



PURDUE
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Indiana CTSI Retreat at Purdue University

“Engineering Giant Leaps in Medicine”

Retreat Booklet

January 31, 2020

Martin C. Jischke Hall of Biomedical Engineering

Schedule

10:00 am	Welcome and CTSI overview George Wodicka – <i>Dane A. Miller Head of Biomedical Engineering, Purdue University</i>	MJIS 1097*
10:15 am	Greetings from Theresa Mayer – <i>Executive Vice President for Research and Partnerships, Purdue University</i>	MJIS 1097*
10:30 am	The Engineering-Medicine Partnership Mark S. Lundstrom – <i>Acting Dean of Engineering, Purdue University</i>	MJIS 1097*
10:45 am	Keynote Address Bruce J. Tromberg – <i>Director, National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institutes of Health</i>	MJIS 1097*
12:00 pm	Lunch and Thematic Poster Sessions David Umulis – <i>Associate Head for Research, Biomedical Engineering, Purdue University</i>	MJIS Atrium + 2nd floor
2:00 pm	Parallel Engineering Breakout Sessions I. Wearable Technologies Chi Hwan Lee – <i>Assistant Professor of Biomedical Engineering and Mechanical Engineering, Purdue University</i> Shreyas Sen – <i>Assistant Professor of Electrical and Computer Engineering, Purdue University</i> II. Point-of-Care Diagnostics and Treatment Tamara Kinzer-Ursem – <i>Marta E. Gross Associate Professor of Biomedical Engineering, Purdue University</i> Jackie Linnes – <i>Marta E. Gross Assistant Professor of Biomedical Engineering, Purdue University</i> III. Healthcare Data Connectivity Eneida Mendonca – <i>Vice President for Research Development, Regenstrief Institute</i> Pavlos Vlachos – <i>Professor of Mechanical Engineering, Purdue University</i> IV. Personalized Medicine and Precision Health Sherry Harbin – <i>Professor of Biomedical Engineering and Basic Medical Sciences, Purdue University</i> Bryan Schneider – <i>Vera Bradley Professor of Oncology, Indiana University School of Medicine</i>	MJIS 1001 MJIS 1083 MJIS 2001 MJIS 1097

Automated breast segmentation and breast density quantification using deep learning

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Breast cancer remains the most commonly diagnosed cancer among women in the United States; about 1 in 8 women will develop invasive breast cancer over the course of her lifetime. Mammographic density, also known as the breast fibroglandular tissue (FGT) density, indicates the FGT density (FGT%) and background parenchymal enhancement (BPE) are different measures, each with different Breast Imaging Reporting and Data System (BI-RADS) scales. The FGT% is a strong prediction of breast cancer risk. The American College of Radiology (ACR) recommend using the BI-RADS final assessment categories for MR examination [1]. There is a great potential ahead to employ the deep-learning method for automated breast FGT% quantification. In this study, we demonstrate the application of the convolutional neural network model U-net to breast MRI, yielding accurate tissue segmentation, FGT quantification.

Our work demonstrated the application of convolutional neural networks, e.g., U-nets, adding value for breast image segmentation, tissue composition quantification, and abbreviation of breast MRI protocols while complying with safety guidelines.

1. Morris E, Comstock C, Lee C. ACR BI-RADS® magnetic resonance imaging. ACR BI-RADS® Atlas, Breast Imaging Reporting and Data System Reston, VA, American College of Radiology. 2013.

Speaker Biographies

George R. Wodicka is the Dane A. Miller Head and Professor of the Weldon School of Biomedical Engineering, and Professor of Electrical and Computer Engineering. Professor Wodicka attended The Johns Hopkins University where he received the B.E.S. degree in biomedical engineering and the Massachusetts Institute of Technology, where he received the S.M. degree in electrical engineering and computer science and the Ph.D. degree in medical engineering. Through his founding and leadership, the Weldon School has achieved tremendous programmatic growth and translational research impact. Professor Wodicka spearheaded the resourcing and building of this dynamic enterprise that fosters a unique academic environment for experiential learning and technology development through tight connections to industrial and clinical partners. Burgeoning accomplishments include unprecedented faculty and student contributions in the medical device, biotechnology, and pharmaceutical fields with more than three million patients worldwide directly helped through the licensing of the School's innovative healthcare technologies.



Professor Wodicka conducts an active translational research program in the area of biomedical acoustics - the application of sonic technologies toward the solution of problems in clinical medicine. His current efforts include the design of acoustic guidance systems for clinical catheters and tubes, the development of acoustic biosensors to quantify airway obstruction, and computational modeling and analysis of speech production in both health and disease. He co-founded with his graduate students SonarMed, Inc. which markets the only FDA-approved endotracheal tube monitoring system for advanced neonatal and adult intensive care. He has been recognized for his instructional innovations at Purdue through the Ruth and Joel Spira Outstanding Teacher Award, the Eta Kappa Nu Outstanding Teacher Award twice, the Honeywell Excellence in Teaching Award, the A.A. Potter Outstanding Teaching Award, and the D.D. Ewing Outstanding Teacher Award. He also has received the Faculty Award of Excellence in Leadership from the Purdue College of Engineering and the Outstanding Commercialization Award for Purdue University Faculty. Professor Wodicka was the recipient of a Young Investigator Award from the U.S. National Science Foundation and was a Guggenheim Fellow. He is a member of Tau Beta Pi and Eta Kappa Nu, and a Fellow of the Institute of Electrical and Electronics Engineers (IEEE) and the American Institute for Medical and Biological Engineering (AIMBE).

Theresa S. Mayer is the executive vice president for research and partnerships and a professor of Electrical and Computer Engineering at Purdue University. She is internationally recognized for her research in applications of nanoscience and technology to electronic and photonic devices with new and previously unexplored functions. Her work in directed and self-assembly of nanoparticles has been used to expand the types and complexity of materials that can be integrated into devices beyond standard lithographic approaches, enabling a wide range of novel structures from low-power integrated nanosensor circuits to nanostructured gradient index optical components. Mayer's research, education, and translational programs have been supported by diverse agencies, including the National Science Foundation, Department of Defense, National Institutes of Health, Department of Energy, and industry.



Mayer joined the executive team of President Daniels at Purdue in 2019. In addition to her prior academic leadership positions as Vice President for Research and Innovation at Virginia Tech and Associate Dean for Research and Innovation at Penn State, she served as the Penn State site director of the NSF National Nanotechnology Infrastructure Network and director of the Materials Research Institute Nanofabrication Laboratory, which enabled cutting-edge materials and techniques to be shared among researchers in academia and industry. She has more than 350 technical publications, invited presentations and tutorials, and holds nine patents. Several of her co-inventions have been transitioned into commercial products. She is a fellow of the Institute for Electrical and Electronics Engineers and has received numerous awards for her teaching and research, including the NSF CAREER award. Throughout her career, she has supported the advancement of women in science and engineering.

Mark S. Lundstrom is the Acting Dean of Engineering and Don and Carol Scifres Distinguished Professor of Electrical and Computer Engineering at Purdue University. He earned his bachelor's and master's degrees from the University of Minnesota in 1973 and 1974, respectively and joined the Purdue faculty upon completing his doctorate on the West Lafayette campus in 1980. Before attending Purdue, he worked at Hewlett-Packard Corporation on NMOS process development and manufacturing. At Purdue, his research has explored solar cells, heterostructure devices, carrier transport physics, and the physics and technology of nanoscale devices. His current focus is on energy conversion devices such as solar cells and thermoelectric devices. In the 1990s, Lundstrom co-founded (with his colleagues, Nirav Kapadia and Jose Fortes, the PUNCH project, which provided online simulation services for research and education in micro and nanoelectronics. That work led to the NCN and nanoHUB.org, which now serves the nanotechnology community worldwide. He is the author of *Fundamentals of Carrier Transport* (2nd Ed., Cambridge, 2000) and *Nanoscale Transistors: Device Physics, Modeling, and Simulation* (Springer, 2005). Lundstrom is a fellow of the Institute of Electrical and Electronic Engineers (IEEE), the American Physical Society, and the American Association for the Advancement of Science (AAAS). He has received several awards for his teaching and research and is a member of the U.S. National Academy of Engineering.



Sonodelivery of targeted IL-27 for treatment of prostate cancer

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Systemic, long-lasting therapies are required to effectively combat metastatic prostate cancer. Immune-stimulation therapy can be used to treat tumors and their microenvironment by recruiting immune effectors to tumors. Prior studies have shown that gene therapy using the cytokine interleukin 27 (IL-27), delivered to muscle cells via ultrasound-mediated gene delivery (sonodelivery), can reduce tumor growth. In a recent (unpublished) study from our lab, a plasmid was created that encodes for IL-27 fused to a peptide targeting IL-6R α , which is upregulated on prostate cancer cells. The data suggested that the IL-27-peptide compound exhibited improved antitumorigenic effects compared to IL-27 with a non-targeting peptide. The focus of the present work was to develop new plasmids, each encoding for IL-27 linked to one of three targeting peptides by a flexible linker in a backbone plasmid that is more conducive to *in vivo* expression. The efficacy of the new plasmids was tested *in vitro* by qPCR analysis of genes commonly upregulated by IL-27 (Tbx21, XCL1, and IFN γ) on cDNA derived from TC2Ras cells that had been transfected with the new plasmids. An *in vivo* study was conducted in twenty-one C57BL/6 mice. Mice received TC2Ras cells subcutaneously to produce tumors. The treatment group received intramuscular IL-27-peptide plasmid via sonodelivery and the control group received a non-targeted IL-27 plasmid. Preliminary qPCR analysis of the tumors suggested that there was an increase of IL-27 in the treatment group tumors. Current efforts in this project include optimizing the intramuscular sonoporation protocol and the treatment timeframe and frequency, as well as optimizing polymer formulations and vector design for improving gene delivery *in vitro* and *in vivo*. We anticipate decreased tumor growth and enhanced immune effector infiltration in the tumors of mice in the treatment group due to an increase of cytokine expression and improved tumor targeting.

Research Grant: 1R01CA196947

Keywords: Sonoporation, gene therapy, interleukin 27, prostate cancer

In-vitro characterization of cells present in heterogenous tumors

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Breast cancer continues to be a prevalent condition affecting women worldwide, and its metastasis is a leading cause of death in patients. A recent study has shown that fibronectin producing mesenchymal tumor cells stabilize the tumor microenvironment to promote the metastasis and survival of epithelial tumor cells. It is also known that stem cells play a key role in migration and invasion during metastasis. We investigated the stem-cell like properties of the fibronectin producing mesenchymal tumor cells, and their ability to enhance survival. Epithelial, mesenchymal, and fibronectin knockdown mesenchymal tumor cells were characterized using a stem cell marker flow cytometry panel, including CD90, CD105, and CD73. An increase in CD90, which has been shown to upregulate fibronectin in ovarian cancer, was seen in the mesenchymal tumor cells. CD90 knockdowns will be created, and cocultured with epithelial and mesenchymal tumor cells. Survival and growth within these cultures will be tracked using a generalized multi-parametric particle tracking algorithm. We expect that the CD90 knockdowns will decrease survival and growth within a coculture with epithelial cells, when compared with a coculture of fibronectin producing mesenchymal cells and epithelial cells. A deeper understanding of the metastatic process will aid in the development of future cancer therapies.

Keywords: Breast Cancer, Metastasis, Stem Cells

Bruce J. Tromberg, is Director of the National Institute of Biomedical Imaging and Bioengineering (NIBIB) at NIH, a post he assumed in January 2019. He is a pioneering leader in the field of biophotonics.



Prior to his appointment at NIBIB, Dr. Tromberg held dual appointments as professor in the Departments of Biomedical Engineering and Surgery at the University of California at Irvine (UCI). He also directed UCI's Beckman Laser Institute and Medical Clinic, an interdisciplinary research, teaching and clinical center for optics and photonics in biology and medicine.

In his 30-plus-year academic and scientific career, Dr. Tromberg conducted extensive NIH-supported research, and was the principal investigator (PI) for multiple NIH grants going back as far as 1994. This includes 20 years as PI for the Laser Microbeam and Medical Program (LAMMP), an NIH National Biomedical Technology Resource Center where several cutting-edge technologies have been developed and disseminated to laboratories and clinics around the world. In addition to advisory committee appointments with numerous national and international entities, Dr. Tromberg provided expertise on NIH working groups, review committees, and boards, including the NIBIB National Advisory Council from 2012-2016.

Dr. Tromberg's research spans biophotonics and biomedical optics, two rapidly growing fields that use light to image and conduct therapy at the molecular, cellular and tissue levels. He has co-authored more than 450 publications and holds 18 patents for biophotonics technologies and their applications in areas such as cancer, neuroscience and vascular disease. He specializes in new technology development as well as the "bench to bedside" clinical translation, validation and commercialization of promising methods designed to improve human health.

