and media volume, maximum media volume, and rate of expansion of the cell mass were critical factors to consider in planning future implantation experiments using the plastic ring method. Furthermore, an implantation workflow was developed to aid future students in executing the implantation experiments and imaging ex vivo cell growth over time. Lastly, we identified histological staining considerations to aid in tissue analysis of future implantation experiments. In summary, our study provides insights into the development of implantation methods for CAM research.

DYSREGULATION OF PROLINE PATHWAY ENZYMES IN FL-HCC VS HCC

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Fibrolamellar Hepatocellular Carcinoma (FLHCC) is a rare liver cancer that typically impacts juveniles between the ages of 18 and 40. The disease is caused through a deletion on each of the genes PRKACA and DNAJB1 that leads to the formation of a functional chimera. We received preliminary proteomic data from our Mayo Clinic collaborator outlining changes in metabolic pathway enzymes caused by this chimera. The data indicates significant dysregulation of proline pathway enzymes in FL-HCC samples. We have utilized Western Blot analysis to verify results of this proteomic data by comparing the expression levels of these proline pathway enzymes in FL-HCC samples and normal liver samples. The data collected over the past several years has been summarized and then utilized to create a heat map. The results collected will be crucial in developing immunotherapy drug targets for FL-HCC, and the heat map can be utilized as initial data towards understanding tumor or stage specific dysregulation of proline pathway enzymes.

FINDING ANTIBIOTIC PRODUCERS FROM SELECTED SOIL SAMPLES

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Tiny Earth, a universal network supporting antibiotics research, inspired this project. The discovery of antibiotics has been an innovation for modern medicine, whether for treating diseases or preventing infections. Unfortunately, some bacteria have developed resistance to antibiotics and further, the overconsumption of antibiotics means they are no longer as effective as before. Thus the discovery of new antibiotics is vital today. The aim of this project is to find antibiotic producers from soil at Crown College. Samples were collected from two different areas, diluted and plated. The resulting isolates were tested against *B. subtilis*, a relative of the ESKAPE pathogen *E. faecium*. Four isolates formed zones of inhibition against *B. subtilis* suggesting that antibiotics were produced. Using DNA sequences from the isolates, two were identified from the NCBI data base as *Bacillus* and *Lysinibacillus*, and the other two had no matches. Our results suggest that some antibiotic producers from our soil samples were likely discovered. Future studies could include confirmation of these findings and testing our soil against other ESKAPE pathogens.

HELA CELLS' INTERACTION WITH NANOPARTICLES

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Interest in nanoparticles has grown significantly as concerns of their potential toxicity to human cells and aquatic life have been raised. Engineered nanoparticles are of great interest as they are used in many common products from pharmaceuticals to sunscreen. Nanoparticles are materials with a diameter less than 100 nanometers, which makes their penetration of cell membranes quick and efficient. HeLa cells are excellent human cell models in research and were chosen because of their immortality. This work explored the impacts of zinc oxide (ZnO) and titanium dioxide (TiO_2) nanoparticles on living HeLa cells. The goal of the experiment was to determine whether cell mortality would increase with increasing nanoparticle concentration, and if a crucial nanoparticle concentration existed to signal cell mortality. Cells were prepared, cultured, subcultured, and analyzed observing strict aseptic technique. After one month of successful HeLa cell replication, ZnO and TiO_2 nanoparticle dispersions were prepared at a range of dilutions and introduced to the HeLa cells. The nanoparticles' effects on cells were established using hemocytometry to determine cell viability and cell mortality. The results suggest that cell mortality increased monotonically with higher concentrations of TiO_2. In contrast, lower concentrations of ZnO increased cell viability, while increased cell mortality was observed at the higher concentrations of ZnO. Given that results with ZnO were less conclusive, more research is needed to address the impact of ZnO on HeLa cells. Future studies involving nanoparticles will aid to further understand the implications of these materials on living cells.

IDENTIFICATION OF THE PRESENCE OF STAPHYLOCOCCUS ENTEROTOXIN A IN POSITIVE *S. aureus* SAMPLES

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Staphylococcus aureus is a commensal bacterium found in the nares, skin, and mucosal membranes. In most cases, it causes no harm, but there are instances where it can become pathogenic and produce toxins that have superantigenic activity. Staphylococcus enterotoxin A (SEA), when ingested, is the causative agent responsible for classical food poisoning symptoms such as emesis, nausea, and others. When SEA enters the bloodstream, it can cause toxic shock due to mass T cell proliferation and cytokine storm. In order to determine if positive *Staphylococcus aureus* samples contain the SEA gene, DNA was purified and amplified using PCR. DNA gel electrophoresis was conducted to analyze whether positive samples contain the SEA gene. We found that out of 56 samples tested, 0.07% tested positive for the SEA gene.

