ITMAT EDUCATION PRESENTS THE

2016 CTSA UNDERGRADUATE SYMPOSIUM ON REGENERATIVE MEDICINE

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2015 Summer Undergraduate Intern Organizing Committee

SCHEDULE

Time	*Participants*	*Event*
12:30 – 1:00	Attendees	Registration and Poster Setup
1:00 - 1:15	Carsten Skarke, MD	Welcome and Introduction
1:15-2:00	Susan Margulies, PhD	Keynote Presentation #1 *Translational Research in Ventilator Induced Lung Injury*
2:00-2:45	Profs. Julie Blendy, PhD; Lawrence "Skip" Brass, MD/PhD; Cindy Christian, MD; and David Manning, PhD	PhD, MD/PhD, MD, Alternative Career Panel
2:45-3:15	Faculty Panel and Students	Q&A with Panel
3:15-4:00	Faculty Reviewers/Panel/Students	Poster Session, Informal Q&A with panel speakers. *Appetizers Available*
4:00-4:45	Edward Morrisey, PhD	Keynote Presentation #2 *Generation and Regeneration of the Respiratory System*
4:45-5:00	Carsten Skarke, MD	Closing and Announcement of poster award winners

KEYNOTE SPEAKERS

SUSAN MARGULIES, PHD

Dr. Susan Margulies is a Professor in Bioengineering, in the School of Engineering and Applied Science at the University of Pennsylvania, with over 30 years of experience in the area of traumatic brain injury research, and over 25 years in pulmonary biomechanics. Dr. Margulies received her BSE in Mechanical and Aerospace Engineering at Princeton University and her PhD in Bioengineering at the University of Pennsylvania, and was a post-doctoral fellow in Thoracic Diseases at Mayo. Her first faculty position was as an Assistant Professor in Biophysics and Physiology at Mayo, and she joined the faculty at Penn in 1993. The overall goal of her research program is to determine the mechanical thresholds associated with functional and structural injury in the brain and lung, ultimately to open avenues for injury prevention, intervention and treatment. The research thrusts are united by a common, integrated approach consisting of experiments to measure cell or tissue function/structure under carefully controlled loading conditions (deformations or forces), complemented by mathematical models that extend these microscopic findings to a broad range of real-world, macroscopic environments. By integrating across scale and species and across theory and experiments in our novel interdisciplinary platform, her research translates basic research to clinical injury prevention and treatment strategies. Dr. Margulies is a Fellow of the American Society of Mechanical Engineers, Biomedical Engineering Society, and American Institute for Medical and Biological Engineering. With funding from industry, NIH, NSF, CDC, and the Department of Transportation, she has published over 141 peer-reviewed papers, and has trained 25 post-doctoral clinicians, engineers, and scientists, 26 graduate students, and dozens of undergraduates in her laboratory. She has just launched the NSF-funded Penn Pathfinder program, designed to foster career awareness and skill development for PhD students interested in nontraditional science and engineering career paths in industry and academia.

EDWARD MORRISEY, PHD

Edward E. Morrisey, Ph.D. is the Robinette Professor of Medicine and a Professor of Cell and Developmental Biology at the University of Pennsylvania. Dr. Morrisey received his B.S. degree from the University of Illinois and his Ph.D. from Northwestern University. Dr. Morrisey is the founding Director of the Penn Center for Pulmonary Biology (PCPB), a new organization on the Penn-CHOP campus that is focused on both basic and translational aspects of pulmonary research. Dr. Morrisey is also the Scientific Director of the Penn Institute for Regenerative Medicine (IRM). Since starting his lab at the University of Pennsylvania almost 18 years ago, Dr. Morrisey has made seminal discoveries in pulmonary and cardiovascular development and regenerative biology and has become an international leader in the lung development field. He has published more than 100 papers and authored numerous review articles on lung and cardiovascular developmental and regenerative biology. Dr. Morrisey's Lab focuses on how signaling and transcription factor pathways integrate to promote the expansion and differentiation of tissue specific progenitors within the lung and heart. His lab was the first to demonstrate the importance of Wnt signaling in lung specification and early cardiac development and his lab continues to have a strong focus in this area. Findings from the Morrisey Lab have outlined many of the important pathways currently used to generate lung specific epithelial lineages from pluripotent stem cells used by several labs. The overall goal of his lab is to better understand how the lung and heart respond to injury, whether pathways important for their development are reactivated or suppressed after injury, and use this knowledge to identify ways to promote proper repair and regeneration in these tissues.

