First Annual Maine Research Symposium on Biomedical Science and Engineering

Students Poster Abstracts

October 13-15, 2022

100

Thursday October 13; 10:00 am – 12:00 pm
Basic and Applied Research in Biological Sciences
Biology
Physiology/ pathophysiology
Audrie Langlais BS

GSBSE/MaineHealth Institute for Research

Sertraline Positively Regulates Trabecular Bone Microarchitecture in Morphine-Treated Male C57BL/6J Mice

Osteopenia and increased fracture risk are unwanted side effects of drugs targeting the nervous system, including opioids and selective serotonin reuptake inhibitors (SSRIs). Furthermore, combination of opioids and SSRIs has been found to increase fracture risk greater than the use of either drug alone. Our lab has recently developed a mouse model of opioid-induced bone loss. Specifically, 8-week-old C57BL/6J (B6) male mice implanted with minipumps containing morphine (17 mg/kg) have significantly decreased trabecular bone volume and bone formation compared to vehicle (0.9% saline). We hypothesized mice receiving morphine and the SSRI sertraline would have increased bone loss relative to morphine alone. To test this, 8-week-old male B6 mice received morphine with or without sertraline in drinking water (0.05 mg/mL) for 4 weeks (N = 11-13 per group). In contrast to our hypothesis, mice receiving morphine and sertraline tended to have greater femoral trabecular bone volume fraction (BV/TV) than those treated with morphine alone measured by micro-computed tomography. Similarly, morphine decreased vertebral BV/TV, but this loss was rescued with sertraline. To understand how bone remodeling was impacted by morphine-sertraline co-treatment, we measured gene expression in the tibia. There was a significant interaction between morphine and sertraline to increase expression of osteoblast and osteocyte genes compared to sertraline alone, suggesting co-treatment may alter the effects of each drug to promote bone formation. Additionally, expression of receptor activator of nuclear factor kappa-B ligand trended lower in morphine-sertraline treated mice compared to morphine alone, indicative of suppressed osteoclastogenesis, which may also contribute to the rescue of bone loss by morphine. While we did not observe a negative impact of morphine-sertraline co-treatment on bone mass in mice, it's possible other SSRIs could reduce bone density when combined with morphine. Ultimately, this work may aid in prescribing practices to mitigate risk of osteopenia and fracture.

101

Thursday October 13; 10:00 am – 12:00 pm
Basic and Applied Research in Biological Sciences
Biology
Chenhao Yang, Ph.D.

GSBSE/MaineHealth Institute for Research

Notch signaling regulates PVAT function during diet-induced obesity

Obesity is an established risk factor for cardiovascular diseases (CVD) and possibly shares molecular and cellular disease mechanisms as CVD. As a component of the vasculature, perivascular adipose tissue (PVAT) is as a critical regulator of vascular function due to its anatomical proximity to the vascular wall. In addition to a broad role in embryonic development, Notch/Rbp-jk signaling plays a crucial role in regulating metabolic homeostasis. Suppression of Notch signaling components was reported to induce beige phenotypes within white adipose tissue. We studied how Notch/Rbp-jk signaling and potential downstream pathways regulate PVAT phenotype. We generated mouse models with adipose tissue constitutive activation of Notch intracellular domain using Adipoq-Cre driver and examined their physiology, histology, and expression of metabolic and vascular relaxation pathway components as compared to control non-Cre mice. Our data showed that Notch signaling was activated in PVAT during high fat diet (HFD) treatment. Expression of Notch signaling component was increased during differentiation of PVAT. Moreover, PVAT from adipocyte-specific N1ICD overexpression mice showed alterations in the vasorelaxation as demonstrated by vessel wire myography experiment. Our studies further show that Notch signaling regulates mitochondrial biogenesis and metabolism in PVAT. This was indicated by alter oxygen consumption rate and expression of mitophagy markers.

102

Basic and Applied Research in Biological Sciences
Molecular biology
Ashley Soucy, BS

GSBSE/MaineHealth Institute for Research

RAB27a-mediated regulation of the vascular microenvironment

Ras-related protein RAB27a is a regulator of exosome secretion. Studies show that exosome signaling dysregulation may promote the development of human cardiovascular disease (CVD). It is documented that exosome secretion is increased in humans during obesity, a major risk factor for CVD. However, the mechanism of how exosome signaling becomes dysregulated is poorly understood. Our lab has shown that in mice with obesity, the perivascular adipose tissue (PVAT) exhibits increased RAB27a expression. These data suggest that obesity may promote CVD through altered communication between PVAT and the vasculature. Improving our understanding of the importance of RAB27a in regulating communication within the vascular microenvironment will help identify novel targets for preventative therapies for obesity-induced CVD.
To better study the role of RAB27a in vascular physiology, we have established a novel RAB27a global knockout (null) mouse on a C57BL/6J background. Null mice show no changes in development compared to wildtype (WT) mice, aside from the characteristic ashen coat color. To understand the impact of Rab27a loss on the vasculature, we utilized proteomic methods to identify altered signaling pathways and associated phenotypes in the null mice. We found that the significantly affected proteins were associated with cardiac contraction, metabolism, and adipose-specific processes. To understand the impact of Rab27a loss of function on the vasculature, we evaluated the structure of the thoracic aortae and lipid accumulation within PVAT. Morphological analysis revealed that while the PVAT of WT mice exhibited increased lipid accumulation with age, no such change was observed in the null mice. On-going studies suggest that null male mice are showing changes in thoracic aorta reactivity. Together, this work suggests that global expression of RAB27a may regulate vascular physiology.

103

Basic and Applied Research in Biological Sciences
Molecular Biology
Marissa McGilvrey

GSBSE/MaineHealth Institute for Research

Mechanisms of the health-promoting effect of methionine restriction on adipose

Increasing rates of obesity have a significant impact on cardiovascular disease, dyslipidemia, and diabetes. Our research focuses on perivascular adipose tissue (PVAT) which surrounds blood vessels and controls vasoreactivity by secretion of signaling factors. PVAT is interesting because it has characteristics of thermogenic, calorie burning, and heat generating adipose tissue, which is commonly associated with expression of uncoupling protein 1 (UCP1). In the mouse, activation of the thermogenic phenotype of PVAT suppresses vascular pathology associated with obesity. Dietary methionine restriction improves health of animals, prevents accumulation of fat mass, and induces browning of white adipose tissue. Our preliminary studies in obese C57BL/6 mice maintained on a high fat diet revealed that a reduction of methionine in the diet, despite high levels of fat, caused the mice to become lean, with improved metabolic measures, and restored PVAT to a thermogenic phenotype. Furthermore, we observed methionine restriction to be effective at improving PVAT phenotype in young, middle-aged, and old mice. While methionine restriction in young mice was associated with increased UCP1 expression in PVAT, this associated was lost in middle-aged mice. Thus we are utilizing Ucp1 null mice to study the effects of methionine restriction without the capability of mitochondrial thermogenic uncoupling provided by UCP1. In vivo, Ucp1 null mice were sensitive to a methionine restricted diet, as indicated by significant reduction in total body weight and lipid accumulation in PVAT. In vitro, adipose progenitor cells derived from inguinal white adipose tissue of Ucp1 null mice accumulated more lipids than wildtype adipocytes in response to standard methionine concentrations, while methionine depletion similarly reduced lipid content in both Ucp1 null and WT adipocytes. This provides a model for our future studies to elucidate cellular mechanisms that are influenced by methionine restriction and contribute to a healthy PVAT adipocyte phenotype in a UCP1-independent manner.

104

Basic and Applied Research in Biological Sciences
Physiology/pathophysiology
Samantha Costa, MS

GSBSE/MaineHealth Institute for Research

PD-L1 Expression Promotes Bone Marrow Immunosuppression and Bone Loss with Diet-Induced Obesity

Diet-induced obesity (DIO) causes bone loss and impaired skeletal micro-architecture, but the bone marrow (BM) changes driving obesity-related bone loss are not fully understood. Programmed death-ligand 1 (PD-L1) is an essential immune checkpoint protein that regulates immune response through inhibitory signaling. The relationship between obesity and BM immunosuppression through PD-L1 signaling is still ambiguous. To date, no preclinical studies have investigated the possible link between BM immunosuppression and obesity-related bone loss in non-tumor-bearing mice. We hypothesized DIO would generate PD-L1+ myeloid cells with immunosuppressive and pro-osteoclastogenic activities. To test this, 8-week-old male C57BL/6J mice were fed a high-fat (HFD; 60% kcal) or sucrose-matched low-fat (LFD; 10% kcal) diet for 12 weeks. At a molecular level, isolated BM adipocytes from HFD-fed mice did not have increased pro-inflammatory cytokine expression (IL1-alpha, IL6, TGF-beta, TNF-alpha, IFN-gamma) compared to LFD controls. However, there was a significant increase in MCP-1 and IL10 expression ($p<0.0001$ and $p=0.0130$), the combination contributes to the recruitment and expansion of myeloid-derived suppressor cells. The lack of inflammation within stroma of HFD-fed mice was accompanied by a significant increase in CD11bhiCD11chi mature myeloid cells ($p<0.0001$) and an increase in a PD-L1 expressing subset (CD11bhiCD11chiMHCIInegF4/80+PD-L1+, $p<0.0001$). In addition, µCT analysis revealed HFD-fed mice had significantly decreased trabecular bone volume (-32.35%, $p<0.0001$) that was accompanied by an increase in PD-1+ osteoclast precursors ($p=0.0042$). In conclusion, these data suggest DIO promotes an immunosuppressive BM microenvironment that is linked to the stimulation of PD-1+ osteoclast precursors towards osteoclastogenesis. The downstream skeletal consequences of obesity-induced BM immunosuppression remain to be elucidated as well as the impact on peripheral tolerance.

105

Basic and Applied Research in Biological Sciences
Physiology/pathophysiology
Victoria DeMambro, MS

GSBSE/MaineHealth Institute for Research

Alkaline Phosphatase is More Than a Pro-mineralizing Enemy: Effecting Adipogenesis and Mitochondrial Function

Hypophosphatasia (HPP) is caused by loss of function mutations in the tissue-nonspecific alkaline phosphatase (TNAP) gene (ALPL) resulting in rickets, osteomalacia, bone fragility and lean body mass in severe childhood forms. In vitro, inhibition of TNAP leads to decreased lipid accumulation. To further delineate the role of TNAP in adipogenesis and mitochondrial function a global AlplKO mouse model was utilized. Alpl-/- and +/+ male and female pups were collected at postnatal D9, weighed and IWAT depots collected for histology, analysis of adipogenesis, gene expression and mitochondrial function utilizing a XF96 Seahorse analyzer. Alpl-/- pups weighed less at D9 vs +/+ ($p= 0.001$). Alpl-/- IWAT depots had reduced weight ($p< 0.0001$), including when normalized for body weight ($p= 0.0001$) and exhibited smaller adipocyte size vs +/+ ($p= 0.001$). In vitro, Alpl-/- IWAT cells had decreased ORO staining at D8 of adipogenic differentiation ($p= 0.001$) coupled with downregulation of key pro-adipogenic genes such as Pparg ($p< 0.0001$) and Cebpa ($p< 0.0001$). Expression of Plin1/2 ($p< 0.0001$), Lipe ($p< 0.0001$) and Ppara ($p< 0.0001$) were also decreased suggesting defects in lipid droplet formation and energy metabolism. Seahorse analysis of IWAT cultures at D0 and D8 of adipogenesis revealed lower mitochondrial respiration ($p= 0.001$) and ATP production rates ($p= 0.001$) indicative of defective mitochondrial function in cells lacking TNAP. Studies are ongoing to test our hypothesis that the lack of TNAP in adipocyte progenitor cells leads to decreases in adipogenic differentiation and lipid accumulation due to defects in mitochondrial function. In sum, we observed significant downregulation of lipid accumulation and mitochondrial function in Alpl-/- pups and in vitro SVF adipogenic cultures. Understanding the role of TNAP, a known pro-mineralizing enzyme, in adipogenesis and energy homeostasis will expand our knowledge of the pathophysiology and treatment options for HPP patients.

106

Basic and Applied Research in Biological Sciences
Neuroscience
Madison Mueth

GSBSE/University of New England

Sex differences in susceptibility to develop advanced osteoarthritis pain

Osteoarthritis (OA) patients develop different pain phenotypes that are characteristic of either mid-stage or advanced OA. Patients with mid-stage OA report pain during joint use that subsides during rest and that can be managed with non-steroidal anti-inflammatory agents (NSAIDs), while advanced OA patients report NSAID resistant ongoing joint pain. We examined the hypothesis that females show increased susceptibility to develop the advanced OA pain phenotype. We administered intra-articular injections of monosodium iodoacetate (MIA) to produce concentration-dependent knee joint OA in mice. Behavioral assays showed females presented with advanced OA pain at a lower MIA concentration (16 mg/mL), whereas this concentration produced weight asymmetry without ongoing pain in males indicating mid-stage OA. In males, higher MIA concentrations (80 mg/mL) were required to induce both weight asymmetry and ongoing pain indicating advanced OA. Assessment of joint pathology using Hematoxylin and Eosin (H&E) and toluidine blue demonstrated that there are no significant differences in MIA induced joint pathology between males and females at the 16 mg/mL MIA dose. In addition, analysis of a marker of neuronal damage, activating transcription factor 3 (ATF3), in lumbar DRG using RT-qPCR demonstrated lack of ATF3 expression in male or female samples from the 16 mg/mL MIA treated knee joints. Increased ATF3 expression was observed in male DRGs from the 80 mg/mL MIA treatment group. These data support the hypothesis that females have a greater susceptibility to develop ongoing pain indicative of advanced OA compared to males. This sex difference is not dependent on MIA-induced joint pathology or development of nerve

damage as indicated by similar knee joint pathology and lack of ATF3 expression in DRGs from male and female mice treated with 16 mg/mL MIA that produced mid-stage OA in males and advanced OA in females

107

Basic and Applied Research in Biological Sciences
Biology
Molecular Biology
Mary Astumian

GSBSE/University of Maine

Localization of Dystroglycan and Integrin Proteins Within Muscle Cell Membranes

Muscular dystrophies and dystroglycanopathies are progressive diseases that affect muscle and neurological health. In many of these diseases, the actin cytoskeleton inside the muscle cell does not connect correctly to the extracellular matrix (ECM) outside the muscle cell. As a result, muscle fibers detach from the ECM and degenerate. Over time, muscle degeneration leads to stem cell depletion, decreased regeneration, increased fat infiltration, and fibrosis, which all lead to decreased muscle function. In skeletal muscle, Integrin alpha 7 (Itga7) and dystroglycan (Dag) are the most prominent transmembrane receptors that anchor actin to the ECM. We previously showed that the muscle ECM and muscle health improved after NAD+ treatment in zebrafish deficient for Itga7 or Dag. The purpose of this project is to discover how NAD+ impacts membrane localization of Itga7 and Dag in normal versus mutant muscle. We hypothesize that the membrane localization of Dag and Itga7 proteins relative to each other is important. In Dag mutants treated with NAD+, we anticipate more Itga7 clustering compared to untreated. In Itga7 mutants treated with NAD+, we predict more Dag clustering compared to untreated. However, in dystroglycanopathy mutants where Dag is present but not correctly glycosylated, we hypothesize NAD+ may not alter Itga7 and Dag localization. By using super resolution microscopy, we can measure nanoscale differences in protein localizations which is not possible with confocal microscopy. The cluster size and average distance between Dag and Itga7 molecules in zebrafish muscle membranes and at the myotendinous junction will be assessed in NAD+ treated mutants and untreated controls. Preliminary data have been acquired for dystroglycan clustering in 2- and 3-day old zebrafish.

108

Basic and Applied Research in Biological Sciences
Biology
Physiology/ pathophysiology
Rebecca Peters, BS

GSBSE/University of Maine

Investigating the Direct Effects of β2-Adrenergic Receptor on Osteoclasts

The sympathetic nervous system (SNS) is important for maintaining bone homeostasis through β-adrenergic signaling. Studies have traditionally focused on sympathetic signaling directly to the osteoblast, then promoting osteoclast activity through RANKL-mediated mechanisms. However, our recent work using the non-selective β-blocker, propranolol, has suggested the SNS may also signal to osteoclasts directly. To directly examine the effects of β2-adrenergic receptors (β2AR) on osteoclast differentiation and resorption, we have developed an osteoclast specific β2AR knockout mouse model. We found deletion of Adrb2 was comparable in both Adrb2fl/flLyz2Cre/+ (Cre/+) and Adrb2fl/flLyz2Cre/Cre (Cre/Cre) mice using gene expression and RNAScope™. Additionally, we measured areal bone mineral density in Cre/+, Cre/Cre, and +/+ mice at 8 weeks of age using DXA and bone microarchitecture with µCT in +/+ and Cre/+ mice. No significant changes were observed among any of the genotypes in male or female mice, but bone density parameters were not affected in Adrb2-/- mice until six months of age. We next used this model to test if the β2-selective agonist, salbutamol (affinity is 28-fold more for β2 vs β1 and 61-fold more for β2 vs β3), increases bone resorption by acting directly through osteoclasts. We found that salbutamol increased bone resorption in C57BL/6J female mice, but also in Cre/+ mice, indicating that 2AR in osteoclasts was not required for salbutamol's effects. We have also examined the

effects of salbutamol on osteoclasts in vitro and found no difference in osteoclast number, however, the size of the osteoclasts differed in a dose-dependent manner with salbutamol treatment. Future work will use this model to study differences in osteoclast differentiation and resorption with the loss of β2AR at six months of age and test whether the anti-resorptive effects of β-blockers may be mediated in part by direct osteoclast-mediated mechanisms.

109

Basic and Applied Research in Biological Sciences
Biology
Physiology/pathophysiology
Molecular Biology
Marissa Ruzga, B.S.

GSBSE/University of Maine

Establishing a high throughput screen for genes required to activate muscle gene expression downstream of mTOR/low translation in C. elegans

Dietary restriction (DR) increases healthful longevity in multiple species, including C. elegans. In part, this is due to reduced signaling through the nutrient-sensing mechanistic target of rapamycin (mTOR) pathway resulting in reduced activity of the cap-binding complex (CBC) governing mRNA translation. One subunit of this complex is IFG-1, referred to as eukaryotic translation initiation factor (eIF)4G in mammals. Downregulating ifg-1 gene expression in the whole animal or selectively in specific tissues increases C. elegans longevity (Howard et al., 2021). We find that lowering ifg-1 selectively in neurons or the germline, in addition to increasing lifespan, preserves motility in a body-muscle specific proteotoxicity model that results in paralysis during aging and increases expression of muscle-specific genes encoding structural and regulatory factors. Interestingly, the same motility phenotype and gene expression signature are not observed when ifg-1 is selectively attenuated in body muscle. Results indicate that adaptive changes in muscle function are driven by a cell nonautonomous mechanism and suggest that long-term preservation of muscle under low mTOR/translation conditions may be coordinated, at least in part, by distal tissues. Our goal is to develop a fluorescence reporter screen to identify genes required for adaptive increases in muscle gene expression under low translation conditions, which can then be used to determine whether the enhanced muscle expression signature is required for enhanced proteostasis in this tissue. We are in the process of crossing an ifg-1 loss-of-function mutant with GFP reporter strains driven by promoters of the body muscle genes myo-3 and pat-10, which are upregulated in low translation conditions.