INVESTIGATING THE ROLE OF MAST CELLS IN MURINE MODEL OF VULVODYNIA

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Mast cells are tissue-resident immune cells implicated in local inflammatory responses by influencing nearby immune and tissue cells. They are primarily located in connective tissue which allows them to quickly detect extracellular pathogens resulting in immune-defensive, and allergic, responses through IgE-mediated activation. Mast cells can also be activated through alternative pathways upon detection of noxious stimuli, thereby initiating protective pain responses. Recent studies also implicate mast cells in the development of maladaptive pain, underlying chronic pain conditions such as vulvodynia. Vulvodynia, affecting ~10% of individuals with female anatomy, is characterized by persistent vulvar pain despite the absence of tissue injury or infection. Increased mast cell density in the painful vulvar tissue has been reported in individuals. Additionally, there is an increased vulnerability to developing vulvodynia in individuals with allergies which suggests a link between mast-cell derived immune mediators and vulvodynia. In our murine model of vulvodynia, repeated exposure to an allergen, MI, leads to prolonged mast cell-dependent genital hypersensitivity. Thus, in the present study, we examined mast cell functions to elucidate how exposure to MI can lead to the establishment of chronic pain by measuring the release of inflammatory, analgesic mediators TNF-α and IL-6. We also examined the impact of therapeutic Gleevec and THC, that decrease tactile sensitivity, on mast cell function.

MAPPING MICROBIOME TURNOVER WITH BACTEROIDES

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Members of the human gut microbiome can be classified as transient (short-lived) or resident (long-lived), depending on how often they are gained and lost (i.e., turnover). Based on metagenomic sequencing, the likelihood of microbiome turnover differs between certain bacterial taxa, but this phenomenon remains largely unknown even for taxa that are abundant in most people. The overall goal of this project was to use a combination of cultivation and genetic characterization to quantify turnover at the individual bacterial clone level. Frozen stool samples from a previous longitudinal study of healthy adults were cultured on selective and differential growth media (Bacteroides Bile Esculin Agar). Single colonies from samples taken from 2016 to 2018 were taken from plates and subcultured in rich media, followed by a highly discriminant DNA fingerprinting GTG5 PCR to identify individual clones. Next, PCR targeting the full-length 16S rRNA encoding gene (8F/1492R) was conducted and resulting amplicons were sequenced using Sanger sequencing (forward and reverse reads). Reads were aligned using an R computer program and used to identify representative clones. Most clones in our collection belonged to the *Bacteroides* genus, which is one of the most abundant genera in humans. Also, only a few clones were observed at timepoints separated by more than a few months, suggesting that turnover is quite common in the human gut. The protocol developed here is repeatable for quantifying microbiome turnover in the human microbiome and ongoing work will provide better estimates of this phenomenon in other microbiome taxa and additional human subjects.

***Staphylococcus aureus* STRAINS WITH SELX PRESENCE OFTEN HAVE DIFFERENT TOXIN PROFILES THAN THOSE WITH ABC CARRIAGE**

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Staphylococcus areus is a bacteria that can exist asymptomatically, but can be dangerous due to its individual toxin profile. These various toxins, however, seem to be mutually exclusive from another in some cases. Samples of *S. aureus* often have superantigen SELX, a genome-encoded toxin responsible for necrotizing pneumonia, and some samples have Staphylococcal Enterotoxin (SE) A, B, or C, which are known as the number 4 causes of food poisoning. Through the use of Airtable and various methods of data organization and comparison on the strains we've collected on campus, we found that SELX exhibits an inverse relationship with SE A/B/C, and that SE A/B/C also exhibits exclusivity between the three toxins. Through thorough research on *Staphylococcus aureus*, we also found that this exclusivity is due to the presence of mobile genetic elements and the differences in phylogenetic development of this species.