As a high school student, Dr. Tromberg volunteered in a National Cancer Institute laboratory on the NIH Bethesda campus, graduating in 1974 from Woodrow Wilson High School, Washington, D.C. He earned a B.A. in chemistry and psychology in 1979 from Vanderbilt University, Nashville, Tennessee, and a Ph.D. in chemistry in 1988 from the University of Tennessee in Knoxville. While completing his Ph.D., he conducted research as a Department of Energy predoctoral fellow at the Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Posters Arranged by Impact Areas

Each impact area of is making a difference through multidisciplinary teams that integrate diverse expertise in engineering and medicine to improve health and combat disease. While our faculty work in diverse areas of medical impact, we have particular strength in the following applications:

Drug and substance abuse | 1-3

Each year, over 1,000 Hoosiers die from drug overdoses (1,245 in 2015). We are a team of faculty focused on addressing this crisis by: developing new formulations for slow release of opioids to improve therapeutic pain relief; using engineering tools to reduce the number of prescriptions and pills that make it into the community; and develop devices to automatically sense and reverse overdose.

Musculoskeletal health | 4-8

We are a multidisciplinary team that combines imaging, modeling, and mechanical testing to reduce pain, improve aging, and overcomes sports related injuries that impact how our bodies move. Engineering and clinical faculty are discovering new interactions between muscle and bone and developing new technology to improve tissue repair, noninvasive imaging, and patient outcomes.

Hearing loss and other degenerative diseases | 9-12

Purdue has one of the largest concentrations of faculty and researchers focused on hearing science in the world. Through this partnership, we are supported by a NIH training grant that combines engineering and medicine to discover how molecular and cellular processes control hearing and auditory perception. This is leading to new understanding of how hearing loss due to damage, disease, and congenital disorders can be improved or reversed.

Infectious diseases | 13-20

Treatment optimization and technology development for rapid detection of pathogens are two of the many ways our biomedical research partnerships are having an impact on infectious diseases and global health. We are a team of multidisciplinary engineers that couple modeling, engineering device design, engagement with the global community and healthcare management to make a difference in treatment, prevention, and detection of infectious diseases.

Abstract page number corresponds to the poster number.

Identification of ECM proteins involved in metastatic breast cancer

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Breast cancer is the most commonly diagnosed cancer in women. Distant metastatic breast cancer accounts for a majority of deaths in breast cancer patients. Studies have shown that lung metastasis is extremely common, being found in about 50-77% of metastatic breast cancer patients. However, the mechanism for why breast cancer cells metastasize to the lungs, bones, and lymph nodes remains unclear. This study aims to understand and identify changes in the extracellular matrix (ECM) of the metastatic niche, specifically in the lungs of a 4T1 breast cancer model in Balb/Cj mice. Liquid chromatography- tandem mass spectrometry was used to identify and quantify key ECM proteins in the primary tumors, lungs, and metastatic tumors during cancer progression. Further characterization of ECM protein changes throughout metastasis of breast cancer will aid in development of future therapies, *in vitro* and *in vivo* models, ultimately enhancing the knowledge of this phenomenon.

Keywords: Breast Cancer, Metastasis, Mass Spectrometry, Protein

Network approach to identifying DNA damage response pathway modifications in cancer through scaffold proteins

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The advancement of personalized drug therapies in oncology could benefit from more effective methods of translation in drug development. Many drug candidates and experimental agents must be discarded, or at the least are severely limited in their use, due to the necessary dosage to achieve efficacy as single agents. Many of these agents work through damaging DNA. Emerging targeted therapies have been able to sensitize various tumors to radiation or chemotherapy through disrupting these pathways specifically to the disease's effects on these pathways. Many effects occur scaffold proteins that are involved in multiple complexes throughout the process.

In many cancers, including breast, prostate and bladder, there is evidence of dysregulation of DNA replication and repair pathways that involve scaffold proteins including PCNA. However, due to universal necessity of PCNA, and other scaffold proteins, direct inhibition of all its functions would reduce selective targeting. The main hypothesis is that identifying disease specific dysregulation of PCNA and other scaffold proteins that will provide a basis for antagonism to these pathways that are highly specific to tumor subtypes. Disease specific networks are created by mining the TCGA Data Portal for gene expression profiles of breast tumor subclasses. A scaffold network is composed of all of its primary and secondary interactors and was derived from BioGrid and HIPPIE databases. Subnetworks of the scaffold protein network are created based on gene ontology terms and differential expression of genes in the disease state versus the normal tissue. By investigating these subnetworks for pathway dysregulation leading to losses in redundancy and the emergence of addiction, favored pathways are identified. Potential combinations of existing therapeutics could be derived from these networks based on the detection of aberrant network connectivity.

Keywords: informatics, network-theory, DNA damage repair

Brain health and function | 21-35

From leading research on traumatic brain and spinal cord injury to controlling seizures to unlocking the causes of neurodegenerative diseases, we are a multidisciplinary team working on problems at the interface between medicine and engineering for improved brain health, rehabilitation, functional ability, and quality of life. This group is strengthened by numerous collaborations with clinical faculty throughout Indiana and researchers part of the Purdue Institute of Integrative Neuroscience.

Diabetes and cardiovascular disease | 36-50

We are applying tissue engineering, mathematical modeling, and quantitative noninvasive imaging to improve outcomes in diabetes and cardiovascular health. This group is supported by numerous clinical collaborators and a NIH training grant centered around improving diabetes outcomes through engineering and medicine.

Technology and healthcare | 51-71

We are working to improve patient-centered healthcare delivery, increase wellness, and integrate technology and devices for human health. Technology and device development, internet of things, electronic medical record management, and data science drive the modernization of healthcare and improved patient outcomes. This is in cooperation with the Regenstrief Center for Healthcare Engineering.

Cancer and developmental disorders | 72-91

We are a multidisciplinary team of engineers and clinicians that collaborate to develop new strategies to detect, understand, and combat cancer in order to improve treatment or improve recovery. Computational modeling of cell differentiation and disease progression, technology development for characterizing metastasis and cancer cell proliferation, are just a few examples of the ways engineering is engaged in the work to fight cancer.

Abstract page number corresponds to the poster number.

Wearables sensors for monitoring substance use disorder

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Substance use disorder is an increasing concern in our communities. In 2016, approximately one drug overdose death occurred each week in Tippecanoe County and 174 deaths occurred each day nationwide. As a result, developing new technologies to monitor substance use disorder is a major public health priority. Current methods for monitoring substance use include urinalyses and blood testing. However, urinalyses and blood tests are incapable of mitigating drug overdose deaths as such tests are limited to in-patient clinical settings where overdosing is not a concern. In out-patient settings, however, patients are much more vulnerable to overdosing as they are not under the supervision of a physician. In response to this need, we are engineering new portable health devices for monitoring substance use disorder patients in out-patient settings. Our devices will inform physicians of their patients' substance use habits allowing for personalized treatment strategies for each patient. We have two major approaches: (1) monitoring changes in vital signs due to substance use and (2) detecting illicit drugs excreted through sweat. Drug use is known to affect cardiopulmonary activity, making heart rate, respiration, and blood oxygen monitoring potential indirect measures of drug use. Currently, we have developed a smartwatch capable of measuring heart rate and respiration and have validated the device against an FDA cleared reference standard. Secondly, we are utilizing an aptamer-based biosensor to detect drugs in sweat. The biosensor generates an electrical current when bound to a target drug of interest and the electrical current is measured by a custom-designed sensing circuit. Presently, we have shown a proof-of-concept of our device by detecting micromolar concentrations of cocaine dissolved in buffer solutions. We envision that our biometric monitor and sweat biosensor will allow for continuous monitoring of substance use disorder patients in out-patient settings, reducing the occurrence of drug overdose deaths.

Keywords: wearables, point-of-care, substance use disorder, personalized medicine, predictive medicine

An exemplary combination of miRNAs for differentiation between diffuse large B-cell Lymphoma (DLBCL) and healthy tissue

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The diffuse large B-cell lymphoma (DLBCL) is the most common cause of cancer-related deaths in dogs. It accounts for up to half of lymphomas. Despite advances in molecular characterization of cancers, the current approach to diagnosing lymphomas relies on the histopathologic evaluation of a biopsy of the affected organ, which can take up to two weeks to produce comprehensive diagnostic results. Given the heterogeneity nature of lymphomas and differences in prognosis, additional immunophenotyping through immunocytochemistry (IHC) or flow cytometry and/or polymerase chain reaction (PCR) for antigen receptor rearrangements (PARR) is needed. Therefore, comprehensive and rapid molecular tests are highly desired. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression. MiRNAs critically affect normal cellular homeostasis by controlling cellular processes such as differentiation, progression, and death. Aberrant miRNA expression usually occurs in diseases, especially cancer. MiRNAs' stability and abundance in tissue and body fluids make them promising biomarkers for diagnosing and monitoring diseases. This study aimed to identify miRNA signatures of DLBCL in canine patients to develop rapid and specific molecular diagnostic tools. Total RNA was extracted from formalin-fixed paraffin-embedded tissue (FFPE) lymph nodes from 22 DLBCL samples and 14 controls. MiRNAs relative gene expression were quantified through RT-qPCR. Eight tumor-regulating miRNAs were tested and RNU6 used as a normalizer. Welch two-sample t-tests showed downregulation of the let-7 family ($P \leq 0.001$), and mir-155 ($P = 0.02739$), whereas mir-34a was upregulated in DLBCL ($P = 0.002373$) compared to the control samples. Besides, the Akaike information criterion (AIC) was used to demonstrate the possible combinations of miRNAs to diagnose DLBCL. An example of these promising combinations, mir-34a and let-7f, can differentiate DLBCL from healthy tissue in 100% of the samples. Our results demonstrate a potential forward step in search of rapid and accurate diagnostic miRNAs biomarkers for DLBCL in dogs.

Keywords: microRNA, Biomarkers, Lymphoma, Large B-Cell, Diffuse, Dogs.

Disease-on-a-chip platform for screening anti-cancer drugs

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Presenter: **Apekshya Chhetri** (Email: chhetri@purdue.edu, +12347160048)

Abstract Preclinical models that recapitulate human cancer microenvironments are necessary to improve anticancer candidate drug testing. We have developed a Disease-on-a-chip (DOC), in which breast tumors are cultured on a curved surface (obtained through microfabrication of hemichannels) in the presence of a monolayer of non-neoplastic epithelial cells, hence mimicking the ductal environment. We are hypothesizing that the phenotype of cancer cells in the hemichannel mimics the situation *in vivo* because of the impact of tissue geometry on the cell nucleus. This impact was assessed by comparing nuclear morphometry (size and circularity) of cells in tumors formed in the DOC and in nodules produced by aggregation of cancer cells in hydrogel and via hanging drop systems that have been proposed for drug screening. Only nodules in the DOC displayed similar nuclear morphometry as real tumors of the same cancer type. High sensitivity of triple negative breast cancer cells to cytotoxic drug cisplatin, measured by caspase-3 staining, and low sensitivity to antiproliferative drug SAHA, measured by Ki67 staining, were observed in the DOC model, but these *in vivo*-life efficacy results were not entirely reproduced in the other systems. A “hydrophobicized methylcellulose” matrix spin-coated on the DOC enabled tumor production from single cells without the presence of non-neoplastic cells, which reduced the length of culture by 50 %; yet, nuclear morphometry did not exactly compare to that of the real tumors. The tuning of the mechanical properties of such coating and the paracrine influence of the non-neoplastic epithelium on cancer phenotypes are being investigated.

Uncovering longitudinal healthcare utilization data for identifying opioid addiction behavioral patterns

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We are currently facing a public health crisis of opioid use disorder (OUD), for which people will benefit the most from an integrated system of care. However, access to treatment for OUD is limited by social stigma, as well as underserved geographical locations, underutilized medical settings, and outdated service delivery infrastructure. Utilizing Medicaid health insurance claims data from the Indiana Family and Social Services Administration database, we apply data mining techniques to generate insights on health outcomes and behavioral patterns for people diagnosed with OUD. We present opioid addiction patterns through visualization of longitudinal utilization behavior data to reduce opioid-related overdoses.

Keywords: opioid use disorder, behavioral patterns, claims data

The disengaging brain: Dynamic transitions from cognitive engagement and alcoholism risk

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Human functional brain connectivity is usually measured either at “rest” or during cognitive tasks, ignoring life’s moments of mental transition. We propose a different approach to understanding brain network transitions. We applied a novel independent component analysis of functional connectivity during motor inhibition (stop signal task) and during the continuous transition to an immediately ensuing rest. A functional network reconfiguration process emerged that: (i) was most prominent in those without familial alcoholism risk, (ii) encompassed brain areas engaged by the task, yet (iii) emerged only transiently after task cessation. The pattern was not present in a pre-task rest scan or in the remaining minutes of post-task rest. Finally, this transient network reconfiguration related to a key behavioral trait of addiction risk: reward delay discounting. These novel findings illustrate how dynamic brain functional reconfiguration during normally unstudied periods of cognitive transition might reflect addiction vulnerability, and potentially other forms of brain dysfunction.