110

Basic and Applied Research in Biological Sciences
Biology
Ahmed Almaghasilah, MS

GSBSE/University of Maine

Quantifying Birefringence Images of Zebrafish Using a Mix of Deep Neural Networks and Image Analysis Tools

In Henry's lab at University of Maine, we study muscular dystrophies such as Duchenne Muscular Dystrophy, a genetic disorder that results in progressive weakening and loss of muscle fibers and can lead to early death in children. We use birefringence, a non-invasive, live-imaging technique that uses polarized light to visualize muscle fibers of zebrafish. This technique allows us to assess the effectiveness of several therapies, which attempt to improve the functionality and structure of muscle fibers, applied on zebrafish with muscular dystrophies. The birefringence images are quantified by highlighting the zebrafish first and then calculating the mean gray value. However, the experiments typically generate a huge volume of images and quantifying these images manually can be an extremely time-consuming process. Analyzing data manually is also subjective and can result in variation, which can lead to the wrong conclusion. In addition, to the best of our knowledge, no one has ever developed a

software nor an algorithm to automate the quantification of birefringence images. In the past, we trained a convolutional neural network (CNN) to automate the process but CNN did not yield reliable and high accuracy outcomes. For those reasons, we decided to apply image analysis techniques such as background contrast and several built-in functions on MATLAB while keeping the CNN predictions to enhance the final results. This novel approach has delivered better results than using CNN alone. The developed algorithm is able to discard noises that are usually mislabeled as zebrafish by the CNN and thus increasing the accuracy of the mean gray value. We believe our method will one day be the new standard in evaluating and quantifying birefringence images in the field of biomedical research.

111

Basic and Applied Research in Biological Sciences
Biology
Amanda Ignacz, BS

GSBSE/University of Maine

Neuromuscular characterization and development in DPM3-associated secondary dystroglycanopathies

Secondary dystroglycanopathies are a group of muscular dystrophies which result from mutations in proteins involved in the glycosylation of dystroglycan, a link between the cytoskeleton and extracellular matrix in skeletal muscle that allows for muscle fiber adhesion to occur. Interesting clinical phenotypic variation is observed in individuals with secondary dystroglycanopathies, ranging from mild with progressive muscle weakness to severe with central nervous system defects. Individuals with DPM3-associated mutations, which affect a protein in the dystroglycan glycosylation pathway, are shown to have dystroglycanopathy with variable onset and severity. Recently, we have developed a DPM3-associated dystroglycanopathy zebrafish model through CRISPR/Cas9 gene editing which recapitulates the phenotypic variation observed in human dystroglycanopathy patients. While motility behavior was unaffected, birefringence imaging of muscle structure shows mild dystrophy in DPM3 mutants. This dystrophy has variable onset and severity even within the same embryo, and regions of muscle damage seem to heal over time at varying rates. Since the regions of dystrophy observed in birefringence imaging were localized to small regions of the embryo as opposed to the entire embryo body, confocal imaging, a more refined technique for visualizing muscle damage and DPM3 mutation-induced defects, was used to assess muscle structure, neuromuscular junctions (NMJs), myotendinous junctions (MTJs), and fiber adhesion. Through this we observed an increase in the number of myotomes with fiber detachments and disruption of NMJs and MTJs. Interestingly, while these neuromuscular defects are observed in DPM3 mutants, they are also observed in heterozygotes of this mutation, suggesting this may be a dominant negative mutation. Use of this model can provide further mechanistic insight into secondary dystroglycanopathies and can aid in the development of therapeutic treatments for this group of diseases.

112

Basic and Applied Research in Biological Sciences
Biology
Computer Modeling
John Butts, MS, and Ryan Tewhey

GSBSE/University of Maine/ The Jackson Laboratory

Prioritization of Non-Coding Cancer Drivers using an MPRA Model

As cells age, they accumulate somatic mutations through several means including exposure to cigarette smoke, UV light damage, and DNA replication errors. These mutations are predominantly benign, passenger mutations", however when mutations confer a selective advantage they are known as "drivers" and contribute to tumorigenesis. Given the high background of passenger mutations, often thousands of passengers per driver, the prioritization of drivers for validation is challenging. Studies of driver mutations have largely focused on mutations in coding sequences, where the impact of a mutation is readily assigned.

Advancements in whole-genome sequencing (WGS) have expanded the search for drivers to the non-coding genome but this comes with significant challenges. Unlike coding mutations, the impacts of non-coding mutations are difficult to discern. For example, if a mutation lies in a cis-regulatory element (CRE) one must determine the gene it regulates and even then, gene expression changes may be small or buffered by compensatory regulatory mechanisms. Massively Parallel Reporter Assays (MPRA) allow researchers to test thousands of sequences for their regulatory potential and are sensitive enough to quantify the impact of single nucleotide changes. Our group has generated a model from thousands of MPRA experiments capable of predicting MPRA activity from sequence data. Using this model we have generated predictions for all non-coding mutations from WGS in the Catalog of Somatic Mutations in Cancer (COSMIC) and find enrichments for active elements in promoters, enrichment of expression modulating variants in recurrent promoter mutations and more highlighting the power of reporter assay models to prioritize non-coding cancer drivers in-silico.

113

Basic and Applied Research in Biological Sciences
Microbiology/Virology
Bailey Blair

GSBSE/University of Maine

Uncovering Candida albicans Factors that Modulate the Host Phagocyte Response

In 2020 Candida was the most frequent cause of bloodstream infections accounting for 28% of these infections in the U.S. The first line of defense against these infections, is the innate immune system. Previous work suggests that early immune response is critical in controlling C. albicans infection. However, it has been seen that C. albicans has strategies to evade the host immune system. Evidence suggests that the ability to transition from yeast to hyphal growth may facilitate immune evasion by limiting early phagocyte recruitment and uptake of Candida albicans. Reduced containment of C. albicans can lead to uncontrolled hyphal growth, causing damage that can lead to death. However, the mechanism by which C. albicans limits recruitment or containment is unknown. To uncover factors important in innate immune evasion we utilized the transparent larval zebrafish infection model to screen C. albicans mutants for altered virulence and immune response. Ten mutants with markedly reduced virulence were identified. Many of these mutants also induced an altered immune response. RIM101 and NMD5 were found to play a role in limiting phagocytosis, while CHT2 and RBT1 were found to limit the recruitment of macrophages and or neutrophils to the infection site. These results highlight the ability of C. albicans to use multiple strategies that allow it to impair the different steps of the immune response such as recruitment and uptake. This work will provide valuable insight into the mechanisms that C. albicans uses to evade the host immune response to cause disease.

114

Basic and Applied Research in Biological Sciences
Molecular biology
Cell Regeneration
Michayla Moore, B.A.

GSBSE/University of Maine

Knockdown of Activin A receptor like type 1 (ALK1) in Human Cardiac Progenitor Cells Affects Angiogenic Secretome

Myocardial infarction (MI) is the number one cause of cardiovascular disease mortality and is characterized by a decrease of blood flow to the heart, pathological remodeling, and irreversible cellular necrosis. Recent evidence has highlighted the protective role of transplanted cardiac progenitor cells (CPCs) in the regulation of cardiac repair, with an emerging role of CPC paracrine

response and secreted proteins in this process. However, the molecular mechanisms for CPC paracrine effects on cardiac function are poorly understood. Our lab has isolated a novel class of CPCs from the human epicardium (hHiPCs). hHiPC clonal isolates are characterized by their high proliferation rate, CD90 and CD105 (Endoglin) expression, and variable expression of CD31. We found that Activin receptor-like kinase 1 (ALK1) is expressed in hHiPC. Further, we have found that pre-treatment of hHiPC with the ALK1 ligand, Bone morphogenic protein-9 (BMP9), increases hHiPC transcription and secretion of pro-angiogenic and BMP-regulated secreted proteins, including Sclerostin (SOST) and CD105, in vitro. Using lentiviral mediated knockdown in hHiPC we found that knockdown of ALK1 significantly decreased RNA expression of CD105 and SOST following BMP9 treatment. Similarly, using LC-MS/MS quantitative protein analysis we show that CD105 expression is decreased in clones that did not improve cardiac function following transplantation into the murine myocardium after MI. The decrease in pro-angiogenic factors in the absence of BMP9/ALK1 signaling may suggest the potential relevance of BMP9/ALK1 signaling in cardiac progenitor cell secretome mediated repair and will be investigated using knockdown of ALK1 of transplanted hHiPC following in vivo MI in the future.

115

Basic and Applied Research in Biological Sciences
Molecular biology
Omodasola Adekeye, MS

GSBSE/University of Maine

A screen for novel genetic requirements for the development of podocyte structure and function in zebrafish

The podocyte is the key unit of the glomerular filtration barrier. Thus, a loss of structure and function of the podocyte plays a vital role in the pathogenesis of various nephritic glomerular diseases which progress to end-stage kidney disease. We recently found genes that are highly enriched in the developing pronephric glomeruli of zebrafish by differential gene expression profiling. Many highly enriched glomerular genes were zebrafish orthologs of known human kidney disease genes however many enriched genes have not been characterized in kidney function. Our goal is to functionally characterize genes that may have novel activities in glomerular development or function and identify novel causes of genetic kidney disease. We identified 17 genes expressed in glomeruli but of unknown function. These genes included three transcription factors, eight genes involved in cell signaling, and six genes identified as interacting proteins involved in protein binding. Next, we conducted a zebrafish CRISPR G0 screen to test gene function in glomerular development. Of six genes tested so far, knock out of ptpqr, naalad2, and enpp6 caused whole body edema which is a primary kidney failed osmoregulation phenotype. This suggests that the genes might be involved in podocyte function. Presently, we are performing histological analysis on the knockout embryos to identify changes in the structure of the podocyte as a result of the gene knockout. Also, we will perform in situ hybridization to confirm cell-type specific expression, and a glomerulus filtration assay to screen for proteinuria in the zebrafish-a test to determine the loss of high molecular weight proteins from the blood during filtration. Our research would provide insight for further analysis of human kidney disease.

116

Basic and Applied Research in Biological Sciences
Molecular biology
Connor Murphy, B.S.

GSBSE/University of Maine

The Long-Chain Acyl-CoA Synthetase (ACSL) Family Supports Multiple Myeloma Cell Proliferation, Survival and Mitochondrial Respiratory Function

Multiple myeloma (MM), defined by the clonal expansion of malignant plasma cells in the bone marrow, has a relative 5-year survival rate of 57.9%. The development of drug resistance is a major factor why MM remains incurable; therefore, it is critical to investigate novel treatments and the mechanisms of drug resistance in MM cells. Changes in fatty acid (FA) metabolism have

been shown to support the proliferation, migration, and the development of drug resistance in other blood cancers and solid tumors. However, the role of FA metabolism in MM cells has been understudied. To better understand what FA metabolism genes support MM cells, we queried the Cancer Dependency Map, a genome wide CRISPR screen of various human cancer cell lines for genes within the Hallmark FA Metabolism gene set. We found that most of the long-chain acyl-CoA synthetase (ACSL) family members supported MM cell line fitness. The ACSLs are a family of enzymes that are crucial to FA metabolism because they activate FAs with a CoA group so FAs can be metabolized. Thus, the purpose of this study is to test the hypothesis that the ACSL family is supportive of MM cell proliferation and survival.
In order to define the role of the ACSLs in MM cell proliferation and survival, we treated human MM cell lines with an inhibitor (triacsin C, triC) of the ACSL family. TriC treatment significantly decreased MM cell proliferation and increased apoptosis in a dose-dependent manner. We also found triC treatment significantly reduced basal, maximal and ATP-dependent respiration as well as proton leak. Additionally, triC treatment significantly reduced total ATP and mitochondrial ATP production rate. We can conclude from our data that targeting the ACSL family decreases proliferation, survival and mitochondrial respiratory function in MM cells and is a promising target for mechanistic studies.

117

Basic and Applied Research in Biological Sciences
Molecular biology
Madeleine Nowak, BA

GSBSE/University of Maine

Understanding the Mechanisms Driving Mest Variability in Adipocytes

There are numerous ways in which an individual's environment and their genome interact to affect their overall metabolic health. Variable expression of mesoderm specific transcript (Mest), an imprinted gene only expressed from the paternal allele, is one example of this interaction. In an otherwise genetically identical inbred mouse population, raised in carefully controlled environmental conditions, we showed not only a dramatic inter-individual variation in their development of diet-induced obesity, but also in their expression of Mest. Furthermore, Mest expression in adipose biopsies of mice prior to diet-induced obesity was predictive for future development of obesity. This suggests that the epigenome has a role in the regulation of Mest. Mest is primarily expressed in mature adipocytes; however, adipose tissue is heterogeneous with only 10-50% of cells being adipocytes. To enhance the likelihood of identifying epigenomic features controlling Mest in adipocytes, we used the NuTrap mouse model developed by Roh et al. (2017), which allows for isolation of mature adipocyte nuclei using a nuclear fluorescent marker tied to the expression of adipocyte-specific adiponectin (Adipoq), to collect nuclei via fluorescence activated cell sorting (FACS). We plan to use these nuclei for both chromatin immunoprecipitation (ChIP) and bisulfite sequencing studies to identify differences within the epigenome associated with variable expression of adipocyte Mest in mice. To identify nuclear fractions with low and high Mest expression prior to analyses, we isolated RNA from the adipose tissue and quantified Mest expression using RT-qPCR. Ultimately, we aim to determine the epigenetic source of variable Mest expression and shed light on one way in which the environment and the genome interact to cause obesity and other metabolic disorders.

118

Basic and Applied Research in Biological Sciences
Molecular biology
Shivangi Pande, MS

GSBSE/University of Maine

Interleukin-17 Receptor D regulates endothelial cell activation during inflammation

Endothelial cell activation is a critical phenomenon underlying the pathophysiology of several inflammatory diseases including Crohn's disease, Systemic lupus erythematosus, sepsis, and cardiovascular disease. Given the complex etiology and the widespread incidence of these diseases, the molecular mechanisms regulating endothelial cell activation still require further

elucidation. For our investigative purposes, we focused on the role of Interleukin-17 receptor D (IL17RD), an orphan receptor belonging to the Interleukin-17 signaling family, during endothelial cell activation in mice and humans. Our results suggest that upon proinflammatory cytokine stimulation, IL17RD modulates the expression of endothelial adhesion markers in human endothelial cells in vitro and consequently promotes the adhesion of primary monocytes. We further characterized its role in regulating endothelial cell activation in vivo using an IL17RD global loss of function mouse model. Our findings demonstrate that IL17RD modulates aortic endothelial cell activation and promotes the infiltration of proinflammatory monocytes into the aorta under western diet induced inflammatory conditions in vivo. Next, in order to characterize disease relevance, we induced atherogenesis in an IL17RD knockout mouse model using a mutant gain of function AAV-PCSK9 model of atherogenesis. Our results suggest that IL17RD does not significantly affect plaque size in vivo. Future studies will focus on characterization of the role of IL17RD in other disease models involving endothelial cell activation.

119

Basic and Applied Research in Biological Sciences
Molecular biology
Kodey Silknitter

GSBSE/University of Maine

Investigating the impacts of disrupted axon guidance in dystroglycanopathy

A new phenotype presented by motor neurons may contribute to the pathology of muscular dystrophy. The dystroglycan complex is a glycosylated, transmembrane receptor that binds to extracellular proteins and is critical for extracellular matrix protein-myofiber interaction. Dystroglycanopathy is a form of muscular dystrophy in which 1 of the 18 proteins responsible for glycosylating dystroglycan is non-functional. If one of the genes responsible for dystroglycan-glycosylation contains a mutation resulting in a disrupted protein, the result is dystroglycanopathy. Patients diagnosed with dystroglycanopathies can experience muscle wasting, Walker-Warburg syndrome, and a shortened lifespan. Recent studies have found that when dystroglycan is knocked-out, axon guidance and subsequent muscle innervation are disrupted. While dystroglycanopathies clearly compromise the function of the neuromuscular system, the role of the dystroglycan glycosylation proteins on axon guidance is not well understood. Our preliminary data suggest that primary motor neuron axon guidance and subsequent neuromuscular junction formation are variably disrupted in multiple forms of dystroglycanopathy. We have generated multiple, novel, dystroglycanopathy zebrafish models, including zebrafish that harbor a mutation in either ispd or b4gat1, both of which are responsible for dystroglycan glycosylation. Our current hypothesis is that ispd and b4gat1 are both required for proper axon guidance. Additionally, a zebrafish model with a mutation in another protein needed for dystroglycan glycosylation, gmppb, exhibits differentially expressed genes associated with axon guidance and formation. Of these genes, neurexin, a known binding partner of dystroglycan, was found to be significantly expressed. To better understand the impact these genes have on dystroglycanopathy pathology, we will continue characterizing these models using molecular and computational techniques.

120

Basic and Applied Research in Biological Sciences
Molecular Biology
Michael Babcock, MS

GSBSE/University of Maine

Integrating Molecular Profiles with Clinical Outcomes in Cancer Patients from Rural Maine

Colorectal cancer (CRC) is the second most deadly cancer and symptoms do not manifest until tumors are in advanced stages and despite diagnostic advancements and new treatment regimens, patient survival rates remain low. Extended molecular profiling reveals a large amount of genomic data during colon cancer subtyping; however not all findings are incorporated for patient clinical management. In a previous study, we found that established prognostic/therapeutic markers and additional molecular

features correlate with lung cancer patient outcomes. Here we propose that a similar stratification model can be used to classify colorectal adenocarcinoma patients to improve prognostic/therapeutic outcome once biomarkers that affect survival are identified. Clinical pathology data from CRC patient tumor specimens were analyzed for mismatch repair deficiency, microsatellite instability status, and genomic profiles. Consecutive CRCs from 2017-2018 were analyzed by Next Generation Sequencing (NGS; Oncomine, Thermo-Fisher, CA) and findings correlated with clinical characteristics and overall survival (OS). NGS results were integrated with clinicopathological data using standard R software. Kaplan-Meier survival curves demonstrated the prognostic value of individual mutations to be used in new categorical classifications. Mutations occurred most frequently in TP53 (61%), APC (41%), KRAS (34%), PIK3CA (21%), BRAF 17%), and SMAD4 (10%) genes. Overall survival was 66% at median follow-up of 37 months. Advanced clinical stage ($p<0.001$), concurrent mutations in APC, KRAS and TP53 genes ($p=0.008$), and AKT mutations ($p=0.06$), showed independent association with adverse OS on multivariate analysis. Extended NGS identifies adverse CRC patients in community oncology practice. Outside of the NCCN-endorsed testing, APC and TP53 mutations are associated with adverse outcomes, when occurring concurrently with KRAS mutation. This is consistent with stepwise CRC biologic progression, hereby supported by real-time patient data from a single geographic region. The identification of a novel colon cancer molecular classification could be used to improve patient outcomes.