STUDYING THE EFFECTS OF ALTERED CYTOSOLIC CALCIUM LEVELS ON THE INTERACTION OF SERCA2A AND PHOSPHOLAMBAN (PLB) OBSERVED THROUGH THE FLUORESCENT YIELD OF A CIRCULARLY PERMUTATED GREEN FLUORESCENT PROTEIN (CPGFP)

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Heart failure with a reduced ejection fraction (HFrEF) is characterized by left ventricular enlargement and an ejection fraction <40%. HFrEF is associated with decreased cardiac pump function and diminished cardiac contractility. Cardiac contractility is regulated by phospholamban (PLB) inhibition of the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a), a pump protein that translocates calcium ions into the sarcoplasmic reticulum. We studied the interaction of SERCA2a and PLB protein with an interposed circularly permutated green fluorescent protein (S-cpGFP-P = SGP). PLB-mediated disinhibition of SERCA2a may be directly mediated by higher calcium concentrations in the cytoplasm. HEK 293 cells were transfected with SGP-cDNA to confirm SGP integrity, cultured, and observed using an inverted tissue culture fluorescence microscope (Olympus IX73). Sprague-Dawley rat adult cardiomyocytes were isolated and transfected with the SGP construct, and fluorescence was assessed. Cardiomyocytes were cultured. Using several alternative approaches i.e., electrical stimulation, ionomycin, and extracellular calcium modifications, we found the intracellular calcium concentration is increased in HEK 293 cells, H9 cells, and adult rat cardiac myocytes. With a rise of intracellular calcium, the fluorescent yield of SGP increases to directly reveal a dissociation of PLB from SERCA2a which translates into increased contractility. This mechanism is independent of PLB phosphorylation. This is the first documentation in living cells that the interaction of SERCA2a and PLB is directly regulated by cytoplasmatic calcium levels suggesting an autoregulatory mechanism that likely plays a substantial role in physiology as well as heart failure as it increases sarcoplasmatic calcium loads and contractility.

TEMPERATURE DEPENDENCE OF THE XANTHOPHYLL CYCLE ENZYMES

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Every plant engages in photosynthesis, requiring a certain amount of light energy, however too much light can be harmful. To avoid this, all plants use the zeaxanthin cycle, wherein during high-light conditions violaxanthin is converted into zeaxanthin using violaxanthin de-epoxidase (hereafter referred to as VDE). Zeaxanthin Epoxidase (ZE) preforms the opposite function, allowing for a resetting of the process. This allows the plant to tolerate light conditions it might normally find damaging, for relatively short periods of time. Additionally, Zeaxanthin has photoprotective qualities, allowing the plant to further regulate itself. It has been observed that during winter there is a large buildup of zeaxanthin in many plants, indicating that the plant is experiencing high-light conditions. This obviously cannot be the case as winter typically has lower light levels than that of summer. We hypothesized that VDE functions far better than ZE when at lower temperatures. Spinach leaves were dark treated, then had their thylakoids isolated before being subjected to an enzyme assay. The results showed that VDE lowers in its reaction time as temperatures decreases, but there does not seem to be a temperature above freezing that would render the enzyme entirely inactive. Due to time constraints, we were unable to measure the activity of ZE at various temperatures. We plan to continue method development in the future, when we hope to be able to show how the two enzymes change in reactivity as temperature decreases.

TSST-1 SUPERANTIGEN PRESENCE IN *Staphylococcus aureus*

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Staphylococcus aureus is an opportunistic bacteria that is carried commensally by 30% of the population. Superantigens like TSST-1 are toxins produced by *S. aureus* and are important contributors to its pathogenicity. In order to examine the superantigenic profile of nasally carried commensal isolates, DNA from multiple *S. aureus* positive samples were isolated and then amplified using polymerase chain reaction (PCR). The amplified DNA was visualized through DNA gel electrophoresis to see if each sample contained the TSST-1 superantigen. Results showed that few strains contained TSST-1.

CHEMISTRY

DYE LOADING OF AND RELEASE FROM SWELLING POLYMERS FOR AGRICULTURAL APPLICATIONS

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To achieve the goal of eradicating hunger by 2030, it is necessary to improve crop production through sustainable and cost-effective solutions. Nanoparticles are increasingly being used to improve agricultural crop production because they can promote nutrient absorption, lower soil and water contamination, and increase the plant's resilience in non-ideal environments. Polymeric nanoparticles like poly-2-(diethylamino)ethyl methacrylate (pDEAEMA) have been used extensively for drug delivery in medical applications. The objective of our study was to use pDEAEMA to deliver beneficial cargo to plants. pDEAEMA is a pH-responsive polymer that reversibly swells in acidic media, a property that can serve as a cargo release mechanism, and is synthesized via a free-radical polymerization. We used dynamic light scattering to determine the polymer nanoparticle size and swelling behavior. We present the optimization of pDEAEMA loading using rhodamine-B isothiocyanate as model fluorescent cargo by taking advantage of the polymer's swelling and deswelling mechanism. In parallel, we synthesized poly-methyl methacrylate (pMMA) nanoparticles were synthesized, which we used as a non-swelling control to confirm the loading mechanism. A plate reader was used to determine the fluorescence of the dye-loaded pDEAEMA system, and confocal microscopy images were taken to confirm the uptake and release. Preliminary results show that the polymer takes up the dye of interest, and future experiments will focus on the loading and release of beneficial cargo.

