Welcome to the Science2011 Undergraduate Research Poster Reception at the University of Pittsburgh

We would like to take this opportunity to thank the undergraduate researchers and their faculty mentors for sharing their scholarship with the University community at this event.

Undergraduates from the School of Arts and Sciences, the Swanson School of Engineering, the School of Health and Rehabilitation Sciences, the School of Nursing and the Honors College are presenting at this reception.

We hope you enjoy the presentations and take the opportunity to engage our undergraduate researchers in conversation about their work. Inside this program you will find research abstracts written by the undergraduates.

We wish to thank Science2011 for graciously including this undergraduate research event in its 2011 program.

Office of the Provost
University of Pittsburgh
For more information about Undergraduate Research at Pitt, www.undergradresearch.pitt.edu
DETECTION OF BONE ALKALINE PHOSPHATASE ACTIVITY IN ANIMAL BONE SAMPLES

Bone Alkaline Phosphatase (BALP) is a widely accepted marker for osteoblast activity and bone growth. In biomaterials research, BALP is used to analyze a biomaterial’s ability to induce regenerative bone growth. However, direct comparisons between biomaterials are challenging because biomaterial in vivo animal studies have involved testing different animals with different biomaterials. Our study hopes to find a method for extracting BALP from Sheep femur, Rabbit arm, Rat femur, and Mouse femur in order to find baseline BALP activity levels in animals commonly used in such in vivo studies so that results from in vivo test can be normalized. Homogenized and crushed Spongy and Cortical bone samples were incubated in .01% TritonX-100 for 48 hours to extract BALP. From the supernatant of the samples, activity was calculated using spectrophotometer kinetic assays and total protein concentration was found using the BCA test. Activity levels, Protein Concentration, and Normalized Activity were compared between animal types, bone preparation methods, and bone types. Crushed bone yielded the best overall results for detecting BALP activity. Homogenized bone samples were generally inconclusive and suggested interference by the released hydroxyapatite on BALP extraction. Spongy bone consistently yielded higher values for Activity, Protein Concentration, and Normalized Activity than Cortical bone. BALP activity increased with decreasing animal size while normalized activity remained similar for most animals, implying a correlation between BALP activity and total protein content. With results providing preliminary insight to baseline BALP activity, we hope further research can yield more comprehensive results to allow for more direct and accurate comparisons between biomaterials.

NSF Engineering Research Center
UMBILICAL CORD STEM CELL DIFFERENTIATION MEDIATED BY SMALL MOLECULE DRUG

INTRODUCTION
Control of stem cell differentiation is critical to harness the potential of the cells for regenerative medicine. One target is nuclear factor-kappa B (NF-κB). SOD mimics were shown to reduce NF-κB binding to DNA, cause release of cytokines and chemokines, and PARP activation in human islet cells, resulting in higher survival and better insulin release (1). The drug protects cells from oxidative stress while function and phenotypic cell characteristics were preserved, as was the capacity to rescue diabetic mice (2).

We investigate the use of a small molecule SOD mimic drug to control cell differentiation and act as a protectant during ex vivo biomanufacturing of human umbilical cord mesenchymal stem cells (UC-MSCs).

METHODS
UC-MSCs were isolated and cultured. Cells were plated in the presence of small molecule drug and in the absence of the drug. Live cell time-lapsed imaging was used to examine proliferation and apoptosis. Osteogenic differentiation was stimulated using previously described methods; Alkaline Phosphatase (ALP) staining was used to determine stem cell differentiation (3). Cell phenotype was examined by flow cytometry (3). Levels of intracellular reactive oxygen species (ROS) were examined using dihydroxyrhodamine 123.

RESULTS
Treatment of the human UC-MSCs with the drug did not affect cell phenotype or expression of MSC markers CD44, CD73, CD105 or CD90. The addition of the drug led to significantly reduced ROS. UC-MSCs grown in the presence of the drug have significantly reduced levels of differentiation as compared to cells grown in the absence of the drug.

DISCUSSION
Addition of SOD mimic helps retain the stem cell phenotype of UC-MSCs longer by delaying stem cell differentiation. Controlling differentiation allows for larger ex vivo expansion yields through self-renewal of the cells. In vivo control of the timing of differentiation through small molecule drugs is critical for therapeutic function of the cells.

One of the most readily observable phenomena in nature is the phenotypic variation that can occur between related species. The underlying question inquires after the cause by which these differences arise. I examined expression levels and patterns for target genes in four sister Drosophila species—melanogaster, sechellia, simulans, and mauritiana. By comparing RNA seq data for these 4 species, candidate genes were selected based on those which indicated a significant level of upregulation in one species compared to the other three. I visualized the expression patterns by performing in situ hybridization and antibody staining on larvae carcasses, followed by dissection and imaging of the imaginal discs. Repeatable patterns that occur in only one species' discs suggest that gene's expression evolved a novel pattern. The explanatory mechanism for this evolved novelty can be explored via subsequent in situ hybridizations of adjacent genes and comparative sequencing to look for mutations. Once the question of how is answered, the data can then be used to examine why the species in question has evolved this unique expression pattern.

Lab funding from Pitt
Alison Austin

Biology

School of Arts and Sciences

Dr. Nancy Kaufmann

EXPRESSION OF AQUAGLYCEROPORIN AQP17664 IN LIPID STORAGE SITES SUGGESTS A ROLE IN FAT METABOLISM

Intercellular glycerol transport is crucial for an animal’s ability to maintain homeostasis and is necessary for survival. However, little is known about possible mechanisms in the cell membrane that transport glycerol. Evidence suggests that specific types of water channels called aquaglyceroporins allow glycerol molecules in and out of the cell. We are interested in learning more about the role of aquaglyceroporins in fat metabolism during early life stages by using Drosophila melanogaster as a model organism. I am investigating the expression of the aquaglyceroporin AQP17664 in adipocytes found in the fat bodies. Reverse Transcriptase-PCR was used to compare expression of AQP17664 in young life cycle stages, and showed highest expression in third-instar larvae (a life cycle stage when the insect is actively feeding and storing fat). I am currently using immunohistology technique to test for protein localization and to elucidate the structure of the fat body. By learning more about how AQP17664 functions in the transport of glycerol, we can further understand crucial mechanisms of fat metabolism.

HHMI, University of Pittsburgh
Ingrid Avendano

Electrical Engineering

Swanson School of Engineering

Dr Steven Levitan

In the field of circuit development engineers depend on writing code in textual hardware description languages, such as Verilog, to simulate and test a circuit’s functionality before creating a prototype. This research project focused on developing a visualization tool in C++ of circuits written in Verilog to debug, understand, and trace the logic of schematic. The first stage of the project required creating a program that would transform Verilog code into C++ code using parsing tools from the open source software Veripool. To test the integrity of a parsed circuit schematic simple images were created of a circuit’s tree like data structures using the open source graph visualization software Graphviz. Objects and 2D images were then created in C++ of the pins, cells, ports, nets and modules of a circuit’s netlist using the visual art tools from the openFrameworks library. The Reingold-Tildford algorithm used for social network analysis was implement in connecting the nodes of circuit elements to the wires of edges. To make the schematics easy to observe the amount of congestion and density within a select area, and ability to trace input signals on the left to output signals right of a schematic was focused on.

Swanson School of Engineering
Stacey Beasock
Health and Community Systems
School of Nursing
Dr. Jennifer Lingler

PILOT TEST OF A COMMUNICATION SKILL-BUILDING PROTOCOL FOR DEMENTIA CAREGIVERS THROUGH TWO TRAINING APPROACHES: WEB-BASED V. FACE-TO-FACE.

BACKGROUND: Successful communication among patients with dementia, their caregivers, and healthcare providers is critical, yet effective and easily accessible training programs are not widely available.

PURPOSE: To explore the effectiveness of a communication skill-building protocol for dementia caregivers, comparing a Web-based seminar to face-to-face instruction.

METHODS: An interactive skill-building training was delivered to twelve participants in person. Participants developed specific goals for communication with healthcare providers and follow-up assessments were completed. The same instructional design was used for thirteen participants of the web-based intervention through a webinar medium. Goal achievement was defined as a caregiver discussing a pre-identified issue during a medical visit as planned AND perceiving oneself to have been “very” or “extremely” successful at communicating during discussion of that issue.

RESULTS: Forty-two percent of participants in the face-to-face and 69% of those in the web-based training achieved at least one goal during their loved one’s medical visit. Face-to-face trainees were less likely than online trainees to achieve a second (25% vs. 69%; \( \chi^2 = 4.89 \ p = .03 \)) or third (8% vs. 54%; \( \chi^2 = 5.91; p = .02 \)) goal.

CONCLUSION: Web-based instruction may be more effective than face-to-face training in enhancing caregivers’ communication skills for achieving multiple goals.

Brookdale Foundation and Steven Manners Faculty Development Award
Highly Nonlinear Solitary Waves (HNSWs) are compact, non-dispersive waves that can form and propagate in highly nonlinear systems such as granular, layered, or porous materials. The feasibility of using a new (Pittâ€™s patent pending) HNSW transducer to assess the stability of dental implants was investigated. A dental endosteal implant is an artificial device that is placed in contact with oral connective tissues, with the purpose of replacing the missing natural tooth. After the typical three to six month healing time required for completing osseointegration, a crown is then placed on the implant.

The transducer described above was designed to provide the reliable noninvasive method to measure the stability of implants during osseointegration not previously available. Such a method reduces the time between the surgery and the placement of the crown, with undisputable cosmetic, economical, and clinical advantages for the patient in addition to avoiding premature restoration of the implant, which may cause failure.

HNSWs were created and monitored as they propagated into and reflected from a dental implant embedded into a sample of bone similar to jawbone in a nitric acid solution. The experiment was designed, on recommendation of Dr. Mark Ochs, DMD, to simulate bone degradation in order to determine a relationship between the change in stiffness of a material and changes in the mechanical properties of an HNSW. It was found that by studying the trends in mechanical property changes of the HNSW interactions, an assessment of the stability of the screw/implant could be qualitatively defined.
Examining Assembly Formation in Inhibitory Neural Networks

Understanding and predicting the behavior of neural networks is a key goal of mathematical neuroscience. When coupled, two inhibitory neurons will alternate firing. In larger networks, this activity can give rise to sequentially switching assemblies of neurons, as well as waves of activity. We predict the formation of these patterns of activity based on network topology and connectivity. Groups form among neurons with low connectivity between them, and group size and connection weights influence firing dynamics. We find that dynamics at each network node are insignificant compared to the topology of the network as a whole. This work was supported by NSF Award # 1004555.
IDENTIFICATION OF NOVEL SPT16 MUTANTS THAT ARE DEFECTIVE FOR TRANSCRIPTION DEPENDENT NUCLEOSOME OCCUPANCY

Recently, transcription of intergenic DNA, leading to a non-coding RNA (ncRNA) product, has been shown to be a major player in the regulation of coding genes. Our lab has identified one ncRNA, SRG1, which regulates the expression of an adjacent gene, SER3, where transcription of the ncDNA assembles nucleosomes over the regulatory regions of SER3. In addition to SRG1, the essential yeast protein Spt16 plays a part in the repression of SER3 by repositioning nucleosomes around RNA Polymerase II during active transcription. The act of maintaining nucleosome architecture is important in denying other transcription factors access to SER3 promoter elements. To investigate the mechanism by which Spt16 represses SER3, a genetic screen was performed to isolate mutations in SPT16 that derepress SER3. We identified 26 unique spt16 mutants that derepress SER3 while having no effect on SRG1 mRNA levels or Spt16 protein levels. The spt16 mutants cause a reduction in nucleosome occupancy over SRG1 correlating with the severity of SER3 derepression. By ChIP analysis, we found that histone H3 and Spt16 levels were also reduced over SER3. This trend was exhibited over other highly transcribed genes, however not over lowly transcribed genes. Taken together, we have identified novel spt16 mutants that are specific to transcription regulation and chromatin dynamics.
Shear forces are present in a variety of industrial and clinical applications with the potential to influence enzyme kinetics and stability. Existing studies have focused almost exclusively on free enzyme solutions exposed to shear and generally conclude that any kinetic inhibition is the result of agitation at air-liquid interfaces. A bioactive coating of Carbonic Anhydrase (CA) is utilized on a hollow fiber membrane based Percutaneous Respiratory Assistive Catheter (PRAC) capable of accelerating CO2 removal from blood for therapeutic lung failure applications. During operation, the PRAC device is exposed to shear forces resulting from impeller mixing. In this study, we hypothesized that shear rates relevant to the PRAC device were not expected to have a significant effect on the CA coating’s kinetic activity. Amine groups were deposited via Plasma Enhanced Chemical Vapor Deposition (PECVD) and glutaraldehyde (GA) and CA were co-incubated to crosslink the enzyme to the surface. A numerically designed parallel plate flow chamber was modified to provide a controlled shear regime at 8300 s⁻¹ for two hours. An esterase activity assay demonstrated an 11% increase in activity between the control samples and shear-exposed samples with statistical equivalence between both sets (p = 0.327). The CA coating retained its activity after exposure to shear rates comparable to those of the PRAC device and not suffer from shear-related loss of activity during bench-top catheter testing.
By studying sexual systems in the Fragaria genus, it may be possible to more fully understand the evolution of separate sexes. For a gynodioecious population (comprised of both pollen-sterile females and pollen-bearing hermaphrodites) to be sustained, females must demonstrate either a seed quantity or quality advantage to compensate for loss of male function. Fragaria vesca ssp. bracteata is a gynodioecious wild strawberry species; previous studies indicate maintenance of females may not be attributed to reallocation of resources to seed quantity. Therefore, female maintenance may result from superior seed quality due to avoidance of inbreeding depression (IBD) or enhanced maternal provisioning. Self-fertilization is common in the field, and the progeny resulting from a selfing hermaphrodite plant may show reduced fitness compared to its outcrossed siblings. Data was collected on various fitness traits of selfed (hermaphrodite) and outcrossed seeds (hermaphrodite and female) in order to investigate potential mechanisms of female advantage. Thus far, the progeny of outcrossed hermaphrodite plants are not significantly more fit than their selfed siblings, indicating low levels of IBD in the early life stages. Seeds of females, by demonstrating higher probability of germination, have a quality advantage over those produced by hermaphrodites. Considering IBD is often expressed in harsh environments, the progeny are currently growing in the greenhouse under various ecological conditions. Continuation of this research will help determine whether IBD avoidance and/or maternal provisioning are adequate mechanisms for sustaining females.