121

Basic and Applied Research in Biological Sciences
Neuroscience
Zaid Al-Abbasi, Ph.D.

GSBSE/University of Maine

Comparative analysis of Gs-coupled receptor signaling in mouse and human sensory neuron models

Evaluation of target translatability from rodent to human is a major challenge to advancing novel pain therapeutics into the clinic. We report here an ongoing project to understand molecular mechanisms initiated by Gs-coupled receptors (GsPCRs) that sensitize nociceptors, requiring large amounts of protein samples to characterize cAMP-mediated signaling pathways. To analyze receptor signaling in human cells, we are characterizing GsPCRs in the human male DRG neuron-derived cell line (HD10.6). We have systematically analyzed GPCR expression in published RNA-seq datasets from mouse and human DRG to identify GsPCRs highly expressed either in both species. We have observed through PCR validation an apparent underrepresentation of some GPCRs that may lead to the false exclusion of some receptors from further study, which others have also observed. Several receptors that are highly expressed in human DRG show low or no expression in mouse, including the P2YR11 (a gene absent in rodents), tachykinin receptor TACR2, lysophosphatidic acid receptor LPAR6, calcitonin gene-related peptide/adrenomedullin receptor CALCRL and beta-adrenergic receptor ADRB2. Other receptors are highly expressed in both species, including the prostaglandin receptors. All these receptors are also expressed in HD10.6 cells. We profiled total, PKA- or PKC-dependent phospho-protein by gel electrophoresis after stimulating cells with receptor agonists, including NF546 (P2YR11), neurokinin A (TACR2), isoproterenol (ADRB1/2), beraprost and PGE2 (prostaglandin receptors). Banding profiles differed across species and showed several bands unique to individual receptors. Thus, the results demonstrate the existence of some cAMP signaling components that differ between mouse DRG and HD10.6 cells. We propose that HD10.6 cells, in combination with human DRG RNA-seq data, provide a valuable resource to investigate intracellular signaling mechanisms involved in human nociceptor sensitization.

122

Basic and Applied Research in Biological Sciences
Neuroscience
Cory Diemler, MS

GSBSE/University of Maine

Deciphering the Role of Microglia in Glaucomatous Neurodegeneration

Microglial activity has been shown to play an essential role in the pathogenesis of Glaucoma and other neurodegenerative diseases, as well as retinal ganglion cell (RGC) survival, with relatively unknown mechanisms. In recent years, microglial activation has been correlated with later glaucoma severity, however, the specific role(s) of microglia throughout the progression of glaucoma has been under-studied. Here, we investigated the role of microglia in glaucoma pathogenesis by depleting microglia from 9.5-month-old male and female DBA/2J mice using dietary PLX5622, a CSF1R inhibitor, and aging them to 12 months. Preliminary data suggest, PLX5622 administration results in the near complete elimination of microglia in the retina of DBA/2J mice. Microglial depletion significantly worsened optic nerve damage, and RGC survival in DBA/2J. These data suggest a model where microglia serve a neuroprotective role in the window of ocular hypertension in DBA/2J mice. To further test our model, ongoing studies are evaluating iris pigment disease, intraocular pressure, retinal health, blood pressure and heart function in DBA/2J mice exposed to PLX5622 compared to controls. In addition, to explore the relationship between glial cells and the pathogenesis of glaucoma Immunohistochemistry of retina and optic nerve head samples is being performed. The long-term goal is to decipher the mechanisms by which microglia play a neuroprotective role during glaucomatous neurodegeneration.

123

Basic and Applied Research in Biological Sciences
Neuroscience
Clinical Research
Megan Tomasch, BS

GSBSE/University of Maine

Investigating corticotropin releasing hormone's role in neonatal trauma-induced adolescent pain-vulnerability

Although neonatal intensive care units (NICUs) provide life-saving care for preterm and sick neonates, many of these procedures are painful and stress-inducing. Neonatal medical trauma results in increased susceptibility to chronic pain, anxiety disorders, and depression that manifests in later life following a subsequent injury or psychological stressor. Using a "2-hit model" of juvenile pain vulnerability, our lab has successfully modeled this in rats. We subjected neonatal rats to a common NICU manipulation (e.g., painful needle-prickings from PD 1 to PD 7), followed by an activating stressor (e.g., foot shock) on PD 23, and observed a tactile hypersensitivity in both sexes. Although the neurobiological mechanisms remain unclear, neonatal pain has been shown to drive activation of corticotropin-releasing factor expressing cells (CRF+ cells). Later in life, neonatal pain is associated with a significant reduction in the number of CRF+ cells in the central amygdala (CeA), but only in males. Therefore, we hypothesize that the immediate and lasting consequences of neonatal trauma in males are the result of changes to CeA-CRF cell function. We will test this hypothesis using patch-clamp electrophysiology (ex vivo) and optogenetics (in vivo). To determine if CeA-CRF cell activation is sufficient for inducing hypersensitivity in rats that experienced neonatal trauma, we will use optogenetic activation of CeA-CRF cells in lieu of a second hit and observe its impact on tactile sensitivity. Furthermore, with preliminary electrophysiology data suggesting that CeA-CRF cells are hyperexcitable and hyperactive in our male neonatal pain group, we will use patch-clamp electrophysiology to examine the mechanism(s) through which neonatal pain affects CeA-CRF cells. Together, these experiments will elucidate the neurobiological mechanism(s) that contribute to juvenile anxiety- and pain-vulnerability in males following neonatal trauma.

124

Basic and Applied Research in Biological Sciences
Neuroscience
Brianna Gurdon, BS

GSBSE/University of Maine

Brain-wide spatial analysis reveals cell-type-specific genetic modifiers of Alzheimer's disease progression

Alzheimer's disease (AD) is a progressive neurodegenerative disease that is characterized by severe neuropathology and cognitive decline. Despite strong genetic underpinnings, the specific variants, target genes, and cell types that drive AD-related deterioration remain elusive. Recent advances in brain-wide cell and pathology quantification within AD model mice provide new opportunities to identify genetic factors that act in a cell-type-specific fashion to modify disease progression. These efforts link, for the first time, genetics and regional cell composition to characterize susceptibility versus resilience to AD.
The QUNIT workflow was implemented to evaluate neurodegeneration (NeuN), gliosis (Iba1&GFAP), and AD-specific pathology(AB1-42) among 126 mice from the AD-BXD panel. Immunohistochemistry-stained brain slices were segmented and registered to the Allen Brain Mouse Atlas to quantify regional stain coverage. Quantitative trait loci(QTL) mapping was completed using cellular load as our trait, and cell composition changes were compared to frontal cortex bulk RNAseq data to identify genetic correlates of resilience to AD.
At 6m and 14m of age, AD-BXD animals exhibited reduced cortical neuronal cell coverage, and a significant QTL peak on chromosome 17 associated with cortical neurodegeneration was identified. Overall, there were 46 variants under this peak and variant rs33120395 was detected as the most significantly associated loci with our trait. 5XFAD individuals with the BB genotype at this location had less neuron coverage than those with the BD genotype. Differential gene expression analysis between individuals with the BB vs BD genotype at rs33120395 revealed Leucine Rich Repeat and Fibronectin Type III Domain Containing 2(Lrfn2) to be the only differential expressed gene between these populations.
In future experiments, we will validate the relationship between Lrfn2 expression and cortical AD-related neurodegeneration by creating an Lrfn2 overexpression mouse model. Levels of neurodegeneration, dendritic spine morphology, and memory performance will be measured to characterize the effect of this gene.

125

Basic and Applied Research in Biological Sciences
Physiology/Pathophysiology
Managing large data sets
Food science
Lola Holcomb

GSBSE/University of Maine

Interaction of Broccoli Bioactives and Gut Microbiota: Using Bioinformatics to Study Microbial Ecology and Inflammatory Bowel Disease

Inflammatory Bowel Diseases (IBD) are chronic conditions characterized by inflammation of the gastrointestinal (GI) tract. IBDs can have debilitating symptoms that bear great burdens to daily life and may eventually result in other systemic complications such as autoimmune disorders, and disruption to a person's taxonomic and functional structure of the gut microbiome. Much of the current research on IBD is focused on identifying lifestyle and/or environmental factors that may contribute to etiology, and how making lifestyle changes may reduce disease morbidity. However, the influence of gut microbial ecology in IBD, and its adaptations to lifestyle and dietary changes are still poorly understood. The purpose of this study was to use large dataset DNA sequencing analysis and techniques in bioinformatics to investigate quantitative and qualitative changes to host mouse gut microbial communities in response to a broccoli diet intervention for IBD. Based on current available literature, it was hypothesized that a diet containing cruciferous vegetables may have anti-inflammatory effects on the GI tract, due to bioactives within said vegetables. C57BL/6 IL-10 knockout (a genotype that models inflammation) mice, aged 4 or 7 weeks, were fed either a control chow diet or an intervention diet containing 10% broccoli sprouts. Key findings of this study suggest that the broccoli diet increases bacterial species richness in the gut, especially in the younger group of mice. Furthermore, our results show that the control diet group has greater prevalence and abundance of inflammation-causing bacteria, namely, Helicobacter. Taken together, our study indicates that a broccoli diet promotes a rich and diverse gut microbiome, while potentially protecting against inflammation-causing bacteria.

126

Basic and Applied Research in Biological Sciences
Physiology/pathophysiology
Molecular biology
Obesity
Matthew Siviski, BS

GSBSE/University of Maine

CTHRC1 Suppresses Adipogenesis in a YAP/SOX9-Dependent Manner

The development of improved therapeutic approaches to prevent obesity is imperative. Whether attributed to genetic predispositions or other risk factors, obesity is the result of an energy imbalance culminating in the vast production of fat cells (adipocytes) over time, putting obese individuals at an increased comorbidity risk including stroke, heart disease, and diabetes. In this respect, the study of collagen triple helix repeat-containing 1 (CTHRC1) is a relevant area of basic science research given our finding that it suppresses adipocyte formation (adipogenesis). To define the mechanism by which CTHRC1 attenuates adipogenesis we have developed in vitro model systems to study the effect of CTHRC1 on the regulation of adipogenic gene expression and related signaling networks, as well as utilized flow cytometry to discern its expression among adipocyte progenitor cell populations in vivo. Accordingly, we have shown that CTHRC1 is a negative regulator of the critical adipogenic genes, including C/EBPα and PPARγ. Corresponding signaling data support that CTHRC1 enhances the activation of Rho-like GTPases that drive an effector YAP-SOX9 axis of signaling to directly inhibit adipogenic gene expression. Significantly, among adipose tissues, CTHRC1 expression is restricted to a PDGFRα-positive/CD24-negative population of adipocyte progenitor cells, which are specifically defined as preadipocytes given their unique ability to differentiate into mature, functional adipocytes. In direct comparison to Cthrc1-null mice, wildtype mice are characterized by adipose tissues that possess a statistically larger pool of preadipocytes, yet mature adipocytes that are markedly smaller both in terms of overall size and number. These collective data support that a novel CTHRC1-YAP-SOX9 signaling axis functions to restrict precocious adipogenesis and regulate a healthy degree of preadipocyte-to-adipocyte differentiation.

127 n/a

128

Basic and Applied Research in Biological Sciences
Biology
Caryl Young

GSBSE/University of Maine

Mybl2 establishes a boundary between progenitors and the sensory epithelium in the developing mammalian cochlea

The cochlea is an asymmetric sensory organ patterned by morphogen gradients and cell-cell interactions that guide progenitors to adopt different cell fates during development. The sensory epithelium of the cochlea, the organ of Corti, has one row of inner hair cells (IHCs) that detect sound and three rows of outer hair cells (OHCs) that amplify sound. On embryonic day (E)14.5 the sensory progenitors reside in the prosensory domain and are distinct from the cochlear stem cells that reside in the future inner sulcus (IS) domain. A boundary will keep these two populations spatially segregated during development until cells are ready to differentiate into different epithelia, or the sensory cells. Previous studies suggest the Wnt pathway establishes the boundary between the IS and sensory domain. We define a role for a Wnt-regulated gene, Mybl2 in regulating normal proliferation of progenitors in the IS and the expression of Jag1 to establish a boundary between the IS and the organ of Corti.

We hypothesize that MYBL2 represses Jag1 to segregate the progenitor population from the JAG1+ prosensory domain. We generated β-catenin and Mybl2 conditional knockouts and analyzed changes in proliferation in the IS and JAG1 in the sensory domain, and HC differentiation at later stages.
Analysis of β-catenin cKOs on E14.5 show that Jag1 and Mybl2 are regulated by the Wnt pathway. β-catenin cKOs and Mybl2 cKOs also showed JAG1 failed to be refined to the sensory domain. We observed a decrease in Ki67 (marker for M-phase proliferating cells) in the IS domain. On E18.5, Mybl2 cKOs showed additional IHCs, which we predict is caused by an expanded JAG1 domain. We found the HCs were abnormal showing evidence of aneuploidy. We surmise, Mybl2 is an important developmental control gene that specifies cochlear stem cells and regulates DNA repair mechanisms prior to cell differentiation.

129

Biomedical Engineering & Medical Physics
Diagnostics
Clinical Research
Liza White, BS

GSBSE/University of Maine

Industrial-Scale Fabrication of Low-Cost Microdroplet Generators for Massively Multiplexed Biological Assays

Microdroplet generators have the capacity to effectively generate large quantities of nanoliter-sized droplets, which can be highly useful for massively multiplexed single-cell biological assays among other uses. However, the current fabrication methods of these devices is complex and expensive, limiting their use to specialized laboratories. Leveraging paper industry technology in Maine, we have developed a cost-efficient and reusable water-in-oil microfluidic microdroplet generator system which costs orders of magnitude less than current microdroplet generation options and can be easily assembled by a non-expert. The system consists of a single water/cell solution inlet channel and two oil matrix inlet channels, all 30 µm deep x 60 µm wide which can create nanoliter-sized drops. The design was then cast at a Maine paper company on their industrial-scale roll-to-roll casting equipment. The resulting 50-lb roll of transparent microdroplet generator channel systems were cut into individual pieces and mounted in a custom acrylic chamber which used uniform pressure to contain the liquid inside the channels. Water and oil flow rates of up to 1,500 µL/hour were achievable. The channel networks within the housing could either be used, washed, and re-used, or disposed of as the application required. The system was also found to be resistant to ethanol, making it easy to sterilize. These results demonstrate that microdroplet generators can be mass-manufactured using Maine-based industrial manufacturing technology, allowing simpler and more cost-efficient fabrication and more widespread use of microdroplet assays in biotechnology.

130

Biomedical Engineering & Medical Physics
Jeremy Grant, MS

GSBSE/University of Maine

Nanoparticles as Local Reporters in Biological Systems: Modeling Signal and Understanding Limitations

As early as 2001, advances in engineered nanotechnologies have caused great excitement about the possibility of using SERS-active nanoparticles as inert, non-toxic, non-bleaching alternatives to fluorescent molecules and quantum dots. Now, nearly 20 years later, there exist few practical examples of the use of SERS-active nanoprobes, despite significant levels of funding and many related publications. These previous works failed because they ubiquitously assumed near-optimal behavior in several key probe factors including Raman cross-section (enhancement), nanoparticle surface loading (number of probe species present), and available nanoparticle concentration (number of particles), all of which are attributable to working in a complex, highly non-

ideal biological environment. Here we present a simple scalable accounting model which makes it possible to understand the signal limitations apparent in previous attempts at imaging these probes in biology and allows for determination of likely signal limitations, a key consideration for planning future Raman based imaging experiments.

140

Basic and Applied Research in Biological Sciences
Neuroscience
Clinical Research
Emma Noel, BS

Bowdoin College

Early life adversity in a rat model induces sex-specific and developmental changes to corticosterone reactivity and DNA methylation patterning in parvalbumin-containing interneurons

Early life adversity (ELA), such as abuse or neglect in childhood, can result in adverse neurological outcomes in brain regions connected to emotional processing. Individuals with a history of ELA exhibit higher rates of mental illness later in life (i.e., anxiety, depression), with females showing a higher prevalence; however, the mechanisms underlying these outcomes remain poorly understood. DNA methylation (DNAm), an epigenetic modification of DNA readout, may contribute to ELA-induced vulnerability by altering neural development through epigenomic pathways, particularly in certain neural subtypes. GABAergic parvalbumin (PV) containing cells are sensitive to ELA and show sex-related outcomes that are linked to affective processing, and therefore these neurons may be uniquely vulnerable to methylation outcomes. To investigate the degree to which changes in DNAm – particularly in PV cells – may underlie ELA-related sex disparities, both male and female Sprague-Dawley rats experienced ELA in the form of pre-weaning maternal separation. Rats were evaluated at two developmental timepoints (postnatal day (P)25 or P45) for corticosterone (stress hormone) and DNAm levels both globally and in PV cells. Results were gathered using both immunohistochemistry and quantitative ELISA to discern patterns of DNAm in brain regions associated with anxiety, with the goal of understanding how DNAm may connect to ELA. The epigenetic results suggest an age and sex-dependent increase in 5-methylcytosine (5-mc) in PV cells in prefrontal cortex. Additionally, females displayed a relationship between developmental age, rearing condition and stress hormone reactivity, with P25 ELA females showing blunted corticosterone reactivity compared to control animals. Overall, by characterizing regional and cell-specific differences in DNAm with respect to sex and age, critical windows for epigenetic alterations, symptom onset, and even treatment intervention may be discerned.

141

Basic and Applied Research in Biological Sciences
Neuroscience
Sydney Bonauto

Bowdoin College

Leveraging rat ultrasonic vocalization playback to expose sex-specific outcomes following early life adversity

Investigating the effects of early life adversity (ELA) through maternal separation in rats provides a translational window to study affective (dys)function related to adversity. In rats, ultrasonic vocalizations (USVs) communicate affective social information. These USVs are within-species cues that may serve as analogues to human facial expressions. Specifically, 22 kHz USVs are characterized as aversive and negatively valenced, while 55 kHz appetitive calls are positively valenced. By exposing ELA and control rats to 22 kHz USVs as a probe for hypervigilance and/or alterations in affective processing, we can examine how ELA impacts behavioral responses to emotionally valenced stimuli in a translational manner. Here, juvenile, and young adult male and female rats were evaluated in an open field test (OFT) during USV playback to assess whether responses to playback may be

influenced by an interaction between development and ELA. Results show sex differences across development and ELA, with ELA females most influenced by playback. Findings also indicate that there are distinct and sex-dependent behavioral responses to multiple frequencies of USV playback over time in the OFT. Differences in behavior as a function of sex and/or ELA exposure suggest the likelihood of different behavioral approaches to affective stimuli, as well as different neural recruitment dependent on these factors. Activity of brain regions associated with affective processing, quantified via density of cFos+ cells, may indicate condition- and sex-dependent neural recruitment during playback. Further, examining changes in parvalbumin cell functionality in these regions may point to the specific altered neural circuitry that underlies affective dysfunction due to ELA experiences. Taken together, this model provides an opportunity to characterize patterns of activity in the brain that correspond with behavioral output while furthering understanding of treatment options for at-risk populations.