IMPACT OF STORAGE METHOD ON VITAMIN C CONTENT IN PINEAPPLE

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Vitamin C is an essential nutrient that plays a major role in supporting cellular health and the immune system. Vitamin C can be supplemented through either a daily diet or dietary supplements. For those who get their Vitamin C primarily from their diet, how food is stored plays a significant role in how much Vitamin C is available once you are ready to eat it. The purpose of this study was to examine the effect of various storage conditions (room temperature, refrigerated, frozen, and canned) on the concentration of vitamin C in sample servings of pineapple over time and determine which method should be recommended when purchasing and storing pineapple. Vitamin C (L-ascorbic acid) was measured through titration of the pineapple sample with potassium iodate using a potassium iodide and starch indicator. Through this method, the mass of vitamin C in the sample along with the percent recommended daily allowance (RDA) in a serving was calculated. Across trials, it was found that frozen outperformed refrigerated, canned, and room temperature storage conditions in terms of Vitamin C concentration and retention.

FIBROUS MYCELIUM MATS AS ABSORBENT MATERIALS

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The development of absorbent materials for use in medical applications is crucial in producing wound dressings with improved water absorbency, antimicrobial qualities, and elasticity. Mycelium (mushroom extracellular matrix) possesses noteworthy attributes due to the biopolymers that constitute its structure. We hypothesized that lyophilization of base-treated mycelium fibers would yield a strong, low-density cryogel-like material. The mycelium was grown on potato dextrose agar (PDA) plates until uniform mats formed. The mats were treated with concentrations of NaOH varying from 0.05% to 0.15%. After base treatment and washing, the samples were lyophilized to generate a cryogel-like material. To assess the impact of culture conditions on fiber properties, the standard PDA growth conditions were compared to agar modified with additives (corn husk, orange oil, silver nanoparticles, and silver nitrate). Examination of the physical properties of the mats using tensile and water absorption testing was used to assess their potential as wound dressings. Modifying the growth conditions with additives did not result in significant differences in material strength or water absorption when compared to the control. The mycelium samples that underwent 0.05% base treatment endured more stress before breaking when compared to the 0.10% and 0.15% treatments. Lyophilization dramatically improved water absorption and strength compared to air-drying. This study demonstrates that cultivated mycelium with cryogel-production methods exhibit increased porosity and absorbency; qualities that can simulate and accelerate wound healing.

ECOLOGY AND ENVIRONMENTAL SCIENCE

EAT, PREY, LIVE: THE ROLE OF FORAGE AND PREDATION IN SHAPING BEAVER ACTIVITY

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Indirect effects of predation can alter prey behavior and resource use, thus having rippling consequences on community structure. Beavers are iconic herbivores of the boreal forests; they engineer ecosystems through their construction of dams and lodges. Wolves rely on beavers for their diets in the ice-free seasons, and beavers are vulnerable to wolf predation when they venture on land. Using carefully designed camera-trap surveys of identified beaver colonies as well as radiotelemetry data on wolves from different habitats that vary in wolf-predation intensity, we investigated the role of forage and risk of predation in modulating terrestrial beaver activity in the ice-free seasons in Voyageurs Ecosystem in MN, USA. We quantified forage availability for beavers at each site from high-resolution satellite imagery coupled with ground data, and developed wolf and beaver activity patterns from telemetry and camera-trap images. Our results show that beaver colonies under no wolf predation threat show different optimal foraging trends than beavers that live under chronic predation threat. Wolves appear to maximize encounters with beavers in habitats where it is relatively easy to ambush them, by syncing their activity patterns with that of beavers. Our results highlight the role of predation risk and forage availability in shaping the activity of a keystone herbivore.