POSTER SESSION ABSTRACTS

1-Effects of Prostaglandin E2 on T Lymphocytes

Chuprin, J., Scholler, J., McGettigan S., Bhojnagarwala, P., June, C.

University of Pennsylvania

Prostaglandin E2 (PGE2) is a prostanoid lipid that is synthesized by cyclooxygenase 1 and 2 (COX 1 and 2). It is the most abundantly synthesized prostaglandin downstream of the COX2 pathway, and elevated levels of COX1 and 2 have been linked to premalignant and malignant tissue. PGE2 has been shown to induce angiogenesis through the growth factor VEGF, causes hyperalgesia, and is involved in inflammation. In addition, PGE2 has been shown to suppress T lymphocyte (T cell) function by suppressing their proliferation upon activation. EP2 and EP4, two G-protein coupled receptors for PGE2 signaling, are known to be expressed on T cells. We explored timing and duration of PGE2 exposure affected T cell inhibition. In this study, we demonstrate T cell proliferation following an anti CD3x28 activation was suppressed by PGE2 in a dose dependent manner if present before or within the first 24 hour of bead stimulation. Unexpectantly, we found proliferation was unaffected if expanding T cells were exposed to PGE2 after the initial 24 hours after stimulation. Additionally, we observed that PGE2 had decreased the expression CD25, an activation marker and IL-2 alpha receptor, on only the CD8+ T cell subset. We are currently investigating the mechanism underlying the lack of PGE2 inhibition on T cell proliferation after the initial 24hour of stimulation.

2-Sleep plasticity due to sexual experience in Drosophila melanogaster

Dove, A., Irgebay, Z., Vecsey, C.

Swarthmore College

Sleep behavior is a nearly universal biological phenomenon, but its precise purpose and underlying regulatory mechanisms remain largely unknown. Besides the ability of sleep to influence an organism's waking behavior, wakeful experiences can alter the nature of subsequent sleep. This is nicely exemplified by the fruit fly Drosophila melanogaster, a species wherein various social conditions can induce sleep behavioral plasticity. It was recently demonstrated that female flies experience a post-copulatory reduction in daytime sleep that is mediated by sex peptide (SP), a molecule transferred from the male to the female via seminal fluid. Using activity monitors to measure the sleep of individual flies to further characterize this phenomenon, we discovered that the daytime sleep reduction lasted 6 days post-mating and had no apparent critical period with regard to female age or previous mating experience. Interestingly, we cast doubt upon the existing evolutionary model that the female sleep reduction is an adaptive response that promotes foraging behavior for egg-laying sites and report that SP may not be the sole regulator of this "mating effect"; unexpectedly, courtship alone, without copulation or the transmission of seminal fluid proteins, is sufficient to induce this behavioral effect. This work provides evidence that sexual experience can temporarily modify an organism's sleep behavior. Due to the neurochemical similarities between fly and mammalian sleep, a biochemical understanding of the mechanisms underlying this form of plasticity in Drosophila sleep may inform us about the mechanisms underlying social control of our own sleep.

3-Monitoring Melanoma Patients on Pembrolizumab through Next Generation Sequencing of Circulating Tumor Material

Fan, R., Yee, S.Y., Xu, W., Harmon, S.R., Schuchter, L.M., Gangadhar, T.C. and Carpenter, E.L.