142

Basic and Applied Research in Biological Sciences
Neuroscience
Lucia O'Sullivan

Bowdoin College

Using Artificial Intelligence Software to Investigate the Effects of Early-Life Adversity on Anxiety-Like Behavior

Early life adversity (ELA) is a reliable model to induce long term anxiety-like effects in rats. The present study used a maternal separation ELA paradigm to observe how pre-weaning adversity can lead to adult anxiety-like behavior. However, behavioral assays to measure affective states, such as the Open Field Test (OFT), rely on precedents set by data derived from historically male subjects, despite the fact that many affective disorders are predominantly diagnosed in women. In addition, commercial softwares (i.e., EthoVision) are often inaccurate and neglect the rich ethological behaviors that rats display. The present study used computational techniques to construct a bottom-up assessment tool for anxiety-like behavior in rats. Supervised and unsupervised machine learning softwares were used to detect subtle behavioral patterns in the OFT and connect them to the rat's sex and early-life experience. DeepLabCut is a pose estimation software that was used to track thirteen salient points along the rat's frame in the OFT. This study employed k-means clustering on R and B-SOiD to gather statistically significant sequences of poses into subtle, sub-second behaviors. The present study found several significant clusters of sub-second behaviors, which may shed light on subtle sex differences in anxiety-like behavior. Some behaviors, such as rearing and turning, have been previously characterized, while others appeared to be novel. The results from this study will contribute to a behavioral database similar to those for genomes and transcriptomes. Future studies can use this foundation to systematically and reproducibly determine the effectiveness of care interventions such as psychiatric drugs, as well as evaluate the impact of psychiatric behavioral profiles in pre-clinical models.

143

Basic and Applied Research in Biological Sciences
Physiology/Pathophysiology
Neuroscience
Isabel Petropoulos

Bowdoin College

Peripheral modulation of cardiac contractions by the neuropeptide myosuppressin in the American lobster, Homarus americanus, is mediated by effects on the cardiac muscle itself

The American lobster's (Hormarus americanus) cardiac neuromuscular system, which includes the neuromuscular junctions (NMJ) and the peripheral cardiac muscle itself, is driven by a central pattern generator (CPG) called the cardiac ganglion (CG).

Generally, CPGs are networks of neurons that produce patterned motor outputs and drive behaviors like walking, breathing, and swimming. Modulation of CPGs, most widely by peptides, allows systems to produce flexible outputs that allow organisms to adapt to changes in the environment; peptides can alter muscle dynamics peripherally both via the neuromuscular junction and by acting on the muscle itself. The present study focuses on the highly conserved peptide Myosuppressin (pQDLDHVFLRFamide), which is endogenous to H. americanus. In the isolated CG myosuppressin is known to increase the duration of action potential bursts and decrease cycle frequency, and peripherally, to increase contraction amplitude through a mechanism previously unknown. Here, we investigated the remaining question: does myosuppressin exert its peripheral effects on the cardiac muscle, the NMJ, or both? Regarding the question of myosuppressin acting on the NMJ, excitatory junction potentials (EJPs) were evoked by direct stimulation of the motor nerve and recorded with a microelectrode inserted into a single muscle fiber in control saline and myosuppressin. Myosuppressin did not modulate the amplitude of EJPs. To elucidate if myosuppressin acts on the cardiac muscle, the CG was removed, and muscle contractions were stimulated with L- glutamate while superfusing myosuppressin. Myosuppressin significantly increased glutamate-evoked contraction amplitude in the isolated muscle, suggesting that myosuppressin exerts its peripheral effects directly on the cardiac muscle.

144

Basic and Applied Research in Biological Sciences
Clinical Research
Sydney Bonauto

Bowdoin College

Leveraging rat ultrasonic vocalization playback to expose sex-specific outcomes following early life adversity

Investigating the effects of early life adversity (ELA) through maternal separation in rats provides a translational window to study affective (dys)function related to adversity. In rats, ultrasonic vocalizations (USVs) communicate affective social information. These USVs are within-species cues that may serve as analogues to human facial expressions. Specifically, 22 kHz USVs are characterized as aversive and negatively valenced, while 55 kHz appetitive calls are positively valenced. By exposing ELA and control rats to 22 kHz USVs as a probe for hypervigilance and/or alterations in affective processing, we can examine how ELA impacts behavioral responses to emotionally valenced stimuli in a translational manner. Here, juvenile and young adult male and female rats were evaluated in an open field test (OFT) during USV playback to assess whether responses to playback may be influenced by an interaction between development and ELA. Results show sex differences across development and ELA, with ELA females most influenced by playback. Findings also indicate that there are distinct and sex-dependent behavioral responses to multiple frequencies of USV playback over time in the OFT. Differences in behavior as a function of sex and/or ELA exposure suggest the likelihood of different behavioral approaches to affective stimuli, as well as different neural recruitment dependent on these factors. Activity of brain regions associated with affective processing, quantified via density of cFos+ cells, may indicate condition- and sex-dependent neural recruitment during playback. Further, examining changes in parvalbumin cell functionality in these regions may point to the specific altered neural circuitry that underlies affective dysfunction due to ELA experiences. Taken together, this model provides an opportunity to characterize patterns of activity in the brain that correspond with behavioral output while furthering understanding of treatment options for at-risk populations.

145

Basic and Applied Research in Biological Sciences
Neuroscience
Atalay Ata, MS

MDI Biological Laboratory

Tau induced ribosome dysfunction as a driver of neurodegeneration in a C. elegans model of Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disease characterized by an early loss of memory formation and impaired movement. Tau is an intrinsically disordered microtubule binding protein subject to extensive post-translational modifications (PTMs). Some PTMs of Tau have been shown to alter the localization of Tau and result in the disruption of protein translation; a process needed for memory formation. As protein interactome studies have indicated that Tau binds to ribosomal proteins, we hypothesized that Tau is causing ribosomal dysfunction via direct interaction with active ribosomes as an early event in AD. Furthermore, we hypothesized that, this interaction is dependent on Tau's PTMs. We used C. elegans expressing human Tau as a model for AD to measure its effect on lifespan and locomotion. The C. elegans strains tested expressed single copy insertion fluorescently tagged Tau engineered to mimic PTMs thought to be casual to AD pathology. With our assay to measure tissue specific translation, we showed that phosphomimic Tau (T231E) led to a significant decrease in neuronal translation. This mimetic strain also showed significant decrease in median lifespan and locomotion. Unexpectedly, in all Tau expressing strains, we detected a significant decrease in whole worm translation suggesting that Tau triggers a signal from the neurons to other tissues to regulate them cell non-autonomously. Current efforts are underway to demonstrate direct Tau-ribosome association via fluorescent polysome profiling and that this association leads to memory loss. Together, these results suggest that Tau-ribosome association is increased by its phosphorylation at T231 and is likely causal to an early pathology of AD.

146

Basic and Applied Research in Biological Sciences
Physiology/pathophysiology
Blanca de Juan Mora, MS

MDIBL

Zebrafish Larvae Xenotransplantation: A Novel Tool to Study Cellular Senescence and Senolysis

Introduction: Cellular senescence has a physiological function during the development but was identified as a key driver of age-related diseases, amongst others due to the production of the Senescence Associated Secretory Phenotype (SASP). Due to this, the search for new drugs to selectively ablate senescent cells is currently a strong focus. Our zebrafish (zf) xenotransplantation model will be a cost-effective in vivo model and will allow high throughput in studying senescence and senolysis.
Methods: A temperature assay was performed to rule out the effect of increasing the incubation temperature from 28 to 34 °C to enhance the murine cell survival in the yolk. Non-senescent (D6 after extraction) and senescent (D16, cell culture stress) murine Primary Tubular Epithelial Cells (PTEC) were labelled with fluorophores (eFluor/NucBlue) and injected into the yolk sac (Nacre background, n=300 cells/zf) at 2 days post fertilization (dpf). Injected zf were evaluated for survival and morphology until 7 dpf when SA-β-Gal whole mount was performed. Preliminary data of the effect of the senolytics ABT-263 and ABT-737 was obtained via microinjections in the yolk sac and diffusion (tank water) in cell-uninjected 3 dpf zf.
Results: The temperature assay showed no significant difference in survival or malformation rates when the temperature was increased to 34°C at 2 dpf. The preliminary results of zf injected with senescent PTEC showed a tendency of poorer survival than those injected with non-senescent PTECs (71,68% vs 90,04% at 7 dpf), but no differences in morphology or SA-β-Gal staining were found. Zf treated with ABT-263 and ABT-737 showed similar survival rates independent of the way of drug delivery.
Outlook: Further experiments are needed to prove the effect of known senolytic drugs to finally start the testing of potential new ones.

147

Basic and Applied Research in Biological Sciences

Biology
Molecular biology
Sofia-Christina Papadopoulos, MSc

MDIBL/ MHH

The role and regulation of the thyroid pathway during axolotl limb regeneration

Mammals, display limited regenerative abilities during early embryogenesis which are lost with the onset of thyroid gland organogenesis. Similarly, amphibian thyroid hormone-driven metamorphosis correlates with loss or decline of regenerative ability present in premetamorphic stages. Axolotls are a special model system because they spend their adulthood in neoteny, retaining low levels of systemic thyroid hormone and a remarkable ability of tissue regeneration. However, upon thyroid hormone injection, axolotls are capable of metamorphosis. This correlates with reduction of regenerative rate and fidelity. We are utilizing this ability to study the role of the thyroid hormone pathway in regeneration. We have characterized the hallmarks of axolotl metamorphosis which include a decrease of body water content and extensive changes in the architecture of the epidermis and dermis. Comparative proteome analysis of neotenic and metamorphic upper arm skin showed downregulation of proteases and upregulation of protease inhibitors in metamorphic samples. Further, we have previously shown that dedifferentiating fibroblasts drive axolotl limb regeneration. Molecular profiling of these cells showed increased expression of the deiodinase-3 enzyme that suppresses the thyroid pathway. Application of a Dio3-inhibitor drug during regeneration showed delayed blastema formation. In addition, we have developed a transgenic thyroid responsive element reporter line to track the activity of the thyroid pathway during axolotl development and regeneration. Lastly, we performed skin grafting experiments with a neotenic host and metamorphic donor and vice versa. We showed that a neotenic skin graft heals more efficiently on a metamorphic host than the opposite and that a metamorphic skin graft on a neotenic host does not participate in regeneration. In summary, our results suggest that thyroid-driven metamorphosis causes extensive phenotypic changes and impacts tissues regeneration negatively.

148

Basic and Applied Research in Biological Sciences
Physiology/pathophysiology
Yannic Becker, MS

Mount Desert Island Biological Laboratory

The Role of Heparanase 2 for Vascular Integrity in Zebrafish

The endothelial glycocalxy (eGCX) is a brush-like glycan layer covering the luminal surface of the endothelium contributing critical functions for the circulatory system. Amongst others it is involved in vascular permeability regulation, mechanosensing, and the controlled release and storage of growth factors. Microvascular diseases such as diabetes, sepsis, and systemic coronavirus infection coincide with a diminished and altered eGCX. Heparan sulfate (HS) is the central glycan structure of an intact, healthy eGCX and its direct degradation from the vascular surface is limited to one endoglycosidase, well known as heparanase. Scarcely known is its homologue heparanase 2 (HPSE2). Noteworthy, no catalytic activity of HPSE2 towards HS has been reported. In fact, due to its vigorous binding affinity towards HS, HPSE2 inhibits HS degradation. We consider HPSE2 as a potential protective molecule for the vascular system due to prevention of eGCX degradation and ultimately the development of a dysfunctional endothelium. Here we utilized zebrafish larvae as a vertebrate model to study the vascular role of HPSE2 in vivo. CRISPR Cas9-induced knockout (genomic level) as well as morpholino-induced knockdown (mRNA level) techniques were applied to diminish hpse2 expression in zebrafish larvae. Vascular integrity after hpse2 downregulation was measured by the capacity to keep fluorescent-labeled protein or dextran inside the blood vessels. Both methods for hpse2 downregulation decreased the amount of labeled macromolecules inside the vasculature. Furthermore, spatial and temporal expression analysis of hpse2 in zebrafish larvae by in situ hybridization revealed pronounced expression in the liver. We conclude that hpse2 downregulation increases the vascular permeability in early zebrafish larvae development. Mechanistically we propose that HPSE2 protein is secreted by the liver into the vascular system where it eventually binds HS on the luminal site of the endothelium and preserves a physiological HS pattern, which in turn maintains the endothelial barrier function.

149

Health and Social Sciences
Nursing
Clinical Research
Vikram Rao, MS

Northeastern University

Characterizing nurses with advanced nursing degrees in Maine and Massachusetts using the National Sample Survey of Registered Nurses

Background: Filling vacant faculty roles means targeting advanced degree nursing programs to the right audience, which necessitates understanding who already has masters or doctoral nurse education.
Purpose: To understand key characteristics associated with nurses who have an earned master or doctoral degree.
Methods: Using the 2018 National Sample Survey of Registered Nurses that was limited to Maine and Massachusetts nurses with higher degrees, weighted multivariable logistic regression, stratified by state and degree, demonstrated characteristics associated with advanced nursing degrees.
Results: We found n=742 (weighted n=17794) nurses from Maine and n=989 (weighted n=90644) nurses from Massachusetts, where the mean age was 48 (sd=13) and 47 (sd=13), respectively. Approximately 90% were female, and >80% were White. In Maine n=337 had a masters, n=19 had a doctorate. In Massachusetts n=589 had a masters, n=51 had a doctorate. Males had higher odds of a doctorate (OR 1.61, 95% CI [1.23,2.10]) in Maine, but lower odds for all other degrees. Having dependents was associated with higher odds of all degrees except a masters in Maine (OR 0.76 [0.71, 0.81]). Having self-financed their first nursing degree meant higher odds of all degrees except a masters in Massachusetts (OR 0.62 [0.58, 0.66]). There were higher odds of all degrees for nurses with >=31 years of experience compared to those with <16 years.
Conclusion: Barriers to advanced nursing degrees may include personal and financial characteristics potentially influenced by each state's social climate. Targeting early career nurses and considering these barriers will be critical for filling nurse faculty roles.

150

Basic and Applied Research in Biological Sciences
Biology
Emily Cornell du Houx, Undergraduate Student

Southern Maine Community College

Spatiotemporal Analysis of the Stem Cell Response to Injury in Planarians

Spatiotemporal changes in stem cell division are hallmarks of regeneration in a variety of model organisms, yet the mechanisms governing these responses are not well understood. We are addressing this problem in Schmidtea mediterranea, an experimentally tractable planarian flatworm with the remarkable ability to regenerate any lost body part in just over a week. The foundational work of Wenemoser and Reddien revealed that amputation triggers a biphasic increase in the rate of division among the adult stem cells that drive formation of new planarian tissues during both homeostasis and regeneration. To further characterize these responses, we developed an automated image-analysis script for the open-source Fiji program and used it to quantify dividing stem cells labeled by whole-mount, phospho-histone H3 (H3P) immunostaining. After analyzing the staining patterns in over 2,000 animals fixed at multiple timepoints following a variety of different injuries, we determined that both the early and late mitotic peaks described by Wenemoser and Reddien exhibit pronounced gradation, with a significantly greater increase in cell division occurring proximal to the wound site. Unexpectedly, animals fixed at two hours, the earliest timepoint at which increased proliferation was evident, did not show the same pattern, instead exhibiting a more uniform, systemic response. We speculate that the nervous system may trigger a rapid, body-wide increase in the rate of stem cell division following injury, as has previously been documented in axolotls. Consistent with this scenario, we find that treatment of planarians with

anesthetics decreases H3P labeling. In summary, our ongoing work on this project has provided new insight into how stem cell division changes after injury and may support the involvement of deeply conserved regulatory mechanisms.

151

Health and Social Sciences
Psychiatry, Psychology
Clinical Research
Courtney Hatton

The Jackson Laboratory and Geisinger Commonwealth School of Medicine

The Relationship Between Treatment Center Services and Number of Opioid-related Deaths in the United States Before and After a Declaration of a National Opioid Crisis

Opioid-related deaths are a national problem that have increased over the past two decades. Multiple policy interventions have been enacted to decrease opioid misuse and expand treatment. The Comprehensive Addiction and Recovery Act (CARA) was passed in July 2016, just before declaring the opioid epidemic a National Emergency in 2017. CARA was enacted to combat the opioid epidemic by providing more funding yearly for items including but not limited to prevention, treatment, and opioid overdose reversal. To evaluate the impact of these policy changes, we carried out secondary data analysis for the period 2011-2019 using the CDC's Wide-ranging Online Data for Epidemiologic Research and National Survey of Substance Abuse Treatment Services databases.
Research variables included: a comparison of the 50 states across the 2011-2019 timeframe, the number of opioid treatment centers, the percentage of government funding for facilities per state, percentage of opioid treatment facilities which offer free/low-income services and the opioid death rate. We also assessed differences in low-income access to opioid treatment services by comparing Medicaid expansion states versus non-Medicaid expansion states.
While both the number of treatment facilities per state and opioid death rates nearly doubled during this time, there was little to no association between them (R2 ranging from: 0.094-0.188 for years 2013-2019). Our research suggests that while state-level differences in opioid use disorder treatment facility characteristics related to access to care, they were only weakly associated with opioid-related deaths. This analysis may be used in the planning of subsequent actions against the national opioid epidemic and invites further inquiry into the impact of state Medicaid expansion on drug-specific opioid usage and mortality.

152

Basic and Applied Research in Biological Sciences
Microbiology/Virology
Avery Bond, BS

The University of Maine

Blocking host-cell calcium signaling pathways: a potential target for JC polyomavirus infection

JC polyomavirus (JCPyV) is the causative agent of the fatal demyelinating disease progressive multifocal leukoencephalopathy (PML). JCPyV infects the majority of the human population and causes a lifelong, asymptomatic infection in the kidneys of immunocompetent individuals. Those with severe immunosuppression, such as individuals with HIV or those receiving immunomodulatory treatments for diseases such as multiple sclerosis, are at risk for JCPyV spread from the kidneys to the brain. JCPyV lyses glial cells, leading to the debilitating, and ultimately deadly, disease PML. Unfortunately, there are no approved treatments for PML, highlighting the need for continued research on this disease. The Maginnis laboratory performed a large-scale drug screen using the National Institutes of Health Clinical Collection (NIH-CC) to identify potential antiviral candidates. SVGA cells, a transformed human glial cell line, were treated with drugs and small molecule inhibitors, challenged with JCPyV,

and assessed for viral infectivity by high-throughput In-Cell Western assays. Multiple FDA-approved drugs from different drug classes demonstrated potential to reduce JCPyV infection. Drug categories with the most “hits” include receptor agonists/antagonists, calcium signaling-related drugs, and enzyme inhibitors. Further characterization of calcium channel blockers and related calmodulin inhibitors through viral infectivity assays supports a role for calcium signaling during JCPyV infection. Additional detailed characterization of drugs that block JCPyV infection could reveal potential antivirals to treat and prevent JCPyV and PML. Since repurposing existing drugs for new diseases is an effective method for discovering new antiviral therapies, exploring pre-approved drugs from the NIH-CC is a promising avenue to identify treatments for PML.