Keywords: network neuroscience; brain connectomics; familial alcoholism risk; functional magnetic resonance imaging; dynamical functional connectivity

Mapping protein dynamics in developing mice using non-canonical amino acid tagging

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Introduction: Mapping the temporal dynamics of proteins during development is imperative for proper understanding of mechanisms of tissue repair as a consequence of disease or injury. This goal is however hampered by lack of tools. To address this gap, we developed a technique that enables labeling the new proteins while they are being synthesized based on systemic injection of the non-canonical amino acid azidohomoalanine (Aha). The azide functionality of Aha allows selective enrichment of labeled proteins via click chemistry using alkyne-bearing affinity tags. Using this technique, we were able to examine the dynamics of protein synthesis and turnover in various cellular compartments in developing murine tissues. **Methods:** Pregnant C57Bl/6 mice were injected with Aha or PBS (control) at different embryonic timepoints. Embryos were homogenized and Aha-labeled proteins were conjugated to biotin-alkyne via click chemistry. Biotinylated proteins were enriched with NeutrAvidin beads and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). For turnover studies, embryos were harvested at different time points following Aha injection, fractionated into different cellular compartments based on their solubility profiles, clicked to biotin-alkyne, and then analyzed via western blotting. **Results:** Using this technique, we were able to selectively enrich for newly synthesized proteins with minimal background in developing embryos. In addition, we were able to probe the dynamics of protein synthesis and turnover in different cellular fractions. Our data indicates that the rate of protein turnover differs significantly between various fractions. **Conclusions:** Probing protein dynamics as a function of development will ultimately have a significant impact on our understanding of protein regulation during functional tissue assembly and will guide the development of regenerative therapies.

Keywords: Development, Protein dynamics, Click chemistry, Mass spectrometry

Mapping changes in the 3D distribution and composition of the extracellular matrix during murine renal development

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The extracellular matrix (ECM) is a network of proteins and glycosaminoglycans that provides structural and biochemical cues to cells. In the kidney, the ECM is critical for different aspects of nephrogenesis, including initiation, branching, and function of the glomerulus and tubule cells; however, the overall protein composition and 3D structure of the ECM during murine development is unknown. Using embryonic day (E)14.5, E18.5, postnatal day (P)3, and adult kidneys, we fractionated proteins based on differential solubilities and performed liquid chromatography tandem-mass spectrometry (LC-MS/MS). Decellularized kidneys from the same timepoints were stained for ECM proteins and imaged in 3D using confocal microscopy. Using LC-MS/MS, we resolved an elevation in basement membrane proteins critical for metanephric induction (FRAS1, FREM1, and FREM2) at earlier timepoints and an increase in proteins critical for glomerular basement membrane integrity (COL4A3, COL4A4, COL4A5, and LAMB2) in the adult. The relative composition of interstitial matrix proteins also changed with development. Surprisingly, we observed a transient upregulation of proteins from the elastic-microfibril axis (ELN, FBN1, EMID1, FBLN5, and COL26A1) at E18.5 and P3. The 3D visualization of kidneys revealed a complex ECM structure, including fibers surrounding and running parallel to medullary rays at E18.5 and P3. By correlating 3D ECM spatiotemporal organization with global protein abundance, we are gaining more insight into how ECM changes may regulate murine kidney development. The composition of developing kidneys and native structure can be used by tissue engineers as a guide for the design of regenerative scaffolds.

Keywords: mass spectrometry, 3D imaging, interstitial matrix, basement membrane

Musculoskeletal Health

Purdue-IUSoM collaborative research in bone mechanobiology

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This poster presents results of collaborative research between researchers at Purdue University, the IU Medical School and IUPUI under funding from a COLLOBRATIVE EAGER Award from NSF. As a result of this collaboration we will present new findings on the biomechanics and mechanobiology of microcrack formation and growth in trabecular bone as well as on bone remodeling activity. The overarching outcome of this work is the finding that bone tissue anisotropy is a key factor in the bone tissue response. While biological imaging with light-based approaches have long demonstrated bone tissue anisotropy, this influence has all but ignored in the bone biomechanics and mechanobiology literature. This work demonstrates a combined effort on 3D bone staining, coordinated high resolution microCT imaging, and image processing, conducted at IUSoM, together with bone tissue orientation modeling and finite element simulation of bone deformation conducted at Purdue University. The outcomes of this work are relevant to our understanding of bone toughness and microcrack damage resistance. The broader impact of this work is a contribution to advancing understanding of aging bone and osteoporosis.

Keywords: Bone, Aging, Osteoporosis, Finite Element Analysis, Advanced 3D microCT

Effect of novel molecules on the pro-inflammatory activity of fibroblast-like synoviocytes: An equine osteoarthritis model

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Equine osteoarthritis (OA) is a progressive, chronic disease that causes the degeneration of articular cartilage in the joint, resulting in pain, stiffness, and lameness in equine of any age. OA can result from a single traumatic incident but is often caused by repetitive injurious occurrences or mechanical strain. The disease process of OA can be career-limiting, and potentially career-ending for equine athletes. The pathogenesis of OA is complex, but inflammation is a common characteristic and has been studied extensively in naturally occurring OA. Equine fibroblast-like synoviocytes (eqFLS) have shown to contribute to joint disease and participate in the pathogenesis of OA by producing pro-inflammatory cytokines and cartilage-degrading mediators. In our recent research, the inflammation activity of eqFLS, isolated from the synovium of the metacarpophalangeal (MCP) joint, has been characterized *in vitro* by analyzing the expression of genes representing acute inflammation, such as interleukin (IL)-1 β , IL-6, IL-8 and matrix metalloproteinases (MMPs), after being stimulated with tumor necrosis factor alpha (TNF- α). The gene expression of IL-6 has been shown to be an ideal marker of pro-inflammatory activity of eqFLS. Beyond understanding the pro-inflammatory responses of eqFLS, novel molecules have been developed to counteract the stimuli of TNF- α , resulting in anti-inflammatory activity of eqFLS. The novel molecules were synthesized to mimic the interaction region between Pigment Epithelium Derived Factor (PEDF) and its receptor, Laminin Receptor 1 (LAMR1), and show promising effects. **The overall aim of the study is to develop new therapeutics for osteoarthritis that can reduce inflammation via eqFLS and promote cartilage repair.** In future directions, second generation molecules are to be developed to enhance drug affinity for the LR receptor and to lower the effective dosage amount, making the molecules better-suited for clinical translation and ideal for therapeutic methods that would control inflammation and promote cartilage repair within the OA joint.

Keywords: osteoarthritis, fibroblast-like synoviocytes, tumor necrosis factor alpha

Fibronectin-expressing mesenchymal tumor cells promote breast cancer metastasis

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The multifaceted interactions between heterogeneous tumor cell types and the extracellular matrix (ECM) within the tumor microenvironment play an important role in mediating and regulating many processes during tumor metastasis. Numerous studies have implicated that elevated levels of the ECM protein fibronectin (FN) correlate strongly with decreased survival in breast cancer patients. A recent study by our group demonstrated that FN-producing mesenchymal tumor cells cannot complete the metastatic process by themselves, but can facilitate the metastasis of responder epithelial tumor cells in a paracrine manner (Shinde et. al, 2018). Further, the genetic depletion of FN from these mesenchymal tumor cells initiated them to regain epithelial characteristics while losing their stromal-like features. Despite these recent findings, a better understanding of the role of secreted FN within heterogeneous tumors is critical. In this study, we utilized a microfluidic-based assay to capture a heterogeneous tumor microenvironment *in vitro*, which consists of epithelial, mesenchymal, and FN-depleted mesenchymal tumor cells embedded within a 3D hydrogel matrix. A generalized multi-parametric particle tracking algorithm was used to assess the metastatic potential of FN-dependent heterogeneous tumor cells. Here, we demonstrate that growth rate, motility, and overall metastasis of epithelial tumor cells is enhanced by at least two-fold when in the presence of FN-expressing mesenchymal tumor cells. Additionally, homogeneously cultured epithelial tumor cells with exogenous FN showed higher growth rate and motility than the control group of homogeneously cultured epithelial tumor cells. Only the epithelial tumor cells cultured with either FN-expressing mesenchymal cells or exogenous FN showed continuous increase in their growth rate and motility beyond the second day during five days of live cell-culturing. These epithelial tumor cells not only showed greater survival but migrated longer distance faster than any other phenotypes, demonstrating their increased invasiveness. Our findings indicate that FN signaling stimulates molecular mechanisms that facilitate the survival and metastasis of epithelial tumor cells.

Keywords: Breast Cancer, Tumor Microenvironment, Fibronectin, Microfluidics, Cell migration

Targeted Interleukin-27 gene therapy development for disrupting key malignant crosstalk between metastatic prostate cancer and bone

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Prostate cancer (PCa) bone metastases have been reported in up to 90% of patients with advanced disease. Prostate cancer cells ‘hijack’ bone homeostasis by disrupting the crosstalk between critical cells within the tumor/bone microenvironment (osteoblasts, osteoclasts, and immune cells), and utilize this effector-rich environment for cancer cell survival and growth. Crosstalk pathways primarily involve Interleukins (IL-6 and IL-11) and transforming growth factor β (TGF β). Therefore, a key therapeutic objective in malignant skeletal disease management is to eliminate tumors while restoring bone homeostasis. Current treatments are limited to palliative radiotherapy, chemotherapy, or anti-RANK treatments, all of which have considerable side effects such as osteonecrosis of the jaw or enhanced tumor invasion. An emerging approach to treating skeletal malignancies, osteoimmunology, investigates new multifunctional stimulatory agents that can simultaneously combat tumor growth, and promote bone regeneration. IL-27 is an immunomodulatory cytokine with a great potential as a multifunctional cancer therapeutic. Its regulatory roles include antitumor immune responses (promoting Th1 differentiation), inhibition of tumor growth, and direct modification of tumor/bone crosstalk to support bone regeneration. Thus, **we hypothesize that a tumor-targeted IL-27 gene therapy approach would facilitate the accumulation of IL-27 in the bone/tumor microenvironment and enhance its therapeutic efficacy.** To test this hypothesis, we created a tumor-targeted IL-27 construct by incorporating a novel peptide (LSLITRL); an IL-6 receptor antagonist known to inhibit angiogenesis and tumor growth; or a scrambled control peptide at the C-terminus. To detect the expression, secretion, and tumor recognition specificity, IL-27 constructs were fused to a secreted NanoLuciferase expression vector. We have also assessed the potential for our proposed gene therapy in disrupting malignant characteristics of PCa *in vitro* in by assessing STAT-1/-3 activation and will be assessing bone cell mineralization in PCa/bone co-cultures. Future studies will examine the therapeutic efficacy of secreted, tumor-targeted IL-27 *in vivo*.

Keywords: Cytokines, Gene Therapy, Prostate Cancer.

Chemical homing for localized delivery of extracellular matrix cues to bone fractures to accelerate repair

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Delayed fracture healing is a major health issue involved with aging. Therefore, strategies to improve the pace of repair and prevent non-union are needed in order to improve patient outcomes and lower healthcare costs. Current investigative strategies using Extracellular matrix fragments (ECM) for bone fracture repair have relied on one-time invasive surgical application of ECM peptide-coated implants or -impregnated cements, or demineralized bone to stimulate the accelerated repair which have proven only somewhat effective. We hypothesized that hydroxyapatite binding acidic oligopeptides which home to bone fractures surfaces could be used to deliver ECM fragments systemically. This would open the possibility for the development of these compounds as pharmaceuticals that could be delivered noninvasively and have sustained dosing throughout the fracture repair, thus increasing the efficacy of the treatment without surgical implantation. This bone fracture-targeted platform also allows for greater specificity of the drug’s accumulation leading to lower required doses and lower side effects.

The best-performing ECM cue was ITGA5—a fibronectin memetic that binds to the integrin alpha 5 integrin on mesenchymal stem cells and promotes differentiation into osteoblasts. Mice with femur fractures treated with Targeted ITGA had an 89% increase in portion of mineralized tissue of the fracture representing a dramatic structural improvement after 3 weeks. Not only were we able to improve the fracture healing structurally but we were also able to achieve a significant 216% increase in work-to-fracture in a 4-point bend analysis representing a dramatic mechanical improvement in the healing. In the targeted drugs, no side effects were observed even at very high doses. No off-target skeletal effects were observed. The targeted ECM fragments were able to improve fracture healing and stimulate a more rapid bone formation at the site of injury. These compounds represent promising nontoxic noninvasive therapeutics to accelerate bone fracture repair.