Department of Medicine, Division of Hematology-Oncology, Abramson Cancer Center, Perelman School of Medicine at the University of Pennsylvania

Background: The analysis of circulating tumor material from metastatic cancers is a rapidly expanding field of personalized medicine with the potential to improve clinical tests for early detection, enable customized drug therapies, and allow genomic sequencing of cancers without invasive biopsy. Pembrolizumab, an antibody-mediated tumor immunotherapy, was recently approved by the FDA and designed for treatment of advanced metastatic melanoma with ~40% response rate. Pembrolizumab targets and blocks the PD-1 (programmed cell death 1) receptor on the surface of T-cells, thus enabling their cytotoxic capabilities. Despite its potential, oncologists continue to investigate novel ways to improve the drug's efficacy.

Hypothesis: Genetic analysis of circulating tumor material can be used to measure patient response to treatment by pembrolizumab.

Methods: Whole blood was collected from melanoma patients undergoing immunotherapy at the following time points: 1) Prior to commencement of pembrolizumab treatment (T0), and (2) approximately 3 weeks after beginning treatment immediately before surgical resection (T1). Circulating tumor cells (CTCs) and circulating cell-free tumor DNA (ctDNA) were isolated from patient plasma and subjected to genetic analysis using next generation sequencing. Circulating tumor material data will be correlated to tumor burden and other patient outcome data which can potentially provide important insight on therapy response and prognosis.

Results: In total, 7 melanoma patient blood samples have been collected with a goal of 30 patients by the end of the study.

Discussion: Circulating tumor material data will be correlated to tumor burden and other patient outcome data which can potentially provide important insight on therapy response and prognosis.

Conclusion: Patient sample collection is estimated to conclude December 2016, at which time genetic analysis of CTC and ctDNA will commence.

4-Synthesis and Characterization of an Arylbicycloheptylamine Intermediate

Filemban, N., Colestock, T., Wallach, J., Adejare, A.

University of the Sciences in Philadelphia

The N-methyl-D- aspartate receptor (NMDAR) plays a role in synaptic plasticity, a process required for cognitive functions such as memory and learning. NMDAR antagonists may aid symptomatic and behavioral relief in many CNS disorders including Alzheimer's disease, depression, and neuropathic pain. Few drugs with this mechanism of action are clinically approved; many fail in clinical trials due to poor tolerability. We have been investigating the arylbicycloheptylamine class of compounds which bind NMDAR. Functional group manipulation has led to discovery of lead compounds that are tolerable in mice and rats, including 3,4-methylenedioxyphenylbicycloheptylamine (3,4-MD-PBCHA). Our hypothesis is that chemical properties necessary for the desired activities can be separated from those leading to poor tolerabilties. The goal of the project is to synthesize 3,4-MD-PBCHA and derivatives and examine their pharmacological properties. The compounds are to be synthesized using modifications of the Geneste route as previously reported by our laboratory. Mass spectroscopy and NMR are used to characterize structures. NMDAR bindings of final compounds are assessed using [3H]MK-801 competitive binding assays. We now report synthesis of 3,4-MD-PBCHA. The analytical results are consistent with the desired compound. After obtaining gram quantities of the 3,4-MD-PBCHA intermediate, numerous options exist for creating final compounds. It can be reacted with alkyl halides to form secondary or tertiary amines. Enantiomeric resolution can be utilized because the compound is chiral, leading to twice as many potential products to screen at NMAR. This project will enable us to generate numerous pharmacological tools which can help us probe the NMDAR channel.

5-Dextran-Coated, Doped Iron-Oxide Nanoparticles as Anti-Biofilm Agents

Jonnakuti, V.S., Naha, P.C., Gaoc, L., Kooc, H., Cormode, D.P.