Howard Hughes Medical Institute Fellowship
Seismic waves traveling through porous rock are subject to velocity dispersion due to various factors including confining pressure, temperature, and pore filling fluids. Seismic waves are critically important to subsurface imaging related to energy exploration, geothermal resources, and studying earthquakes. Seismic surveys and wave dispersion is extremely important in understanding fluid flow in porous rock as well as tracking fluids sequestrated in rock such as CO2. Working in cooperation with the University of Pittsburgh Department of Geology and Planetary Science and the National Energy Technology Laboratory (NETL) of the U.S. Department of Energy, I have studied the effects of varying geophysical parameters on primary and shear wave spectrum traveling through and across various rocks including quartz sandstone and rhyolite. Measurements were made using the NER 1500 device and data has been organized into a rock physic database. The program Matlab was used to perform a Fourier analysis on both the primary and shear wave-types. This analysis provides us with insight into the attenuation of the each different wave-type and calculations were performed to calculate the attenuation/quality factor Q for each core sample and the effects of varying temperatures, pressures, and other relevant rock properties on seismic wave attenuation.
STEPUP: IMPROVING RELATIVE PHOTOMETRY TO DETECT SUB-JUPITER SIZE PLANETS AROUND OTHER STARS

Extrasolar planets are planets orbiting stars outside our own Solar System. The STEPUP team searches for exoplanets by looking for the slight dimming of the light from a distant star as one of its planets transits in between the star and our telescope. We collect data using a 16” Meade telescope at the Allegheny Observatory of the University of Pittsburgh and we then measure the brightness over time for both known and unconfirmed transiting systems. Data analysis is performed using a method called differential photometry, which compares the light received from the target star to the light received from one or more reference stars. We have demonstrated that many factors affect the probability of a star being a good choice for a reference star. These factors include: the star’s angular distance from the target star, the star’s magnitude, and the stability of the telescope tracking throughout the time of observation. These findings are closely related to conclusions about how the focus of stars changes differently across our focal plane. Fully accounting for these effects will lead us to better data analysis and allow us to detect ever more distant and smaller exoplanets.

University of Pittsburgh
ROLE OF C-TERMINUS IN DROSOPHILA AQUAPORIN LOCALIZATION

Aquaporin water channels are an important part of the renal system, facilitating the passage of water through cellular membranes and maintaining fluid balance. We do not understand why the family of aquaporins is so large – there are 12 aquaporin genes in humans and 7 in Drosophila melanogaster. Our hypothesis is that this variety is maintained in order to send different aquaporin proteins to different locations in a polarized cell. This polarized trafficking can be achieved through an interaction between the aquaporin protein in the transport vesicle and a cellular transport protein. We predict that the C-terminus of each aquaporin protein directs trafficking to its particular location, as a mutation in this region of human Aquaporin 2 causes a mislocation of the protein. This results in the disease diabetes insipidus. We have created fusion proteins that contain different lengths of the C-terminal end of various Drosophila aquaporins fused to an affinity domain protein, and purified the fusion protein using affinity chromatography. We performed whole fly pull downs to identify cellular binding partners of the different aquaporin C-terminals. If these binding partners are known transport proteins, that would support our hypothesis that the C-terminus of aquaporin proteins are directly involved in polarized trafficking.

HHMI
Emotional Ramifications of Unmet Needs in Neuro-Oncology Caregivers

Family members who provide care to someone with cancer are at high risk for depression and burden. Interventions to alleviate these outcomes have focused on providers’ perceptions of caregivers’ needs, rather than proven associations between specific needs and distress. In caregivers of persons with a brain tumor, we examined the relationship between 52 care needs and levels of depressive symptoms and burden. As part of an ongoing study (R01CA118711), 46 family caregivers >18 years, not currently providing care for another adult, were recruited within one month of patients’ diagnosis. Telephone interviews were conducted at 4-month intervals to identify the presence/absence of each need and to measure depressive symptoms and caregiver burden. Independent t-tests showed that, overall, caregivers reporting more depressive symptoms reported significantly (p=0.0301) more unmet needs (M=18.6, SD=9.9 versus M=11.2, SD=9.6) than those who reported fewer depressive symptoms. These results were mirrored when analyzing caregiver burden (p=0.01). Specifically, Fisher’s exact tests showed that depressive symptoms were significantly associated with: safety issues, social support, managing medications, managing uncertainty/worry, and recognizing signs of disease progression. Caregiver burden was significantly associated with: patients’ difficulty understanding, remembering, feeling distressed, and irritability. Interventions to improve outcomes for neuro-oncology caregivers should specifically address these issues.

National Cancer Institute
Nausea is a common symptom in patients with chronic disease (e.g., cancer, diabetes) and is also a side effect of general anesthesia and chemotherapy. A major obstacle in developing better strategies to treat nausea is our lack of knowledge of forebrain pathways responsible for the perception of nausea. The goal of the current study was to identify the inputs to the insular cortex (IC), a putative region for nausea processing, and specifically the input of the hindbrain emetic circuitry. Here we used musk shrews because these small laboratory mammals have a vomiting response (rodents lack this reflex). We injected the IC of musk shrews (n = 4) with pseudo-rabies virus, which is transported retrogradely from the injection site, to label multiple synaptic levels. Preliminary immunohistochemical analysis of viral transport indicates labeling in the midbrain (periaqueductal gray, parabrachial nucleus, locus coeruleus) and the hindbrain (nucleus of solitary tract, NTS; dorsal motor nucleus, DMN). These results suggest that the IC is connected to key hindbrain areas in musk shrews that are components of the emetic circuitry (NTS and DMN). A better understanding of this hindbrain-forebrain connection could potentially lead to strategies to inhibit nausea and improve the quality of life for patients.
Matthew Dye

Department of Neuroscience

School of Arts and Sciences

IDENTIFYING NEURAL PROJECTIONS TO THE INSULAR CORTEX USING PSEUDO-RABIES VIRUS IN MUSK SHREWS, A SMALL ANIMAL MODEL FOR EMESIS RESEARCH

Nausea is a common symptom in patients with chronic disease (e.g., cancer, diabetes) and is also a side effect of general anesthesia and chemotherapy. A major obstacle in developing better strategies to treat nausea is our lack of knowledge of forebrain pathways responsible for the perception of nausea. The goal of the current study was to identify the inputs to the insular cortex (IC), a putative region for nausea processing, and specifically the input of the hindbrain emetic circuitry. Here we used musk shrews because these small laboratory mammals have a vomiting response (rodents lack this reflex). We injected the IC of musk shrews (n = 4) with pseudo-rabies virus, which is transported retrogradely from the injection site, to label multiple synaptic levels. Preliminary immunohistochemical analysis of viral transport indicates labeling in the midbrain (periaqueductal gray, parabrachial nucleus, locus coeruleus) and the hindbrain (nucleus of solitary tract, NTS; dorsal motor nucleus, DMN). These results suggest that the IC is connected to key hindbrain areas in musk shrews that are components of the emetic circuitry (NTS and DMN). A better understanding of this hindbrain-forebrain connection could potentially lead to strategies to inhibit nausea and improve the quality of life for patients.

University of Pittsburgh Cancer Institute
IN VITRO EVALUATION OF THE EFFECTIVENESS OF A MAGNESIUM-BASED RING IN BRIDGING A TRANSECTED GOAT ACL

Advances in functional tissue engineering have shown promise in healing a torn anterior cruciate ligament (ACL). However, these techniques do not provide enough initial support to the knee and the ACL insertion sites deteriorate over time. Our lab plans to use a combination of suture augmentation along with a form of mechanical augmentation, a magnesium (Mg)-based ring, to enhance ACL healing. This study focuses on the structural properties, stiffness and ultimate load, of the femur-ACL-tibia complex (FATC) after transection and treatment with suture augmentation and Mg-based ring repair, and these properties will be compared to those of suture augmentation alone and bone-patellar tendon-bone (BPTB) reconstruction which is the current gold standard for ACL treatment. Cadaveric goat stifle joints were used to perform the testing, and the Mg-based ring was designed based off the dimensions of the goat ACL. Initial results show that the stiffness of suture augmentation with Mg-based ring repair is 22.1±4.7 N/mm and that the ultimate load is 211±37.5. The stiffness and ultimate load of suture augmentation alone is 14.8±0.4 N/mm and 149±3.5 N. The values for both stiffness and ultimate load for the suture augmentation with Mg-based ring repair are superior to suture augmentation alone. However, while the values for ultimate load of the suture augmentation with Mg-based ring repair are comparable to BPTB, the stiffness is lower. These results are promising, and if the stiffness for suture augmentation with Mg-based ring repair can be increased to comparable levels with reconstruction then this new technique could be a viable surgical alternative to reconstruction.

National Science Foundation Engineering Research Center “Revolutionizing Metallic Biomaterials” (ERC-RMB) and Swanson School of Engineering
Pelvic Organ Prolapse (POP) is the descent of pelvic organs into the vagina due to a loss of connective tissue and muscular support. There is no clear cause for POP but some risk factors include multiple pregnancies, chronic cough or constipation, and obesity. This condition affects approximately 50% of menopausal women nationwide and 11% of women undergo surgery by age 80. Sacrocolpopexy is the current gold standard surgical treatment and it involves implanting a synthetic mesh to support the pelvic floor. However, roughly 15% of these surgeries result in complications directly associated with the mesh including erosion of the vagina or adjacent structures. In this experiment, three meshes are being studied: Gynemesh, the most widely used in sacrocolpopexy, and UltraPro and Smartmesh, two “new generation” meshes that are ultra light weight, with distinct knit patterns and higher porosity than Gynemesh. The aims of the study were twofold: 1) To determine the effect of mesh type and ovariectomy on histology. 2) To determine the effect of mesh type and ovariectomy on apoptosis.

Two groups of nulliparous C57Bl6J mice were used employing an abdominal hernia model for testing meshes. Group 1 did not receive ovariectomy (OVX) while Group 2 received OVX. Both groups had Gynemesh, UltraPro and Smartmesh implanted in the abdomen for a one-week period. In addition, sham surgeries were performed in both groups to improve understanding of how menopause impacts surgical outcomes following mesh implantation. Control mice were only included in Group 1 and had no surgical manipulation. After one week, the mesh-tissue complex or equivalent was explanted, imbedded in Optimal Cutting Temperature (OCT), quick frozen in liquid nitrogen, and sliced into 7 Åµm sections using a cryostat. Hematoxylin and Eosin (H&E) staining method was used for histology while the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) was used to quantitate apoptosis.

As hypothesized, more apoptosis was seen in the grafted tissue, especially near the mesh holes. However, tissue degeneration occured with grafted and ungrafted tissue.
Within S. cerevisiae, a region of noncoding DNA (ncDNA), SER3 Regulatory Gene 1 (SRG1) has been identified in the regulation of its adjacent gene SER3, an integral part of the biosynthetic pathway for serine. The intergenic transcription of SRG1 has been shown to repress SER3 through the reorganization of nucleosomes over the promoter of SER3. To identify factors involved in the regulation and activation of SER3, we screened for genes that activate SER3 expression when expressed at high copy. A reporter gene composed of the SER3 promoter and the HIS3 ORF was constructed. The strain was transformed with a library of plasmids that overexpress large fragments of the yeast genome. Activation of the reporter gene by the fragments on the overexpression plasmids was then assayed by the growth of transformants in the presence of 3-aminotriazole (3-AT), a competitive inhibitor of the His3. Transformants resistant to 3-AT were digested to distinguish plasmids containing distinct regions of the genome. Each unique plasmid was sequenced to identify the region. Screening plasmid libraries that cover 95% of yeast genomic DNA identified 82 plasmids that confer resistance to 3-AT, three of which have now been sequenced. Once all of the 3-AT resistant plasmids have been sequenced, we will identify the particular genes on these fragments that activate SER3 when expressed at high copy by standard subcloning methods. Northern Analyses will be performed to ensure that the potential activator upregulates endogenous SER3 in addition to the reporter gene, indicating the presence of a true activator of SER3.
Brandon Fields

Biological Sciences

School of Arts and Sciences

Dr. Lewis Jacobson

GROWTH OF MUSCLE ATTACHMENT COMPLEXES DURING POSTEMBRYONIC DEVELOPMENT

About one hundred muscle expressed genes of C. elegans showed decreased expression after prolonged spaceflight. One of these (unc-112) encodes a protein of the dense body, an integrin-containing complex that forms muscle-muscle attachments, anchors contractile fibers to the hypodermis, and is highly homologous to human focal adhesion complexes. Knockdown via mutation or RNAi of any one of eleven members of this complex provokes protein degradation in muscle cytosol. In an unc-112ts mutant, paralysis occurs within 24 hours of disruption. RNAi against pat-2 (Î±-integrin) or pat-3 (ÂŸ-integrin) caused myofiber disruption, but only pat-2 RNAi caused myosin fiber defects. Both treatments produced muscle lacking dense bodies, with M-line disruption by pat-2 but not pat-3 RNAi, corresponding to the myosin fiber defects. Acute RNAi treatment of adults causes myofiber disruption and soluble protein degradation in 24h, implying a continuing requirement for new dense body proteins. Does this reflect addition of new dense bodies, or protein accretion to existing dense bodies? Confocal microscopy shows that the number of dense bodies per muscle cell remains constant as the worm grows, while the mean size of each dense body increases. This implies that new proteins are added to existing dense bodies postembryonically.