EFFECTS OF LEAF LITTER AND ROAD SALT ON AQUATIC MACROINVERTEBRATE COLONIZATION AND SURVIVAL

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Aquatic macroinvertebrates are vital to lentic ecosystem functioning because of their importance in food webs, trophic interactions, and complex life cycles. Because adult aquatic macroinvertebrates are often highly mobile, colonization of new wetlands happens often, and environmental characteristics can act as environmental filters on community composition. Road infrastructure located near or through wetlands also poses a significant threat to macroinvertebrates due to a multitude of stressors including, habitat fragmentation and degradation, barriers of movement and gene flow, noise pollution, direct mortality, light pollution, and chemical pollution. Road salt application for deicing during the winter is increasing the salinity of freshwater ecosystems near roads, and many taxa of macroinvertebrates are extremely sensitive to water quality. Human development can reduce littoral inputs into aquatic systems via tree removal and deforestation. Using a mesocosm approach with a multi-factorial design, it was found that treatment (control, +salt/CT litter, CT salt/+litter, +salt/+litter) had no significant statistical effects on macroinvertebrate community of individual family abundance. Additionally, no significant differences between treatments were found for functional diversity. These results suggest that the presence of road salt in this study was either at too low of a concentration, the exposure time was too short, or that these taxa do not respond to salinity or leaf litter presence in newly colonized habitat patches.

LITTLE ARACHNIDS, BIG IMPACT: OPILIONES IN THE BOCAS DEL TORO ARCHIPELAGO

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Harvesters (commonly known as daddy long legs) are arachnids that have very specific moisture and microhabitat requirements. Therefore, they have the potential to be good bioindicator species for habitat health. We sampled harvesters in four islands in the Bocas del Toro archipelago in Panama between April 18 and May 2, 2022, to understand their diversity and distribution. We also compared the species distribution across two sites on each island that varied in their level of disturbance. In addition, we evaluated whether the femur length of the species was correlated to the height at which they were found. We sampled over 900 individuals and found 20 species from 10 families. We found that there was no significant difference in harvester biodiversity across the islands or across habitat disturbance levels. However, we did find that leg length was positively correlated with the height at which they were found. The three species with the longest legs were found most often on high vegetation and tree trunks (~1-2m off the ground). We, therefore, suggest that these species could be used as bioindicators, given that with increased anthropogenic disturbance, these habitats will likely be severely imperiled. Long-term evaluation of population trends of these three species of harvesters can thus offer a feasible and moderately inexpensive way to monitor habitat quality.

PLANT COMMUNITY CHANGE IN ROADSIDE REMNANT PRAIRIE FRAGMENTS OVER A 22-YEAR TIME SPAN

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The prairie ecosystem in Minnesota is severely endangered with less than 1% of original prairie remaining. What remains are small fragments often located in areas that were unsuitable for agriculture. These areas can include roadsides; however these remnants face serious threats from human caused disturbances, woody species encroachment causing land cover change, and spread of invasive species. In the summer of 2000, 56 roadside remnant prairie sites were located and surveyed within Rice County in southern Minnesota. In the fall of 2022, 9 sites were resurveyed to evaluate changes within the plant community. From the 9 sites sampled, there has been a decrease in the overall species richness, decrease in the presence of native species, increase in presence of non-native species, and loss of native forbs. These results suggest that small remnant fragments, which remain largely unmanaged, face increasingly serious threats which will contribute to a decline in species diversity, especially of specialist and rare native species.

THE LION'S SHARE: IMPLICATIONS OF LION DIET ON THE CONSERVATION OF A CRITICALLY ENDANGERED HERBIVORE IN TSAVO

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Through predation, apex mammalian carnivores can have cascading effects across ecological communities, while also maintaining ecosystem balance. However, predator populations are witnessing massive declines worldwide, compromising such balancing interactions. A thorough understanding of predator diet can therefore provide crucial information regarding their ecology and conservation, as well as promote effective management of prey species. Using scats collected between 2019 and 2022 and estimation of prey abundance through aerial surveys, we characterized lion prey-consumption and preference in Tsavo, Kenya. Biomass models applied to prey frequencies in scats revealed that nearly 80% of lion diet consisted of large ungulates weighing over 150kg. While large ungulates were also preferred prey items in terms of their availability, we found a strong preference by lions for the critically endangered hirola—an antelope that was introduced in Tsavo as part of an ex-situ conservation program. Our results have far-reaching effects on the management of hirola because disproportionate lion predation on this small but crucial population, as well as the high availability of alternative prey, may trap hirola within a predator-pit. These findings have strong implications for understanding lion diet, optimal foraging, and the potential effects predators have on endangered prey species in a landscape of critical conservation importance.