Department of Radiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

Oral biofilms are groups of bacteria that are embedded in a matrix formed from exopolysaccharides. These biofilms cause dental caries that eventually lead to cavities. Currently, no clinically effective treatments for oral biofilms exist, as drugs fail to penetrate the acidic EPS-rich matrix and kill the bacteria – motivating the search for more potent anti-biofilm therapies. We have recently found that iron-oxide nanoparticles (IONPs) are capable of penetrating biofilms and, in the presence of H2O2, generating free radicals that lead to destruction of the bacterial biofilm. We herein aim to improve the effectiveness of the IONP/H2O2 system for biofilm control in vitro by varying the dextran coating size and by adding different dopant metals to potentially increase the catalytic activity of IONPs. A small library of IONPs with different dextran coatings and dopant metals were synthesized and subsequently characterized using a range of physical methods. Hydrodynamic diameters and zeta potential values remained consistent for all IONP formulations, at around 41 nm and -17 mV respectively. ICP-MS revealed substantial amounts of dopant metal within the core of doped IONPs; furthermore, the core sizes of doped IONPs decreased, as evidenced by TEM images. We also conducted a colorimetric assay using 3,3',5,5'-tetramethylbenzidine and 0.5 mg/ml and 0.5% H2O2 to measure the catalytic activity of different IONP formulations over time. This assay showed that decreasing the dextran

coating and doping the core with Mn yielded the highest catalytic activity. Ongoing efforts are to understand the mechanisms of IONP/H2O2 action in situ within intact biofilms.

6-Validation of MRI-based Assessment of Mechanical Competence of Distal Tibia using Cadaveric Human Bone

Kobe, E.A., Teter, O.M., Slinger, M., Miller, R., Rajapakse,C.S.

Perelman School of Medicine Departments of Radiology and Orthopaedic Surgery

Background: It is now possible to non-invasively estimate bone strength in human subjects using magnetic resonance imaging (MRI) guided finite element modeling. The present study validates this approach by comparing to mechanical testing of bone specimens.
Hypothesis: MRI-derived bone stiffness is highly correlated with stiffness obtained by direct mechanical testing.
Methods: 20-mm thick segments from human cadaver tibia specimens of 13 male and 5 female donors aged 33-88 years were imaged on a 3T MRI scanner using the same protocol used for patient imaging (voxel size: 0.137x0.137x0.410mm3). The images were processed to generate a finite element model. Simulated compression was applied to mimic loading conditions for standing. Axial stiffness was computed as the ratio of the reaction force to applied displacement. Specimens then underwent uniaxial compression tests at a displacement rate of 1mm/min using a servo-hydraulic material testing machine. Stiffness was calculated as the initial tangent of the force-displacement curve.
Results: MRI-predicted stiffness was highly correlated with values obtained by mechanical testing (R2=0.84).
Discussion: We provide a direct validation of MRI-based strength assessment of bone. The current study did not investigate non-linear bone properties, which is the focus of an ongoing study.
Conclusion: The findings from this study support the use of MRI-based finite element analysis to reliably predict the mechanical competence of distal extremities in human subjects in clinical settings.

7-Hormonal and molecular effects of restraint stress on formalin-induced pain-like behavior in male and female mice

Long, C., Sadler, K., and Kolber, B.

Duquesne University

The evolutionary advantages to the suppression of pain during a stressful event (stress-induced analgesia [SIA]) are obvious, yet sex-differences in the expression of this pain reduction are not. How males and females integrate physiological stress responses and descending pain inhibition are unclear. A potential supraspinal modulator of SIA is the central nucleus of the amygdala (CeA). This limbic brain region is involved in the processing of stress and pain; the CeA is anatomically and molecularly linked to the HPA axis and descending pain network. Sex-based differences in behavioral and molecular indices of SIA were examined following noxious stimulation. Acute restraint stress in male and female mice was performed prior to intraplantar injections of formalin, a noxious inflammatory agent. Spontaneous pain-like behaviors were measured for 60 min following formalin injection and mechanical hypersensitivity was evaluated using von Frey microfilaments 180 min post-injection. Restraint stress decreases centrally mediated formalin-induced spontaneous behavior and formalin-induced mechanical hypersensitivity in only male mice. To assess molecular indices of SIA, tissue samples from the CeA and blood samples were collected at the 180 min time point. Restraint stress prevented formalin-induced extracellular signal regulated kinase 1/2 phosphorylation in the male CeA, but not in the female. In male mice, restraint and formalin injection significantly increased blood corticosterone concentrations 180 min post injection compared to naïve and non-restrained formalin-injected males. These results demonstrate sex-based differences in behavioral, molecular, and hormonal indices of acute stress in mice that extend for 180 min after stress and noxious stimulation.