Keywords: Bone Fracture Healing, Targeted Therapeutics, Precision Medicine, Bone Targeting, Regenerative Medicine

Chondrogenic differentiation of mesenchymal stem cells in collagen blend hydrogels

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Since it is a major component of articular cartilage and plays a key role in chondrocyte function, collagen type II is a promising scaffold candidate to repair cartilage defects. However, compared to collagen type I, collagen type II exhibits poor mechanical properties when forming a physically crosslinked hydrogel. Thus, our lab previously developed and characterized hydrogel scaffolds made of a 3:1 collagen type I to collagen type II ratio (referred to as Col I/II gels) to harness the biological activity of collagen type II and the superior gelation of collagen type I. In the current study, we investigated the chondrogenic differentiation potential of bone marrow-derived mesenchymal stem cells (MSCs) embedded within a Col I/II gel or an all collagen type I hydrogel (Col I) *in vitro* and the ability of MSCs encapsulated in a Col I/II gel to repair cartilage defects *in vivo*. The *in vitro* studies demonstrated that glycosaminoglycan (GAG) production in Col I/II hydrogels was statistically higher than in Col I hydrogels or pellet culture, and these results suggested that adding collagen type II promoted production of GAGs, a key component in cartilage extracellular matrix. Col I/II hydrogels had statistically lower alkaline phosphatase (AP) activity, an early bone indicator, than pellets cultured in chondrogenic medium (CM). The *in vivo* results demonstrated that the cellular morphology and proteoglycan staining for the Col I/II hydrogels matched that of articular cartilage tissue surrounding the repair tissue. We saw a greater degree of cartilage repair for the Col I/II hydrogels compared to the Col I hydrogels and the empty defect controls in both the medial condyle and the trochlear groove. Results from this study suggest that there is a clinical value in the cartilage repair capabilities of our Col I/II hydrogel with encapsulated MSCs.

Keywords: cartilage, tissue engineering, and hydrogels

Tracking the process of treatment seeking in breast cancer patients

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Higher mortality in black versus white breast cancer patients is a historical and continuing problem. Black and white incidence rate has converged but the mortality rate between black and white patients has increased. [1] Many studies have verified the existence of racial disparities in breast cancer mortality; [2-4] however, one study found that racial differences were fully attenuated when adjusting for socioeconomic status (SES), age, and stages of breast cancer; [5] while others found that racial disparities were not eliminated regardless of covariates in the model. [6] Some of the potential reasons for these disparities may be black/white differences in risk factors such as higher rates of obesity, diabetes, hypertension, less than adequate treatment, and the fact that blacks have been historically underrepresented in randomized trials. For example, obese women with breast cancer often receive intentionally reduced dosing because physicians mistakenly believe obese patients are at-risk for adverse effects of chemotherapy. [7-8] Finally, black compared to white patients are less likely to have hormone positive tumors for which chemotherapy is more effective. [2]

Most disparity studies focused on descriptive analysis on incident rate, mortality rate, late-stage diagnosis, and their key factors. This study focuses on the disparity on processes for breast cancer patients seeking diagnosis and treatment. We propose to use care process map and simulation model to understand the variability of the care system. With the simulation model, we can identify critical paths in the care system and set target performance in those areas to achieve systemic improvement in accessibility. The ultimate goal of this project is to reduce the disparity of access for breast cancer patients in order to reduce unnecessary missed opportunity for care.

Keywords: Breast Cancer, Process Mapping, Simulation, Diagnosis, Treatment

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Effect of delivery format and microenvironment on type 1 diabetes reversal with oligomer-encapsulated islets

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Islet/ β cell replacement with long-lasting glucose-sensing and insulin-releasing functions has the potential to eliminate the need for insulin injections and minimize complications for individuals with type I diabetes. However, limitations remain that preclude this procedure from widespread clinical use, including i) limited donor supply, ii) significant loss of functional islet mass upon transplantation, iv) limited functional longevity of islet/ β cell grafts, and v) need for life-long systemic immunosuppression. Using a novel type I oligomeric collagen (Oligomer) for in situ encapsulation, we have previously shown rapid (<24 hours) reversal of hyperglycemia with maintenance of euglycemia for 90 days following subcutaneous delivery of 500 syngeneic islets in streptozotocin-induced diabetic mice. Here, we extend this work by performing studies to better define the mechanisms underlying oligomer-islet-recipient interactions, with the goal of providing additional preclinical evidence supporting the utility of natural collagen polymers for islet/ β cell replacement strategies. Specifically, we define how the host tissue response and glycemic control was affected by i) in-situ injectable versus preformed implantable macrocapsule formats and ii) intraperitoneal (IP) versus subcutaneous delivery microenvironments when using 500 allogeneic or xenogeneic islets. For the two delivery formats, euglycemic curves were statistically similar; however, a modest improvement was observed in the percentage of mice remaining euglycemic after 90 days for both allogeneic (50% performed vs 25% in situ) and xenogeneic (67% preformed vs 50% in situ) islets. Interestingly, in-situ injection within the IP space showed a reduction in percent euglycemia and average euglycemia times for both allogeneic and xenogeneic islets, with euglycemic curves for the Allo IP group (17%) significantly different from the Allo SubQ (25%) group. In summary, study results highlight the potential of Oligomer macroencapsulation for islet/ β cell replacement, documenting the versatility of delivery format and importance of delivery site.

Wearable and implantable paper-based electronics

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Traditional manufacturing methods and materials used to fabricate epidermal electronics for physiological monitoring, transdermal stimulation, and therapeutics are complex and expensive, preventing their adoption as single-use medical devices. This work describes the fabrication of epidermal, paper-based electronic devices (EPEDs) for wearable and implantable applications by combining the spray-based deposition of silanizing agents, highly conductive nanoparticles, and encapsulating polymers with laser micromachining. EPEDs are inexpensive, stretchable, easy to apply, and disposable by burning. The omniphobic character and fibrous structure of EPEDs make them breathable, mechanically stable upon stretching, and facilitate their use as electrophysiological sensors to record electrocardiograms, electromyograms, and electrooculograms, even under water. EPEDs can also be used to provide thermotherapeutic treatments to joints, map temperature spatially, and as wirelessly powered implantable devices for stimulation and therapeutics. This work makes epidermal electronic devices accessible to high throughput manufacturing technologies and will enable the fabrication of a variety of wearable medical devices at a low cost.

Hearing Loss & Other Degenerative Diseases

TBI-on-a-chip: A novel tool for investigating mechanisms of post-trauma neurodegeneration

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A clinical correlation between Traumatic Brain Injuries (TBI) and the development of neurodegenerative diseases such as Alzheimer's Disease (AD) has been well established. Patients who experience a single TBI-event during their lifetime are estimated to have a 35% higher probability of developing dementia. Unfortunately, the underlying pathophysiological mechanisms have not yet been uncovered. While current *in vivo* models provide suitable testing platforms for verifying potential treatments and identifying biomarkers, they are somewhat limited in their ability to perform sub-cellular mechanistic investigations. Recently, our group developed a model capable of mimicking closed head injuries *in vitro*, with the intent of investigating damage on the cellular and subcellular levels. This novel system utilizes a pendulum (BPA) to subject neuronal networks cultured on Multielectrode Arrays (MEAs) to forces within clinically relevant ranges (30-300g), while providing simultaneous electrophysiological and morphological assessments. Initial studies revealed significantly consistent network activity impact-response profiles. To further validate the system, clinical biomarkers previously implicated in TBI and AD were investigated post-impact using immunocytochemistry. Briefly, when compared with age and procedurally matched controls, injury-exposed networks displayed significant increases in the following proteins at 24 hours post-impact: amyloid precursor protein, amyloid beta, phosphorylated tau, and α -synuclein. In addition, we also report significantly elevated levels of the aldehyde acrolein, which is known to increase following mechanical CNS trauma and contribute to secondary injuries. These results demonstrate proof of concept, and indicate that such a device could be important in improving our understanding of the basic pathophysiological mechanisms involved in linking TBI and AD by allowing the visualization of early-stage protein aggregation and physiochemical changes initiated via force (solo) in real-time.

Keywords: Traumatic Brain Injury, Alzheimer's Disease, neurotrauma, neurodegeneration, *in vitro*

Non-canonical methods for restoring the levels of tumor suppressor *let-7*: Development of an RNA-based cancer therapeutic

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Based on the knowledge that miRNAs are dysregulated in diseases such as cancer, there have been multiple attempts to develop miRNA-based cancer therapeutics. Because a single miRNA can bind to multiple mRNA targets, therapeutically, miRNAs act as a multi-drug cocktail. Many strategies have been used to restore the levels of therapeutically relevant miRNAs. However, clinical delivery of the processed or mature miRNA to cancer cells still remains a challenge due to lack of efficient vehicles. To overcome this challenge, innovative methods are being explored to increase the pools of tumor suppressive miRNAs. This is especially true for the *let-7* family of tumor suppressive miRNAs. Loss of mature *let-7*, often observed in cancer, predisposes the cells to a tumorigenic fate. Therefore, it is important to restore the levels of *let-7* maintaining cellular differentiation and preventing uncontrollable cellular proliferation. We propose that *let-7* levels can be restored (1) by enhancing miRNA *let-7* biogenesis by small molecule regulators or (2) through targeted and efficient delivery of *let-7* using a ligand-mediated delivery approach. We have conducted a cell-based high-throughput screen to identify small molecule regulators that will increase *let-7* activity. The screen was performed against 23,680 compounds. Compounds identified as positive hits will be further evaluated to identify the mechanism involved in increasing *let-7* activity – i.e. elevated transcription, enhanced biogenesis, increased stability, etc. Additionally, exogenous *let-7* is delivered specifically to cancerous cells using a relevant ligand whose receptor is overexpressed on tumor cells. In this case *let-7* conjugated to folate is delivered to acute myeloid leukemia cells, which overexpress folate receptor. This is the first attempt to deliver an unprotected miRNA-conjugate specifically and robustly to a hematological malignancy. Results from our study are expected to have significant positive impact in cancer treatment by providing new opportunities to advance the next phase of miRNA-based therapeutics.

Keywords: miRNA, cancer, miRNA *let-7*, small molecule, ligand-mediated delivery

Sensitizing prostate cancer cells to IL-27 immunotherapy by chemotherapy induced immunogenic modulation

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One of the most common metastatic sites for advanced prostate cancer is bone tissue. Treatment options for bone-metastatic prostate cancer are currently limited, with immunogenic modulation (IMO) emerging as a possible therapeutic strategy. IMO involves modulation of cancer cell phenotype by sub-lethal doses of chemotherapy, making them more susceptible to an immune attack. Such modulation can improve the efficacy of immunotherapeutic agents such as interleukin-27 (IL-27). By this approach, one can reduce chemotherapy toxicity and the associated side-effects. Moreover, an IMO approach may enable one to treat tumors unresponsive or resistant to chemotherapy. Our main goals for this project are to investigate if prostate cancer cells respond to sub-lethal doses of chemotherapy drugs through IMO and if IL-27 immunotherapy can enhance tumor cell killing following IMO. To characterize the response of prostate cancer cells to sub-lethal doses of chemotherapy drugs, we examined gene expression changes in cancer cells *in vitro* following exposure to varying sub-lethal concentrations of chemotherapeutics and optimized cellular response with respect to duration of treatment, drug concentrations, and different cell types by using quantitative Polymerase Chain Reaction (qPCR). Expression levels of five markers of IMO were investigated in two prostate cancer cell lines. For example, the carcinoembryonic (CEA) gene, which is a tumor antigen and previously associated with IMO, was significantly upregulated in response to sub-lethal doses of chemotherapy. The results suggest that sub-lethal doses of chemotherapy might induce tumor cell sensitivity to CEA-specific T-cell mediated killing. Further studies in *in vivo* models to profile tumor-infiltrating immune effectors will help characterize the associated immune response and examine whether chemotherapy and immunotherapy (IL-27) can act in synergy to enhance the anti-tumor immune response. We anticipate that such combinational therapy will open new avenues for treating bone-metastatic prostate cancer while reducing chemotherapy-associated toxicity and promoting healthy musculoskeletal tissue repair.

Keywords: Prostate cancer, immunogenic modulation, chemotherapy, Interleukin-27.