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arsenopyrite (FeAsS) is an iron (Fe) arsenic (As) sulfide mineral associated with metal ore that contributes to arsenic contamination in ground and drinking water around the world. A series of leaching experiments were run to investigate how changes in pH and oxygen content of ground and surface waters could influence the release of As and Fe from arsenopyrite. In these dissolution-oxidation experiments, samples of arsenopyrite immersed in varying aqueous solutions were agitated continuously over a period of 45 hours, except when the solution was periodically decanted and collected for analysis. To vary the oxygen content, 0.16M and 0.5M hydrogen peroxide (H2O2) was used. Hydrochloric acid (HCl) was added to lower the pH. Elemental analysis of the leachates by ICP-AES indicate an inverse relationship between pH and mole percent of arsenopyrite leached. In contrast, as the concentration of H2O2 increased, the amount of As and Fe leached from the mineral increased as well. In cases where the solutions were both acidic and O2-rich, the percent leached and the dissolution rate were higher than the two variables tested alone. Fe isotopes are currently being studied to see how and if isotopic fractionation occurs during this oxidative dissolution.

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THE ANALYSIS OF TITANIUM IMPLANT SURFACE COATINGS

The purpose of the project is to analyze the histomorphology of the bone-implant interface to determine whether a new biomimetic coating for titanium implants is as good as or better than the standard coating, BONIT matrix. The samples collected were small bone fragments of rabbit distal femur with calcium phosphate coated titanium implants imbedded in the tissue. To quantify the presence of osteointegration, Micro-Ct data was collected to measure bone contact volume at the implant surface. To maintain the integrity of the harvested tissue for further histological evaluation the titanium implant was dissolved using an electrochemical process developed in the lab. Once the implant was removed, the sample was re-imbedded using plastic technovit to conserve the biological structure of calcified bone in order to investigate the molecular mechanisms evoked by wear debris or degradation products derived from biodegradable polymers or metal alloys. After re-imbedding, the samples were evaluated using classic histological staining. These stains were analyzed with light microscopy and axiovision to observe the quality of tissue at the interface. Under analysis, the new coating seemed to show increased dissolution kinetics temporally thereby enhancing resorption around the implant interface for osseointegration. However, with the amount of data collected we could not prove or disprove the proficiency of the new coating because osteoblast and osteoclast activity appeared to be normal and low macrophage count showed little inflammation occurred in both coating types.
COMPUTATIONAL MODELING OF A BETA SHEET AS A CONNECTION IN A NANOGEL LATTICE

Previous research has shown that the introduction of parallel bonds into a lattice of nanogels can significantly improve both the mechanical strength and the ability of the material to repair itself after experiencing tensile deformation. We now investigate the introduction of protein structures called ß-sheets as connections between two discrete gel clusters. The entangled hierarchical structure of the ß-sheet allows for several stages of deformation that ultimately allow the connections to stretch to much greater lengths while retaining the self healing behavior exhibited by a single chemical bond. In this research, a single ß-sheet connection is modeled computationally using the Hierarchical Bell Model. The chemical properties of the proteins are varied to provide insight towards creating entire systems of nanogels connected by ß-sheets, both computationally and experimentally. Specifically, by experimenting with different equilibrium distances, stiffness, and number of entangled connections within the ß-sheet, a balance can be found between a material that is too strong and stiff to allow bond reformation and a material that is too elastic and weak to prevent mechanical failure.
PROFILIN1 DOWNREGULATION LEADS TO TRANSCRIPTIONAL UPREGULATION OF MMP9 IN BREAST CANCER CELLS

Profilin1, a key regulator of actin polymerization and cell migration, is down-regulated in breast cancer. It has been previously shown that in stark contrast to its inhibitory effect on motility and invasion of various normal cell types, profilin1 depletion facilitates breast cancer cell motility and invasion. Matrix metalloproteases (MMPs) play a key role in ECM degradation during tumor cell invasion. The overall goal of this study was to investigate whether perturbing profilin1 expression has any effect on MMP secretion in breast cancer cells. We showed that stably depleting profilin1 expression in MDA-MB-231 breast cancer cells by RNA interference leads to increased secretion of matrix metalloprotease 9 (an MMP that plays a key role in promoting breast cancer metastasis). This was followed by RT-PCR experiments which revealed that profilin1 knockdown causes transcriptional upregulation of MMP9. Finally, our preliminary studies suggested that profilin1 knockdown increases MMP9 promoter activity in MDA-MB-231 cells, and may involve signaling pathway downstream of PI3-kinase activity. Together, these findings imply a novel role of profilin1 in MMP9 regulation.
By 2020, 90% of healthcare should be evidence-based practice (EBP), but clinical translation barriers exist. To facilitate translation, legislation prompted the creation of Provider-Based Research Networks (PBRN). Of the 85,000 Nurse Practitioners (NP) nationwide, 77% specialize in Primary Care. Yet 2% of established PBRNs are NP-based. NPs are vital and instrumental to implementing EBP guidelines into clinical practice. A cross-sectional survey was conducted on 1158 Primary Care NPs in western Pennsylvania to identify 1) awareness/interest in University of Pittsburgh’s School of Nursing PBRN (NP-PittNet), 2) perceived barriers/facilitators for translation of EBP. A reliable and valid questionnaire identified EBP barriers/facilitators. Descriptive statistics were used to analyze the precoded paper and web-based Teleforms. Surveys (344=30% response rate) indicated 77% (n=244) were interested in NP-PittNet, 92% (n=316) currently practicing, 66% (n=210) specializing in Family Health, 87% (n=276) maintaining specialty certification, 87% (n=274) Master’s Degree, and 82% (n=260) had >5yrs experience. While 96% (n=304) indicated some use of EBP, two main barriers for translation were 53% (n=167) “lack of time to search for evidence,” and 31% (n=96) “organizational constraints and lack of administrative support or incentives.” Primary facilitator for translation was 91% (n=289) “keeping up-to-date with Continuing Education programs.” NPs were interested in membership to NP-PittNet, and identified barriers and facilitators to translate evidence into practice for future strategies.

Clinical Translational Science Institute: University of Pittsburgh: 5UL1RR02413
EVOLUTION OF COMPLIMENTARY, NON-OVERLAPPING GENE EXPRESSION PATTERNS IN DROSOPHILA

The vast majority of genes are conserved across species; fruit flies and humans share approximately 60% of their genes. Therefore, it is the expression of these genes, not the genes themselves, which contributes most to phenotypic diversity. However, we know very little about the process by which networks of genes evolve to create complex phenotypes. We sought to characterize a regulatory network that contributes to phenotypic diversity by looking at abdominal pigmentation in two closely related species of Drosophila, D. prostipennis and D. takahashii, which differ in the number of pigmented abdominal segments present in the males of each species. We found that the core genes involved in pigmentation are highly conserved; still, the two species exhibit significant differences in pigmentation patterns. Utilizing in situ hybridization we observed spatial differences in the expression patterns of both tan and ebony genes between D. prostipennis and D. takahashii that correlate with the differences in phenotype. Of particular interest, we found that tan and ebony maintain their complimentary non-overlapping pattern of expression as the number of abdominal segments expressing these genes evolved. These results will allow us to look more closely at the regulatory regions of these genes and elucidate the molecular mechanism that drove the evolution of this network, shedding light on how complimentary patterns of expression evolve.

HHMI and University of Pittsburgh Startup funds
Looking for Expression Patterns and Relative Abundance of the Splice Variants Found in the Fruit Fly Gene Aqp7777

Alternative splicing is a process that can increase the amount of protein products made from a single gene. Although this can be beneficial in increasing complexity of eukaryotes, it has also been found that over half of human diseases are caused by mutations that affect splicing (Lopez-Bigas 2005). The Drosophila melanogaster gene, Aqp7777, is predicted to be spliced into three isoforms, which translate into three different proteins. This gene codes for an aquaporin, which is a membrane-bound water channel belonging to a family of proteins regulating fluid homeostasis. To understand the splicing regulation of this aquaporin we aim to test the presence of each variant in flies and then determine the relative abundance of each variant. First RT-PCR with varied specific primers using adult cDNA supports the hypothesis that all three variants are expressed. Northern analysis will be used to find the relative abundance of the three splice variants. This will allow for future experiments that may give a better understanding of the purpose of splicing by helping us know which variants are expressed and in which tissues.
HASSLES, UPLIFTS, CAREGIVER VIGILANCE, AND DEPRESSIVE SYMPTOMS AMONG CAREGIVERS OF PERSONS WITH MEMORY LOSS

Aims: Caring for an individual with memory loss can be burdensome. This preliminary study examines associations among caregivers’ daily hassles and uplifts, caregiver vigilance, and depressive symptoms at baseline in a clinical trial. Methods: Participants were 36 caregivers from an ongoing randomized control trial examining an intervention targeting caregivers of individuals with memory loss living in the community. Standard measures assessed daily hassles and uplifts (Lazarus & Folkman), caregiver vigilance (Mahoney), and depressive symptoms (Beck). The sample was 76.5% female, 79.4% Caucasian, with average age 66 years (SD=12.7). Over half (55.6%) of the caregivers were spouses. Analysis included descriptive statistics and Pearson product moment correlations. Results: Findings demonstrated a significant positive association between severity of daily hassles and depressive symptoms, \( r = .396, p = .025 \). Caregiver age was found to correlate negatively with number of daily hassles, \( r = -.404, p = .024 \), severity of daily hassles, \( r = -.573, p = .001 \), and number of daily uplifts, \( r = -.464, p = .009 \). There was a trend toward an association between caregiver vigilance and depressive symptoms, \( r = .303, p = .072 \). There were no significant correlations between uplifts and depressive symptoms, \( r = -.127, p = .488 \), uplifts and vigilance, \( r = .170, p = .352 \), or hassles and vigilance, \( r = .028, p = .880 \). Conclusions: The association between increased daily hassles and depressive symptoms suggests caregivers are burdened by their responsibilities. Surprisingly, we did not see an association between reported severity of daily hassles and caregiver vigilance among caregivers. Notably, age was negatively associated with daily hassles perhaps reflecting increased caregiver acceptance or adaption to this caregiver role.

NIH-NINR #P01NR010949
Finding the Most Effective Barrier Glove for Prevention of Needlesticks in Musculoskeletal Injection Procedures

Needlestick injuries to medical personnel are estimated at 600,000-800,000 per year. Physicians who practice and perform treatments involving repeated needle penetrations of the skin have an increased risk of sustaining inadvertent needlestick; however, the density of current surgical gloves designed to protect from puncture interfere with the necessary ability to palpate and manipulate during musculoskeletal injections. The goal of this research is to lower the incidence of accidental needlestick by testing the effectiveness of gloves that act as barriers without compromising dexterity. A NANO-17 force/torque sensor (ATI Industrial Automation) was used to measure the force required to penetrate a variety of gloves by 21 and 25 gauge hypodermic needles. The Semmes Weinstein monofilament test and Grooved Pegboard Test were used to assess pressure sensitivity and dexterity of an individual wearing the gloves. The data shows that of the gloves tested, the cut-resistant glove provided the least sensitivity and dexterity. Single layers of the disposable Nitrile and PVC exam gloves provided the greatest sensitivity and dexterity but were least resistant to puncture. From the data, we can extrapolate that effectiveness of gloves as a barrier to needlestick is inversely proportional to their allowance for sensitivity and dexterity, though thin leather gloves provided the most resistance to puncture with only a small decrease in dexterity. Future work will focus on the development of two-sided gloves to minimize the risk of puncture while maintaining sensitivity and dexterity.
A growing number of newly discovered small RNA molecules of unknown function (sRNA) has piqued the interest of many scientists. A few of these sRNAs have been characterized, but most have not. An sRNA known as ‘rna3’ that is found in bacteriophage T5 was chosen for study due to its similarities to a tRNA molecule. A close relative of T5 known as bacteriophage ‘Awesome’ was found to have a similar sequence ‘rna3 region’ like T5, and it is hypothesized that Awesome also transcribes this enigmatic rna3 molecule. The first step of the experiment involves attempting to transcribe rna3 from Awesome and extract the molecule. Two approaches were chosen – plasmid expression and phage infection. First a plasmid was built that could be induced to transcribe the inserted rna3 region. The plasmid was made, sent for sequencing, and soon will be used to express rna3. Second, bacteriophage Awesome was grown in quantity and concentrated. The high titer bacteriophage stock was used to infect E. coli cells. The RNA that accumulated during phage infection was extracted and recovered. A northern blot will be performed with rna3 probes, and the results will be compared to the pattern from plasmid expression and the known size of rna3 from bacteriophage T5.

Howard Hughes Medical Institute
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Biology, Mathematics

School of Arts and Sciences

Theodore Huppert

FUNCTIONAL BRAIN IMAGING OF MULTI-SENSORY VESTIBULAR PROCESSING DURING COMPUTERIZED DYNAMIC POSTUROGRAPHY USING NEAR-INFRINGEMENT SPECTROSCOPY

Functional near-infrared spectroscopy (fNIRS) is a noninvasive, brain imaging technology that uses low levels of non-ionizing light to record changes in cerebral blood flow in the brain through optical sensors placed on the surface of the scalp. This portable system allows neuroimaging while participants perform tasks such as standing or walking. This exploratory study investigated the relationship between postural balance and the hemodynamic response recorded using fNIRS. An Equitestâ„¢ posture platform (Neurocom, Inc.) was used to create conditions corresponded to the Sensory Organization Test (SOT) conditions used clinically in dynamic posturography protocols. This is similar to a clinical test to evaluate balance disorders. A 32-channel fNIRS system was used for all testing. Four sets of postural conditions were paired to independently identify the effects of visual and proprioceptive feedback on bilateral cerebral activation in the temporal and parietal gyri. Ten healthy, right-handed subjects (6 M/4 F; age 18 to 42) with no history of vestibular disorders were evaluated. Subjects exhibited an increase in postural sway velocity and root mean square (RMS) of postural displacement after transition from the baseline conditions. All subjects similarly exhibited increased cerebral activation after the transition from baseline to non-baseline conditions. This study is the first to successfully measure blood flow response to dynamic posturography in free standing participants. Results suggest that the cortical areas in the brain may be involved in active balancing.