153

Health and Social Sciences
Infectious Disease Public Health
Clinical Research
Fajar Alam, OMS-II, Meghan May

The University of New England College of Osteopathic Medicine

Pandemic Parallels: Shared features in the emergence of AIDS and COVID-19 and the lessons learned

Introduction: Two pandemics faced by the world over the last century, COVID-19 and AIDS, have striking similarities. Both are caused by RNA viruses with zoonotic origins. AIDS is the associated disease of HIV (human immunodeficiency virus) which is a retrovirus and COVID-19 is the associated disease of SARS-CoV-2, a coronavirus. There are several analogous themes in the emergence of these two pandemics, how they were managed and responded to and their societal impacts. This analysis will explore the parallels between the unfolding of AIDS and COVID-19 in an effort to highlight the similarities that enabled these epidemics and the lessons to be learned.
Methods & Materials: Our laboratory developed a core set of ten questions focused on common features found in major disease epidemics, including: the emergence and detection of the disease, societal responses and control measures, scapegoated populations, major discoveries from research of the pathogens, and the lasting impacts on society and the affected patient populations. We utilized primary literature, contemporary news coverage, documentary and written accounts to fully answer the core questions.
Results: Several of the core questions identified parallels between the AIDS and COVID-19 pandemics. These include lack of government urgency evidenced by slow policy responses and implementation of control measures, disproportionate impact on marginalized populations, global disparities in treatment and in testing and vaccination roll outs, significant scientific advancements, continued economic and social impacts.
Conclusion: This analysis identifies multiple commonalities in the emergence of the COVID-19 and HIV/AIDS epidemics. There are several lessons to be learned from the unfolding of both of these diseases. To prepare policies and responses for control of potential infectious disease outbreaks and pandemics in the future, it is important to reflect on the even"

154

Basic and Applied Research in Biological Sciences
Biochemistry
Molecular Biology
Sudati Shrestha, Ph.D.

University of Maine

The Anillin-Like Protein Boi2 is Required for Septin Localization During the Yeast Pheromone Response

G-protein coupled receptors (GPCRs) play vital roles in human health ranging from tissue development and metabolic control to neurotransmission. In Saccharomyces cerevisiae, GPCR signaling detects pheromone and drives growth towards mating partners. At high pheromone concentrations, yeast form a mating projection, called a shmoo, while at intermediate pheromone concentrations, yeast elongate and grow towards the source of the pheromone. Both mating projection morphogenesis and gradient tracking require septins, conserved cytoskeletal proteins that form the barrier at the mother-daughter bud neck and stabilize curved membranes. Hence, septin localization is controlled by GPCR signaling, yet the molecular mechanism is unknown. Boi1 and Boi2 are yeast anillin-like proteins that interact with polarity machinery and are involved in the central cellular processes of cytokinesis and exocytosis, but with no characterized role in mating. We hypothesized that Boi1 and Boi2 may regulate septins during the pheromone response. Here, using fluorescence microscopy and computational image analysis we examine the role of Boi1 and Boi2 in septin localization during the pheromone response. Despite their reported redundancy, we find that Boi1 and Boi2 proteins localize to distinct areas in addition to sites of colocalization, suggesting unique functions. Additionally, Boi2 undergoes a pheromone dose dependent change in localization from the center of the polarity site (at low dose) to the periphery at the base of the mating projection (at high dose), where septin structures are formed when cells shmoo. This led us to investigate the role of Boi2 in septin structure formation. Cells lacking Boi2 show aberrant morphology consistent with septin defects. When we then examined receptor-controlled septin localization, we found that septins accumulate at the polar cap, rather than establishing structures at the periphery. Thus, the anillin-like protein Boi2 is required for receptor control of septin structures during pheromone-induced morphogenesis.

155

Basic and Applied Research in Biological Sciences
Biochemistry
Molecular Biology
Nicholas Leclerc, Graduate Student

University of Maine

GPCR levels on the plasma membrane are controlled by autophagy in Saccharomyces cerevisiae

G-protein Coupled Receptors (GPCRs) are key players in many sensory processes such as intercellular communication, gradient tracking, and nutrient detection. The yeast GPCR, Ste2, activates Gα and Gβγ signaling cascades to alter gene expression and cell morphology in response to mating pheromone. Gα-mediated signaling activates selective autophagy during the pheromone response, but the reason for this is unknown. Autophagy is a metabolic recycling process that promotes cell survival in response to starvation. This occurs through selective or bulk encapsulation of biomolecules and organelles for vacuolar delivery and subsequent degradation. Under nutrient-starved conditions, we have observed reduced Ste2 abundance at the plasma membrane, suggesting that activation of autophagic pathways may promote internalization of the receptor. To test this, we induced autophagy pharmacologically with the Tor inhibitor, rapamycin, mimicking the effect of nutrient starvation. This caused reduction of receptor at the plasma membrane. To test if this response is specific to Ste2, we tested whether rapamycin affects Ste3, the mating GPCR in yeast of the opposite mating type. We found that a reduction of Ste3 at the cell periphery is also seen with rapamycin treatment. Thus, both GPCRs used for mating in yeast are downregulated in response to starvation conditions by an autophagic process. This may serve as a mechanism to delay or inhibit mating during times of nutrient deprivation.

156

Basic and Applied Research in Biological Sciences
Biochemistry
Bright Obeng, BSc

University of Maine

Pharmaceutical Agent Cetylpyridinium Chloride Inhibits Immune Mast Cell Function by Interfering with Calcium Mobilization

Cetylpyridinium chloride (CPC), the positively charged broad-spectrum antimicrobial, has been used widely in consumer products and agricultural processes at concentrations up to 3 mM, thus exposing much of the U.S. populace to significant levels of CPC. However, minimal information exists on the eukaryotic toxicology of CPC; hence, there is an urgent need for information. Mast cells, ubiquitous throughout the human body, particularly at interfaces, are implicated in many diseases and key players in normal immune and nervous system functioning. We have demonstrated that CPC potently (low-µM) inhibits antigen (Ag)-stimulated function of RBL-2H3 mast cells, including degranulation. We have interrogated the molecular mechanism underlying CPC's inhibition of degranulation. Following 30 min pre-treatment, CPC drastically inhibits Ag-stimulated store-operated Ca2+ entry (SOCE) into the cytosol, a core mediator of the degranulation pathway. Inhibited SOCE may be caused by CPC's inhibition of Ca2+ efflux from the endoplasmic reticulum (ER) into the cytosol, a trigger of SOCE. In turn, Ag-stimulated mitochondrial Ca2+ uptake from the ER is reduced by CPC. Inhibited ER Ca2+ efflux may be caused by CPC's interference with Ag-stimulated tyrosine phosphorylation and phosphatidylinositol 4,5 bisphosphate, which together provide the trigger signal for ER Ca2+ efflux. Plasma membrane potential (PMP) and cytosolic pH, contributors to SOCE, are not affected by CPC. Dampened cytosolic Ca2+ leads to CPC inhibition of microtubule polymerization, necessary for degranulation. This work outlines biochemical mechanisms underlying the effects of CPC on immune signaling and allows the prediction of CPC effects on disparate cell types that share similar signaling elements.

157

Basic and Applied Research in Biological Sciences
Biology
Alyssa Marini, MS

University of Maine

Silvicultural treatments affect adult mosquito (Diptera: Culicidae) abundance and species diversity in a managed forest

Mosquito-borne disease is a serious public health concern worldwide, and transmission may be facilitated by the creation of favorable habitat conditions by human activities. There are many important disease vector species inhabiting forested ecosystems, and timber harvesting treatments may play a role in altering the abundance of vector species. Silvicultural systems are defined as a plan that integrates specific harvesting, regeneration, and tending methods contributing to a healthy forest stand. Timber harvesting treatments can alter the diversity or abundance of adult mosquito species through a variety of mechanisms, such as heavily harvested stands may have fewer tree holes, providing habitat for developing larvae. The goal of this study was to analyze the response of adult mosquito abundance and diversity to different timber harvesting treatments. To test our hypotheses, mosquito surveillance was conducted from 6 June to 23 August 2022 in the DeMeritt University Forest in Old Town, Maine, across five different treatments: 1) hardwood stands with no recent harvest, and softwood stands with 2) no recent harvest, 3) overstory removal, 4) thinning, and 5) shelterwood establishment cuts. Light traps and infusion baited gravid traps were deployed to collect a diversity of adult mosquitoes in 13 forest stands. We collected a total of 4,843 mosquitoes with three dominant taxa that were Ochlerotatus japonicus (6.48%), an invasive mosquito, Anopheles puntipennis (6.48%), a vector species for West Nile virus, and Coquillettidia perturbans 67.33(%), a vector species for both WNV and Eastern Equine Encephalitis. The no recent harvest in the hardwood stands treatment had a higher mosquito abundance compared to the other treatments, while the softwood thinning treatment had the lowest number of mosquitoes. Overall, the results suggest that the risk of arboviruses transmitted by vector species may be inhibited by forest management practices because of reduced Oc. japonicus,

158

Basic and Applied Research in Biological Sciences

Biology
Elizabeth Dabek, MD

University of Maine

The Effects of Microclimate Variation on Blacklegged Tick (Ixodes scapularis) Host Seeking

Ticks within the genus Ixodes are able to transmit several human pathogens including the agents that cause Lyme disease, anaplasmosis, and babesiosis. Recently, the northern range and incidence of diseases caused by I. scapularis have increased, yet current studies on questing - a host-seeking behavior - focus primarily on physiological constraints. Frequently, these studies test why ticks react to specific stimuli (i.e. production of heat shock proteins at high temperatures) and forgo observation and analysis on behavior. This project presents the first phase of a diurnal questing study where constraints on nymphal I. scapularis behavior and survival were evaluated. We were interested in testing if specific types of weather events and times of day correspond to specific questing behavior. We believe that ticks will reduce questing in adverse weather (i.e. rain) and will prefer questing at night due to the nocturnal nature of common I. scapularis hosts. From June - August 2022, tree stands were selected in the Demeritt Experimental Forest at the University of Maine in Orono to represent a range of environments and microhabitat conditions where encounters with ticks may occur. Enclosures housing both lab-reared (sourced from a Rhode Island population) and field-collected (sourced from a population in Maine) nymphal ticks were observed during randomized hours of the day and night and survival was checked in August. Initial exploratory analysis shows differences in questing based on weather events, with most ticks preferring to quest during days of high humidity but not during rain, and no differences in questing preference by time of day. By informing the public on tick behavior and preference this research increases our understanding of female tick questing ecology and may have implications for public health risk for tick-borne disease.

159

Basic and Applied Research in Biological Sciences
Disease Ecology
Public Health/ Disease Ecology
Megan Schierer, B.S.

University of Maine

Environmental DNA (eDNA) Methods for Mosquito Surveillance

Vector-borne diseases account for 30% of global emerging infectious diseases, with arboviruses (i.e., viruses transmitted via mosquito bite) contributing the highest risk to public health. Effective mosquito-borne disease (MBD) prevention in human populations relies on surveillance of the distribution of MBD vector species. Current mosquito surveillance methods consist of trapping adult and larval mosquitoes, and habitat identification for larval mosquitoes. These methods are labor and cost intensive, relying on taxonomic expertise for species identification. Additionally, these methods are ineffective for the surveillance of invasive species, which may only be detected at extremely fine spatial scales. A potential alternative to current surveillance methods for disease vectors and invasive mosquitoes is the application of environmental DNA (eDNA) methods, an established tool used in aquatic systems. Organisms shed DNA into their environment, and environmental samples (e.g., water samples) can be tested for known DNA sequences. The purpose of this study is to operationalize eDNA methods for surveillance of two important disease vector species in the Northeast US, targeting the aquatic larval stage and their preferred “artificial-container” habitats. Three experiments were deployed between June-August 2022. First, optimization of water sample collection was tested across residential properties located within the known target species distribution. Second, quantification of mosquitoes from eDNA was tested by sampling buckets with known concentrations of mosquito larvae. Third, a natural experiment was deployed across residential sites, encompassing an urban-rural gradient to validate the optimization and quantification experiments. DNA extraction and qPCR amplification will be performed to test the 802 samples for target DNA. Presently, an assay for target species is under development, and can detect the target genus. Experimental conclusions have the potential for application in widespread surveillance efforts, without the need for taxonomic expertise, contributing to the protection of public health against MBD.

160

Basic and Applied Research in Biological Sciences
Microbiology/Virology
Sarah McCallister

University of Maine

Interactions between co-habitating prophages increases expression of mycobacterial intrinsic resistance gene, whiB7

Prophage, integrated viral genomes, are known to increase antibiotic resistance of bacterial pathogens. Non-tuberculosis mycobacteria such as Mycobacterium abscessus, causes pulmonary and disseminating infections that are often totally drug resistant. Most M. abscessus isolates carry one or more prophages but their role in intrinsic antibiotic resistance is not yet known. We have demonstrated that M. chelonae, a close relative of M. abscessus, has higher antibiotic resistance and expression of a conserved mycobacterial regulator of antibiotic resistance genes, whiB7, increases in the presence of two prophage genomes. The first prophage, McProf, only carries out lysogenic infection of M. chelonae. The second prophage, BPs, is capable of lysogenic infection but also undergoes induction and lytic infection. We hypothesize that BPs induction activates McProf gene products, such as polymorphic toxin systems, to increase expression of whiB7. We have demonstrated that strictly lytic infections by BPs increases whiB7 expression in the presence of McProf. Inhibiting BPs induction in the M. chelonae double lysogen (BPs, McProf) decreases whiB7 expression. We don't know whether whiB7 expression increases in the BPs induced cells or through signaling in neighboring lysogenic cells. To determine if BPs induction increases whiB7 expression in cis or in trans we have constructed M. chelonae strains with an mCherry-whiB7 promoter reporter and a BPs-GFP fluorophage that reports lytic gene expression. Using fluorescent microscopy we will monitor BPs induction events and whiB7 expression in double lysogen strains of M. chelonae (BPs, McProf) and in a super M. chelonae lysogen (BPs, McProf) (pMHBPsgp33) strain that has diminished induction events.

161

Basic and Applied Research in Biological Sciences
Microbiology/Virology
Sarah McCallister

University of Maine

Interactions between co-habitating prophages increases expression of mycobacterial intrinsic resistance gene, whiB7

Prophage, integrated viral genomes, are known to increase antibiotic resistance of bacterial pathogens. Non-tuberculosis mycobacteria such as Mycobacterium abscessus, causes pulmonary and disseminating infections that are often totally drug resistant. Most M. abscessus isolates carry one or more prophages but their role in intrinsic antibiotic resistance is not yet known. We have demonstrated that M. chelonae, a close relative of M. abscessus, has higher antibiotic resistance and expression of a conserved mycobacterial regulator of antibiotic resistance genes, whiB7, increases in the presence of two prophage genomes. The first prophage, McProf, only carries out lysogenic infection of M. chelonae. The second prophage, BPs, is capable of lysogenic infection but also undergoes induction and lytic infection. We hypothesize that BPs induction activates McProf gene products, such as polymorphic toxin systems, to increase expression of whiB7. We have demonstrated that strictly lytic infections by BPs increases whiB7 expression in the presence of McProf. Inhibiting BPs induction in the M. chelonae double lysogen (BPs, McProf) decreases whiB7 expression. We don't know whether whiB7 expression increases in the BPs induced cells or through signaling in neighboring lysogenic cells. To determine if BPs induction increases whiB7 expression in cis or in trans we have constructed M. chelonae strains with an mCherry-whiB7 promoter reporter and a BPs-GFP fluorophage that reports lytic gene expression. Using fluorescent microscopy we will monitor BPs induction events and whiB7 expression in double lysogen strains of M. chelonae (BPs, McProf) and in a super M. chelonae lysogen (BPs, McProf) (pMHBPsgp33) strain that has diminished induction events.

162

Basic and Applied Research in Biological Sciences
Microbiology/Virology
Molecular biology
Meg Caron

University of Maine

Manipulating Virulence of C. albicans Through Administration of RBT1 Peptides

Candida albicans is a pathogenic yeast responsible for the fungal infection candidiasis. This fungus resides in the microflora of the majority of the population, in some cases entering into vulnerable areas of the body or overgrowing to cause worsened infection. With a 40% mortality rate of candidiasis, the virulence of C. albicans has proven a severe threat to immunocompromised individuals. A gene, RBT1, in C. albicans' genome, encodes two secreted peptides that may be associated with Candida virulence. A mutation in RBT1 has been shown to lack virulence. To determine whether RBT1 is a possible target for drug therapy, I've investigated whether peptides from RBT1 mediate Candida virulence. Zebrafish models are used in a range of studies to mimic human host-pathogen interactions. Injections into uninfected fish demonstrated that the Rbt1p-derived peptides are not toxic at up to a dose of 2 mg/ml. In C. albicans-infected fish, administering the peptides did not significantly alter fungal virulence, although preliminary results show a trend towards decreased virulence of up to 11.1% when peptides are added to infection at the full concentration (2 mg/ml). Additionally, differences in CFU (colony-forming units) per fish and mean fungal area amongst the Candida-infected and Candida with peptide groups suggest that Rbt1p-derived peptides may also lead to reduced fungal burden. This is promising evidence that the peptides may lessen candidiasis infection levels or play a critical role in limiting fungal growth of C. albicans. This might occur through enhanced immune responses. Further research will involve intravital imaging to monitor immune recruitment amidst infection in the presence and absence of Rbt1p-derived peptides. The peptides will also be administered to other RBT1 mutants to determine their effect on C. albicans strains of varying virulence levels.