USING PASSIVE ACOUSTICS TO STUDY ENDANGERED BALEEN WHALES OFF THE COAST OF SENEGAL

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Marine mammals use vocalization as a primary means of interacting with their environment and engaging in social activities such as foraging, intra-group communication, and courtship. Passive acoustic monitoring enables us to study these behaviors as well as species occurrence and distribution using sound received on stationary receivers. Recently, a new monitoring program was initiated in the eastern North Atlantic off the coast of Senegal, an area that may include humpback whales (*Megaptera novaeangliae*) from the endangered Cape Verde/West Africa distinct population segment. Using six months of recordings taken from receivers deployed at the head of the Dakar Canyon, this study seeks to document the distribution of humpback whales and the other baleen whale species in this ecologically critical habitat. Acoustic data were auditorily and visually analyzed in Raven Pro version 1.6.3 using spectrograms showing frequency (0.0 to 500.0 Hz) and amplitude on a temporal scale. Characteristic vocalization signals were identified from prior studies and published literature. The cetacean species that were readily identifiable in the first dataset spanning early June to early November 2021 included humpback, sperm (*Physeter macrocephalus*), blue (*Balaenoptera musculus*), and killer whales (*Orcinus orca*). Furthermore, the presence of other cetacean signals, including possible Omura's (*Balaenoptera omurai*), Bryde's (*Balaenoptera brydei*) whale song, and unidentified small odontocete signals suggest that species diversity exceeds what our current findings reveal. These results stress the importance of West Africa as habitat for large whale species while also underlining the need for more exhaustive studies documenting cetacean stock structure and distribution in the region.

ENGINEERING

100 HOURS: THE BRIDGE FROM CONNECTIVITY TO ACCESSIBILITY

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This research aimed to address the gap between accessibility and connectivity in modern technology for individuals with limited limb dexterity. Various methods were explored to provide accessible connectivity while considering security protocols. Video experimentation of oscilloscope activity on PS2 and USB devices were observed to examine the security aspects of connectivity and data transmission. Findings revealed that device functions and features are directly tied to each device type's permissions. Additionally, it was discovered that the Host (computer) is responsible for all communication with the Peripheral (Keyboard/Mouse) and that USB devices are queried by the Host to set those functionalities and permissions. Furthermore, the type of USB device is hard written into the device's machine kernel code. Changing USB device types would require a rewrite of the kernel code. Configuring device types for enhanced features would not be possible over USB. Other forms of connectivity technology were explored, as Wi-Fi and Bluetooth technical specifications were reviewed. Configuring Bluetooth device types through Bluetooth profiles was identified as a viable solution to configurable device types and functionalities. However, Bluetooth pairing was identified as an accessibility hurdle to connectivity. To address this, potential devices with configurable Bluetooth profiles were examined, and open-source projects with similar ambitions were reviewed. A Raspberry Pi 4b was purchased to experiment with a potential solution to set multiple profiles, providing multiple device functionalities for accessible connectivity. This ongoing research emphasizes the need for inclusive technology development to ensure accessibility for all individuals.

AN INTERACTIVE WEB-BASED DASHBOARD REPRESENTING MEDICARE-ACCEPTING RURAL HEALTH CLINICS AND GENERAL HOSPITALS IN MINNESOTA

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In the state of Minnesota, rural residents face great difficulties in seeking medical care. Minnesota Rural Health Connect (MNRHC) is an internet-hosted webpage designed to enable rural Minnesotans to seek out Medicare-accepting rural health clinics and hospitals through an interactive map. Following MNRHC's establishment, follow-up research was performed through the use of a questionnaire hosted for site users to analyze its effectiveness. An analysis of the corresponding questionnaire results suggested that the target demographic was successfully reached, with 36.4% and 29.7% of respondents being residents of rural and semi-rural areas, respectively. Of the respondents, 36.8% identified as over the age of 65, and 44.5% of the respondents identified as being between 35 and 65, showing that MNRHC was heavily used among Minnesotans of older age groups. The page was also shown to be well received among users, with user feedback reflecting a satisfaction score of $\mu = 4.18$ ($\sigma^2 = .70$, $n = 209$), on a 1-5 scale. Promising feedback provides evidence of the value of a web-based interactive map representing Medicare-accepting rural health clinics and hospitals in Minnesota.