Optineurin-E50K mutant astrocytes demonstrate neurodegenerative phenotypes when derived from human pluripotent stem cells

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Retinal ganglion cells (RGCs) provide the vital connection between the eye and the brain, with damage to these cells severing this connection and resulting in loss of vision or blindness as found in diseases such as glaucoma. While the degeneration of RGCs is a primary characteristic of glaucoma, other cell types that interact with RGCs may influence this degenerative process in a non-cell autonomous manner. Astrocytes are specifically found in close association with RGCs in the nerve fiber layer of the retina and optic nerve where they play important homeostatic roles, but can also respond to damage and disease affecting the retina. Human pluripotent stem cells (hPSCs) provide an advantageous model system for studying the interactions of these cells, as populations of highly purified RGCs and astrocytes can be differentiated using established protocols. Previous studies from the Meyer lab have demonstrated the importance of RGC-astrocyte interactions in healthy conditions, revealing enhanced functional and morphological maturation of RGCs. Thus, given their important role in RGC maturation, astrocytes may also play a crucial role in glaucomatous neurodegeneration. Thus, efforts of the current study were initially focused on studying disease-related phenotypes in astrocytes derived from OPTN(E50K) glaucomatous hPSCs as well as their isogenic control cell lines. Results indicated morphological and protein expression differences in OPTN(E50K)-astrocytes. Additionally, OPTN(E50K)-astrocytes revealed deficits in mitochondrial dynamics as well as disruptions to the autophagy pathway. Co-cultures of healthy and glaucomatous RGCs and astrocytes will reveal the non-cell autonomous effects of astrocytes upon RGCs in both healthy and glaucomatous states. The results of the current study are the first of its kind to identify neurodegenerative phenotypes in hPSC-derived OPTN(E50K)-astrocytes, as well as study the interactions of these cells with hPSC-RGCs in a co-culture environment and provide a new target for therapeutic intervention and cell replacement for glaucoma.

Keywords: stem cell, retinal ganglion cells, glaucoma, neurodegeneration, Optineurin

HYPE-mediated AMPylation as a novel therapeutic target for neurodegeneration

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A major hallmark of Parkinson's disease (PD) is the deposition of the intrinsically disordered protein α -synuclein (α Syn) into intracellular inclusions termed Lewy bodies. HYPE—the sole human representative of a conserved family of adenylyltransferase enzymes—has been shown to covalently modify (AMPylate) α Syn in vitro. Remarkably, HYPE-mediated AMPylation ameliorates many of the neurotoxic phenotypes of α Syn implicated in the progression of PD, such as α Syn fibrillation and membrane permeability. These potentially cytoprotective phenomena conferred by HYPE's adenylyltransferase activity make it an attractive therapeutic target. Unfortunately, wild-type HYPE is intrinsically inhibited, showing only basal AMPylation levels relative to a constitutively active mutant (E234G-HYPE). To this end, we set out to screen FDA-approved, natural, and semi-synthetic small-molecule compound libraries towards the identification of novel manipulators of HYPE AMPylation. Employing fluorescence polarization (FP) of a labelled ATP analogue on a 384-well microplate platform, we've developed a robust, high-throughput assay suitable for monitoring changes in AMPylation. First-pass selection of our combined ~10,000 compound libraries yielded promising hit percentages: 0.3 for activators of WT HYPE, and 0.9 for inhibitors of E234G-HYPE. Challenging neuronal cell culture models of PD with these hits provides molecular insights into α Syn-induced neurotoxicity, and paves the path for novel therapeutic strategies in combating PD.

Keywords: HYPE, AMPylation, α -synuclein, Parkinson's disease, drug discovery

Laryngeal reconstruction using tissue engineered implants in pigs: A pilot study

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There are currently no treatments available that restore dynamic laryngeal function after hemilaryngectomy. In previous studies, we have shown that dynamic function can be restored post hemilaryngectomy in a rat model. Here, we report in a first of its kind, proof of concept study, that this previously published technique is scalable to a porcine model. Briefly, muscle and fat biopsies were taken from Yucatan minipigs and muscle progenitor cells (MPCs) and adipose stemcells (ASCs) were isolated and cultured for 3 weeks. The minipigs underwent a left laterovertical partial laryngectomy sparing the left arytenoid cartilage and transecting the recurrent laryngeal nerve. Each layer was replaced with a tissue engineered implant:(1)an acellular epithelial layer composed of densified Type I oligomeric collagen,(2)a skeletal muscle layer composed of autologous MPCs and aligned oligomeric collagen differentiated and induced to express motor endplates (MEE), and(3)a cartilage layer composed of autologous ASCs and densified oligomeric collagen differentiated to cartilage. Healing was monitored at 2 and 4 weeks post-op, and at the 8 week study endpoint. Animals demonstrated appropriate weight gain, no aspiration events, and audible phonation. Video laryngoscopy showed progressive healing with vascularization and re-epithelialization present at 4 weeks. On histology, there was no immune reaction to the implants and there was complete integration into host tissue with nerve and vascular ingrowth. This pilot study represents a first of its kind technique in which a transmural vertical partial laryngectomy was performed and successfully repaired with a customized, autologous stem cell-derived multi-layered tissue engineered implant.

Keywords: stem cells, regenerative medicine, otolaryngology

The role of the innate immune response in ERG positive prostate cancer

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Prostate cancer is the second leading cause of cancer-related deaths in American men. Half of all prostate cancers harbor a gene fusion between the highly expressed, androgen driven *TMPRSS2* gene and the silent *ERG* gene. This *TMPRSS2/ERG* fusion gene results in aberrant ERG expression. ERG expression in prostate cells increases migration and invasion and coupled with secondary mutations, drives tumorigenesis.

To investigate the regulation of ERG, we performed an shRNA screen for genes required for ERG-mediated migration in prostate cells. This screen revealed that genes involved in the toll-like receptor 4 (TLR4) signaling pathway are important in ERG-mediated migration. We tested the effect of a specific TLR4 inhibitor on ERG function and observed decreased migration and clonogenic survival exclusively in ERG-positive cells, indicating specificity.

To address the mechanism by which TLR4 inhibition reduces ERG function, we investigated signaling pathway activity upon inhibition of TLR4. No changes in expression were observed for pAKT or pERK, but pMEK and pERG levels were reduced when TLR4 was inhibited. This suggests a mechanism in which TLR4 upregulates pMEK, leading to the phosphorylation and activation of ERG. This is supported by functional assays in which cells expressing a phosphomimetic ERG are resistant to the inhibitor.

We also demonstrated that ERG increases the transcription of *TLR4* and its ligands. Therefore, ERG can sensitize the cell to TLR4 activation. This produces a positive feedback loop in which ERG increases the activity of TLR4, which then activates ERG through MAPK-mediated phosphorylation.

Here we proposed a mechanism in which ERG can be regulated by TLR4. Stimulation of TLR4 can lead to phosphorylation of ERG through the MAPK pathway. This increases expression of ERG's target genes, including *TLR4* and its ligands. This mechanism allows ERG to be indirectly targeted by inhibiting TLR4, opening new therapeutic pathways for ERG-positive prostate cancer.

Keywords: Prostate Cancer, Innate Immunity, TLR4, ERG

Decoding cochlea development to facilitate sensorineural hearing loss therapies

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Noise-induced sensorineural hearing loss affects nearly one in five adults over 20 years old with five or more years of occupational loud noise exposure. This is due to death or damage done to hair cells and other structures necessary for auditory signal transduction. Hearing loss is an irreversible disorder. Characterizing the principles guiding embryonic development of this system is crucial for the design of therapies meant to regenerate a working system in adults. The mature sensory epithelium of the inner ear, the organ of Corti (OC), demonstrates exquisitely resolved patterning crucial for its function in auditory signal transduction. The radial pattern—consisting of 13 to 15 adjacent cells, each with a unique functional purpose—repeats approximately 3500 times with little variation. This pattern emerges from progenitors exposed to morphogenic signals. An incompletely resolved network involving Bmp, Wnt, Notch, Fgf, RA, and Shh pathways is active during the earliest cochlear developmental stages. As a first step towards a quantitative understanding of this system, we explore the Bmp4 pathway at E12.5 by characterizing its contribution to positional information (in an information theoretic sense) and exploring mechanisms by which its profile may arise through simulation. Preliminary results show a tightly distributed P-Smad signal (the Bmp secondary messenger). P-Smad takes the form optimized for information content of a linear profile over a domain spanning the future OC, mimicking the classically described profile of an ideal source-sink system. Simulations using *Bmp4* transcript measurements suggest underexplored contributions from variable receptor densities and/or extracellular ligand modulators, which are implicated in many other developmental systems. However, Bmp4 alone is insufficient to specify position along the axis, corroborating partial patterning achieved within in vitro organ explants inhibited for Bmp signaling. Integrating this information with Jag1 and Wnt ligands will inform hypotheses of information transference in the developing cochlea.

Keywords: Positional information, morphogenesis, development, cochlea, modeling

Infectious Diseases

Structural determinants of immunogenicity for peptide-based immunotherapy

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T cells can initiate an immune response upon the binding of the T cell receptor (TCR) to “non-self” peptide antigens loaded onto major histocompatibility complex (MHC) proteins. This process can be utilized in cancer therapeutics by targeting cancer-specific peptide/MHC antigens (referred to as neoantigens). A given cancer can present hundreds to thousands of neoantigens, many of which do not induce an immune response. Predicting which neoantigens that promote an immune response would improve the cost and timeline of therapies. Here, we are seeking to identify structural features that might lead a neoantigen to be an effective immune target. Our collaborators identified a neoantigen that was recognized by T cells in a human ovarian cancer tumor. We determined that immune recognition of the neoantigen was due to a stronger interaction between the TCR and the neoantigen compared to the corresponding self-peptide. Crystal structures and molecular dynamics simulations demonstrated that the mutant residue imparts a different shape in the peptide, which impacts the conformation of an adjacent tryptophan residue important in TCR recognition. Additionally, the shape of the neoantigen may be better accommodated by the TCR. Altogether, we aim to demonstrate that TCR recognition of a neoantigen can be linked to structural differences between the neoantigen and the corresponding self-peptide. Demonstrating a connection between structural differences and immune recognition would support efforts to utilize structural modeling to predict candidate neoantigens for therapy.

Keywords: structure, affinity, mutation, prediction

Polarization of adipose-derived mesenchymal stem/stromal cells via Toll-Like receptor priming

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Mesenchymal stem/stromal cells (MSCs) are a multipotent stromal cell population of interest for various clinical applications due to their ability to home to injury, and participate in tissue repair and immunomodulation by differentiating or secreting paracrine factors. Although how MSCs modulate their many functions is incompletely understood, evidence suggests toll-like receptor (TLR) signaling can affect MSC activity. Promoting desired effector properties of MSCs represents a potential strategy for using these cells against different diseases. It has been reported in the past that human bone marrow-derived MSCs (hBM-MSCs) can be “polarized” by TLR priming, into contrasting immunomodulatory phenotypes. TLR4 priming leads to an immune activating MSC1 phenotype with roles in tumor repression, whereas TLR3 priming induces a strong immune suppressive MSC2 phenotype associated with support of tumor progression, and reduction of inflammation in a model of diabetic peripheral neuropathy. An alternative subpopulation of MSCs for therapeutic development are human adipose-derived MSCs (hASCs), with the advantages of a relatively ease of isolation and higher abundance compared to their bone marrow counterpart. Although the MSC1/MSC2 polarization has not been reported in hASCs, **we hypothesize that hASCs can be polarized upon short term and low level TLR stimulation.** To test this, we evaluated gene expression profiles of primed hASCs. We have identified similarities and differences in the gene expression signatures of polarized hASCs compared to hBM-MSCs. Upregulation of IL8 (TLR4), and CCL5, IDO, and IP10 (TLR3) were detected in primed hASCs, suggesting that hASC polarization may occur similarly to hBM-MSCs. However, other signature genes remained unchanged, suggesting cell lineage differences in TLR stimulation response. Ongoing efforts aim to further characterize the gene expression signature changes on hASCs upon polarization as well as the key signaling pathways associated with these, and to determine if these polarized phenotypes affect the therapeutic potential of hASCs.

Keywords: mesenchymal stem/stromal cells, toll-like receptors, immunomodulation, inflammation

Understanding ECM-based drug resistivity in breast cancer

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Cell-cell (C-C) and cell-matrix interactions (C-M) are known to affect the drug sensitivity of cancer cells, but are not effectively recapitulated using 2D drug screening platforms. This research aims to determine how cell and matrix interactions confer drug resistivity in three distinct culturing models: 2D (no C-M/limited C-C), 3D spheroids (C-C) and 3D fibronectin (both). We analyzed the growth rate and sensitivity to the tyrosine kinase inhibitor, Neratinib (0-1000 nM), in breast cancer cells derived from a non-metastatic primary tumor (HMLE-E2) and overt bone-metastasis (BM). The transglutaminase 2 gene (TGM2), which upregulates the TG2 protein and crosslinks proteins of the extracellular matrix, was upregulated in the BM cells. We therefore established E2-TGM2 upregulated and BM shTGM2 knockdown cell lines. To account for the different transport properties of the 3 distinct culture environments, we are developing a mathematical model for each condition, which allows us to effectively compare true biological resistivity. Our results indicate that increased cellular levels of TGM2 significantly increase the growth rate and drug sensitivity of the cells on 3D fibronectin matrices. Interestingly, in 2D cultures, TGM2 expression was correlated with higher Neratinib resistivity but did not affect growth rates. In spheroid models, that rely solely on C-C interactions and do not contain a significant matrix component, high levels of TGM2 were correlated with lower survival rates. Lower levels of TGM2 are correlated with a more epithelial phenotype, and we theorize that these spheroids have denser packing, which lowers the rate of diffusion and, thus, the effective concentration of the drug to the majority of the cells. Our work clearly indicates a matrix-dependent drug resistivity in the cells of the primary tumor and growth and drug resistivity increases in the metastatic cells in the presence of matrix. This will help highlight the importance of matrix presence, not just a 3D environment, in drug-screening platforms to accurately guide clinical decisions.