University of Pittsburgh Department of Radiology and the National Institutes of Health (NIH-NIA P30AG024827)
QUANTIFYING THRUST PRODUCED BY A THIN, VIBRATING CANTILEVERED BEAM

Although the problem of a vibrating cantilevered beam has been well studied, there is an insufficient understanding of the induced fluid flow and propulsive force. A better understanding of the thrust generated by a vibrating cantilever can bring improvements in propulsion systems and fluid flow control. In this study the thrust produced from thin, elastic cantilever beams is experimentally analyzed. The vibratory motion was actuated by a piezoelectric material bonded to the beam. The thrust force produced was measured using a precision balance, for different vibration frequencies and amplitudes. The performance of a single cantilever beam was characterized in terms of appropriately defined dimensionless parameters. This model was continued by considering two beams aligned edge-to-edge. The performance of the beams coupled through the fluid was accurately characterized in terms of the dimensionless parameters. Enhancement and reduction of the propulsive performance was observed for the coupled beams, depending on the specific operational parameters. The greatest enhancement occurred when the beams were separated by a very small distance and vibrating out of phase. This work can be used to support the development of a comprehensive model to predict the thrust force and flows generated by oscillating cantilevers.
SYNTHESIS AND ACTIVITY OF 5-[[E]-2-{4-[[E]-2-{1H-INDAZOL-5-YL}ETHENYL]-3-METHOXYPHENYL}ETHENYL]-1H-INDAZOLE

Background: In the past, the only reliable diagnosis for Alzheimer’s disease was an autopsy. A test that has the potential to detect the disease in living patients would be beneficial to the study of Alzheimer’s disease and would allow the utility of therapies for treating the disease to be tested. The purpose of the synthesized compound is to bind to and label amyloid-beta deposits that are characteristic of Alzheimer’s disease in the brain of living patients.

Methods: Using the Wittig-Horner reaction, 5-[[E]-2-{4-[[E]-2-{1H-indazol-5-yl}ethenyl]-3-methoxyphenyl}ethenyl]-1H-indazole was synthesized from 5-formyl-1H-indazole and, p-xylylenediphosphonic acid tetraethyl ester. It was characterized by NMR, HPLC, UV/Vis spectroscopy, and fluorescence spectroscopy. A binding assay was done against [H-3]Chrysamine G.

Results: The proton NMR and HPLC results indicated that the compound was pure. The absorbance maximum of the UV/Vis spectrum was at 382 nm. The excitation maximum of the fluorescence spectrum was at 383 nm and the emission maximum was at 448 nm. The binding assay showed that the compound binds weakly (Ki=8.3 micromolar) to the same sites on amyloid-beta sheets as does [H-3]Chrysamine G (Ki=1 nanomolar).

Conclusions: The compound was successfully synthesized but is a weak candidate for binding to amyloid deposits in the brain.

Cure Alzheimer's Fund
Approximately 50% of the 33.2 million people living with HIV worldwide are women. There is a great need to develop a female controlled method for protection against HIV infection. Microbicide drugs like Tenofovir (TFV) are meant to prevent or minimize HIV infection. The overall goal for this project is to develop a quick dissolving vaginal film containing TFV. The first step in development of quick-dissolve films is the selection of appropriate polymer(s), which is currently based on lengthy experimentation. Our efforts were focused towards the development of a quicker process for polymer selection. Solubility parameters were calculated for TFV and various potential polymers using group contribution method. From this data, a theoretical prediction of TFV solubility in respective polymers was made. The theoretical prediction was cross checked by X-ray diffraction experiments. Overall a good correlation was obtained between theoretical prediction and experimental observations. All the polymers reduced TFV crystallinity with increasing polymer to TFV ratio. The qualitative analysis of data showed the extent of reduction in TFV crystallinity was higher in presence of hydroxyethyl cellulose and polyvinylpyrrolidone-K90 than other polymers. Efforts are underway to deduce a quantitative relationship between various polymer to TFV ratios and reduction in TFV crystallinity.

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Seeding stem cells onto electrospun fibrous biomaterial scaffolds with structures that mimic the native extracellular environment has gained recent experimental attention in the development of cell-based tissue engineered cartilage. Manipulating electrospinning parameters allows for the creation of fibers of various sizes from the nano- to micro-scale. Several studies have investigated the effects that varying fiber sizes have on chondrogenesis with conflicting results. Due to cell-cell interactions being key in mesenchymal chondrogenic differentiation, initial cell-seeding density on the biomaterial scaffold is likely a key variable. In this study, both the effects of scaffold fiber diameter and seeding density were examined. Adult human MSCs were isolated with IRB approval from patients undergoing total hip arthroplasty. Poly-e-caprolactone (PCL) nanofiber (NF) and microfiber (MF) mats were prepared by electrospinning. MSCs were seeded onto scaffolds at 1E5, 5E5, 2E6, or 4E6 cells/cm³ and maintained in chondrogenic differentiation medium containing 10 ng/ml TGF-b3 for 21 days. Scaffolds were then collected for real-time RT-PCR, biochemical assays to quantify DNA, collagen and glycosaminoglycan (GAG) content, and histological staining. Cells seeded at higher densities had higher cell numbers and high chondrogenic gene and protein expression. At low densities, MF constructs produced more collagen than NFs. Results were confirmed by histological results. These results suggest there may be a threshold in NF constructs where effective cell density overcomes effects of fiber diameter. Further investigation is needed into the cellular mechanisms of chondrogenesis on NF and MF to develop effective tissue engineered cartilage replacement.
L-cysteine, a thiol that enhances the opening of T-type Ca2+ channels in sensory neurons has an excitatory effect on neonatal but not adult bladders. Neonatal bladders also exhibit large amplitude, coordinated spontaneous bladder contractions (SBCs); whereas adult bladders exhibit small amplitude, uncoordinated SBCs. Therefore, T-type Ca2+ channels might be over expressed in neonatal bladders and contribute to the bladder overactivity in these preparations. The present experiments examined the effect of a selective T-type Ca2+ blocker (3,5-dichloro-N-[1-(2,2-dimethyl-tetrahydro-pyran-4-ylmethyl)-4-fluoro-piperidin-4-ylmethyl]-benzamide, TTA-P2) on SBCs and on the excitatory effects of agonists in bladder strips from neonatal and adult rats. Bladders were removed from isoflurane anesthetized rats, cut into four strips and maintained at 37°C in organ baths, initially under 1 gm tension. Neonatal strips exhibited large amplitude, rhythmic contractions that were suppressed by TTA-P2 in a concentration (0.050-1 uM) dependent manner. TTA-P2 also reduced baseline tone but did not suppress the contractions induced by L-cysteine (1mM), carbachol (0.5-1 uM) or K+ (80 mM). Nifedpine (1 uM) an L-type Ca2+ channel blocker completely blocked SBCs and the contractions elicited by the agonists. TTA-P2 did not alter SBCs, baseline tone or contractions elicited by agonists in bladder strips from adult rats. These results indicate that T-type Ca2+ channels contribute to SBCs in neonatal rat bladders but not in normal adult bladders. This raises the possibility that T-type Ca2+ channels blockers might be useful for treating certain bladder disorders, such as spinal cord injury, that cause re-emergence of neonatal-like pattern of bladder over activity.

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SYNTHESIS AND APPLICATIONS OF BIOLOGICALLY-COMPATIBLE METAL-ORGANIC FRAMEWORKS

Metal-organic frameworks (MOFs) are an exciting class of new materials akin to natural zeolites synthesized by the linking together of metal ions or clusters by multi-functional ligands. MOFs demonstrate many useful properties such as high porosity, crystalline and tunable structures, and easy functionalization of pores. Because of these properties, MOFs are potentially useful in the absorption of gases such as hydrogen and carbon dioxide, making them useful for fuel storage and carbon sequestration. In addition, MOFs are also being investigated for use in separations of complex mixtures, drug delivery, and catalysis. Our group has focused on the synthesis of so-called biologically-compatible metal-organic frameworks (bioMOFs), beginning with the synthesis of bioMOF 1 and its mesoporous analogue bioMOF 100 in 2009. These materials are based around a zinc-adenine cluster linked together with benzenediphenylcarboxylate ligands. The zinc-adenine combination is less toxic than many other starting materials used in the synthesis of MOFs, and the lower toxicity might potentially allow for a wide range of biological applications. My poster will describe our group’s work towards developing a series of analogues of both of these materials using different dicarboxylate ligands as well as diaminopurine in the place of adenine. Additionally, my poster will describe our work on the applications of MOFs, specifically the trapping of heavy-metal ions for water purification and the encapsulation of fullerenes for facile pore modification.
RESPONSE OF E. COLI CELLS TO HIGH LEVELS OF ANTIBIOTICS

An understanding of bacterial response to antibiotics is crucial for effective treatment against infection. Important to understanding this relationship is determining how bacteria respond as function of population size. This study investigated expression levels of Escherichia coli containing a plasmid that controls resistance to a specific type of antibiotic, chloramphenicol (Cm). In this study, E. Coli cells were grown in media containing high concentrations of chloramphenicol. The effect of various antibiotic concentrations was analyzed over populations of different sizes to understand how the expression level of individual bacteria changes with bacteria and antibiotic concentrations. It was determined that, on average, the expression of bacteria in environments with high antibiotic concentrations had lower expression levels than those environments without the same type of antibiotics. This suggested that when faced with harsh environmental conditions, bacteria slow their growth. This experiment also showed evidence of a relationship between growth and population density. When the same antibiotic concentrations were added to different, but low, concentrations of bacteria, this resulted in differing responses. It is this understanding of bacteria populations and how they respond to specific stresses that will lead to a more comprehensive understanding regarding how to combat bacterial infection.
LIPOCALIN 2 MEDIATES HOST SURVIVAL RESPONSE TO KLEBSIELLA PNEUMONIA INFECTION BY MODULATING MACROPHAGE POLARIZATION.

The recent exponential increase in antibiotic resistance compels researchers to explore alternatives to antibiotic therapy. A better understanding of antimicrobial proteins and their role in our immune system can lead to a novel way to combat pathogens. Lung injury in the pneumonia model between wild type and Lcn2 knockout mice were assessed to study the antimicrobial protein, lipocalin 2. Survival tests and rectal temperatures revealed a significant difference between wild type and knockout in their ability to fight pathogens. Lipocalin 2 is known to fight pathogens by actively inhibiting bacteria’s iron intake. Further investigation via histology, bronchoalveolar lavage, western blot, and real time PCR led us to discover that macrophages in knockout mice were wrongly polarized. Based on these data, we believe that lipocalin 2 is not only responsible for preventing bacterial growth, but also play a role in our adaptive immune system by polarizing the macrophages.

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Anticipatory psychological stress increases chemotherapy-induced nausea in cancer patients, but the mechanisms responsible are not known. Musk shrews have been widely used as an animal model in nausea and vomiting research; however, unlike rats and mice (which do not vomit), shrews have not previously been used in stress research. We examined a classic stress response in shrews, using contextual fear conditioning, a procedure that produces freezing—a “fear” response—in rodents. For 1 or 3 successive days, shrews in the experimental groups were placed in a novel chamber and received repeated footshocks, while control animals received no shock (n’s=7-10/grp). On the test day, each animal was placed in the chamber for 5 min without shock. Behavior was recorded by digital camera and analyzed with computer software. As hypothesized, contextual fear conditioning resulted in less locomotion and more immobility (freezing). Fear conditioning also suppressed exploratory behavior in standard anxiety tests (e.g., open field, elevated plus maze, light-dark box), but did not cause vomiting. These data provide the first evidence in the literature that musk shrews show similar stress responses to rodents and suggest the utility of this species for mechanistic studies of stress effects on chemotherapy-induced nausea and vomiting.
Tobacco smoking is a major risk factor for head and neck squamous cell carcinoma (HNSCC); one of the leading causes of cancer mortality. Nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) a major product of cigarette smoke and is a well known carcinogen. Approximately 20 - 40% of former smokers continue to smoke 1 year post-HNSCC diagnosis. The role of NNK in tumor progression remains unknown. We hypothesize that exposure to carcinogens such as NNK contributes to the invasive and metastatic ability of HNSCC. In addition we believe it affects the surrounding stromal tissue, facilitating tumor progression. In order to examine the effects of NNK on HNSCC invasion, we used Matrigel-coated transwell invasion chambers, where the cells were plated on top of the Matrigel for 24 h following which the invaded cells were fixed, stained, and counted. Our data demonstrate that NNK (10 nM) increases invasion of HNSCC cells. NNK-induced HNSCC invasion is increased further in the presence of stromal fibroblasts. Neutralizing antibodies to the hepatocyte growth factor (HGF) mitigates HNSCC invasion induced by NNK and stromal fibroblasts. These findings provide important insights into mechanism whereby NNK contributes to HNSCC tumor progression.

University of Pittsburgh head and neck SPORE career development award to SMT and Department of Otolaryngology, University of Pittsburgh
Nonalcoholic steatohepatitis (NASH) recurs in almost all patients who undergo orthotopic liver transplantation. To identify proteins involved in the pathogenesis of recurrent NASH, we performed a retrospective proteomic analysis on paraffin-embedded donor, allograft, and explanted liver tissue. Six patients who underwent orthotopic liver transplantation for NASH were divided into two experimental groups. Pre-transplant donor biopsies and post-transplant allograft liver biopsies with recurrent NASH were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) yielding 6119 total protein identifications across the 12 samples. The one-way repeated measures ANOVA test, applied to the robust primary peptide spectral count data, revealed 42 proteins that were differentially expressed between the pre- and post-transplant samples. A supervised cluster analysis, utilizing these significant proteins (p < 0.05), discriminated the two groups (see figure). Using Ingenuity Pathway Analysis, major biologic function network included Molecular Transport, Energy Production, and Nucleic Acid Metabolism. Of 42 differentially abundant proteins, 24 were increased in abundance and 18 were decreased in the pre-transplant donor liver samples. Two particularly interesting proteins include COX5A and CPN60, which are related to fatty acid oxidation. This study demonstrated that LC-MS/MS analysis can effectively determine protein expression patterns from needle core biopsy specimens and, more importantly, was capable of identifying dysregulation of protein expression in recurrent nonalcoholic fatty liver disease following liver transplantation. Confirmatory immunohistochemical analysis to identify the location of protein expression will be performed. Important candidate proteins identified in the analysis involved in the pathogenesis of NASH can be targeted for future genetic studies and pharmacologic therapy.
VISUALIZING NEURAL TRAJECTORIES IN CONTINUOUS MOTION BCI CONTROL

For those with deficits in arm function, robotic prosthetic arms offer an opportunity to restore motor function. Previously, we demonstrated the use of a seven degree-of-freedom (DOF) arm by a non-human primate. We are now working to provide control of a seventeen-DOF, human-like robotic arm that has four DOFs in the upper arm, three in the wrist, and ten in the fingers and is capable of participating in a variety of grasping procedures. Our ability to finely control the robotic arm is only as good as our ability to correctly interpret neural signals. The aim of this project is to create a graphical user interface that will enable the user to visualize neural responses in real time during an experiment. As an immediate goal, we intend to use this to identify neurons that poorly fit our model and remove them from the decode--hopefully improving prosthetic arm function. In the long-term, we hope that this visualization method will contribute to the basic understanding of motor-cortical function.