8-Polypharmacological effects of arylcycloalkylamines

Mansi, M., Colestock, T., Wallach, J., and Adejare, A.

University of the Sciences in Philadelphia

N-methyl-D-aspartate receptor (NMDAR) antagonism is a therapeutically relevant target in numerous neurological diseases such as depression, neuropathic pain,

neurodegenerative diseases, and epilepsy. A major impediment to the clinical development of NMDAR antagonist drugs is tolerability. Prior research into the mechanisms of NMDAR antagonists and tolerability has identified several promising leads from the arylcycloalkylamines class. One important variable which appears to influence tolerability of the arylcycloalkylamines is polypharmacology. In particular, affinity for monoamine reuptake transporters including those for serotonin and norepinephrine appears to confer a greater protective index to NMDAR antagonists. Our current goal is the rational design of agents with desirable polypharmacology. A series of novel N-alkyl substituted arylcycloalkylamines were designed and synthesized. In general, we first made needed Grignard reagent by adding magnesium to a bromine substituted compound such as 3-bromotoluene. Next, alkyl ketone was added to create the alcohol. That alcohol was converted to azide using sodium azide under acidic conditions. The azide was then reduced to the primary amine using lithium aluminum hydride. The primary amine was further reacted to the tertiary amine with a di-halo substituted reagent such as 1,4-dibromopentane. The final product was purified using chromatography and crystallization techniques. The compounds were characterized by melting point, NMR, MS and elemental analyses as need be. Affinities for NMDAR, monoamine transporters and other off target central nervous system receptors were/are being determined as well as the anticonvulsant and adverse effects in vivo. Acknowledgment: NIMH Psychoactive Drug Screening Program and NINDS Anticonvulsant Screening Program.

9-The Role of Clot Contraction on Plasma Leakage

Nettey, L., Tomaiuolo, M., Welsh, J., Sinno, T., Stalker, T.J., Brass, L.F.

University of Pennsylvania, Philadelphia, PA

Hemostasis is a process that stops the leakage of blood when blood vessels are injured. Blood-circulating cells known as platelets are activated during this process. Activated platelets bind to the site of injury and adhere to other platelets, forming a physical block over the damaged blood vessel. Using intravital imaging in mouse laser injury models, it has been shown that hemostatic clots are formed by primarily by platelets. As the clot consolidates, the gaps between the platelets become narrower, reducing plasma extravasation and protein loss. Here, the data from these *in vivo* experiments was combined with a computational approach to create a 3D simulation that reproduces the time-dependent reduction of plasma leakage into extravascular space in the presence of a clot.

Results: Following calibration of the *in silico* model to the *in vivo* data the plasma leakage profiles of wild type (WT) mice and diYF mice, which have a mutation that hinders clot consolidation, were analyzed. We find that the time-dependent reduction in effective diffusivity within the platelet clot is the primary predictor of plasma leakage, and show that the diffusivity within diYF clots is consistently greater than in WT clots.

Conclusions: Our results, combined with the in vivo studies, examine the kinetics of clot consolidation and demonstrate the role of clot consolidation for plasma extravasation. This suggests that the narrowing of the gaps between platelets, and thus the microenvironment formed within the clot, is an important factor in hemostasis.

10-Characterizing Estrogen Receptor Positive Circulating Tumor Cells in Metastatic Breast Cancer Patients

Powers, C.A.[1], Kakrecha, C.[2,3,8], Yee, S.S.[2,3,8], Soucier-Ernst, D.[7,8], Goodman, N.[7,8], Colameco, C.[7,8], Clark, C.[7,8], Clark, A.S.[2,3,7,8], Mankoff, D.A.[2,4,7,8], Chodosh, L.A.[5,7,8], DeMichele, A.M.[2,3,6,7,8], Carpenter, E.L.[2,3,7,8]

[1]College of Arts and Sciences, [2]Department of Medicine, [3]Division of Hematology-Oncology, [4]Department of Radiology, [5]Department of Cancer Biology, [6]Department of Biostatistics and Epidemiology, [7]Abramson Cancer Center, [8]Perelman School of Medicine at the University of Pennsylvania