Keywords: Fibronectin, Matrix, Resistivity, Precision Medicine

An agent-based model of host response to biofilm-forming *Mycobacterium avium* in the lungs

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Nontuberculous mycobacteria (NTM) are environmental microbes, capable of colonizing and infecting humans following inhalation of the bacteria. Both incidence and prevalence of NTM pulmonary disease have been increasing, with estimates as high as a 2-5-fold increase over the last decade (1). *Mycobacterium avium* complex (MAC) are the most common disease-causing NTM, are known to form biofilms in the environment, and biofilms have been observed in lungs of COPD patients (2). However, the potential role and dynamics of biofilms *in vivo* remains unclear.

We take a computational approach to explore the initial dynamics of biofilm-forming mycobacteria and the associated innate immune response in patient lungs. We use a spatio-temporal agent-based computational model to simulate the dynamics of host innate immune defenses (macrophage phagocytosis, recruitment, apoptosis and antibacterial properties), as well as bacterial dynamics (growth, replication, and biofilm formation). The model tracks fates of individual cells, as well as quantifying cellular scale events on tissue scale infection dynamics.

To isolate the effect of biofilm on host-pathogen interactions, we performed a sensitivity analysis that revealed the importance of bacterial ability to switch phenotype between growing or producing biofilm. These dynamics have been examined experimentally *in vitro*, and adding them into the computational model will allow further quantification of those dynamics and interactions with the host. Additionally, the model reveals sensitivity to macrophage probability of killing a bacterium it has phagocytosed (rather than becoming infected). Further iterations of this model will also include antibiotic regimens to provide a basis for optimizing treatment of these infections.

A better understanding of the dynamics in this disease will allow us to better inform treatment development taking biofilm into account. Biofilms could contribute to low cure rates, and by quantifying their influence infection and treatment, we can help increase cure rates, shorten treatments and lower reinfection rates.

Keywords: NTM, Agent based models, biofilm, MAC pulmonary disease

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Investigation of a series of 1,4-diaryl-pyrazolo-pyridinones as anti-leishmanial agents

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Leishmaniasis is a grouping of diseases caused by the protozoan parasites *Leishmania spp.*, affecting 12 million people per year with almost 310 million people at risk. Recently, the WHO has declared leishmaniasis a Category I Neglected Tropical Disease. Leishmaniasis has a range of clinical manifestations, from self-healing skin lesions to hepatomegaly to fatality. The first-line chemotherapies used to treat leishmaniasis are intravenous pentavalent antimonials; however, these drugs are highly toxic. Oral treatment options such as paromomycin and miltefosine have been used more as incidences of disease relapse and drug resistance to antimonials develop, emphasizing the importance of identifying new chemotherapies. We developed a novel, target-free fluorometric high-throughput screen (HTS), with an average Z-score of 0.73 +/- 0.13, to identify small molecules with anti-leishmanial activity. Screening of 10,000 small molecules from the ChemBridge DIVERset-EXP™ library cassette #5 yielded 210 compounds that killed 80 percent of parasites, resulting in a hit rate of 2.1 percent. One hundred nine (109) molecular scaffolds were represented within the hit compounds; one scaffold that exhibits potent anti-leishmanial activity was 1,4-diaryl-pyrazolo-pyridinone (1,4-DAPP). A total of 27 novel 1,4-DAPP compounds were synthesized and anti-leishmanial efficacy and host cell toxicity was determined using *L. donovani* mCherry expressing amastigotes and THP-1 macrophages; successful drug treatment was considered when the IC₅₀ value was less than 10µM and the CC₅₀ value was greater than 50µM. Additional pharmacokinetic analyses of a potent 1,4-DAPP compound identified in the HTS were conducted, revealing potential compound metabolism sites. Future studies include *in vitro* and *in vivo* characterization of these novel compounds and their metabolites.

Keywords: leishmaniasis; drug discovery; small molecules; parasitology

Cancer & Developmental Disorders

Differential effect of *OCRL1* patient mutations on Lowe syndrome cellular phenotypes

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Lowe Syndrome (LS) is a lethal X-linked genetic disorder caused by mutations in the *OCRL1* gene, which encodes the lipid phosphatase Ocr1l. LS children present with congenital cataracts, low molecular weight proteinuria and mental retardation as characteristic clinical symptoms. Progressive renal dysfunction is the main cause of death. Ocr1l localizes to the *trans* Golgi network (TGN), early endosomes and the plasma membrane and has been shown to participate in vesicle trafficking. We established that LS patient cells are defective in cell spreading, fluid phase uptake and ciliogenesis, which are critical processes required for kidney function. Over 200 *OCRL1* mutations have been identified in LS, but their specific impact on cellular processes is unknown. However, our results indicate that different mutations have diverse effect on Ocr1l localization and on triggering cellular phenotypes.

We expressed GFP-Ocr1l (WT and several patient mutants) in human kidney proximal tubule (HK2) cells and LS fibroblasts lacking endogenous Ocr1l. The co-localization of WT/mutant Ocr1l with various organelle markers was quantitatively analyzed and different cellular processes/phenotypes were monitored. Our data indicates that in general, N-terminal PH domain mutations resulted in redistribution of Ocr1l to the cytoplasm, ASH-RhoGAP domain mutations prevented localization of Ocr1l to TGN and mutations in the phosphatase domain resulted in TGN morphological abnormalities. Further, different mutants are also differentially affected for the cellular phenotypes such as spreading and ciliogenesis.

This study determined for the first time the effect of different patient mutation on the Ocr1l intracellular localization and on various LS-associated cellular phenotypes and suggests a cellular basis for the differential symptom severity that LS patients display. It also contributes to expand our understanding of the molecular mechanism of LS pathogenesis and provide the basis for the development of a LS-specific therapeutic.

First steps for establishing a program for global health reciprocal innovation within a clinical and translational sciences institute

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BACKGROUND: Efforts to address health challenges in the U.S. can benefit from lessons learned through global health (GH) research and innovations designed to address similar challenges in low- and middle-income countries (LMICs). An example is the WeCare Indiana project that uses community health strategies developed in Kenya to reduce infant mortality in Indiana. Traditional concepts of 'reverse innovation', in which new technologies and methodologies are developed and tested in LMICs and brought back to developed countries, are attractive because they provide solutions that are cost-effective, easy to replicate and sustain, require minimal infrastructure, and can be tailored to local needs. However, "reverse" implies a unidirectional process that does not continue to engage global health stakeholders in refining and implementing these approaches. In response, we created a "reciprocal innovation" model that extends the concept of reverse innovation to better engage stakeholders in a reciprocal process.

METHODS: In 2018, Indiana's Clinical and Translational Science Institute (CTSI) established a new reciprocal innovation program to design, demonstrate, replicate, and rapidly disseminate health innovations developed through collaborations with our LMIC partners. We began with an environmental scan of Indiana CTSI institutions to assess interest in reciprocal innovation, resources, research gaps, and potential innovation targets. We included 75 key stakeholders from IU, Purdue, Notre Dame, state government, health practitioners, community groups, and LMIC partners. Semi-structured interviews were conducted to assess awareness and interest in reciprocal innovation. The interviewed stakeholders were then invited to complete an online questionnaire to identify key health priorities that could benefit from reciprocal innovation.

FINDINGS: Preliminary interview results indicate many stakeholders are not familiar with the concept of 'reciprocal innovation'. However, once the concept was defined, the majority of stakeholders could identify opportunities for a reciprocal innovation process to address health challenges in Indiana. Nearly all expressed support for developing a reciprocal innovation agenda for Indiana.

IMPLICATIONS FOR D&I RESEARCH: Using a systematic approach to engage GH stakeholders in the identification and prioritization of a statewide agenda for reciprocal innovation can strengthen buy-in and support for the concept of reciprocal innovation and aid in developing a stakeholder-driven agenda for reciprocal innovation.

Concentration for the detection of food pathogens from food matrices and contaminated water

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According to the CDC, about 48 million Americans fall ill with a foodborne illness each year. Although pathogen detection methods may be rapid (i.e. PCR), most food samples will not contain the requisite concentration of pathogens for detection. Before the detection step, lengthy sample preparation and enrichment processes (often > 24 hours) are necessary to bring pathogens to a detectable level. There exists a need for more rapid methods of enrichment to enable quick detection. Hollow fiber filtration has demonstrated ability to quickly concentrate microorganisms from dilute samples of various food matrices. Here, a review of results across several studies is presented to demonstrate each sample's unique challenges during filtration. Differences in sample composition, preparation, and filtration parameters affect the eventual recovery of pathogens from dilute samples.

Keywords: microfiltration, food pathogen, pathogen concentration, hollow fiber filtration

Shadow-free UV-light based self-disinfecting surfaces

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Ultraviolet light emitting diodes (UV-LEDs) have numerous applications [1] as light sources for disinfection and sterilization, chemical excitation, replacement of UV lamps, etc. Intrinsic large bandgap of hexagonal boron nitride (*h*-BN) [2-3] and the great development in experimental fabrication facilitates the possibility of flexible UV-LEDs. In this work, we perform a series of atomistic simulations to study the core components of *h*-BN-based LED. The multiple quantum wells (MQWs) are created by selectively putting *h*-BN with different stacking orders and thickness together. Quantum confinement for both valence and conduction bands is found. By analyzing the Schottky barrier of contacts between *h*-BN and various metals, promising metal candidates are identified for p- and n-doped *h*-BN [4-5]. We also calculate the doping effect of *h*-BN under varied doping levels. A concrete LED structure based on *h*-BN is proposed, which gives clear guidance to experimentalists.

Keywords: Surface disinfection; modeling nanotechnology; optoelectronics; 2D-materials;

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Cell phone integrated paper microfluidic device for colorimetric multiplexed detection of analyte targets.

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The effective control and reduction of infectious diseases and environmental pollution, are major concerns worldwide. More specifically, Mercury and Arsenic have been recognized as chemical threats for human health because of their atmospheric transport, environmental persistence, capacity to bioaccumulate in living tissue and proven detrimental effects for human health at extremely low concentrations. Overall, the advancement of biosensors development at lab-scale is very promising. However, the fact that most of the emerging technologies won't reach the market is not yet an open topic of discussion within the field. This restriction is mainly imposed by the cost-efficiency, stability of the biomolecules under harsh conditions, and high-scale reproducibility of the novel biosensing platforms.

Microfluidic paper-based analytical devices (μ -PADS) have offered a next-generation of biosensing by combining the well-known advantages of paper strips with the functionality and utility of microfluidics. This technology holds potential promise for instrument-free, portable and multiplexed detection. A number of successful μ -PADS have been developed during the last decade providing different strategies for fabrication as for colorimetric analysis, achieving semi-quantitative response by visually discriminating the colorimetric signal. Despite the promise of the analytical performance introduced by early cell phone integration, the image analysis described in literature lacks of an optimized testing area identification (area of interest segmentation) that assures a controlled variation of the signal acquired by accounting for the intrinsic variation of the background from test to test.

This work present from the best of our knowledge the first cell-phone integrated μ -PAD, for multiplexed aptamer-based detection of heavy metals (Mercury and Arsenic). We introduce the application of highly stable optical labels as a strategy for enhanced colorimetric response. Finally, we present a robust image analysis processing achieving a LOD of 1 ppm and analytical response in DI water and environmental samples.

Keywords: Heavy metal, Image analysis, paper-based, microfluidics.

Improving translatability of biomaterials for 3D printed tissue constructs

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Three-dimensional bioprinting (3DBP) has remained on the forefront of tissue regeneration and engineering for many years, but the technology is far from being able to deliver functional organs to patients in need. While it has progressed as a tool for in vitro tissue modeling, the development of easily printable, biocompatible, extracellular matrix derived materials remains a barrier to scientific research and clinical usage due to the wide variability in natural polymers and the difficulty of developing user and bioink friendly printing techniques. In this work, cyclodextrin-based polymers are investigated as tunable and universally applicable bioinks to lower the barrier to entry to 3DBP and enable increased capabilities for tissue engineers. Due to its open pocket shape, cyclodextrin (CD) can complex with small complimentary hydrophobic molecules such as adamantane (AD) in a ball and socket fashion, a process called “guest-host” affinity binding. When attached to polymers such as collagen, hyaluronic acid, and polyethylene glycol, cyclodextrin and adamantane may facilitate crosslinking for the formation of a 3D printable biomaterials. This project focuses on the development of cyclodextrin polymers and adamantane modified polyethylene glycol (PEG-AD) as two component shear thinning hydrogel mixtures with tunable mechanical properties. Our preliminary data suggests that altering component molar ratios, total polymer concentration, PEG-AD length, and PEG-AD structure influences viscosity and moduli of the resulting hydrogels. Future work will investigate the printability and biocompatibility of these materials under a range of conditions.