Center for Neural Basis of Cognition, DARPA, NIH
Pillared-layered compounds are an exciting class of porous materials which show promise for capturing CO2 and other impurity gases from the flue of a coal fired power plant. They belong to a general class of compounds called Metal Organic Frameworks (MOFs) which are formed by reacting transition metal ions with various organic linker molecules. The chemistry used to synthesize these compounds creates a material whose pore size, network type, and chemical makeup is controlled in a directed and rational manner by the choice of metal ions and organic linkers. A series of functionalized 4,4’ bipyridine linkers were synthesized and incorporated into a pillared-layer MOF system based on a nickel cyanide complex. Specifically, the effect of adding methyl, methoxy, aldehyde, fluorine, and fluorinated methyl functional groups to the 4,4’ bipyridyl linker was evaluated. CO2 and N2 isotherms were collected to determine the porosity and adsorption energetics associated with the addition of these functional groups to the pore wall of the MOF. The heat of adsorption data indicates that none of the functional groups investigated resulted in an increased affinity for CO2 adsorption in an isostructural pore network. This experimental observation may result from a strong electronic interaction between CO2 and the pyridine ring which minimizes the electron donating and withdrawing effect of the functional groups evaluated. The role of these functional groups on N2 adsorption has also been examined.
The ulnar collateral ligament (UCL) of the elbow, primarily an elbow stabilizer, connects the humerus to the ulna. Athletes commonly injure this ligament due to repetitive overhead throwing motions. A study was designed to understand the relationship of the cross-sectional area at the proximal, mid, and distal locations of the UCL at a range of angles to different loading conditions. The angles used were 0°, 30°, 45°, 60° and 90°. Four human cadaveric specimens were dissected to completely expose the UCL and clamped into a 6-axis robot. The UCL was then scanned using a Faro Arm laser scanner and processed using Geomagic12. The proximal and distal cross-section planes were defined by anatomical markers with the mid plane defined as the midpoints between those planes. The resulting cross-sections, averaged over all trials and all specimens, were graphed at each angle for each loading condition at each location. The distal location was calculated to have the greatest area, followed by proximal then mid for all trials. Proximal decreased in area when loaded over all trials except 45°, mid showed no consistent change, and distal decreased for all trials. In the future, cleaner scan data could be obtained by removing any shiny markers, used in other studies, from the ligament and reducing the overall sheen of the tissue. This study can be applied to an MRI or CT scan of an athlete to compare the athlete’s UCL cross-sections to existing baselines to give foresight to whether the athlete’s UCL is experiencing excessive load.

Allegheny General Hospital, West Penn Allegheny Health System, Pittsburgh, PA; University of Pittsburgh, Pittsburgh, PA
THE TRANSCRIPTION FACTOR SOX11 AFFECTS NEURITE OUTGROWTH AND NERVE REGENERATION

It is common for dorsal root injury to occur in major motor vehicle accidents. Regeneration of the central processes of dorsal root ganglion (DRG) neurons after such damage does not occur to the same degree as what ensues after peripheral nerve injury. Therefore, regenerating damaged centrally projecting axons in order to restore sensory function after these accidents is of considerable interest. One of the key differences in the ability of DRG neurons to regenerate their peripherally and centrally projecting axons is in the induction of pro-regeneration gene expression. One particular transcription factor, SRY-Box containing gene 11 (Sox11), has been found to be successfully upregulated after peripheral but not central nerve damage in DRG cells. Further, inhibition of Sox11 has been found to block both neurite growth in vitro and peripheral nerve regeneration in vivo. Therefore, there may be a potential association with the lack of enhanced levels of Sox11 gene expression after central nerve damage and the deficiency in successful regeneration of centrally projecting axons into the spinal cord. This study analyzed neurite growth and changes in gene expression in primary DRG neurons transfected with modified plasmids that overproduce Sox11. We found that overexpression of Sox11 indeed promotes neurite growth and alters gene expression and responsiveness in vitro. These studies may provide a better understanding of whether induction of Sox11 transcription may be beneficial for central nerve regeneration.
Cell mediated immunity is comprised of T-cells which are highly specialized cells that aid in the immune response to foreign and unknown pathogens invading the body. Dysfunctional T-cells are associated with various immune related diseases such as Alzheimer’s (AD) and Parkinson’s disease, amongst others. To better understand the mechanisms of compromised immunity in AD, we are developing a proteomics method to profile proteins in mammalian T-cells. In order to achieve this, we are using positive T-cell isolation from spleen tissue, extraction of proteins, offline strong cation exchange liquid chromatography (SCX-LC) fractionation, and reverse phase LC-tandem mass spectrometry (MS/MS). From preliminary results of T-cells from rat spleen tissue, 25,475 peptides and 755 proteins were identified. This presentation will discuss preliminary results and ongoing method development for mouse T-cells. Future experiments involve the application of the optimized work flow to T-cells from an AD mouse model.
USE OF CP2 COMPOUND AS A THERAPEUTIC APPROACH TO RESTORE MEMORY AND MOTOR FUNCTIONS IN MURINE MODELS OF ALZHEIMER'S DISEASE

Soluble oligomers of amyloid-beta (Aβ) peptide have been shown to directly affect mitochondrial dynamics, trafficking, and function. These factors most likely contribute to memory loss in transgenic animal models and Alzheimer's Disease (AD) patients. CP2, a member of the tricyclic pyrone (TP) family, has been shown to prevent Aβ oligomerization in cellular AD models. We demonstrate that CP2 penetrates the blood brain barrier and specifically accumulates in the brain. Long-term CP2 treatment does not cause noticeable side effects and does not interfere with animal breeding or development. Short and long-term administration of CP2 in multiple mouse models of familial AD leads to significant improvement in memory and motor phenotype. Moreover, CP2 treatment restores mitochondrial trafficking in neurons from AD mice treated with CP2 from birth. Our data indicate high potential of CP2 as an AD therapeutic.
SPASM: STOCHASTIC PARTICLE APPROACH TO SIMULATING MORPHOGENESIS

We construct a stochastic modeling tool, called SPASM, to analyze the dynamics of groups of moving cells. In our modeling framework, cells exist as groups of particles. Each particle represents a fixed volume, and particles interact through fluid and/or elastic forces. Stochastic noise represents dynamics of the cytoskeleton. Our tool includes options for a cell cortex and extracellular matrix, and allows the user to control cell stiffness and the viscosity of the environment. We ran many simulations to test our modeling framework. To analyze the biological process of cell sorting, we distributed cells of different types and varied adhesive strengths between cells. We found that a high ratio of homogeneous adhesion to heterogeneous adhesion caused some cell sorting to occur. We placed cells above an adhesive surface, representing a plate used in experiments, to observe the change in aspect ratio (height/width) of the cells. A higher adhesive ratio caused the cells to round out slightly on top. SPASM can simulate mitosis and cell growth. Although many dynamic cell models already exist, ours is one of the first to include both fine details of individual cells and adaption to large tissues.

Research Experience for Undergraduates in Modeling and Industrial Applied Mathematics (site: North Carolina State University)
Niketh Nair
Computational Biology
Arts & Science
James Faeder

MODELING OF CELL CYCLE CONTROL THROUGH GROWTH FACTOR SIGNALING PATHWAYS

While a number of computational models have been developed that describe the cell cycle (Csikasz-Nagy et al. 2006, Novak, Tyson 2004) and cell signaling dynamics (Stites et al. 2007, Markevich et al. 2004), few computational models predict how cell signaling events effect the cell cycle. Development of such a model would allow us to better understand nonlinear effects of signal transduction pathways on fundamental cell processes as well as to understand how mutations in these pathways change the cell’s behavior. Using the rule-based framework of BioNetGen (Faeder et al., 2009), we have integrated models that describe signal transduction (Stites et al. 2007), transcriptional regulation (Novak, Tyson 2004), and the cell cycle (Novak, Tyson 1993) into one model that describes how an extracellular signal effects the cell cycle and cell proliferation. We apply known oncogenic mutations in the Ras signaling pathway (Stites et al. 2007) to our model in order to observe the effects of the modified signal on cell proliferation. We seek to identify the signal properties that lead Ras activation to trigger unregulated cell growth and proliferation and to relate those to specific genotypic changes.

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The aim of this project was to develop methods to measure functional connectivity using functional imaging and to evaluate changes within these networks in healthy aging. This systematic procedure employs AFNI (Analysis of Functional NeuroImaging) and other techniques to process and display fMRI images. The preprocessing of resting-state data accounts for motion correction, slice timing correction, co-registration, and regression of nuisance variable. Using a seed-based correlation, the relationship between an activity time-series in a single region of the brain and all other voxel time-series was determined to reveal that region’s functional neural network. To examine the differences in global networks and connectivity, a combinational voxel-wise seed-based approach was developed and applied to resting-state data of various ages. This method calculates the number of connections within a brain, accounting for the variability in brain size. The results reveal a slight negative trend in overall connectivity with age, suggesting that the brain becomes more loosely connected with age. This decrease in overall connectivity could contribute to characteristic behavioral changes with aging including slower reaction time and poorer memory recall. The developed analytical methods could be utilized to study other resting-state data of participants with neurodegenerative diseases such as Alzheimer’s disease.
Protein degradation in muscle plays a role in maintaining homeostasis, but this process can proceed to pathological extremes in disuse atrophy, sepsis, cancer cachexia or age-related sarcopenia. In C. elegans, autophagic protein degradation is promoted in muscle by FGF-triggered activation of the Ras-Raf-MEK-MAPK-RSK cascade, which in turn is inhibited by IGF signal via PI3K-PDK-Akt and activated by elevated intramuscular Ca2+ through CaMKII. Protein phosphatases may act as positive or negative modulators of this signaling network, but little is known of their identities or regulation. In C. elegans, the protein phosphatase FEM-2 has 37% amino acid similarity to the human version, hFEM-2, which dephosphorylates CaMKII in vitro (Yu et al., 2001). In fem-2ts mutants, shifting to non-permissive temperature causes acute degradation of muscle-specific LacZ reporter protein and both qualitative and quantitative defects in mobility. RNAi knockdown of CaMKII prevents LacZ degradation at the non-permissive temperature, as well as preventing quantitative movement defects. Active pT286-CaMKII levels are increased in fem-2ts mutants after a shift to non-permissive temperature, along with increases in active P-MAPK and P-MEK-2. P-CaMKII levels follow a non-linear course to the elevated levels observed 48 hours post-shift, with an initial peak ~2-6 hours, followed by a drop from 6-24 hours. This evidence supports the hypothesis that FEM-2 phosphatase negatively regulates the CaMKII-Raf-MEK-MAPK signaling pathway by dephosphorylating CaMKII, and thus negatively regulates muscle protein degradation.
THE CHARACTERIZATION OF MYCOBACTERIOPHAGE LITTLEE MAJOR CAPSID GENE AND PROTEIN

Bacteriophages are viruses that infect bacterial hosts. They are the most common and diverse organisms on Earth, and collectively comprise the single largest pool of genetic information. dsDNA tailed bacteriophage capsids are comprised primarily of many copies of the major capsid protein. The major capsid protein structure, as determined by x-ray crystallography and cryo electron microscopy is conserved among known dsDNA tailed viruses despite a lack of protein sequence similarity. Comparative genomics reveals that the major capsid gene in Mycobacteriophage LittleE has been disrupted by a substantial insertion, which is likely a self-splicing intron: the first such identified in the mycobacteriophages. To characterize the nature of this insertion into the major capsid protein gene of LittleE, we examined the major capsid protein using SDS-PAGE, and determined the N-terminal sequence through electroblotting and Edman degradation. A single band of approximately 43kDA that began DLDRNGG was observed, supporting the conclusion that the major capsid protein is made from a fusion of the two major capsid protein gene products with some N-terminal cleavage due to the use of a Delta-domain during capsid assembly. Isolation of mRNA from LittleE infected cells, RT-PCR and cloning will be performed to determine if the major capsid protein fusion is created through the splicing of an intron in the major capsid gene transcript.
Epidemiological research suggests that cannabis use during adolescence increases the risk for schizophrenia. However, whether there is a cause-effect relationship between cannabis use and schizophrenia is unknown, in part because the effects of cannabis on the brain are poorly understood. Cannabis’ effects are primarily mediated by Δ9-Tetrahydrocannabinol (THC) acting on the cannabinoid 1 receptors. Here, we report data from studies assessing the effects of THC administration (5 days/week, 12 months) on adolescent rhesus monkeys. Interestingly, THC administration was found to impair the monkeys’ performance on a working memory task (Verrico et al., Soc for Neurosci Meeting 2010). To investigate potential neural correlates of this behavioral impairment, at the end of THC administration we performed electrophysiological recordings from pyramidal neurons in in vitro slices from the prefrontal cortex (PFC). To determine if exposure to THC during adolescence affects inhibitory synaptic transmission from GABAergic interneurons, which undergo substantial maturational changes during adolescence, we recorded inhibitory post synaptic currents (IPSCs) using whole-cell patch clamp techniques. We quantified the IPSC amplitude, rise time and decay time to investigate if THC altered GABAA receptor density or subunit composition. We found that THC did not significantly change the IPSC rise time or decay time, indicating that the subunit composition of GABA receptors was not altered by THC administration. However, IPSC amplitudes tended to be larger in the THC-exposed animals, suggesting more GABA receptors on PFC pyramidal cells. To determine whether THC affects the excitation/inhibition balance, we are currently investigating the properties of glutamate-mediated excitatory synaptic transmission.