Background: In 2016, approximately 246,660 women will be diagnosed with breast cancer. About 6-10% of new cases are metastatic, and 20-30% will become metastatic. Approximately 80% of all breast cancers are estrogen receptor (ER) positive. Biopsies and serum assays do not provide a complete understanding of breast cancer and may be difficult to perform for patients with metastatic disease. However, technologies, such as the FDA approved CellSearch system (Janssen Diagnostics), can be used for the detection and characterization of circulating tumor cells (CTCs) in blood and offer an alternative to biopsies.

Hypothesis: With the addition of an ER antibody, the CellSearch can be used to determine the ER status of CTCs.

Methods: Normal donor blood was spiked with ER-positive MCF-7 cell line cells and run on the CellSearch. CTCs were enumerated, and ER positive CTCs were selected based on nuclear ER expression. Next, we applied the same protocol to metastatic breast cancer patient blood samples.
Results: CTCs and ER expression were detected in 8 spiked samples. CTCs were recovered in 6 out of 8 metastatic breast cancer patient samples, with CTC counts ranging from 0 to 321 per 7.5mL blood. Among the samples with detectable CTCs, one or more ER-positive CTCs were found in 4 out of 6 samples.
Discussion: The feasibility of monitoring ER expression in CTCs in blood will be assessed in a currently open clinical study.
Conclusion: ER expression of CTCs can be determined using the CellSearch platform.

11-Role of Interplatelet Signaling in Thrombus Formation

Valluru, G., Brass, L., and Stalker, T.

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After vascular injury, activated platelets release many chemical factors that activate other platelets leading to a heterogeneous core/shell thrombus architecture. On this basis the idea was developed that interplatelet signaling may contribute to the distinctive core/shell architecture during thrombus formation in vivo.
We sought to develop an in vitro assay to investigate the role of interplatelet signaling in thrombus formation at the level of individual platelets. This assay used a fluorescent calcium probe, Fluo-4, and caged calcium chelator, caged EGTA, loaded in platelets. Upon photoactivation with a 405 nm laser, caged EGTA releases Ca2+ into the platelet cytosol, thus allowing single cell targeting via computer-controlled photoactivation laser. Platelet cytosolic [calcium] were measured in near real-time using spinning-disk confocal microscopy.
Using this assay, we found that photoactivation of cEGTA loaded platelets led to shorter duration, but similar peak amplitude of calcium rise to that of traditional platelet stimulation with an agonist, SFLLRN. To prolong the response, platelets were loaded with a SERCA pump inhibitor thapsigargin to prevent DTS reuptake of calcium. Platelets with thapsigargin showed an extended rise in cytosolic calcium levels, thus demonstrating that the rapid decrease in cytoplasmic calcium after photoactivation was partially due to calcium uptake by the DTS. These results establish the utility of an assay which has the ability to activate single platelets precisely in time and space providing a sensitive system in which questions regarding platelet activation events and interplatelet signaling may be addressed at the single cell level.

12-Quantitative Analysis of Tracking in *Ciona* Heart Founder Cells

White, J., Cota, C., Davidson, B.

Swarthmore College

All plants and animals start out as single cells that divide and grow into adults. Each of the cells in a developing embryo has to decide what kind of adult cell to become. There are two ways they decide: by what molecular factors they inherit from their mothers and by what signals they receive from their neighbors. We study how cells in developing tunicates, barnacle-like creatures that are close cousins to the vertebrates, decide to become the heart. The decision occurs when the heart founder cells divide into two daughter cells. Before they divide, the heart founder cells move all of a certain type of signaling receptor to one side. After the division, one set of daughter cells receives all the receptors which allows them to hear the signal to become the heart, a call their sisters are deaf to. We do not know how the founder cells move their receptors. To figure this out, I took videos of dividing founder cells and quantitatively analyzed them. We hope that understanding how hearts develop and cells communicate in tunicate embryos will help medical researchers find treatments for heart birth defects and diseases that arise from errors in cell communication like cancer.