Keywords: 3d bioprinting, biomaterials, tissue engineering

Smartphone diagnostic for asymptomatic detection of malaria

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Malaria is a completely treatable and preventable disease. Yet, there are over 200 million cases and over 400,000 malaria related deaths annually. Malaria, caused by the protozoan parasite *Plasmodium*, is spread by *anopheles* mosquitoes. Current gold standard methods for detection of the *Plasmodium* parasite include microscopy and rapid diagnostic tests (RDTs). Microscopy requires expensive equipment that must be maintained in a clinic or lab. Those in malaria-endemic countries may find these clinics inaccessible or time-consuming. RDTs are more feasible for point-of-care diagnostics; however they do not have sensitivity low enough for asymptomatic cases and may generate false positives. Early and asymptomatic diagnosis of malaria is key to preventing malaria related deaths and providing proper treatment.

Isothermal amplification assays have emerged as the front runner in point-of-care diagnostics due to their simplistic heating and accurate detection. Here I use one such assay and apply it for use in complex sample matrices on a portable smartphone platform. First, I optimize 3 different loop mediated isothermal amplification (LAMP) primer sets for specific and sensitive detection of *Plasmodium falciparum* and *Plasmodium vivax* in blood and urine. Next, I combine this assay with particle diffusometry and analyze the results using a smartphone device. Lastly, I explore a technique using magnetic separation to enrich the sample prior to amplification. The development of this portable platform for the rapid detection of *Plasmodium* parasites from blood and urine is the first of its kind and can easily be modified for the detection of other infectious diseases.

Keywords: Malaria, Particle Diffusometry, LAMP, point-of-care

Construction and operation of a multiplexed microfiltration device to facilitate rapid pathogen detection

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Millions of Americans contract food poisoning or are affected by microbial pathogens each year. Rapid, sensitive detection of dilute levels of pathogens in foods, produce, water, and biomanufacturing process samples is key to consumer protection; however, current enrichment methods require as much as a full day to enrich viable bacterial pathogens to detectable levels. Our lab previously demonstrated the ability to concentrate and detect dilute levels of pathogens, within eight hours, from various food matrices using microfiltration in our continuous cell concentration device (i.e. C3D) with one or two filter modules. Here, we show results from a work recently published as a short communication (Zuponic et al. (2019) *Biotechnol Progress*) describing the design, materials and construction, layout, and operational characteristics of a four filter module multiplexed system based on a 4 channel device. Benefits are a 2x greater sample capacity than an equivalent duplex system (achieving the same time to result of less than 8 hours from sample preparation to detection), simpler operation, and a footprint enabling operation inside a biosafety cabinet instead of requiring a BSL-2 room. Flow rate variability through four channels fit within an operational envelope of $\pm 3\%$; flow rates are reproducible from one run to the next thus ensuring relatively simple, concurrent processing of samples.

Keywords: microfiltration, food pathogen, pathogen concentration, hollow fiber filtration

Development of automated early detection test for preeclampsia

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Preeclampsia, a condition in pregnancy associated with hypertension and preterm birth, affects over 4 million pregnancies and causes more than 60,000 maternal deaths annually. Preeclampsia can induce negative long-term consequences, such as increased risk of cardiovascular disease. To automate a diagnostic tool known as the supine pressor test (SPT), we are integrating a blood pressure (BP) cuff, position sensor, and mobile application into one device. To combat a lack of access to reliable healthcare, our goal is to provide expecting mothers the ability to track their BP and risk of developing preeclampsia from home.

We conducted usability studies with both non-pregnant (n=75) and pregnant women (n=25). For the non-pregnant cohort, we determined the baseline difference in BP when shifting positions and observed how long BP takes to stabilize after light exercise. When shifting positions from lateral to supine, pregnant subjects experienced a BP increase of 14.0 \pm 4.0 mmHg systolic and 12.5 \pm 4.8 mmHg diastolic, whereas non-pregnant subjects experienced an increase of 11.4 \pm 4.7 mmHg systolic and 11.7 \pm 5.4 mmHg diastolic.

We also observed that heart rate and BP stabilized within the first 5 minutes post-exercise in non-pregnant women. Both groups found the device easy to use and required minimal assistance based on survey data we collected. These results suggest that body position alone may lead to significant changes in BP and should be accounted for when measuring systolic and diastolic levels.

Overall, our data shows that 1) a baseline increase in BP occurs simply due to a change in body position, 2) BP is stable within a few minutes once a subject is stationary, and 3) it is feasible for pregnant women to use our integrated SPT autonomously. Future work is needed to demonstrate the effects of home monitoring for preeclampsia to maternal and child health.

Keywords: preeclampsia, pregnancy, blood, pressure, device

Service dogs as complementary intervention for the symptom severity of posttraumatic stress disorder

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Military veterans and service members with posttraumatic stress disorder (PTSD) face a number of psychologically taxing symptoms including anxiety, depression, hypervigilance, and isolation. In the search for effective interventions, anecdotes and emerging research have suggested that service dogs may be effective in reducing symptoms of PTSD. However, skepticism has remained reportedly high in policy makers, medical providers, and the public, possibly due to a limited number of empirical studies and the lack of replication for those that do exist. Therefore, the objective of the present study was to compare PTSD symptom-severity in veterans and military members with and without a service dog. Among those with a service dog, the secondary objective was to explore the relationship between symptom-severity and length of time since service dog placement.

The present study used a cross-sectional design to compare PTSD symptom-severity measured by the PTSD Checklist version five (PCL-5). Participants included 186 post-9/11 veterans and military members; 112 receiving usual care in addition to having a service dog and 74 receiving usual care alone while on waitlist for a service dog. Among participants with a service dog, the mean amount of time since placement was 1.8 years, with a range of less than 1 month to 7 years.

Controlling for age, gender, relationship status, children, and pet dog-ownership, analysis identified that participants with a service dog reported significantly lower PTSD symptom-severity than those without a service dog ($p < .001$). Further, within the service dog group, there was no linear relationship between symptom-severity and length of time with the service dog ($p = .14$). These results replicated and expanded upon prior evidence for the efficacy of service dogs as complementary intervention for PTSD. In conclusion, the present study adds to the groundwork and justification for large-scale clinical trials.

Keywords: PTSD, service dogs, military veterans

Understanding complex single molecule emission patterns with deep learning

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Analyzing single molecule emission patterns plays a critical role in retrieving the structural and physiological information of their tagged targets and, further, understanding their interactions and cellular context. These emission patterns of tiny light sources (i.e. point spread functions, PSFs) simultaneously encode information such as molecule's location, orientation, environment within the specimen and the paths the emitted photons took before captured by the camera. However, to date, retrieving multiple classes of information beyond 3D position from complex or high-dimensional single molecule data remains challenging, due to the difficulties in perceiving and summarizing a comprehensive yet succinct model. We developed smNet, a deep neural network that can extract multiplexed information near the theoretical limit from both complex and high-dimensional point spread functions. Through simulated and experimental data, we demonstrated that smNet can be trained to efficiently extract both molecular and specimen information, such as molecule location, dipole orientation and wavefront distortions from complex and subtle features of the PSFs, which otherwise are considered too complex for established algorithms. The capability of smNet in extracting sample induced aberration through the raw single-molecule blinking data itself allows wavefront measurement deep into the specimen without guide star and will further allow continuous feedback to a wavefront-control element during single molecule imaging of a living specimen. We expect that smNet will pave the way for multiplexed physical and physiological measurements through the emission pattern of a single molecule.

Keywords: Single molecule, deep learning, multiplexed measurements, wavefront, feedback

Role of PSD95 and nNOS interaction in gene regulation following fear conditioning and implications for molecular mechanisms underlying PTSD

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Fear and anxiety are evolutionarily developed responses to perceived or anticipated threats. Normal learning can produce avoidance behavior that promotes survival, but excessive and persistent fear after trauma can lead to development of phobias and post-traumatic stress disorder (PTSD). Involvement of the amygdala in fear acquisition is very well described and requires activation of N-methyl-D-aspartic acid receptors (NMDARs). At a cellular level, NMDAR activation leads to production of nitric oxide (NO) by a process that is mediated by interaction between postsynaptic density protein 95 (PSD95) and nitric oxide synthase (nNOS). Our laboratory has previously shown that fear conditioning enhances PSD95-nNOS interaction and that the small-molecule ZL006 inhibits this interaction. Treatment with ZL006 attenuates rodent cued-fear consolidation, impairs long-term potentiation of neurons, and prevents fear-mediated shifts in glutamatergic receptor current densities in the basolateral amygdala (BLA). In addition, we have demonstrated that treatment with ZL006 avoids adverse effects on cognition that are observed following direct antagonism of NMDARs. To further elucidate mechanisms underlying the role of the PSD95-nNOS-NO pathway in conditioned fear, the current study utilized auditory cue paired-fear conditioning and RNA-sequencing of BLA tissue to examine fear conditioning-mediated gene changes. Expression of 516 genes was altered in the BLA following fear expression, and of these genes, 83 were restored by systemic treatment with ZL006. Network data analysis and gene ontology enrichment analysis of these genes with Ingenuity Pathway Analysis and DAVID software found that cell-cell interaction, cognition-related pathways, and insulin-like growth factor binding were significantly altered. Our results reveal novel genetic targets that underlie plasticity of fear-memory circuitry via their contribution of NMDAR-mediated fear consolidation and can inform future strategies for targeting fear related disorders like PTSD.

Keywords: PTSD - Posttraumatic Stress Disorder, Basolateral Amygdala, NMDA receptor, RNA-sequencing, nitric oxide synthase

Measurement precision bounds on aberrated single molecule emission patterns

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Single molecule localization microscopy (SMLM) has been widely used in investigating subcellular structures and mechanisms due to its nanoscale resolution and has been extended to three dimensions (3D). However, its achievable resolution is limited due to aberrations when imaging deep into the thick specimens. Aberrations distort and blur the single molecule emission pattern (i.e. PSF), lower the information carried by PSFs, which results in deterioration of localization precision and imaging resolution. Here, we systematically investigate the effects of various aberration modes on 3D localization precision based on Fisher information matrix and Cramér–Rao Lower Bound (CRLB). We also examine the effects of aberrations on the accuracy of aberration measurement, which should assist in aberration correction design.

Keywords: Single molecule localization, Fisher information matrix, Cramér–Rao Lower Bound, measurement precision

Build a nanoscale anatomy map of fission yeast contractile ring with 4Pi single-molecule switching nanoscopy

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Cytokinesis is the physical procedure that divides one cell into two daughter cells. Failure of cytokinesis will lead to aneuploidy and instability of the genome. However, little is known about the spacial organization of cytokinetic proteins within the fission yeast contractile ring. Because conventional methods are hard to resolve multiple protein species (>4) within a single cell under tens nanometer resolution. The emerging of super-resolution techniques provide us valuable tools to attack this issue. Among these methods, 4Pi single-molecule switching nanoscopy (4Pi-SMSN) exhibits supreme resolving power in the axial direction and allows 10-15 nm isotropic 3D resolution. Furthermore, Integrating the latest labeling techniques such as DNA-PAINT allows us, for the first time, to build high-resolution anatomy map of the contractile ring directly from observation. This study will improve the previous contractile ring model and provide insights on the mechanism of cytokinesis, which can be utilized to help us understand cell proliferation and abnormal cytokinesis induced tumorigenesis.