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ENDOTHELIAL CELL DERIVED EXTRACELLULAR MATRIX SCAFFOLD FOR PANCREATIC ISLET CELLS  

Endothelial Cell Derived Extracellular Matrix Scaffold for Pancreatic Islet Cells  

Phillip Olsen, Saik Kia Goh, Ipsita Banerjee  

Endothelial cells are known to play a central role in organ development by providing developmental cues. Recently, extracellular matrix (ECM) has also been identified for supporting in-vitro cell growth and differentiation. However, the exact mechanism of cell-ECM interaction is still largely unknown. We hypothesize the ECM to be an important mediator in cell development, particularly endothelial cell derived ECM for pancreatic islets cells which do not produce ECM and have been reported to rely on endothelial cells for ECM support. We first test this hypothesis by culturing the β2-TC-6 islet cells on decellularized endothelial cell matrix. Optimal decellularization efficiently removes cellular and nuclear material from tissue while preserving important ECM proteins such as collagen IV, laminin, and fibronectin that are necessary for cellular functionality. The protocol used to create decellularized endothelial cell derived matrix was optimized by systematically applying different chemical detergents to confluent cell culture plates followed by qualitative immunohistochemistry analysis and DNA quantification. An optimized decellularization protocol for endothelial cells utilizing sodium dodecyl sulfate was developed. The pancreatic islet cells were then cultured on the decellularized ECM and analyzed for survival, proliferation, and functionality.

Swanson School of Engineering Undergraduate Research Summer Internship
Reviews of papers by peers have a whole range of quality, and there are many different aspects that make reviews helpful. One such aspect is problem-localization (pLocalization), which is part of a model by (Xiong, 2010) created with a computer program in mind, SWoRD (Scaffolded Writing and Rewriting in the Discipline), an online peer-reviewing interface paired with an online community, to automatically predict a peer review's helpfulness. pLocalization is determined via a decision tree, which is also modeled by (Xiong, 2010), and the second node of the tree involves utilizing domain words. The domain words are a list of words related to a certain topic with a certain quantifiable confidence associated with them. Topic Signatures, created by (Lin, 2000), is a program that automatically extracts domain words, and was used to generate the list. By varying certain features used by Topic Signatures and by varying the use of Xiong's pLocalization features, an attempt was made to automatically recognize pLocalization more accurately. Improving the detection of pLocalization through improving the list of domain words could potentially improve the automation process of determining review helpfulness, and in turn could provide feedback to the reviewer on how to incorporate pLocalization in his reviews.
DEVELOPMENT OF NEAR INFRARED SPECTROSCOPY FOR USE IN EVALUATING LONG TERM STANDING AND WALKING FATIGUE

Standing or walking for long periods of time has been associated with lower extremity discomfort and fatigue (Bousseman et al, 1982; Cham & Redfern, 2001). In order to gain a better understanding of this fatigue, an objective measure, such as muscle oxygenation, is needed. Near Infrared Spectroscopy (NIRS) is a non-invasive technique used to measure changes in oxygenation over time. NIRS takes advantage of the differences in optical properties of oxygen bound- and unbound-hemoglobin to detect oxygen saturation levels. Previous studies have used NIRS to study short-term muscle fatigue in the back and fingers (McGill, 2000; Crenshaw, 2009). The goal of this project was to determine the feasibility of using NIRS to quantify lower extremity fatigue during long term standing and walking. On separate testing visits, subjects stood for a period of four hours or walked for two hours while NIRS data (ISS, Inc., IL) were recorded from the dominant leg rectus femoris. Subjects filled out a questionnaire rating their tiredness every 30 minutes. Rectus femoris percent oxygen saturation [SO2%] was compared to the discomfort ratings. Preliminary results showed a decrease in SO2% during long-term standing and walking. Additionally, decreased SO2% was associated with increased discomfort ratings. These results suggest that NIRS could be as a measure of long term muscle oxygenation and blood flow changes, and those measures are associated with subjective measures of discomfort. If successful, the impact of interventions such as new footwear or flooring could be evaluated.

Internal funding
Respiratory syncytial virus (RSV) is the leading cause of acute lower respiratory tract infections in children worldwide, yet there is no vaccine and no effective treatment. While adult rodent models have been instrumental in understanding numerous mechanisms of the host immune response to RSV disease, many aspects of the immature infant immune system cannot be recapitulated in adult animal models. An infant mouse model of primary RSV infection is critical for determining the role of immature alveolar macrophages in RSV disease. We compared the phenotypic immune responses of lung macrophages among 2 to 4 day-old and 7 day-old neonatal mice to determine the effect of age following primary RSV infection. Using a novel infant mouse model of RSV infection, we determined that age at initial infection has a significant effect on lung macrophage phenotype.
ETHNICITY AS A VARIABLE IN DENTAL RESEARCH

The use of race and ethnicity terms in research continues to generate controversy. The general recommendation is using definitions which basis can be explained and to use those definitions consistently. In our Dental Registry and DNA Repository project, we have access to self-reported ethnicity data. In this project, we invite individuals to be part of a registry and ask them to provide a saliva sample for DNA extraction. The sample is linked to the clinical records and this information is kept available for future research related to dentistry. The purpose of this work is to see whether the self-reported ethnicity data matches with definitions generated from DNA evidence reflected by the genotyping data from the lab. Currently, the registry has recruited approximately 2,800 subjects, and they are asked to report their ethnicity according to the following groups: Caucasians, African Americans, Hispanics, Asians, or other, ethnic groups besides the previously mentioned. Using an Illumina 610- Quad chip, the lab researchers genotype single nucleotide polymorphisms (SNPs) from the saliva samples. From the DNA evidence, the data suggests that the self-reported ethnicity matches with definitions generated from the genotyping data, with a few exceptions. In conclusion, ethnicity should be considered a suitable variable in dental research.

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THE EFFECT OF AGING ON MRI CONTRAST

Typically, diseases associated with aging are characterized in MRI by volumetric changes in gray matter regions of the brain. However, it is often difficult to accurately quantify regional gray matter volumes. Normal aging and neuropsychiatric diseases of aging have been tied to the degradation of brain matter where distinct white or gray matter becomes less distinguishable. This can cause a decrease in the MR image contrast between the gray matter and surrounding white matter, which could be of diagnostic utility. T1-weighted high-resolution volumetric MR images were acquired from 96 subjects (median age=71, 68 female). Images were intensity normalized with an N3 filter, and regions of interest (ROIs) in the sub-lobar regions were extracted using atlas-based segmentation (with a non-linear registration) and masked so that only white matter appeared in the internal capsule and only gray matter appeared in the putamen, caudate, and pallidum. Contrast (C) was calculated for each ROI as the difference in median intensity between white (WM) and gray (GM) divided by the sum. $C = 2(WM - GM) / (WM + GM)$

A significant negative correlation was observed between the regions’ contrast and age, appearing most strongly in the right caudate region ($T(95)=-2.44$, $p<0.0165$). There was no significant correlation of gray matter volume in this region with age ($T(95)=0.91$, $p<0.3628$). These results suggest that contrast is a sensitive neuroimaging marker of brain aging. In future, the method of contrast diagnostics could be tested in patients with neuronal disease or impaired brain function.

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THE GEOMETRY OF UP AND DOWN CORTICAL STATES

The phenomenon of cortical Up and Down states is believed to play some role in working memory and attention. Networks of neurons in the cerebral cortex alternate spontaneously between an Up state, a noisy period with higher potential, and a Down state, a relatively dormant period at lower potential. From experimental data, an external stimulus of sufficient strength can cause the network to transition from the Down state to the Up state. A stronger stimulus can kick the network to the Up state transiently before returning to the Down state. The propagation of the Up state has been observed to move in travelling waves. We construct a Wilson-Cowan model network that explains this recurrent behavior, and determine conditions necessary for bistability. Coupled populations of excitatory and inhibitory neurons are examined via numerical phase-plane analyses with different levels of inhibition. We use the Gillespie algorithm to add stochasticity to the populations. Our model shows that the variance in the Up state is greater than in the Down state. The model also captures the stimulus dependent behavior described above, and transitions from the Down state to the Up state occur in travelling waves. These findings are in accord with experimental data. Developing a spatiotemporal extension of this model, we determine the conditions necessary for stability of the Up state in space, and demonstrate that spontaneous pattern formation is possible only in the Up state.

NSF
IN VITRO BIOMECHANICAL EVALUATION OF MAGNESIUM-BASED SCREWS FOR ACL RECONSTRUCTION

The anterior cruciate ligament (ACL) is one of the most commonly injured ligaments in the knee. For reconstructions, surgeons use polymer or metallic interference screws, but these result in complications for the patient. Therefore, the use of magnesium (Mg)-based screws is being explored due to their potential to degrade at a safe and controlled rate and their acceptable mechanical properties. In a preliminary study, prototypes of Mg-based screws resulted in an unsatisfactory ultimate load and graft slippage. The objective of this study is to evaluate 2nd generation Mg screws in-vitro in terms of initial fixation strength and resistance to graft slippage and to compare those results to commercially-available polymer screws. The first phase of this study involved performing an ACL reconstruction on a goat cadaver stifle joint using polymer screws and evaluating them by performing cyclic creep tests and a load-to-failure test. The data was compared to that of titanium and Mg-based screws in order to suggest improvements in various Mg screw design parameters. Biomechanical data for four femur-ACL graft-tibia complexes (FATC) was collected. The total residual elongation of the FATC was 1.9 ± 1.1 mm, the stiffness was 49.4 ± 4.3 N/mm, the ultimate load was 235 ± 71 N and the ultimate elongation was 9.7 ± 1.6 mm. Thus far, polymer screws result in comparable or superior graft slippage and initial fixation strength when compared to titanium, pure Mg, and alloy AZ31 screws. These results suggest that the polymer screws have an effective design on which that of the 2nd generation Mg screws can be based.
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THE HARDNESS OF MARTENSITE IN DUAL PHASE STEELS (DPS) USED IN THE AUTOMOTIVE INDUSTRY

Dual-phase (DP) steels contain ferrite and martensite and are the most popular of the high strength steels used in the automotive industry. In alloy design and processing of dual-phase steels, understanding the structure-property relationship is essential for the steel to fulfill its function. During the processing of DP steels, all too often the industry accepts equilibrium values and properties leading to inaccurate data. In this study, the behavior of DP steels was investigated during intercritical annealing in the Ferrite + Austenite (\(\alpha + \gamma\)) two phase field of the Fe-Fe3C equilibrium phase diagram. Four small sections of a dual-phase steel were intercritically annealed at 775\(^\circ\)C for different times. Hardness indentations were taken to test the change in properties of both the \(\gamma\) and \(\alpha\) phases, and the measured values were compared to what is predicted from the phase diagram. JMatPro, a thermophysical property program, suggests an equilibrium hardness value of the martensite transforming from the austenite to be 45HRC. Annealing for 1, 10, 40 and 100 minutes resulted in hardness values of 55.07HRC, 52.51HRC, 51.89HRC and 51.46HRC, respectively. These hardness values prove that a higher percentage of carbon exists in the austenite phase than what equilibrium conditions suggest.

BAMPRI
THE EFFECT OF CIDOFOVIR ON THE LIFE CYCLE OF HUMAN POLYMOMAVIRUS BK

Background: Polyomavirus BK lies latent in many healthy subjects. It can be activated if the human immune system is suppressed by medication. Hence, this virus causes kidney disease in patients who undergo transplantation for kidney failure. There is an unmet medical need for developing drugs to treat this virus.

Methods: We tested the anti-viral effect of Cidofovir in-vitro. This drug chemically is a chemical analog of cytosine and is expected to get incorporated into replicating viral DNA molecules. For anti-viral testing, BK virus was grown in a cell-culture system. The rate of viral replication in the presence or absence of the drug being was assessed using a quantitative polymerase chain reaction assay.

Results: The 50% cellular inhibitory concentration (IC50) and 50% effective concentration (EC50) were found to be 63.9+/-17.2 and 36.3+/-11.7 μg/ml respectively. The therapeutic selectivity index calculated as a ratio of the toxic effect to the anti-viral effect was determined to be 2.3. Conclusions: Cidofovir can inhibit BK virus replication by inhibiting DNA synthesis. This inhibitory effect targets viral replication better than cellular DNA replication. Nevertheless, the selectivity index is rather low. Chemical modifications of cidofovir to increase its anti-viral effect are needed to develop it into a more useful drug.
INVESTIGATING THE FUNCTION AND PHENOTYPES OF THE CDC73 C-DOMAIN

The conserved Paf1 complex (Paf1C) is critical to eukaryotic transcriptional regulation. The Paf1C associates with RNA polymerase II (RNAPII) aiding transcription in vivo in part by promoting histone modifications. My work focuses on the Cdc73 subunit of the Paf1C. Mutations in the human CDC73 gene can cause various types of cancer, most notably Hyperparathyroidism Jaw Tumor Syndrome. The VanDemark lab determined the structure of the highly conserved C-domain of CDC73 using X-ray crystallography. From this structure they predicted several residues to be critical to its function. Three different approaches are being used to define the function of the C-domain: an amino acid substitution screen; a yeast two-hybrid screen; and an affinity purification. Using the VanDemark lab’s predictions I performed site-directed mutagenesis of likely important residues, a serial dilution assay was used to screen the amino acid substitutions for loss-of-function phenotypes. Two mutants, Trp-321-Ala, and Glu-306-Ala, Asp-309-Ala, Arg-310-Ala showed phenotypes indicating that they may be important for C-domain function. I am also using affinity purification to purify CDC73 proteins and identify interacting partners utilizing western blots, silver staining, and mass spectrometry. The final results of these experiments will help elucidate the function of the CDC73 C-domain.