163

Basic and Applied Research in Biological Sciences
Molecular Biology
Optical & Non-Optical Image Analysis
Komala Shivanna

University of Maine

The Role of Hemagglutinin in Association with Phosphatidylinositol 4,5-bisphosphate within the Plasma Membrane

Influenza A virus (IAV) is an enveloped respiratory virus infecting people around globe, posing a significant threat to public health. IAV mutates continuously, which requires ongoing modification of vaccines and in some cases resistance to antiviral drugs. The IAV fusion protein hemagglutinin (HA) mediates viral binding and entry. Recently, we found HA co-clusters with host cell phosphatidylinositol 4,5-biphosphate (PIP2) on the plasma membrane (PM), consistent with an interaction (Curthoys 2019). How do HA and PIP2 end up together? Molecular dynamics (MD) simulations show evidence of a direct interaction between HA and PIP2. Using super-resolution microscopy in living cells, HA-Dendra2 and PAmKate-PH frequently increase or decrease together in nanoscopic zones of the PM. We hypothesize that HA and PI4P, the precursor of PIP2, are packaged within the Golgi to be delivered together to the PM. We find PM colocalization between HA and PI4P5K, a kinase which binds PI4P. In fixed cells, we find mutations of the HA CTD affect HA-PIP2 colocalization. Further, HA and PIP2 strongly colocalize on the plasma membrane, but are less colocalized within intracellular membranes. We analyzed Colocalization Index (CI) and Manders' colocalization coefficients (MCC) between HA and PIP2 localizations. The CI and MCC were higher in the PM of the cell and significantly lower inside the cell, suggesting that the PIP2 levels do not increase in the vicinity of HA-containing membranes until they reach (or are very close to) the PM. HA and PIP2 in living cells revealed a time-dependent correlation in their dynamics at the PM and inside cells, demonstrating that HA and PIP2 are together within the PM, but not in general within intracellular membranes. Together,

this evidence suggests a new model for the mechanism of phosphoinositide-dependent HA clustering within the membranes of living cells.

164

Basic and Applied Research in Biological Sciences
Molecular biology
Ryn Harrington, Emily Tomak, and Jared Talbot

University of Maine

Identifying the time window when muscle movement is needed for fin skeletal patterning

Distal Arthrogryposis, or DA, is a human musculoskeletal disorder characterized by contractures in the distal limb due to lack of movement in utero. Affecting 1 in 3000 live births, DA can greatly limit functional movement of the distal joints. Although physical therapy and surgical intervention can mitigate symptoms, it is not successful in recovering full range of movement in affected joints. In order to restore movement, strategies such as stimulating fetal movement have been employed in a laboratory setting. However, in order for this technique to be most effective, the developmental window within which muscle movement is necessary to stimulate joint formation must be determined. To accomplish this, I have used a skeletal muscle myosin inhibitor N-Benzyl-p-toluenesulfonamide (BTS) to paralyze zebrafish embryos during defined developmental phases. Zebrafish serve as an accessible model for muscle and joint manipulation through each phase of development, and allows for easy visualization of tissue structure and function in the context of a whole organism. Previously, when movement of fast-twitch muscle fibers has been inhibited through a gene knockout that causes DA in humans, we observed a reduction in the size of pectoral fin cartilage homologous to the distal limb. In preliminary trials where all muscle is paralyzed by BTS throughout the course of development, a severe defect is observed in pectoral fin cartilage as well as the fin skeleton. For my senior capstone project, I will be applying BTS to zebrafish during different stages of embryonic development in order to identify which stages have the greatest impact on skeletal development in the pectoral fin. This will provide the information necessary to determine when muscle stimulation may be effective in reversing the skeletal effects caused by paralysis in fetal development. Here, we present the preliminary findings.

165

Basic and Applied Research in Biological Sciences
Microbiology/Virology
Molecular biology
Optical & Non-Optical Image Analysis
Lucas Bennet

University of Maine

Utilization of FPALM to understand the localization of 5-HT2 receptors during JCPyV internalization

Super-resolution microscopy techniques are highly specialized forms of microscopy that break the diffraction barrier allowing for single particle resolution of proteins and viral particles. JC polyomavirus (JCPyV) asymptomatically infects the kidney in a large portion of the human population and, in some immunocompromised individuals, can cause a neurodegenerative disease called progressive multifocal leukoencephalopathy (PML). The mechanism by which JCPyV utilizes host-cell receptors to invade host cells is not well characterized. JCPyV utilizes 5-hydroxtrypatime 2 (5-HT2) receptors, G-protein coupled receptors (GPCRs), and beta-arrestin, a cytosolic protein that initiates the internalization of GPCRs, to enter host cells. We utilized Fluorescence Photoactivation Localization Microscopy (FPALM) to better define the spatial and temporal localization of JCPyV with 5-HT2 receptors during infection. These experiments have shown that JCPyV localizes with 5-HT2 receptors at 5 minutes post-infection and further demonstrates that receptors cluster at this time point but not at 0- or 15-minutes post-infection. Interestingly, these observations are congruent with the established timeline of JC particle endocytosis and localization with clathrin. These findings provide insight into the spatial and temporal localization of viruses with cell-surface receptors during infection. Our current and

future research may illuminate targets for the development of new treatments and therapies to potentially prevent or treat JCPyV infection.

166

Biomedical Engineering & Medical Physics
Materials
Clinical Research
Dominic Kugell, BS

University of Maine

Nanocellulose Based Foams for Low-Cost Disposable Medical Applications

Polyurethane foams have been a staple material for their use in medical positioners, such as, post-surgery elevation pillows as well as specific tailored positioners for their use during surgery. Polyurethane and other petroleum-based foams are chosen for this task because of their cost effectiveness, versatility and mechanical properties. However, the environmental impact associated with these foams is immense. Within the waste management pipelines of hospitals these foams are commonly sent to landfills or incinerated with both options being undesirable for the environment. Polyurethanes take decades to see the effects of natural biodegradation and when placed in landfills and the burning of polyurethane produces irritating and toxic fumes. Therefore, there is a need for a biopolymer that can be used to replace these petroleum-based products. Cellulose based foams have become a desirable alternative within the research space within the last 5 years. Cellulose textiles have existed for over a century with viscose rayon fabric. However, the methods for using cellulose in this manner is still undesirable because of its effect on the environment due to the solvents required. It is the goal of this research to design a method of creating porous biodegradable cellulose based foam products with desirable mechanical properties similar in nature to those of polyurethane foams while maintaining the use of green chemistry techniques. In this study, cellulose wood pulp, cellulose nanofibers and other additives will be used to create structure in these products with sodium dodecyl sulfate being utilized for pore generation. These materials in conjunction will create a wet stable cellulose foam which can then be dried using two methods, microwave radiation as well as convection oven drying. The mechanical properties of foams created using this method will be examined and compared to traditional petroleum based foams

167

Biomedical Engineering & Medical Physics
Materials
Evan Leonard

University of Maine

Bio-Inspired Liquid-Infused Surface Coatings Reduce Infection in Catheter-Associated Urinary Tract Infections in vivo

Hospital-acquired infections (HAIs) affect over 1.7 million patients annually and are often treated with antibiotics, which can contribute to antibiotic resistance.1 Catheter-associated urinary tract infections (CAUTIs) are the most common type of HAI, resulting in an estimated $390-450 million in treatment and increased length of stay-associated costs annually.2 In this work, we show how a bio-inspired coating on commercial catheter surfaces can reduce the need for antibiotics by minimizing both protein and bacterial adhesion to the catheter surface as well as the spread of bacteria to other organs. The bio-inspired coating significantly reduced the amount of adherent fibrinogen (Fg), a protein released in response to host tissue damage, on the liquid-infused catheter surfaces compared to unmodified controls. In vivo murine experiments showed significantly reduced amounts of adhesion of CAUTI causing microbes such as E. faecalis, E. coli, P. aeruginosa, K. pneumoniae, A. baumannii and C. albicans on catheter surfaces. The results also showed reduced spread of these microorganisms to organs including the bladder, kidney, spleen, and heart. Given the promise of our bio-inspired coating, we are now working to take the next steps to translate this technology to the market where it can be used for patients. This includes working toward FDA approval as well as partnering with

healthcare providers and commercial catheter manufacturers and vendors. Our goal is to reduce the need for antibiotics by reducing the incidence of CAUTI, improving the lives of patients around the world.

168

Biomedical Engineering & Medical Physics
Materials
Cameron Andrews

University of Maine

The Effects of Chemical Cross-Linking on the Properties of Cellulose Nanofibril Composites

The use of cellulose nanofibrils (CNF) in biomedicine is a recently researched topic, that has many impactful applications. Current research shows that while the material does prove to have many benefits, there are some potential drawbacks. Mechanical and chemical degradation of the material caused by the hydrophilicity of CNF is a problem that needs to be resolved. Potential solution is the use of crosslinking, using polycup, a polyamide-epichlorohydrin. The cross-linking of CNF with its implementations, showed noticeable physical and chemical changes within the CNF. The average percent porosity of CNF was reduced, the water absorption rate decreased when cross-linked, as well as the swelling rate. The young's modulus and tensile stress were also tested, and these properties showed higher mechanical results when compared to pure CNF, after set periods of time. Finally, the contact angle and surface free energy of both bulk material, and thin film material for each sample were tested. The results showed high amounts of error and will need to be tested further before conclusions can be made.

169

Biomedical Engineering & Medical Physics
Systems Development
Other
Ines Khiyara, MS

University of Maine

Engineering the Development of Neuromuscular Circuitry On-Chip

Neuromuscular development happens in a complex interconnected network of biochemical pathways. This very complicated embryonic development follows a strong, functional, and precise neuromuscular network that has interested both scientists and engineers who seek to better understand neuromuscular diseases. These disorders can be inherited or acquired, and their severity and mortality can vary. Researchers first studied the neuromuscular network from an organismal perspective, and more recently from an embryological, cellular/molecular, biochemical, and genetic perspective. From these studies, the fundamental principles of motor neuron pathfinding to muscles are widely understood, but the molecular drivers of specific nerve-muscle pairings remain unknown. Although in vivo experiments provide a precise depiction of the living tissue environment, manipulating in vivo variables in the laboratory is difficult, and the results are frequently intricated by many uncertainties and unquantifiable variables which make it problematic to draw significant and relevant conclusions. In vitro experiments, on the other hand, provide a more controlled, precise, and repeatable motif, but they often lack biological realism. This research employs a novel in vitro, 3D co-culture microfluidic system that can mimic the complex in vivo developmental environment. With this system embryonic stem cells are cultured in two adjacent chambers, where they are supplied with their individual, requisite media, and morphogens to develop into two distinct types of tissues, either motor neurons or muscle fibers, while enabling communication through interconnecting microchannels. Experiments are ongoing to investigate the effects of muscle/neuron signaling in the differentiation of motor neurons into specific motor neuron columnar identity and neuromuscular junction formation. Results are expected to increase our fundamental understanding of developmental processes, neuromuscular junction formation, and neural development in order to research and model degenerative diseases.

170

Computational Biology & Medicine
Computer Modeling
Instrumentation
Materials
Mubarak Khlewee, Ph.D.

University of Maine

The Influence of Heat and Mass Transfer on the Setting Rate of Adhesives between Porous Substrates

With the move to replace plastic packaging with sustainable options, glueing operations are needed to form cellulose-based packaging. In the setting of hot melt adhesives and water-based glues in the production of paper-based packaging, the controlled penetration of the adhesive is important to obtain rapid setting rates and good bond strength. Experiments are designed to understand the extent of penetration of hot melt and water-based adhesives into several porous substrates. Paper surfaces are modified by a range of coatings that have different porosities and pore sizes and contact angles; these surfaces were characterized with a range of techniques. For hot melt adhesives, a layer of the adhesive is pressed against the paper of interest with the carver press, for a known time, pressure, and temperature. The final degree of penetration of both systems is determined with silicone oil, mercury porosimetry, thickness, and weight methods. The strength of the bond at various setting conditions is also measured with the mechanical tester (INSTRON). Using two different press temperatures and pressures, the four methods gave excellent matching results. The mechanical tester (INSTRON) results showed that for a low press temperature, the failure was an adhesive failure mode.
While there is much experimental work discussing various parameters in these operations, little theoretical work has been reported. In this regard, various models are developed to predict the penetration of the adhesive as a function of the fundamental parameters. A finite element method-based model (COMSOL Multiphysics) is used to solve the unsteady-state flow of liquid adhesives into a single pore or a porous medium accounting for temperature or concentration changes, where the fluid viscosity is a function of temperature or concentration. The model predictions are compared to experimental results. Good agreement for different paper types, pressing times, and temperatures are obtained.

171

Computational Biology & Medicine
Managing large data sets
Systems Development
Clinical Research
Dilrukshi Abeyrathne, M.Eng.

University of Maine

Privacy-Based Access Control Model for Blockchain-Stored Healthcare Data

Healthcare data often contain sensitive personal information, which raises various privacy concerns. Additionally, these sensitive data are currently dispersed across several information systems owned by different healthcare organizations, thereby aggravating this matter. At present, patients have limited access and little control over who has access to which part of their healthcare data; individual patients' privacy preferences are not supported. For instance, an HIV patient may prefer to have a higher level of restriction in accessing their healthcare records than a patient with a diabetic condition. In recent years, research studies have proposed using blockchain technology to support the transfer of ownership of medical records to the patient to protect their privacy. However, blockchain technology alone is not adequate to provide fine-grained access control and ensure privacy. In this work, we develop MyHealthChain, a blockchain-based healthcare record system that includes a novel record structure that supports medical records and preserves privacy. As our main contribution, we introduce and implement the MyHealthChain's Privacy-Based Access Control model (PBAC) to help regulate access to blockchain-stored healthcare data based on a privacy

attribute that we define as the Privacy Class. A Privacy Class value is computed based on a function that takes a patient's preferences and the record into account; it is computed per medical record. The record's owner, the patient, can customize a privacy level through this novel function. Thus, instead of having few privacy levels that are the same for all the patients, the proposed PBAC model facilitates the patient to tailor the privacy based on their needs and preferences.

172

Computational Biology & Medicine
Spatial Information and Sensory Science
Food Science
Clinical Research
Meetha Nesam James, MS

University of Maine

Watching Flavors: Enhancing Everyday Eating Behavior for Flavor and Emotional Augmentation

Eating is one of the primary sustenance activities and it is highly individualistic for everyone to make it an enjoyable experience. Eating alone has become common nowadays accounting to lifestyle changes and more recently to the COVID pandemic. People involve in different activities while eating and watching something on the Television or a digital screen (Phone or laptop) is prominent on the list. Research in Human Food Interaction, although underexplored, has recently turned its attention towards studying the impact of digital multisensory stimuli on people's behavioral and cognitive responses. Toward that end, our current user study with 30 participants aimed to assess changes in people's flavor perceptions, liking and elicited emotions based on different videos watched while eating. Mukbang, a genre of food eating video that originated from South Korea, along with a cooking video and a nature video were evaluated in the study. Our research findings indicate that 1) different taste sensations were perceived based on the videos watched while eating (for example, participant felt their white rice was spicy while watching a spicy noodle Mukbang video). This denoted that the visual content digitally augments taste sensations without physically or chemically altering the food, making this a healthier approach. 2) there were changes in the elicited emotions towards a positive note with different videos watched while eating suggesting an enjoyable eating experience. Our findings also discuss insights on how these videos watched while eating could promote digital commensality.

173

Health and Social Sciences
Nutrition and dietary assessment
Computer Modeling
Data acquisition/analysis / Systems Development / Instrumentation
Clinical Research
Chamath Amarasinghe, MS

University of Maine

Automatic Food-Intake Monitoring by Combining Electrical Impedance Spectroscopy and Deep Learning

Food intake monitoring improves understanding of food practices and calorie intake over time, particularly to manage patients' chronic health conditions such as diabetes and obesity. However, recording food intake is challenging and prone to errors since the users have to manually enter the food and beverage data at the end of each meal. Recent literature introduces digital automatic food journaling systems that employ computer vision-based food recognition that demonstrate high performance in discriminating a wide variety of food. However, the technique is ineffective in quantifying the food and recognizing internal their

attributes. Furthermore, the technology underperforms in beverage recognition since liquids have similar colors and take the container's shape. We introduce a novel methodology based on electrical impedance spectroscopy and deep learning to digitally identify beverages and estimate their volumes and sugar content. First, impedances and their respective phase angles of a beverage were acquired using Electrical Impedance Spectroscopy to create a beverage dataset. Then, a multi-task network cascade, deep learning architecture that enables incorporating multiple tasks, was trained with the dataset to develop a proof-of-concept. Finally, our results demonstrated that the multi-task network cascade discriminates a limited number of drinks with an accuracy of 96.32%, estimates volumes with a root mean square error of 13.74ml, and sugar concentration with a root mean square error of 7.99g/dm-3. Our future work will extend this approach to include additional beverage types and their attributes as well as to record solid food intake, enabling a new avenue in automatic food intake monitoring and dietary assessment for improved health and well-being of patients.

174

Basic and Applied Research in Biological Sciences
Biochemistry
Peter Swanson, Undergraduate

University of New England

Toward the Creation of a Novel Interleukin-6 Responsive Biopolymer for Sensing Applications

In response to the fourth industrial revolution and the next generation of regenerative medicine and synthetic organ production, a novel sensor is needed to detect biomolecular analytes specific to monitoring cellular health status. Presented herein is the pathway and methodology used to create an interleukin-6 responsive protein polymer to be used in on-demand real-time surveillance during tissue and organ growth and transport.

175

Basic and Applied Research in Biological Sciences
Biochemistry
Neuroscience
Managing large data sets
Peter Neufeld

University of New England

RNA-Protein Interactions Stimulated by Nerve Growth Factor in PC12 Cells

Gene expression is controlled by interacting layers of regulatory pathways, including transcription, post-transcription, translation, and post-translation. Post-transcriptional mechanisms include the interaction of mRNAs with proteins that control mRNA stability, access to ribosomes, and localization to sub-cellular domains. Individual RNA-binding proteins (RBPs) interact with multiple mRNAs, by binding to interaction site motifs, thereby forming RBP-driven regulons – multiple genes controlled by single proteins. Post-translational regulation of transcripts is especially critical for peripheral neurons. These cells are extremely large, with termini remote from the cell body. Required mRNAs are transported along axons to the termini where they are translated as needed. Changes in transport, stability and the rate of translation are critical for regulating the sensitivity of neurons, and understanding these processes is critical for understanding diseases of the peripheral nervous system, such as maladaptive pain and neuropathies.
PC12 cells are a common cell line used to study neuronal physiology. When cultured with nerve growth factor (NGF), these cells differentiate in a dose-dependent manner, undergoing rapid physiological and morphological changes. Using RNA-Sequencing to profile gene expression changes in response to changing concentrations of NGF, we observed that ~300 RBPs are differentially expressed. Using computational analysis of published binding motifs, we observed that these RBPs bind to differentially regulated transcripts – uncovering potential regulons/networks of RBP-mRNA interactions for future characterization. In this poster we

focus on the most differentially regulated RBP: the neuronal-excitability associated gene, CUGBP Elav-Like Family Member 4 (CELF4). This protein is downregulated by NGF withdrawal, and upregulated by NGF stimulation. Computational analyses predict that this protein binds to >1000 NGF-regulated transcripts, including the high-affinity NGF receptor TRKA (gene name Ntrk1). This interaction was confirmed by RNA-immunoprecipitation using validated CELF4 antibody. These initial observations, and preliminary transfection experiments indicate that CELF4 functions to modulate neuronal plasticity by post-transcriptional regulation of target transcripts.