NEUROSCIENCE

INVESTIGATING CENTRAL SENSITIZATION IN A MURINE MODEL OF VULVODYNIA

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Chronic pain is persistent pain that lasts well beyond typical healing times and may be induced by the overactivation of neurons due to repetitive noxious stimuli. Inflammation has also been reported to play a role in the development of chronic pain through neuronal and immunological cross-talk. Inflammatory mediators can lower the firing threshold for pain neurons leading to their persistent activation and the strengthening of these pathways, which increases pain perception. Our lab seeks to determine the relationship between chronic pain development and inflammation in an allergy-driven mouse model of vulvodynia. Using a standard chemical preservative, methylisothiazolinone (MI), female mice were sensitized with MI on the flank and subsequently challenged with MI or vehicle for ten consecutive days on the labial skin. Repeated challenges induced transient production of inflammatory mediators and long-term sensitivity in the genital region of mice. We have examined the expression of inflammatory mediators that can lead to central sensitization in the spinal cords of challenged mice at various time points after ten challenges. While no significant changes can be seen between MI and control mice, there are differences relative to untreated mice. Trends show an upregulation of inflammatory markers such as IL-6 and IL-1β for the first 21 days after the tenth challenge in both MI and AOO mice. This trend is also seen for markers of central sensitization such as NK-1 and TLR-4. Understanding this process can aid in the study of other inflammation-associated chronic pain conditions and the development of therapeutics for chronic pain.

THE DESENSITIZATION OF FUNERAL DIRECTORS

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Desensitization is the act of the body becoming normalized or having a decrease sensitivity to a specific stimulus. It can occur after exposure to certain topics over a long period of time. In the healthcare field, researchers have found many physicians and nurses to experience desensitization to death or emotional experiences exhibited in their field. In some cases, this has correlated with a decrease in empathy towards patients and patients' families. However, the potential desensitization and reduced empathy of funeral directors has, thus far, been overlooked. This study measures desensitization of funeral directors to death-related topics and if desensitization in these individuals correlates with a decrease in empathy. Funeral directors and age-matched non-funeral directors were exposed to death-related and non-death-related image sets while heart rate variability and skin conductance measurements were obtained. HRV, skin conductance, and Empathetic Concern and Perspective Taking Scale scores were compared between funeral directors and age-matched non-directors. Skin conductance measurements, but not heart rate data, indicated that funeral directors may exhibit less emotional stress when viewing death related images, compared to their non-funeral director counterparts. Additionally, funeral directors scored lower on the empathetic concern scale than the control group. However, they scored slightly higher on the perspective taking scale. The findings of this study build understanding of the emotional impacts of an under-studied field and may give insight on whether funeral directors would benefit from empathy training due to their long- term exposure to death.

ORGANISMAL AND PHYSIOLOGICAL SCIENCES

CHARACTERIZING METHYLISOTHIAZOLINONE-MEDIATED CHANGES IN THE INFLAMMATORY POTENTIAL OF MURINE LABIAL SKIN FIBROBLASTS

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Vulvodynia is a chronic vulvar pain condition of unknown etiology estimated to affect 10-28% of individuals with vulvas. A history of allergies, recurrent vulvovaginal yeast infections, and exposure to solvents and paint are all associated with an increased risk of vulvodynia. Methylisothiazolinone (MI) is a common preservative and studies have shown that MI has cytotoxic and allergenic capacities, and importantly exposure to MI can increase the risk of chronic pain development such as vulvodynia. We are using MI as an allergen in a murine model of allergy-driven chronic pain to model vulvodynia. Interestingly, fibroblast cell lines derived from patients with vulvodynia exhibit heightened cytokine signaling responses upon exposure to yeast and yeast antigens. We are investigating the mechanism behind these inflammatory changes in our model by characterizing fibroblast function following MI and bacterial activation. Given that tissue fibroblasts can produce inflammatory cytokine IL-6 after repeated MI exposure, we are exploring if there is a difference in the production of other cytokines, including IL-1β and TNF-α which are known to have algesic effects. These differences suggest a reprogramming of the local tissue microenvironment following MI exposure and identify tissue fibroblasts as a potential therapeutic target. Additionally, preliminary data show increased fibroblast death following ex-vivo treatment with MI that we are further investigating.

EFFECTS OF INCUBATOR HUMIDITY ON ex ovo EMBRYONIC VIABILITY

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The chorioallantoic membrane (CAM) of the developing chick embryo is a highly vascularized system able to be used for many applications, such as tissue engineering, tumor and stem cell grafts, and drug screening. Scientists often culture chick embryos outside of their shell (ex ovo) to allow easy access to the CAM for these studies. However, published methods for the ideal relative humidity (RH) for ex ovo incubation were found to be inconsistent from various published literatures, ranging from 40%-90%. The goal of this project was to systematically test the effect of RH on embryonic viability to identify the ideal incubation RH for culturing *ex ovo* chick embryos, and to identify the reason behind the impact of humidity on chick embryo viability. To find the ideal incubation RH, trials were conducted through comparing the viability of embryos cultured in two different published vessel types that were placed in varying incubation conditions: 60% RH, 80% RH, and 100% RH. Our findings suggest that higher RH results in higher embryonic viability. To find the reason for humidity's impact on chick embryo viability, we measured the amount of water evaporated from the chicks in the various RH environments. We found that chicks in lower RH environments lost more water than the chicks in the higher RH environments, indicating that a higher RH improves embryonic viability by preventing water loss.