HHMI, NIH
Across all living organisms, regeneration is found in many forms. However, with their ability to reorganize and regenerate virtually all tissues within their body plan, planarians are arguably the masters of this biological process. For this reason, planarians have become an increasingly important model of regeneration. In planarians, the Wnt signaling pathway is critical for axis formation after amputation. Knockdown experiments to manipulate the activity of Beta-catenin, a key transcriptional regulator in Wnt signaling, cause heads to regenerate instead of tails, or visa versa, resulting in double headed or double tailed worms. However, genes involved in Wnt signaling such as the TCF/LEF family as well as a paralog of ß-catenin (ß-catenin-3) have yet to be characterized in planaria. Several methods were used to better understand the role of ß-catenin-3 and the five TCF/LEF genes. To characterize expression patterns, in situ hybridization was performed using riboprobes for each gene. Some lef genes were found in the brain as well as in posterior cut sites while others were present only in the brain. To understand their function, RNAi (via feedings and injections of dsRNA) was targeted against each gene and phenotypes were assayed using known anatomical fluorescent markers. No new phenotypes were discovered as a result of the knockdown experiments. However, double knockdowns may be used in the future to address the possibility of redundancy within the family of genes.
MATERNAL LINEAGE IN HYBRID ZONE POPULATIONS OF FRAGARIA X ANANASSA SSP. CUNEIFOLIA

Hybrid zones may form where two species that are interfertile come into contact. When the fitness of hybrid offspring is not significantly different from that of the parental populations, a stable hybrid zone is formed. One such hybrid zone exists in the northwestern United States where populations of the naturally occurring Fragaria chiloensis and Fragaria virginiana hybridize to form Fragaria x ananassa ssp. cuneifolia (Rosaceae). The composition of the hybrid zone populations at a genetic level has not yet been examined, and thus the relationship between the progenitor species and the hybrid—does one species act as maternal donor more frequently than the other, or are they equally likely—is unclear. The direction of hybridization has consequences for the transfer of adaptive traits or the loss of genes from one of the parental lineages. In addition, by genetically characterizing these hybrid zone populations, a first step will be taken towards establishing the role that the hybrid zone plays in the evolution and differentiation of sex chromosomes in Fragaria. Using restriction digestion, eight populations within the hybrid zone were cytотyped to determine their maternal donor. This was done using single nucleotide polymorphisms present in the chloroplast genomes that differentiate F. chiloensis and F. virginiana. Distribution across the eight populations varied between a single maternal donor for the entire population and up to a fifty-percent split between F. chiloensis and F. virginiana. Wide sampling across the range is in progress and will determine if there is a geographic pattern to maternity.
Objective: The objective of this project was to evaluate a one day program “Advanced Breast Cancer: Living with Health and Wellness” for women from Western Pennsylvania with metastatic breast cancer (MBC). Aims: Evaluation of knowledge of illness and self care strategies among women with metastatic breast cancer pre and immediately post MBC workshop was conducted and the acceptability and satisfaction of the one day workshop was completed. Methods: The conference, held at Pittsburgh’s Gilda’s Club, included one key note speaker focusing on MBC illness and five breakout sessions. A Likert scale of 1 (poor) through 5 (great) was used to measure pre and post knowledge in 5 specific knowledge areas. Open ended questions assessed the workshop’s effectiveness. Results: Twenty-five participants returned evaluations. Post mean knowledge scores improved in all knowledge areas. Overall content and ability to “be open” regarding disease stage among other women with MBC were rated favorably. Participants requested more treatment, symptom management content and information related to employment and disability. Four themes of open-ended response: met needs for information, need for annual conference, interest in specific topic areas, and enjoyed fellowship with women with MBC. Conclusion: The promising results from the MBC conference indicate that this conference was well received and able to address daily life informational needs of women with MBC.
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ANALYSIS OF TILTED SHINGLE ROOFING SYSTEM IN HOT CLIMATE

Houses consume huge amount of energy in heating and cooling. A well designed roof system can significantly reduce the energy use of a house in heating and cooling. In this report, a model of a new system of roof is built, of which the shingles can be tilted up. The model was tested with lifting distance of shingles and shingle base colors as parameters, which may affect the thermal properties during a summer day. Highly lifted shingles with a base color of white is proven to result in the lowest attic temperature, reducing the heat flux through ceiling by up to 32.1%.

Mascaro Center for Sustainable Innovation
Duchenne’s muscular dystrophy (DMD) is an X-linked genetic disorder that affects roughly 1 in 3500 live male births. It is due to mutations in the dystrophin gene, leading to a defective protein product, resulting in degeneration of muscle and walking and breathing difficulties, and eventual death. Inflammatory cells infiltrate muscle and cause increased levels of TNF-α and other cytokines, which induce the upregulation of the NF-κB pathway. This pathway is known to suppress MyoD, a protein required for muscle differentiation. These proteins are required for muscular regeneration and improvement of muscle strength in the mouse model for DMD (mdx). Hence, it is important to inhibit the pathway in muscle to maintain muscle stability and strength. One of the negative regulators of the pathway is the deubiquitinating enzyme, A20, present in muscle. We evaluated the overexpression of A20 in skeletal muscles of mdx mice by injecting an A20 expressing AAV8 virus in mdx mice. We sectioned the quadriceps and diaphragm, staining them with Hematoxylin and Eosin, imaging the sections, and counting the number of necrotic fibers and regenerating fibers in them. The results showed a significant lack of regeneration, but not necrosis in A20-injected mice when compared to the saline-injected mice.
Diffusion imaging is a relatively new MR imaging modality used for the visualization of white matter anatomy. Many different pipelines of diffusion imaging exist but all of them are fundamentally based in measuring the diffusion of water inside a neuron. Using that data, a 3D rendering can be made of all the fiber pathways in the human brain. At the University of Pittsburgh, we use a pipeline called High Definition Fiber Tracking (HDFT) that aims to solve the problem of tracking through fiber crossings. This project’s primary endpoint is to use HDFT to investigate the anatomy of the superior cerebellar peduncle (SCP). The SCP is the principal output for the deep cerebellar nuclei. It originates in the Dentate Nucleus and ascends through the Red Nucleus en route to the Thalamus. Directly anterior to the corpora quadregemini, the SCP undergoes a partial crossing. This project aims to quantify the volume of fibers in the subsegments of the SCP and present an observed and statistically significant lateralization. Also, we have included data on a comparison of methods between HDFT and another diffusion imaging pipeline called Diffusion Spectrum Imaging. The data supports the proposed increase in resolution in the use of HDFT.

Human Connectome Project
EDMONTON SYMPTOM ASSESSMENT (ESAS) SCORES IN HEAVILY PRE TREATED WOMEN WITH METASTATIC BREAST CANCER (HPTWMBC)

Background: Women with MBC are treated aggressively with chemotherapy until death. Symptom control is paramount. HPT is 3 or more MBC chemotherapies. Mean ESAS in historic (all cancers palliative care) control - 28 to 34. Aims: The aims of this project are: To determine the expected level of symptom distress of HPTWMBC. Compare historic ESAS scores to HPTWMBC. Correlate symptom distress and number of previous chemotherapies.

Methods: Retrospective review of medical records, Subjects were women with HPTW MBC from one breast cancer practice, June 2010 - December 2010. Instrument was nine item, 10 point ESAS visual analog scale, range 0 (absent) to 10 (worst possible severity), range 0-90.

Results: Since June, 2010, 30/285 women (9.5%) were HPT MBC. Median age - 52 years. Women received mean 4.6 different chemotherapies (range 3-9) prior to ESAS assessment. Mean total ESAS score was 16.3 (range 0-58). Greatest individual item scores - fatigue (3.4) and pain (2.7). Total ESAS scores showed no significant correlation with previous treatment number.

Discussion/Implications for Nursing: HPTWMBC did not have high distress scores as compared to historic control with no significant relationship between pretreatment and symptom distress. These findings indicate that chemotherapy is well tolerated in HPTWMBC.
Metabolic syndrome is epidemic in the US with the cost of obesity-related cardiovascular complications rising exponentially. Metabolic syndrome, an inflammatory process, is in part mediated by reactive oxygen species (ROS). For example, reports have revealed elevated secondary indicators of tissue ROS production in both animal models of obesity and clinically. To further elucidate this obesity-induced increase in ROS formation, we analyzed murine tissue after 20 weeks on a high fat (HF) diet (60% calories from fat) by in vivo immunospin trapping. Mice treated with the EPR spin trap DMPO demonstrated enhanced DMPO staining in kidney, liver, skeletal muscle, heart and lung tissue compared to age-matched controls on normal chow (NC). This enhancement in DMPO staining is indicative of elevated ROS formation and subsequent protein free radical formation. Importantly, protein expression and enzymatic activity of xanthine oxidase (XO), a key source of tissue and vascular ROS production, was also significantly elevated in the plasma and tissues of HF mice. Experiments are in process to determine the extent of XO’s contribution to the observed differences in DMPO staining. These data demonstrate the utility of immunospin trapping as an indicator of overall oxidative damage under conditions of chronic, nonlethal ROS formation.
Understanding Mechanical Behavior of Hydroxyapatite via Molecular Dynamics Simulation

Hydroxyapatite (HAP) is a bioactive ceramic that has been widely used in orthopedic application. The goal of this research is to investigate fracture toughness of the perfect hydroxyapatite crystal via molecular dynamics (MD) simulation performed in LAMMPS software. Based on the result from the previous studies, modified Buckingham potential with Columbic cut-off is employed to describe the pairwise interaction of HAP crystal. The model was then validated by performing uniaxial and biaxial tensile loading test and compares the pre-existing results. Subsequently, fracture toughness was calculated using cracked specimens in plane strain and plane stress modes; the true value of fracture toughness was obtained by averaging theses two data. Good agreement between calculated fracture toughness and experimental data in literature demonstrated the capability of modified model to predict fracture mechanic of HAP.

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DEVELOPMENT OF LEARNER-ADAPTIVE CAPABILITIES FOR DIALYSIS TRAINING SIMULATOR

Medical simulation is a promising field that is changing how healthcare professionals are trained. By allowing doctors and nurses to practice life-critical procedures before they treat patients, the risk to patients is reduced and the learning process becomes more efficient. Simulation-based training for medical devices is particularly useful, as patient safety depends on the ability of healthcare professionals to operate and troubleshoot these complex machines. Pitt’s Simulation and Medical Technology R&D Center is developing a Dialysis Training Simulator (DTS). This system augments a traditional renal dialysis machine with user interaction sensors and audio-visual feedback to teach nurses how to operate the machine and respond to emergency situations. The goal of this project was to augment DTS with learner-adaptive capability, specifically, to provide automated tutorial feedback based on the skill level of the user. The trainee’s response time to perform a requested action is used as a metric of proficiency. If the time exceeds a preset threshold, an “automated tutor” feature provides the user with customized multimedia guidance and coaching. This capability enables users to learn at their own pace: DTS can assist novices while not hindering the progress of more advanced users, and enables self-learning with automated supervision and support.

Swanson School of Engineering
In the field of smart materials, shape-memory polymers (SMPs) have been given a considerable amount of interest due to their ability undergo significant recoverable and controllable deformation upon the application of an external stimulus such as heat. For the case of thermal activation, SMP have a relatively high elastic modulus (i.e., stiffness) while below a transition temperature (T_{trans}) (that is unique to the polymer composition) and a considerably lower modulus when the SMP are heated above T_{trans} (i.e., activated).

The activated SMP can be deformed into (essentially) any desired shape, and then cooled to "lock" the new shape indefinitely. The primary concern of the current research is to create an approach to optimally design the activation process for an SMP to efficiently achieve a desired shape change. Towards achieving the overall goal, this study presents an approach to model the thermal activation and deformation process (i.e., actuation) of an SMP using the finite element method for two different activation methods: boundary heating and internal wire heating. The two techniques are compared and contrasted based on the ability to perform a specified shape change in the minimal amount of time, least amount of expended energy, and without damaging the material.
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DEFINING THE DEGRADATION OF THE EPITHELIAL SODIUM CHANNEL

The epithelial sodium channel (ENaC) helps regulate the sodium concentration in the blood and osmotic homeostasis in kidney cells. Mutations in ENaC that alter cell surface expression lead to diseases, such as Liddle’s syndrome and pseudohypoaldosteronism. ENaC levels are tightly controlled, and several mechanisms exist by which ENaC can be degraded, including endoplasmic reticulum-associated degradation (ERAD). During ERAD, misfolded proteins are targeted to the cytosolic proteasome where they are destroyed. ENaC can also be degraded in the lysosome/vacuole and possibly by cytosolic proteases. To define how ENaC is degraded, the yeast, Saccharomyces cerevisiae, was transformed with a methionine repressible ENaC expression vector for each subunit (α, β, or γ). Cycloheximide chase experiments tested the effect of specific mutations on ENaC. Our results show that ENaC degradation is proteasome dependent suggesting that ENaC is degraded by ERAD. However, a large percentage of the α subunit is still degraded when the proteasome is inhibited, suggesting this subunit is degraded by another mechanism. When cells lacked a functional vacuole, each subunit’s degradation was proficient, indicating the vacuole is not responsible for degradation. Experiments in which cells lacked a cytosolic protease, known as calpain, indicate that this protease is dispensable for each subunits degradation. Future experiments will be conducted to determine whether each subunit exhibits distinct degradation requirements. Ultimately, we hope to better understand how ENaC is degraded and therefore how its function is modulated.

HHMI
CHARACTERIZATION & GENOMIC ANALYSIS OF MYCOBACTERIOPHAGE GADJET

Mycobacteriophages are viruses that infect mycobacterial hosts, including Mycobacterium tuberculosis and Mycobacteria smegmatis. As the most numerous and genetically diverse organisms on the planet, they are particularly useful in the investigation and exploration of the hosts’ genomes, as well as comparative studies amongst the growing number of bacteriophages. Mycobacteriophage Gadjet was isolated from a soil sample collected in Latrobe, Pennsylvania in 2003, and forms very tiny plaques on lawns of M. smegmatis mc2155, a nonpathogenic relative of M. tuberculosis. Electron microscopy of Gadjet virions indicates that the phage has a long flexible non-contractile tail, and therefore is a member of the Siphoviridae. The genome sequence of Mycobacteriophage Gadjet was completed using 454 pyrosequencing technologies. BLASTN alignment of the complete genome sequence indicates that Mycobacteriophage Gadjet is a member of Cluster B3. This cluster assignment is further supported by restriction digest patterns of the whole genome and bioinformatic analyses of the genome sequence. To date, attempts to recover lysogens of this phage, and other phages of this cluster, have not been successful.
CREATION OF A NON-INVASIVE GLUCOSE SENSOR VIA THE DEVELOPMENT OF A FUNCTIONALIZED POLYMERIZED CRYSTALLINE COLLOIDAL ARRAY

Charged colloidal nanoparticles self assemble into a crystalline lattice that Bragg diffracts light. The lattice will directly determine which wavelength(s) of light is diffracted. These can be polymerized into a hydrogel network in order to maintain this ordering and to keep the diffracted wavelength(s) constant as a result. A polymerized crystalline colloidal array (PCCA) can be functionalized so that the polymer surrounding the colloidal nanoparticles is sensitive to certain chemicals and the chemical environment. This sensitivity results from chemically induced imbalances in the hydrogel that is relieved via swelling or contracting of the hydrogel volume. This volume change will in turn change the colloidal nanoparticle spacing, changing the color of the light diffracted. The goal of this project is to use this sensing motif to create a hydrogel sensitive to changes in glucose concentrations. This technology is intended to be incorporated into a contact lens for real time non-invasive glucose concentration determination.