176

Basic and Applied Research in Biological Sciences
Biology
Microbiology/ Virology
Lauren Cooper

University of New England

Pyrogallol-hydrocarbon hybrid compounds potentiate antibiotic efficacy against staphylococc

Staphylococcus epidermidis and Staphylococcus aureus are opportunistic pathogens and common causes of hospital- and community-acquired infections. Increasing drug resistance in these staphylococci poses a significant health threat, and therefore new therapeutic strategies are needed. One novel approach to treating bacterial infections is the combined use of adjuvant compounds with antibiotics to increase antimicrobial efficacy. Our lab previously found that the phenolic compound pyrogallol increased activity of linezolid against S. aureus and S. epidermidis in a manner dependent on pyrogallol-induced bacterial oxidative stress and increased antibiotic accumulation within the bacterial cells. Here, we sought to determine whether pyrogallol's adjuvant properties could be further enhanced by altering its chemical structure. Using a synthetic chemistry approach, hydrocarbon chains, ranging in length from six to twelve carbons, were added onto pyrogallol to create novel pyrogallol-based hybrid compounds. We hypothesized that the addition of hydrocarbon chains would enhance pyrogallol's adjuvant properties, potentially by enabling greater interaction with the bacterial cell membrane. Checkerboard minimum inhibitory concentration assays were performed to test the effect of a sublethal concentration (25 µg/ml) of each hybrid compound or pyrogallol on MIC of linezolid against S. aureus USA300 and S. epidermidis NRRL-41021. We found that all hybrid compounds decreased the linezolid MIC against S. aureus and S. epidermidis. Fold-reduction in linezolid MIC due hybrid compound addition was comparable to or greater than the fold-reduction in MIC due to addition of pyrogallol. Data suggest potential utility of these hybrid compounds as antimicrobial adjuvants against pathogenic staphylococci. Ongoing work will explore the potential of the hybrid compounds to affect intrabacterial accumulation of linezolid as well as the potential interaction of the hybrid compounds with bacterial cell constituents.

177

Basic and Applied Research in Biological Sciences
Biology
Josephine Nutakki

University of New England

Effects of CD137L on Hind Paw Intraepidermal Innervation Following Sciatic Nerve Crush

CD137 ligand (CD137L) is a member of the tumor necrosis factor superfamily and known to be expressed on monocytes/macrophages and microglia. Previous research in Cao lab demonstrated a pro-nociceptive role of CD137L after peripheral nerve injury, with CD137L knockout (KO) mice displaying reduced pain-like behaviors and faster recovery after nerve injury compared to wildtype (WT) animals. To evaluate pain-related histological changes, in this study we examined the effect of CD137L on hind paw intraepidermal nerve fiber (IENF) density following sciatic nerve crush (SNC). Either sham or SNC surgery was performed on 2 male and 2 female 8-10-week-old CD137L KO mice and corresponding C57Bl/6 WT mice. Both ipsi- and

contra-lateral hind paw skin tissues were collected at days 0 (naive), 7-, 14-, and 28-days post-surgery and then processed for immunohistochemistry with pan-neuronal marker PGP9.5. The average IENF density (number of IENF/mm length of skin) was determined for each animal using ImageJ and analyzed by analysis of variance via SPSS statistical software. Following SNC, the ipsilateral IENF density was reduced in both CD137L KO and WT mice compared to day 0. In WT mice, this reduction was observed at both days 7 and 14 with the greatest reduction occurred at day 14 post-SNC (~17% reduction compared to day 0). In CD137L KO mice, the reduction was only observed at 7-days post-SNC in CD137L KO mice (~17% reduction compared to day 0). We also observed slight decrease in IENF density in the contralateral side, particularly notable in WT mice. Sham surgeries result in slight increases IENF density in both sides and both genotypes, suggesting possible systemic circulation of pro-regenerative factors post-surgery. Consistent with our previous behavioral data, our results showed that absence of CD137L resulted in a faster recovery in hind paw nerve fiber density, supporting a pro-nociceptive role of CD137L.

178

Basic and Applied Research in Biological Sciences
Microbiology/Virology
Katharina Roese, BS

University of New England

Pyrogallol potentiates antibiotic efficacy against Staphylococci

Staphylococcus aureus and Staphylococcus epidermidis are common bacterial pathogens and are leading causes of hospital- and community-acquired infections. Given the global emergence of drug-resistant Staphylococcal strains and their contribution to human morbidity and mortality, there is urgent need for novel therapeutic strategies to treat these serious infections. One potential strategy for treating drug-resistant infections is the use of antimicrobial adjuvants, which are compounds that enhance the antimicrobial activity of existing antibiotics. There is a growing body of evidence that polyphenolic compounds have adjuvant properties and can exert a synergistic effect when combined with common chemotherapeutics. Here, we tested the effect of the polyphenolic compound pyrogallol on the susceptibility of S. aureus and S. epidermidis to the oxazolidinone antibiotic linezolid, which is commonly used to treat infections caused by a range of Gram-positive pathogens. Using broth microdilution assays, we found that a sublethal concentration of pyrogallol (50 µg/ml) significantly decreased the minimum inhibitory concentration (MIC) of linezolid against S. aureus USA300, S. aureus Newman, S. epidermidis RP62A and S. epidermidis NRRL-41021. Given that pyrogallol can exhibit prooxidant properties, we also compared the effect of pyrogallol on linezolid antimicrobial activity in wild-type and a catalase-deficient strain of S. aureus USA300. The catalase-deficient mutant exhibited even greater sensitivity to linezolid in the presence of pyrogallol than did WT USA300. These data suggest that pyrogallol may increase Staphylococcal susceptibility to linezolid in a mechanism dependent on polyphenol-induced prooxidant damage. Ongoing studies aim to further probe the mechanism by which pyrogallol potentiates linezolid anti-Staphylococcal activity, by examining the effect of pyrogallol on intracellular linezolid accumulation.

179 n/a

180

Basic and Applied Research in Biological Sciences
Neuroscience
Talia Lizotte

University of New England

Saphenous Nerve Transection Results in Sensory and Sympathetic Denervation of the Mouse Tibia

The saphenous nerve is primarily a sensory nerve that is thought to innervate the tibia. Injury to this nerve is a common result of ACL repair, varicose vein surgery, and other procedures resulting in numbness and/or pain from denervation. While sensory and

sympathetic input to bone impacts bone homeostasis, little is known about the specific consequences of saphenous nerve injury on tibial innervation and bone mineral density. We hypothesize that saphenous nerve transection will result in a decrease in sensory nerve fibers in the tibia. A greater understanding of factors regulating tibial innervation will help identify risk factors influencing tibial bone mineral density.
To demonstrate that the saphenous nerve innervates the tibia, fast blue dye was injected into the tibia of saphenous nerve transected or sham control mice. Labeling was analyzed in the L2-L5 dorsal root ganglia (DRG). The highest level of retrograde labeling was observed in the L2 and L3 DRGS. Furthermore, retrograde labeling to the L2 DRG was reduced by 75% in mice with saphenous nerve transection, consistent with the paradigm that the saphenous nerve is associated with the L2 DRG. Tibial innervation was also quantified in the proximal, lateral-most periosteum of the ipsilateral tibia from mice with unilateral saphenous nerve transection and compared to the contralateral control tibiae. Calcitonin gene-related peptide (CGRP, sensory fiber marker), tyrosine hydroxylase (TH, sympathetic fiber marker) and βIII-tubulin (β3T, pan-neuronal marker) positive fibers were assessed by immunohistochemistry in cryo-embedded tibiae. Fiber length was quantified and normalized to periosteal volume. CGRP, TH, and β3T positive fibers were reduced by 40-60%. Our findings demonstrate that saphenous nerve denervation reduces innervation of the tibia. Further studies are necessary to determine the impact of tibial denervation on bone mineral density.

181

Basic and Applied Research in Biological Sciences
Neuroscience
Aidan McGrath-Conwell

University of New England

Collateral sprouting of spared nociceptors, proceeds at an accelerated rate in females

Males and females experience pain differently. Women report greater intensity of acute pain, and in addition are more likely to present with chronic/persistent pain. Using the spared dermatome model of pain neuron (nociceptor) collateral sprouting in mice and rats, we observed that female nociceptors sprout at an accelerated rate compared to males. The L2 and L3 dorsal cutaneous nerves were transected and ligated to prevent regeneration, stimulating collateral sprouting of uninjured cutaneous neurons from adjacent segments. Using the Cutaneous Trunci Muscle Reflex (CTMR) to monitor the extent of sprouting we observed that in females 50% of a skin was re-innervated after 14 days, compared to 25% in males. These behavioural data were confirmed using histology with thick skin sections. Studies are currently underway to determine if differences in growth factor concentrations in male and female tissues could be responsible for differences in rate of collateral sprouting of nociceptors. These studies could have wide-ranging implications for our understanding of sex differences in recovery from injuries and the development of chronic pain.

182

Basic and Applied Research in Biological Sciences
Physiology/pathophysiology
Neuroscience
Caitlin Quattrochio

University of New England

Analysis of mid-stage and advanced joint pain states in a murine model of trauma-induced osteoarthritis

Osteoarthritis (OA) is one of the most prevalent causes of chronic pain in US adults. OA pain can be characterized into different types. Mid-stage OA pain is characterized with intermittent joint use associated pain that dissipates with joint rest. Advanced OA

pain is often accompanied by constant dull, aching pain with unpredictable bouts of intense pain. Although NSAIDS are commonly prescribed for mid-stage OA pain and help mitigate mid-stage OA, evidence has shown that NSAIDS are inadequate for treatment of advanced OA pain. To develop new and improved treatments for advanced OA pain, improved understanding of differences in mechanisms underlying advanced and mid-stage OA pain is required. Here we compared behavioral readouts of mid-stage and advanced OA pain at different time-points following OA induced by surgical partial meniscal excision (PMX) in mice. Weight asymmetry was observed within 8-12 weeks post-PMX. PMX treated mice further demonstrated diminished spontaneous rearing behaviors starting 8-12 weeks post-surgery. Preliminary data indicate that persistent ongoing pain develops around 15 weeks post-PMX, indicating that mid-stage OA pain develops 8-12 weeks post PMX and advanced OA pain develops later, 15 weeks post-PMX. These observations indicate that this model can be used to examine mechanisms underlying transition from mid-stage OA pain to advanced OA pain in a model of trauma-induced OA. This work has been supported by the NIH through a National Institute of General Medical Sciences COBRE grant P20-GM-103643 at UNE.

183

Computational Biology & Medicine
Computer Modeling
Megan Greene

University of New England

Ring puckering thermodynamics of sulfated iduronate both in monosaccharide and trisaccharide forms

Glycosaminoglycans are linear polysaccharide chains that attach to proteins to form proteoglycans. Proteoglycans have a variety of important functions in animal cells and are located within the plasma membrane, extracellular matrix, and secretory granules. There are five categories of glycosaminoglycans, two of which contain the monosaccharide iduronate: heparan sulfate and dermatan sulfate. A common modification found within glycosaminoglycans is sulfation of the constituent monosaccharides at specific sites, which affects the structure and biological function of the chain. The variable levels of sulfation and sulfation patterns make it difficult to perform atomic-resolution structural analysis of these polysaccharide chains. Therefore, all-atom explicit-solvent molecular dynamics simulations are a reasonable approach to analyze the atomic-resolution structure of these polysaccharides. This project involves the use of all-atom explicit-solvent molecular dynamics simulations of both mono- and tri-saccharides found in heparan sulfate and dermatan sulfate that contain sulfated iduronate to assess the ring puckering thermodynamics of iduronate when it is sulfated. Each trisaccharide simulated has its own sulfation pattern commonly found in either dermatan or heparan sulfate. Our previous work showed that non-sulfated iduronate ring puckering is closely distributed between 4C1 and 1C4 chair conformations, which is in excellent agreement with available experimental data, and which demonstrates that highly accurate atomic-resolution modeling of iduronate is possible with all-atom explicit-solvent molecular dynamics simulations. In this current work, we compared force field parameters developed for phosphorylated sugars versus those developed for unphosphorylated sugars to determine which set best captures ring puckering thermodynamics of sulfated iduronate. We find that force field parameters developed for unphosphorylated monosaccharides more closely reproduce the existing atomic-resolution structural data for sulfated iduronate found in the Protein Data Bank.

184

Basic and Applied Research in Biological Sciences
Neuroscience
Caitlyn Daly

University of New England College of Dental Medicine

Mechanisms underlying sex differences in development of temporomandibular joint pain

Temporomandibular joint (TMJ) disorder impacts daily activity, psychosocial functioning, and quality of life. Pain is the primary symptom of temporomandibular joint disorder and is typically the reason patients seek medical care. Treatment for TMJ

associated pain is inadequate for many patients and chronic TMJ pain remains an important unmet medical need. TMJ pain is approximately twice as prevalent and reported as more severe in females compared to males. This indicates that there are sex differences in mechanisms driving TMJ pain that may require individualized therapies for females compared to males. We are examining mechanisms underlying sex differences in development of temporomandibular joint pain. In a rat model of TMJOA, female rats develop chronic ongoing pain and central sensitization at a five-fold lower dose of monosodium iodoacetate (MIA) than males. We examined a potential role for sex differences in pain modulation as a potential mechanism underlying enhanced susceptibility of developing TMJOA pain in females compared to males. Counter-stimulation-induced analgesia is used in rodent models to assess endogenous pain inhibition, modeling conditioned pain modulation in humans. Female rats demonstrated less robust counter-stimulation induced analgesia compared to the male rats. These observations support the hypothesis that female rats have lower endogenous pain inhibition compared to males that may underlie enhanced susceptibility to develop moderate to severe TMJOA pain. Future studies are planned to examine biomarkers of pain using non-invasive collection of saliva to further examine sex differences between males and females both within rodent models of chronic TMJ pain and in humans with acute dental pain. This research was supported by a COBRE award and Kahn Family Foundation Fellowships (P20GM103643).

185

Basic and Applied Research in Biological Sciences
Microbiology/Virology
Clinical Research
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University of New England College of Osteopathic Medicine

The Impact of Prophylactic Ceftriaxone Treatment on Antimicrobial Resistance Development by Colonizing Microbes

There are over 350,000 out-of-hospital cardiac arrest (OHCA) cases in the United States annually. Since resuscitation is rarely aseptic, aspiration pneumonia is a frequent complication occurring in up to 65% of patients. Preventing Post-OHCA pneumonia by administering antibiotic prophylaxis would likely reduce mortality and hospitalization costs while improving patient outcomes; however, this practice is counter to antibiotic stewardship principles. We hypothesized that there will be an increase in resistance to ceftriaxone and related antibiotics in the colonizing microbes of OHCA patients undergoing prophylactic ceftriaxone therapy as compared to those who are not. Looking at antibiotic resistance rates would provide the foundation for a cost-benefit ratio study to evaluate the effectiveness of prophylaxis. For this study, bronchiolar lavage (BAL) fluid and rectal swabs were collected from OHCA patients upon enrollment in the “Ceftriaxone to Prevent Pneumonia and Inflammation After Cardiac Arrest (PROTECT)” trial at Maine Medical Center. Samples were collected immediately prior to, and seven days after initiation of ceftriaxone prophylaxis. We analyzed specimens collected between September 2021 and April 2022. Antimicrobial susceptibility testing of cultured isolates was performed using disc diffusion methods on Mueller-Hinton blood agar. This revealed a trend showing the highest resistance to aztreonam and vancomycin respectively. Phenotypic analysis indicates that antibiotic-resistant isolates were detected in many patients. We have also extracted DNA from the samples and are currently amplifying it for high throughput resistome sequencing which is likely to demonstrate the prevalence of antibiotic resistance-associated alleles. The PROTECT trial continues to enroll participants and obtain data. These data will help to provide an understanding of the costs and benefits of prophylactic ceftriaxone use and related improved patient outcomes following OHCA.

186

Basic and Applied Research in Biological Sciences
Molecular Biology
Peter Wilson-Braun, B.S

University Of New England College of Osteopathic Medicine

Nerve Conduction Studies in CD137L Knockout Mice Following Sciatic Nerve Crush Injury-Induced Neuropathic Pain

Introduction: Neuropathic pain is a common and difficult to treat condition around the world. Neuroinflammatory responses within the central nervous system are implicated in the development of neuropathic pain. CD137 ligand (CD137L), a member of the tumor necrosis factor family, has been shown to play a contributing role in the development of pain-like behaviors in sciatic nerve crush (SNC)-induced murine neuropathic pain model. These behavioral changes were significantly reduced in CD137L knockout (KO) mice. We hypothesized that in nerve conduction studies (NCS) CD137L KO mice would exhibit reduced and faster-recovery of SNC-induced electrophysiological changes.
Methods: Adult C57BL/6 wild type (WT) and B6_CD137L KO mice were subjected to SNC or sham surgery at day zero (three to four males and three females per group per genotype). NCS was performed using the UltraPro™ S100 Neurodiagnostic System (Natus Medical Inc., Middleton, WI) following a published protocol. The latency, maximal amplitude, and duration of electrically stimulated responses along the sciatic nerve were recorded and analyzed. NCS was conducted before surgery (baseline), and at regular intervals until eleven weeks post-surgery. Concurrent pain-like behavioral, grip-strength, and pin-prick tests were also conducted to monitor pain-like behaviors in SNC mice. Two-way ANOVA with time and group as main factors, followed by the Holm-Sidak post-hoc analysis was used. $p<0.05$ was statistically significant.
Results: As expected, SNC induced significant reduction of grip strength and loss of pin-prick responses in both WT and CD137L KO mice, while CD137L KO mice showed faster recovery of these changes post-SNC. From the NCS, SNC induced significant increases in latency and duration, but reduced maximal amplitude in both genotypes. CD137L KO mice showed reduced increase in latency early after SNC and potentially faster recovery in maximal amplitude.
Conclusions: Our results so far support our hypothesis that CD137L depletion resulted in improved measures in NCS.