POSITIVE IMMUNE RESPONSE TO EXERCISE AND THE INFLUENZA VACCINE IN YOUNG ADULTS

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Each year, the influenza virus contributes to many serious illnesses and is one of the leading causes of death throughout the world. With influenza being the leading cause of hospitalizations amongst young children over any other vaccine-preventable disease, the need for seasonal influenza vaccines in younger individuals continues to increase in importance. Vaccines offer the body a chance to create antibodies against infectious viruses, which allows the body to fight off the disease before it infects the host and creates symptoms. There are many factors that contribute to the production of antibodies within the immune system, including age, sex, genetics, and comorbidities. This project explored the impact of chronic exercise, sex, and serum vitamin D levels on the humoral immune response to a quadrivalent 2022-23 seasonal influenza vaccine in division III cross country athletes. Our methods investigated the body's response to the H1N1 strain A/Victoria/2570/2019 (H1N1) pdm09-like virus. Our results indicate that exercise provokes a stronger immune response amongst both sexes, with females having a greater overall immune response to the influenza vaccine. We also found that serum vitamin D levels were significantly greater in males who exercise than males who do not. No significance was found between exercise and vitamin D levels in females. The findings presented in this study will be used to evaluate the effectiveness of the influenza vaccine and provide recommendations on methods to increase immune response and vaccine effectiveness in adolescents.

REIMAGINING A BETTER VESSEL FOR ex-ovo CHORIOALLANTOIC MEMBRANE CHICK EMBRYO ASSAYS

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The chorioallantoic membrane (CAM) is a network of blood vessels surrounding the chick embryo during development. This membrane has recently been of interest due to its ability to rapidly vascularize, making it an ideal model to study tumor development, stem cells, and tissue engineering. Normally, the chick embryo develops in-ovo, inside the eggshell, however, access to the CAM is limited and visibility is greatly reduced. Researchers have been using the ex-ovo, outside the eggshell, model to combat these issues, yet survivability of the embryos is reduced. Many novel vessels have been created throughout the field, however, there has yet to be a vessel that both adequately supports the chick embryo and is able to be used for application purposes. The goal of this project was to design a novel ex-ovo CAM vessel that wields this dual function. A novel truncated hexagonal pyramid (THP)/polymethylpentene (PMP) vessel was created, tested, and compared to previously tested ex-ovo vessels. Our findings suggest that the novel THP/PMP vessel can support a high embryonic survivability, comparable to that of a published chick embryo hatching vessel, in addition to being stable enough to begin implantation trial experiments. Testing was also performed to compare how certain design elements affected the survivability of the embryos, and it was found that the inclusion of water in the vessel promoted higher embryonic survivability.

PHYSICS

INCREASING CONDUCTIVITY OF GALLIUM NITRIDE NANOCRYSTAL FILMS

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As demand increases for renewable energy, it becomes especially important to develop alternative materials that will produce high-efficiency energy devices. The semiconductor gallium nitride (GaN) may be one of those materials for dye-sensitized solar cells. Our work attempted to synthesize GaN nanocrystal thin films with desirable electrical properties—specifically high conductivity. To achieve this, the power supplied to the reactor was varied during GaN plasma synthesis to induce nitrogen vacancies in the crystal lattice. These vacancies are believed to contribute to the free electron concentration of GaN. The plasma power varied from the standard 80 W down to 60 W and 40 W. The resulting GaN powder—normally colorless—became more yellow with decreasing plasma power, indicative of defects. However, electrical conductivities were similar to that of the control sample, which implies that the desired defects (nitrogen vacancies) were not induced. Near-infrared absorption spectra did not reveal free carrier absorption, suggesting a low free electron concentration. This further supports a lack of nitrogen vacancies. Analysis of transmission electron microscope images eliminates amorphous crystal structure and unreacted Ga metal nanocrystals as the source of the induced defects. These results demonstrate that varied plasma power does not produce high conductivities in GaN nanocrystal thin films. Possible next steps include varying the amount of nitrogen during synthesis and doping with Si (via silane gas) or Zn metal to increase the free electron concentration of the films.

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