Pennsylvania Space Grant Consortium Research Scholarship
DISTRIBUTION OF NEURONS WITHIN THE BED NUCLEUS OF THE STRIA TERMINALIS THAT PROVIDE DIRECT INPUT TO THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS

Activation of the hypothalamic-pituitary-adrenal (HPA) axis in response to stress leads to the synthesis of glucocorticoids by the adrenal cortex. Dysregulation of the HPA axis is a symptom of many stress-related diseases, including major depressive disorder. HPA activation is initiated in the brain by the recruitment of neuroendocrine neurons within the paraventricular nucleus of the hypothalamus (PVN). Determining the neural circuits that provide input to the PVN is critical for understanding how the HPA axis is recruited by stress, and how this activation is terminated to restore baseline functioning. Neurons within the bed nucleus of the stria terminalis (BNST), part of the limbic forebrain, are known to innervate PVN neuroendocrine neurons that drive HPA axis activity. However, the precise location of PVN-projecting BNST neurons has not been reported. To identify these BNST neurons, we iontophoresed the retrograde tracer cholera toxin subunit B into the neuroendocrine region of the PVN, and mapped the distribution of retrogradely-labeled BNST neurons using Stereoinvestigator mapping software. Results demonstrate that PVN-projecting BNST neurons are most prevalent within the principal subnucleus of the posterior BNST, and within the anteromedial, dorsomedial, and magnocellular subnuclei of the anterior BNST. Relatively few PVN-projecting neurons were located within the transverse, oval, or juxtacapsular subnuclei. The identification of these specific PVN-projecting BNST subnuclei improves our understanding of the central neural control of HPA axis output, which should ultimately contribute to improved treatments for HPA dysregulation in stress-related diseases.

NIMH Grant 59911
Whitney White

Biological Arts and Sciences

Dr. Anthony Schwacha, PhD

UNRAVELING THE FUNCTION OF BETA-HAIRPINS IN THE EUKARYOTIC REPLICATIVE HELICASE

Each day, billions of cells in your body undergo DNA replication. While this common process may seem trivial, failure to correctly perform this task leads to cancer. A critical, regulated step in DNA replication is the unwinding of double-stranded DNA into single strands. In eukaryotes, this process is completed by the replicative helicase, Mcm2-7. Unlike other known helicases composed of six copies of an identical subunit, Mcm2-7 is composed of 6 unique and essential subunits. Together, these subunits form a ring-shaped structure within which DNA unwinding occurs. The exact mechanism of this is currently unknown. My project focuses on 2 questions: 1) what amino acids manipulate the DNA to facilitate unwinding, and 2) which of the 6 different subunits are responsible for this activity? In better studied helicases, it has been shown that DNA binds to the helicase through small loops located on each of the subunits. These loops, known as β-hairpins, contain a conserved lysine that points into the center of the channel. β-hairpin loops appear to be conserved in each Mcm subunit. My goal is to use site-directed mutagenesis to change all six lysines to uncharged alanines, and test these changes in vivo in S. cerevisiae. My work indicates that although each mutant is viable, several have growth defects. In particular, the Mcm7 mutation is nearly inviable, suggesting that its β-hairpin is especially important. Experiments in progress will determine if the growth defects are due to defective DNA replication.

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INTRODUCTION:

Common disorders result in the degeneration of bone tissue within the skeletal system. Umbilical cord mesenchymal stem cells (UC-MSCs) may be beneficial therapeutic cells for use in regenerative medicine to help alleviate the pain and limitations these conditions cause. With techniques from our previous work, beneficial therapy can take place non-controversially and without pain to the donor.

RESULTS: In vitro osteogenic differentiation of UC-MSCs has shown differences between control and stimulated populations. Along with these are also differences in respect to time. After 28 days of stimulation results show a larger mineralized matrix formation vs. 10 days. The Von Kossa staining results supported the micro-CT scan results.

DISCUSSION: Our results support differences in how different populations respond to BMP4, and the amount of mineralized matrix formation after 10 and 28 days of stimulation. After 28 days of stimulation there was a much greater mineralized matrix formation compared to after 10 days of stimulation. Also there was mineralized matrix and calcium deposit differences between control and treatment pellets. The Von Kossa stain revealed a larger percentage positive area for calcium after 28 days. With the support of the Von Kossa stain, our results show there is more bone formation after 28 days. This may imply that more exposure to BMP4 will result in more bone formation. Future experiments will help determine a specific time frame for the optimization of mineralized matrix formation.

NIH, NIAMS, Children's Hospital of Pittsburgh
The Effects of Stimulatory Factors on YKL-40 Expression in Macrophages and Astrocytes

YKL-40 is a chitinase-like protein that lacks enzymatic activity and is elevated in a variety of inflammatory diseases associated with infiltrating macrophages. Paradoxically, we have observed YKL-40 expression in astrocytes but not macrophages in neuroinflammatory conditions. The goal of this project was to determine whether different methods of activating macrophages in vitro stimulate or inhibit YKL-40 production. Since activated microglia/macrophages are a dominant component of the inflamed brain, a second goal was to interrogate whether macrophage-conditioned media (MCM) from these differentially activated macrophages induces YKL-40 expression or phenotypic changes in astrocytes. Monocyte-derived macrophages (MDM) were either classically activated with IFNγ and LPS (M1) or alternatively activated with IL4 (M2). MDM stained with antibodies against YKL-40 and analyzed using flow cytometry suggest that YKL-40 expression is more abundant in M1-activated and non-stimulated controls compared to M2-activated MDM. Similarly, measurement of secreted YKL-40 concentrations in the culture supernatant showed that YKL-40 secretion was higher in M1-activated MDM and non-stimulated controls. Cultured astrocytes treated with MCM from stimulated and non-stimulated MDM cultures for 42 hours were smaller than non-treated astrocytes and became nonadherent. A subpopulation of these astrocytes was induced to produce YKL-40 after culture with M1- and M2-MCM. These results suggest that MDM produce YKL-40 at varying degrees under different stimulatory conditions in vitro and soluble factors secreted from differentially activated MDM have an effect on phenotype and YKL-40 expression in astrocytes.
Teen Dating Violence (TDV) is defined as a pattern of threatened acts that often result in physical, sexual, or verbal abuse amongst adolescents. Many studies have shown TDV is associated with such consequences as: binge drinking, suicidal attempts, and or poor academic achievement. The purpose of this study was to evaluate a community based sponsored teen dating violence program delivered to inner city high school students. We were interested in assessing the impact of the program on students’ knowledge about TDV, one arm of the study’s framework targeting Skills, Knowledge, and Attitudes (SKA). The 40-minute program provides information on TDV risk factors and warning signs (e.g., jealousy and possessives), and shares with students supportive resources (i.e., 24 hour hotlines) available in the community. For the analysis, data were drawn from 262 freshmen and sophomore students attending four schools located in the Northeastern United States. Using a pre-post test design, students completed an eleven item multiple choice test conducted 7-14 days before and after the program. The program was conducted in the health class. Using PSAW version 19, descriptive statistics and T-tests were generated. The sample was comprised of 56% African Americans (52%), and Caucasians (32%) students. Approximately 67.0% of the students were female, and 32% male. The mean pre-test knowledge score was 13.5 + 3.6 compared to a post test score of 16.0 + 4.0 (p = 0.000). The program increased students’ knowledge of TDV. Research is needed to further assess the SKA framework.
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THE EFFECT OF MULTIPLE TROPHIC FACTORS ON AN IN VITRO MODEL OF PARKINSON'S DISEASE

Parkinson’s disease (PD) is a progressive neurodegenerative disorder that affects about 1% of the population worldwide. The motor deficits associated with PD, including slowness of movement, tremor, and rigidity are caused in large part by the loss of dopamine (DA) neurons in the substantia nigra (SN). Neurotrophic factors (NTFs) are a large class of proteins that promote the survival of neurons and many have been shown to increase the basal survival of DA neurons as well as to protect these neurons from toxins, such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA). However, whereas NTFs have been tested individually, they have rarely been examined in combination, although this is presumably how they act under physiological conditions. We examined the effect of various combinations of NTFs on the survival of DA neurons in dissociated postnatal rat midbrain cultures after an acute exposure to 1-methyl-4-phenylpyridinium (MPP+). We then examined individual NTFs and combinations to see if they were neuroprotective against MPP+ in an SN culture. We observed a reduction in MPP+ toxicity of 40-50% by the combination of five neurotrophic factors: glial cell line-derived neurotrophic factor (GDNF), growth/differentiation factor 5 (GDF5), brain-derived neurotrophic factor (BDNF), basic fibroblast growth factor (FGF-2), and transforming growth factor beta (TGF-β), at 100 ng/ml concentrations. We believe that our data suggest that combinations of NTFs might be a more efficacious therapy for Parkinson’s disease than individual NTF species.
THE ROLE OF YBR074 IN YEAST CELL WALL INTEGRITY: GENETIC INTERACTIONS WITH CDC48

In the eukaryotic cell, one third of proteins are targeted to the endoplasmic reticulum (ER) for folding and maturation. Under stress, proteins may misfold and become targeted for degradation by proteases. These degradative enzymes are cytoplasmic, requiring misfolded substrates to be retrotranslocated out of the ER in a process called ER Associated Degradation (ERAD). Sequestering proteases in the cytosol helps protect proteins in the ER that are on the correct folding pathway. Surprisingly, the yeast protease Ybr074 is predicted to have an ER luminal catalytic domain. This uncharacterized transmembrane protease also has a mammalian homolog, Fxna that has been shown to function in ovarian development. By using yeast as a model system, we find that YBR074 interacts with CDC48 under conditions of cell wall stress. Cdc48 is a AAA+ ATPase involved in ERAD as well as in cell wall maintenance during stress. Based on these data, we hypothesized that Ybr074 modulates the cell wall integrity pathway in parallel with CDC48. To test this hypothesis, strains are being examined for cell wall strength and cell wall stress response. Results show that a ybr074Δ cdc48-2 double mutant has reduced cell wall strength. To understand which function of CDC48 acts in parallel to YBR074, current work is focused on examining additional cdc48 mutant alleles. Future work will examine changes in cell wall composition in these mutants to define the role of Ybr074 in cell wall integrity, an effort which may indicate why Fxna is required for ovarian development.

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Siyu Xiao

Biological Sciences

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Dr. Michael Grabe

A QUANTITATIVE APPROACH TO UNDERSTANDING MEMBRANE TARGETING AND ASSOCIATION OF ROP PROTEINS TO THE HOST MEMBRANE DURING TOXOPLASMA INFECTION

Toxoplasma gondii is a parasite that infects animals and humans causing toxoplasmosis in about a third of the world’s population. The infection process involves the secretion of rhoptry proteins (ROPs) into the host cell during invasion. These proteins associate with a nascent parasitophorous vacuole membrane (PVM), which forms about the parasite. The ability of ROP proteins to associate with the PVM is due to a specific arginine-rich domain consisting of three putative Î±-helices. Coarse-grained molecular dynamics simulations were used to elucidate the nature of the interaction between helices from the ROP5 protein with a model membrane. Binding energies and helix orientations were determined by calculating the potential of mean force for helix translation across the lipid bilayer. Our calculations support the experimental claim that helix 2 binds the strongest to the membrane based on fluorescence experiments carried out on ROP5 deletion mutants in vivo.

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Paul Yenerall
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TRANSPOSONS: A SOURCE OF NOVEL INTRONS

Transposons, mobile segments of DNA, are active in most genomes and are known to rapidly remodel the genomic landscape. The insertion of a transposon into the coding sequence of a gene is generally regarded as deleterious. However, one overlooked consequence of this insertion is the possibility of intron generation, which may increase the diversity of protein products of the gene and/or provide finer regulation of gene expression. During an investigation into the mechanisms of intron gain and loss in genomes of 11 Drosophila species, we were able to identify a transposon insertion in the gene GM26034 in Drosophila sechellia that resulted in the formation of a novel intron. This represents the first case of intron gain via transposon insertion in animals and affirms transposons as a source of novel introns.

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INVESTIGATING THE FUNCTION OF HISTONE MUTANTS IDENTIFIED THROUGH A LARGE-SCALE GENETIC SCREEN

Rkr1 is an E3 ubiquitin ligase that exhibits phenotypes associated with defects in transcription and chromatin function. A large-scale genetic screen was performed to identify point mutations in genes encoding histones H2A and H2B, which display sickness or lethality with a deletion of the Rkr1 gene. Ten mutated residues were identified, including the known residue lysine (K) 123 on histone H2B. Many of these histone residues also have published phenotypes associated with defects in transcription and form a surface-exposed patch on the nucleosome. To further investigate the function of these residues, various assays are being performed. The deletion of RKR1 causes severe genetic interactions with mutations that prevent histone H2B K123 ubiquitylation or histone H3 K4 methylation and is synthetically lethal with the deletion of RTF1, a member of the Paf1 complex important for these same histone modifications. Western analysis is being utilized to test for the global presence of H2B ubiquitylation and downstream modifications in the identified mutants. Preliminary data reveals a complex and novel pattern of modification. Since the residues form a patch implicated in histone modification, we hypothesize that it may be a binding site for the histone modification domain (HMD) of Rtf1. Chromatin immunoprecipitation is being performed to test for the presence of the HMD on chromatin with incorporated histone mutants and to verify histone modification results obtained from western blotting.

HHMI, School of Arts & Sciences, Department of Biological Sciences, NIH