187

Basic and Applied Research in Biological Sciences
Neuroscience
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The Role of Spinal Cord Processing of Sensory Input in Maintaining Movement Evoked Breakthrough Pain

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Cancer-induced bone pain is characterized as moderate to severe ongoing pain and is commonly treated with opiates. Approximately 80% of these patients also suffer from episodes of breakthrough pain (BTP) that occur despite medication controlling their background pain. BTP episodes are often triggered by voluntary or involuntary movements, can occur as often as four to six times per day, and last about 30 minutes, dramatically reducing quality of life. In rodent models of cancer-induced bone pain, movement of a tumor bearing bone induces BTP. Blocking peripheral sensory input before movement prevented BTP. In contrast, peripheral nerve block after movement failed to reverse BTP. We hypothesize that movement-induced peripheral input initiates BTP, whereas BTP is maintained within spinal cord circuits through a reverberating circuit. Using a mouse model of cancer induced bone pain, we determined whether spinal administration of lidocaine or muscimol following hindlimb movement blocks movement induced BTP. Hindlimb movement significantly diminished rearing in tumor bearing animals compared to non-movement controls ($p<0.05$). Spinal administration of lidocaine or muscimol five minutes post-movement failed to diminish total distance moved, indicating no motor impairment. Both attenuated the movement-induced depression of rearing behaviors ($p<0.05$ vs movement-saline group). Importantly, spinal administration of lidocaine or muscimol did not alter rearing behaviors in animals that did not receive hindlimb movement. Our findings suggest that movement induced BTP is maintained at the level of the spinal cord. In conjunction with previous studies that demonstrated peripheral nerve block will prevent BTP, but not reverse movement induced BTP, we propose that the BTP is initiated by peripheral input but maintained within the spinal cord. This work

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188

Basic and Applied Research in Biological Sciences
Pharmacology
Neuroscience
Computer Modeling/Modeling
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University of New England College of Osteopathic Medicine

Identifying Candidate Drugs to Treat Tat-associated HIV Sensory Neuropathy Through Bioinformatic Analysis

Purpose: The goal of this research was to utilize bioinformatics analysis to identify drug candidates with the potential to interact with crucial signaling pathways in a Tat-induced HIV-sensory neuropathy model (HIV-SN). We utilized bioinformatics programs to evaluate the potential of current neuropathic pain drugs in their ability to target Tat-induced pathways. Second, we evaluated the potential utility of drugs approved for other conditions in their ability to target Tat-induced pathways.
Significant Results: First, we interrogated the Drug Set Enrichment Analysis (DSEA) database. DSEA was used to screen a list of known neuropathic pain drugs for their potential to affect the Tat-modified signaling pathways. DSEA identified Gabapentin, an anticonvulsant, as a candidate neuropathic pain drug with the potential to interact with the Toll Like Receptor Signaling Pathway. Then, we used Gene2Drug to identify drugs approved for other conditions that might interact with our signaling pathways of interest. Gene2Drug identified Bisoprolol, a Beta-1 Adrenergic receptor blocker, for its potential to dysregulate the Toll Like Receptor Signaling and the Apoptosis Signaling Pathways. Candidate drugs then had their FDA approval status checked. Next, we used Epocrates to detect significant drug-drug interactions between our candidate drugs and those currently used in HIV-antiretroviral therapy. Epocrates showed that Bisoprolol had more drug-drug interactions and thus Bisoprolol was excluded for further analysis. Lastly, Cytoscape, a data visualization platform, was used to illustrate candidate drug interactions with the signaling pathways. Cytoscape showed that Gabapentin interacts with the Toll Like Receptor Signaling Pathway, specifically downstream with MAPK signal transducers.
Conclusion: Using bioinformatics databases, we successfully identified two potential candidate drugs for use in a Tat-induced HIV-SN model, Gabapentin and Bisoprolol. Further screening with Epocrates and Cytoscape revealed that Gabapentin was the safer drug and interacted with the Toll Like Receptor Signaling Pathway downstream with the MAPK Signal Transducers.

189

Basic and Applied Research in Biological Sciences
Pharmacology
Clinical Research
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Mechanistic pharmacology of antipsychotic associated non-alcoholic fatty liver disease (NAFLD): Role of Iron (Fe) metabolism

Atypical antipsychotics (AA) cause metabolic side effects including insulin resistance, obesity and non-alcoholic fatty liver disease (NAFLD). We reported 4-week treatment of mice with olanzapine (OLA) or risperidone (RIS) altered hepatic histopathology and proteomics indicative of NAFLD and disrupted iron (Fe) metabolism. The specific aim of this study was to determine if AA-associated effects on NAFLD and Fe metabolism occur quickly (5-days), before AA-associated weight gain. We hypothesize that AA acutely alters expression of genes that regulate Fe metabolism, insulin signaling and inflammation prior to development of

hepatic steatosis. Plasma and livers were collected from 8-wk male C57BL/6J mice treated qd with vehicle(VEH; 0.1% acetic acid PO), or RIS (1.0 mg/kg PO) for 4-wk VEH, RIS (1.0 mg/kg PO), or OLA (5.0 mg/kg PO) for 5-d. Concentrations of OLA and RIS were determined by LC-MS/MS. Drug effects on hepatic Fe2+, Fe3+, total Fe and triglycerides(TG), and hepatic gene expression (qPCR) were determined. Results: RIS (4 wk) increased total hepatic Fe vs VEH ($P<0.05$). Preliminary data (n=3) show no effect($P>0.05$) of acute(5-d) OLA or RIS on Fe species, ferritin or TG. MMP12 expression, involved in liver remodeling, was reduced in mice with RIS or OLA vs VEH ($P<0.05$) for 5-d while it was increased in RIS mice (4 wk). Conclusion: Sub-chronic RIS or OLA treatment increased hepatic Fe, consistent with drug-associated Fe metabolic dysregulation while preliminary data show no acute drug effect on hepatic Fe or TG content. We posit that drug effects on hepatic gene expression may be altered prior to overt steatosis, as reported for acute AA effects on the cardiac proteome. We also postulate that AA-induced changes in MMP12 expression are temporal in nature, reflecting drug-associated NAFLD progression.

190

Basic and Applied Research in Biological Sciences
Physiology/pathophysiology
Molecular biology
Neuroscience
Diagnostics
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Expression of Inflammation Related Genes in Lumbar Spinal Cord in HIV-Associated Sensory Neuropathy using Doxycycline-Inducible Tat Transgenic (iTat) Mice

Introduction: HIV-associated sensory Neuropathy (HIV-SN) is a common neurological complication of HIV infection. HIV Trans-Activator of Transcription (Tat) facilitates the initiation of viral genomic transcription and contributes to HIV-Associated Neurocognitive Disorder. Previously, using doxycycline-inducible HIV Tat transgenic (iTat) mice, Cao lab demonstrated that Tat induction is associated with the development of neuropathy-like behaviors. A panel of inflammation-related genes was evaluated via NanoString technology in iTat mice, and follow-up bioinformatic analysis identified the apoptosis signaling pathway is one of the major pathways Tat could regulate. This study aims to verify the Tat-induced expression patterns of previously detected inflammation-related genes and attempt to establish potential temporal relationships between these genes following Tat induction.
Methods: 3-4-month-old iTat mice were injected with doxycycline daily for 14 days and lumbar spinal cords were collected at days 0, 3, 7, 14, 21, 28, and 35 days post-initial injection. An additional group of iTat mice was injected with pH-matched phosphate-buffered saline and their lumber spinal cords were collected at day 14 post-initial injection. RNA was isolated from lumbar spinal cord samples. A portion of each sample was subjected to cDNA synthesis and subsequent quantitative real-time PCR (qRT-PCR) in this study. Gene expression was calculated by normalizing the PCR cycle thresholds (CT) for each gene of interest to the housekeeping gene GAPDH (ΔCT) and then converting the values to fold-change. A Multi-variant ANOVA with day and sex as main factors was performed on ΔCT values using SigmaPlot and IBM SPSS to identify the significant changes among cohorts. $p < 0.05$ was statistically significant.
Results: No significant sex differences were detected. Although selected genes did not show similar expression patterns as in the NanoString study, of those that showed similar change patterns, TRAF2 displayed a statistically significant late-elevation (around 21 days post doxycycline injection) following Tat induction.
Conclusion: TRAF2 gene encodes TRAF2 protein (TNF receptor-associated factor 2), which mediates signals transduction through TNF-family receptors, regulates NF-kappa-B/MAPK/JNK pathways, and inhibits apoptosis. Our result indicates a Tat-induced upregulation of TRAF2 in the lumbar spinal cord. TRAF2 involvement in Tat-associated SN requires further investigation.

191

Basic and Applied Research in Biological Sciences
Physiology/pathophysiology
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Translocation of Polystyrene and Polymethacrylate Nanoparticles Across Caco-2 and HT-29 Cell Monolayer

As plastic nanoparticles (NP) travel down the gastrointestinal tract, their physicochemical characteristics may change in response to the change in proteins and pH. These changes may impact their absorption into the body when introduced orally. Furthermore, the amount of mucous locally secreted by the intestinal mucosa may impact the ability of charged nanoparticles to reach the absorptive membrane of the cells. We examined the impact of an in vitro gastrointestinal digestion on the hydrodynamic diameter and surface charge of 50 nm polystyrene (PS) and 25 nm polymethacrylate (PMA) nanoparticles with acid (COOH) and amine (NH2) functional groups on their surfaces. The translocation and cellular effects of the digested nanoparticles were examined after a 72-hour exposure to an in vitro model of the intestine consisting of absorptive (Caco-2) and mucous secreting cells (HT29-MTX) in three Caco-2:HT29 ratios that resulted in low, regular, and high mucous conditions. In vitro digestion changed the hydrodynamic diameter and surface charge of nanoparticles. After exposure to the digested nanoparticles, monolayer integrity and the translocation of nanoparticles were examined. Functionalized particles that passed through chyme led to larger agglomerates, larger particle sizes, than CCM. This demonstrates the effect of digestion on these particles, when digested they are more likely to have proteins form on the surfaces of nanoparticles and consequently larger in size. When looking at the charges on the surfaces of NH2 and TiO2 chyme reduced the net charge in CCM. As for the translocation of these functionalized particles, very little COOH translocated in any of the cell ratio configurations (-0.5 to 0.7%). The NH2 functionalized PMA were translocated across the monolayer to a significantly greater extent (3-fold) than the COOH functionalized PMA. The percent of the NH2 functionalized PMA translocation was not different among the three different ratio configurations. Regions of high concentration of COOH or NH2 may survive digestion, providing the impetus for NH2 to translocate to a greater extent than COOH. The translocation of the NH2 functionalized PMA particles indicates that the integrity of the monolayer have been compromised even at high mucous conditions.

192

Basic and Applied Research in Biological Sciences
Physiology/pathophysiology
Data acquisition/analysis
Clinical Research
Muskaan Chhibber

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Community Health Worker (CHW) Model for Health Care Delivery and Environment Programs focused on Initiatives in USA and India during Post-Covid

Community Health workers are equipped with the tools and resources necessary to bring about a change in the form of a rapid chain reaction. The CHW Model focuses on the practices, strategies, plans and implementation techniques needed to organize a Community Health event based on diverse agendas: health, environment and minorities. This model encompasses past experiences as a guide with tested strategies to assist future health interns with ground framework and resources to showcase the importance of adopting healthy and environment friendly practices.
The basic outline of the program is based on data collected from 15 community health events in Illinois (United States of America) and New Delhi (India). These events included back to the school fairs, cleanliness drives, health camps and educational events with data evaluation based on impressions using post-event surveys and statistical p-test analysis to ensue statistical significance. The next phase of this model highlighted utilizing the data and experience from these events to report to the health ministries of different countries for incorporation of the proposed practices in their health agendas and policies. This model specifically focused on community health engagement in Illinois (United States) and New Delhi (India), allowing the discussion of public health practices in countries with differing developmental stages and cultural ideologies. There was also a further discussion on CHW impact based on area, country, demographics, pertinent issues, and available resources.

This model, if implemented unanimously could bring about a revolution in the arena of health workers. They will be assisted at each step, will establish connections with health workers globally and share their health and environment-based agendas to collaborate and organize important events post a pandemic. This model envisions a strong established network of CHW, display of their agenda and creation of a ripple effect in the society.

193

Basic and Applied Research in Biological Sciences
Biochemistry
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Vitamin D and Bone Health

Purpose: Vitamin D plays a critical role in various physiologic processes throughout the body including metabolism, immune support, and bone formation and mineralization. Vitamin D is chemically modified by enzymatic reactions in the liver and kidney before it can be used by the rest of the body. The purpose of this manuscript was to explore the different functions of Vitamin D in the body and significant clinical effects if these levels are below normal limits.
Results: Vitamin D has significant clinical considerations in orthopedics with its involvement in different bone pathologies such as rickets and osteomalacia. In addition, Vitamin D supplementation has added benefits in patients who suffer from osteoporosis.
Conclusion: The role of Vitamin D in bone health, metabolism, chronic disease and the immune system makes it an attractive therapeutic target. Vitamin D supplements are linked to improvement in chronic pain, obesity, diabetes, autoimmune disease, and even COVID-19. While Vitamin D3 supplements are effective at raising serum calcidiol levels and improving symptoms, more research is needed to understand the exact mechanisms and formulate a comprehensive protocol for supplementation.

194

Basic and Applied Research in Biological Sciences
Biology
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Dynamics of muscle development and regeneration in neotenic axolotl

Our aim is to utilize two transgenic axolotl lines to clarify the roles of satellite cells and muscle cells/fibers in the development and regeneration of muscle. Previous studies of neotenic axolotls and adult newts reveal two modes of muscle regeneration present in neotenic axolotls and adult newts, Pax7+ satellite cells and dedifferentiation of muscle fibers. (Sandoval-Guźman et al., 2014, Cell Stem Cell, Tanaka et al., 2016, Nature Communication).

Using a Pax7 inducible Cre driver that has shown participation of satellite cells in muscle formation (Fei et al., 2017, PNAS) and our new MCK inducible line (MCK: MCK-T2a-GFPnls-ERT2-Cre-ERT2) we are addressing the role of muscle fiber dedifferentiation in limb and tail regeneration and the mechanism of muscle regeneration after metamorphosis.
Characterization of our MCK line suggests accurate and permanent labeling of muscle fibers in limb and tail. Using the double transgenic progeny of the MCK line bred to; Caggs:loxP-GFP-loxP-Cherry, lineage tracing studies were done to understand how new muscles are formed during axolotl tail development.
The results suggest that like teleost (Nguyen et al., 2017, Cell Stem Cell), new muscles are added laterally, whereas old muscles are present medially.
Next, we used the double transgenic progeny of Pax7:Pax7-P2a-memCherry-T2a-ERT2-Cre-ERT2; Caggs:loxP-GFP-loxP-Cherry to trace satellite cells during neotenic tail regeneration. Our data supports previous observations (Sandoval-Guźman et al., 2014,

Cell Stem Cell) that new muscle formation during tail development and muscle formation during limb and tail regeneration is governed by Pax7+ satellite cells (Fei et al., 2017, PNAS).
We support evidence that old muscles are present medially while new muscle fibers are added laterally. In the future, we would like to perform similar experiments with metamorphosed animals to understand lineage potential of satellite cells and muscle fibers.

195

Biomedical Engineering & Medical Physics
Materials

University of Maine

Cellulose nanofibrils-based materials as a substrate for disinfectant wipes

This study investigated a novel biodegradable/disposable substrate for disinfectant wipes composed of cellulose nanofibrils and polyvinyl alcohol. The novel substrate exhibited a high absorption capacity of alcohol-water mixtures. In addition, the substrate significantly reduced the evaporation rate of 70%-isopropyl alcohol compared to commercial cleaning wipes. Furthermore, a disintegration test showed that these substrates are flushable according to the standardized flushability evaluation procedure. Upon further testing, some variations exhibited the ability to self-heal after being torn. The substrates are prepared from renewable and water-soluble materials which reduces the negative environmental impact of the petroleum-based polymer fibers used in commercial products by minimizing the waste that ends up in landfills.

196

Basic and Applied Research in Biological Sciences
Molecular Biology
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Research Assistant III (at MHIR), M.S. candidate (Roux)

3D differentiation of perivascular adipose tissue-derived preadipocytes to explore human vascular disease

Blood vessels are composed of smooth muscle cells and endothelial cells, which are impacted locally by perivascular adipose tissue (PVAT), which surrounds vessels and is thought to play a role in vascular disease pathogenesis. PVAT regulates vascular function through release of bioactive molecules that modulate vascular tone. Our lab is interested in identifying preadipocyte populations existing in human PVAT and exploring their change in response to vascular pathologies including abdominal aortic aneurysm, atherosclerosis, and coronary artery disease. Primary preadipocytes are isolated the stromal vascular fraction of PVAT derived from patients with diverse vascular pathologies. Monolayer cell culture of primary preadipocytes is a standard method to study adipocyte differentiation. Using this method, our lab has successfully differentiated human PVAT-derived stromal progenitor cells into adipocytes and chondrocytes. To further model multicellular adipose tissue, we are optimizing 3D in vitro systems. Human primary preadipocytes spontaneously form spherical structures when cultured in low attachment environments and can be differentiated into mature adipocytes using standard methods. We used immunofluorescence to a validate adipocyte differentiation, detecting lipid accumulation and mature adipocyte markers. Immunoblot was used to compare 2D and 3D systems between progenitors and differentiated cells. 3D differentiation systems demonstrated retention of Pdgfr, a progenitor cell marker, that was lost in cells differentiated in monolayer culture. This suggests that 3D culture retains some progenitors in a differentiating spheroid, similar to PVAT in vivo. Endothelial cells cultured with preadipocytes in

co-culture produced viable structures, showing the interaction between the cell types in 3D. Utilization of the 3D systems appears to be more physiologically relevant and will give insight into mechanisms leading to phenotypic shifts of PVAT during vascular disease.

197

Basic and Applied Research in Biological Sciences
Biochemistry
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University of New England

Research Assistant, Lab Manager

A Novel Insulin-Sensing Genetically Engineered Polymer

Insulin is an important metabolic marker in cellular biomanufacturing. In order to improve the biotechnology associated with insulin, real-time sensing of the peptide hormone is essential. Biosensors are a potential tool that has promise in this area. Utilizing known protein polymers, such as the stimuli-responsive elastin-like polypeptides (ELP), we sought to produce an insulin-responsive protein polymer for applications in biosensors. We present the cloning, expression, and purification of the stimuli-responsive ELP fused to a putative insulin-binding motif.

198

Basic and Applied Research in Biological Sciences
Biology Neuroscience
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Colby College

Research Assistant

Identification and characterization of genetic modifiers of ethanol-induced behaviors in Drosophila

Authors: Li, Yixin, and Sun, Xufeng

Alcohol (ethanol) consumption and its effects have been an important part of our society. Surprisingly, however, the exact mechanism of these effects in the brain is still largely unknown. Using Drosophila, commonly known as fruit flies, as the model organism, we aim to investigate the effect of genetic modifiers on ethanol-induced behaviors. We overexpressed a mutant form of CHMP2B, a protein associated with frontotemporal dementia, in ellipsoid bodies and fan-shaped bodies in the Drosophila brain, which is involved in ethanol sensitivity and tolerance. We measured median sedation time by observing the loss of locomotion. Preliminary analysis suggested that expression of mutant CHMP2B in the targeted neurons resulted in delayed onset of sedation. In particular, the median sedation time for control and experimental flies is 3.5 minutes and 7 minutes, respectively. Our current method of sedation assay is manual and requires subjective interpretation of sedation in real-time. To better analyze sedation behavior, we are developing an automated tracking software that provides a more objective and efficient way to document fly activity. Our tracking method is also easier to implement and cheaper than other available options for fly tracking. Preliminary data from this tracker enabled us to establish baseline characteristics of various aspects of fly locomotion such as distance traveled, velocity, and positional preference in the arena, etc. The tracker will allow us to better characterize the effect of mutant CHMP2B on ethanol-induced behaviors, informing further research on the neurological mechanisms of alcohol consumption and addiction.