Keywords: Super-resolution microscopy, Cytokinesis, Contractile ring, 4Pi-SMSN

Effects and mechanisms of non-invasive brain stimulation with magnetic-electric nanoparticles (MENs)

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Brain stimulation is widely used clinically to diagnose and treat various neurological diseases. These methods, however, are invasive (deep brain stimulation) and have very low spatial and temporal resolutions as well as penetration depth (transcranial magnetic stimulation). Therefore, a non-invasive, efficient, precise, and translational brain stimulation approach will have significant value for disease studies and treatments. Our goal is to develop a non-invasive brain stimulation technique using magneto-electric nanoparticles (MENs) CoFe₂O₄-BaTiO₃ that is capable of both inducing and enhancing neuronal activity with high spatial and temporal resolutions, and minimal toxicity, which will potentially be a new approach in treating refractory neurological diseases. MENs was drawn to a focal brain region with high magnetic field strength ~ 4500 Gauss (G). We then applied much lower field ~ 450G to induce the core (CoFe₂O₄) to vibrate. In response to core's vibration, BaTiO₃ shell surface charges redistributes to produce an electrical dipole, which results in generation of local micro electrical currents, thus in turn activate surrounding neuronal cells. We utilized various imaging and electrophysiological methods to assess MENs effects on neuronal activity both in vitro and in vivo. We also evaluated whether MENs treatment would result in long term toxicity. IBA1 and GFAP staining showed no change in inflammation, i.e. microglial/astrocytic activation at up to one week after MENs treatment. Two-photon imaging revealed MENs distributed within the hemisphere ipsilateral to magnetic application. Both in vitro and in vivo calcium imaging of GCaMP mouse brain showed dramatic increase neuronal activity during the period which the magnet was on. Our data indicates MENs can be localized to a specific cortical region with high efficacy and MENs stimulation efficiently increase neuronal activity.

Keywords: magnetic, electric, stimulation, Calcium imaging, nanoparticles

Biomarkers for risk of psychosis: Neurophysiological measures of cognitive processes

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The study aims to utilize event-related potentials (ERPs) coupled with observable reports of symptoms to comprehensively understand neurological and symptomatic profile of individuals at risk for developing psychosis. The study is a short-term longitudinal design which allows for examination of course as well as structure of illness. We use a combination of well-validated ERPs (P300, N400, ERN) and symptom data to predict variation in symptoms over time. We parse heterogeneity within a high-risk group to create innovative profiles and predict variation in course of symptoms. Data collection is ongoing (n=35; target N=100). Methods include a battery of ERP tasks tracking neural processes associated with attention, language processing, and executive function (P300, N400, ERN), along with assessment of symptom type and severity. Analyses include how ERPs correlate with severity of risk and symptom dimensions (positive, negative, disorganized). We examine whether individual versus global ERP aberrations (P300, N400, ERN) predict individual versus global symptom domain severity (positive, negative, disorganized), or vice versa.

Symptom domain scores were elevated compared to general population on positive (M=1.65, SD=.36), negative (M= 1.9 SD= .42), and depressive (M=1.94, SD=.40) domains. Small to medium effect sizes emerged for P300 profile (r's= -.001 to -.41) and ERN profile (r's = -.03 to -.37), though small effect sizes for N400 profile (r's = -.06 to .29). Analyses were run to determine the degree which profiles of risk were similar: P300/ERN (r = -.09), ERN/N400 (r=-.39), and N400/P3 (r= -.20). Additional analyses suggest potential mediating effects of cognition on neural activity and symptoms. We use well-validated ERPs (i.e. P300, N400, ERN) with behavioral and symptom data to predict variation in symptoms over time. A “fingerprint” physiologic aberration may be exhibited within high-risk individuals and can be used as biomarkers to identify those at risk even before onset of observable symptoms.

Keywords: Event-related potentials, electroencephalography, psychosis risk, epidemiology, symptom profiles

Super resolution volumetric imaging with *in situ* point spread function retrieval

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Single-molecule localization microscopy is a powerful tool in visualizing organelle structures, interactions, and protein functions in biological research. The core of 3D single-molecule localization is to decode the molecular positions in the form of their emission patterns (i.e. point spread functions (PSFs)). Accurate reconstruction of the nanoscale structure of cells and tissues mandates accurate PSF models that reflect the influence of microscope imperfections and, more importantly, aberrations induced by the biological specimen. To date, it is still challenging to obtain the underlying *in situ* PSF generated by single fluorescent probes within a biological specimen. Here we developed an *in situ* PSF retrieval method that enables the construction of an *in situ* 3D response of single emitters directly from single-molecule blinking datasets and therefore allows for pin-pointing their locations with limit-achieving precision and fidelity in whole cells and tissues. We demonstrate this method from various biological samples such as mitochondria, microtubules, and nuclear pore complex in COS-7 cells, fibrillary amyloid- β plaques in mouse brain tissues, and cartilage and tendon fiber in mouse forelimb.

Keywords: Single-molecule localization microscopy, point spread function retrieval

Enhanced 4Pi single-molecule localization microscopy with coherent pupil based localization

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Abstract: Over the last decades, super-resolution techniques have revolutionized the field of fluorescence microscopy. Among them, interferometric or 4Pi microscopy methods exhibit supreme resolving power in the axial dimension. Combined with single-molecule detection/localization and adaptive optics, iPALM/4Pi-SMSN/W-4PiSMSN enabled achieving 10-15 nm isotropic 3D resolution throughout whole cells. However, further improving the achieved 3D resolution poses significantly challenges which, in part, is blocked by the complexity of single-molecule emission pattern generated by these coherent single molecule imaging systems. These complex emission patterns render a large portion of information carrying photons unusable. Here we introduce a localization algorithm that achieves the theoretical precision limit for a 4Pi based single-molecule switching nanoscopy (4Pi-SMSN) system, and demonstrates improvements in localization precision, accuracy as well as stability comparing with state-of-the-art 4Pi-SMSN methods.

Keywords: 4Pi microscopy, phase retrieval, Cramér–Rao lower bound, cavity phase, single molecule localization, maximum likelihood estimator

Twin fingerprints: mapping of heritable and environmental traits in the human functional connectome

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A vast body of recent work has shown that the human functional brain connectivity has an individual fingerprint. This fingerprint can be a result of either genetics, environment, or both. In order to disentangle the phenotype of functional brain connectivity into heritable and environmental influences, we have extended a recently developed identifiability framework based on principal component analysis (Amico and Goñi, 2018) to assess functional connectomes from monozygotic (MZ) and dizygotic (DZ) twins (data from the Human Connectome Project; HCP). First, we showcase through this framework that a fingerprint also exists between the functional connectomes of MZ and DZ twins separately. We observe that this fingerprint is the highest for the test and retest of the subject, followed by MZ twins, and by DZ twins. The level of fingerprint is task-dependent, with Gambling, Language, and Resting state having the highest fingerprints. In the next step we disentangle the functional connectome to find the genetic and environmental influences on it through the classical ACE model frequently used for twin studies. Results indicate that the effects of genetics and environment are dependent on the task being performed.

Keywords: network neuroscience; brain connectomics; heritability; functional magnetic resonance imaging; twin studies

GEFF: Graph Embedding for Functional Fingerprinting

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It has been well established that Functional Connectomes (FCs), as estimated from functional MRI (fMRI) data, have an individual fingerprint that can be used to identify an individual from a population (*subject-identification*)¹. Although identification rate is high when using resting-state FCs, other tasks show moderate to low values. Furthermore, identification rate is task-dependent, and hence is particularly low across cognitive states as captured by different fMRI tasks. Here we propose an embedding framework, GEFF (Graph Embedding for Functional Fingerprinting), based on group-level decomposition of FCs into eigenvectors. GEFF creates an eigenspace representation of a group of subjects using one or more task FCs (*Learning Stage*). This representation is then compared with new instances of FCs for all the *Learning* subjects. This *validation* dataset contains FCs either from the same tasks as the *Learning* dataset or from the tasks that were not included in the *Learning* dataset (*Identification Stage*). This results in 1) significantly increased *subject-identification* rates for all fMRI tasks and, 2) makes the fingerprinting process potentially *task-independent*. Interestingly, including resting-state together with *one* fMRI task for GEFF *Learning Stage* seems to cover most of the cognitive space for subject identification. This information can be useful when deciding on scanning protocols for neuroscientific and clinical experiments. GEFF can also be used to decode cognitive states from FCs i.e. identify the task a given FC is associated with, regardless the subject is already in the *Learning* dataset or not (*subject-independent task-identification*). In addition, we show that eigenvectors can be characterized as task-dominant, subject-dominant or neither, using two-way ANOVA of their corresponding loadings, which provides a deeper insight into the extent of variance of functional connectivity across individuals and cognitive states.

Keywords: network neuroscience; brain connectomics; functional connectome fingerprinting; functional magnetic resonance imaging; principal component analysis

High-resolution volumetric imaging with single molecule switching nanoscopy

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Conventional light microscopy, limited by diffraction, has a lateral resolution of ~250 nm and an axial resolution of ~500 nm, which prevents us from exploring the inner-workings of cells and tissues. The invention of single molecule switching nanoscopy (SMSN) surpasses this limit by stochastically switching single fluorophores on and off so that their localizations can be pinpointed with nanometer precision. This technology has become an important tool in cell biological research, which offers exciting opportunities to address cell biological questions that were previously unanswerable. Here we developed a SMSN with novel algorithm and instrumentation that allows volumetric imaging with high resolution in lateral and axial dimensions. As a demonstration, we used this system to resolve subcellular structures from various biological samples such as mitochondrial networks, microtubules, and nuclear pore complex in 2D and 3D mammalian cultures, to amyloid- β plaques and dendritic spines in brain tissues, and elastic fibers in developing cartilage of mice.

Keywords: fluorescence microscopy, super resolution, three-dimensional microscopy

Precision limit on microscopy image denoising

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Along with the rapid development of microscopy image denoising algorithms, one fundamental question is whether there exists a lower bound on the precision of denoising estimators. Using Fisher information theory and Lagrange multiplier, we show that the bound of microscopy image denoising is influenced by photon count, Gaussian noise variance, detection wavelength and the numerical aperture (NA). By assuming the statistic of each individual pixel is independent, we apply Mixed Poisson Gaussian (MPG) model to the intensity value on camera pixels to generate information matrix. Using low pass filter property of microscopy system, we formulate this universal property of microscopes as a set of constraints in Fourier space and modify the information matrix by taking constraints into account. Our developed method provide a theoretical precision lower-bound of microscopy image denoising. We demonstrated our development by comparing multiple state-of-the-art denoising algorithms to this theoretical denoising limit.

Keywords: Microscopy, Image denoising, Estimation precision, CRLB

A morphospace framework to assess configural breadth based on brain functional networks

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An unresolved question in network neuroscience is the quantification of reconfiguration in functional networks in response to varying cognitive demands. We propose that a mesoscopic generalizable framework would be most apt to investigate the breadth of functional (re-)configurations. We propose a 2D network morphospace using novel mesoscopic metrics, Trapping Efficiency (TE) and Exit Entropy (EE), that characterize the topology of mesoscopic structures and the flow of information within and between them. This framework captures the behavior of a reference set of functional networks (FNs) with changing mental states. We show that this morphospace is sensitive to different FNs, cognitive tasks and subjects. We propose that functional connectivity changes in FNs may be categorized into three different types of reconfigurations: i) Network Configural Breadth, ii) Task-to-Task transitional reconfiguration, and iii) Within-Task reconfiguration; and quantify the Network Configural Breadth across different tasks. In essence, we put forth a framework that can be used to explore the cognitive space in a comprehensive manner, for each individual separately, and at different levels of granularity; a tool that can also quantify the changes that result from such an exploration, as the brain switches between mental states.

Keywords: network neuroscience; brain connectomics; functional magnetic resonance imaging; functional reconfiguration, configural breadth

Brain anatomical fingerprinting: Maximizing differential identifiability in T1-weighted MRI

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Exploration into human brain “fingerprinting” has led to the development of techniques examining the identifiability of individual brain profiles, many of which focus on whole-brain functional connectivity. Here we explore one such technique to evaluate identifiability based on T1-weighted anatomical MRI data. Images (18 subjects, 2 scans at each of 2 different sites, totaling 4 scans per subject) were first registered to a standard anatomical space using nonlinear registration software. Registered image data were then pooled (individually per site) to perform principal component analysis. Registered image data were subsequently reconstructed using varying numbers of principal components to determine the subspace that produced maximum differential identifiability (I_{diff}).

Results indicated the following three points. (i) I_{diff} tended to be maximized when the number of principal components was approximately equal to the number of subjects. (ii) This method was further evaluated by varying two parameters: the nonlinear registration software (including AFNI *auto_warp.py*, FSL *flirt*, or ANTs *antsRegistrationSyNQuick.sh*) and the image resolution (including voxel sizes of 1 mm³, 1.5 mm³, or 2 mm³). The I_{diff} at peak principal component reconstruction tended to be maximized when using AFNI’s registration software, and lower image resolutions resulted in lower I_{diff} . (iii) The method was evaluated by limiting image data to segmented areas of the brain (including gray matter, white matter, or ventricles). Here the segmented area which maximized I_{diff} at peak principal component reconstruction varied between sites: I_{diff} was maximized using ventricles at Site 1 and using gray matter at Site 2.

These findings stress the importance of careful parameter consideration by illustrating the impact of varying parameters on the maximization of I_{diff} using the principal components method.

Keywords: magnetic resonance imaging, anatomical, identifiability, brain fingerprinting

Advanced microrobots with real-time force sensing capabilities for biomedical applications

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Mechanobiology is an emerging field of science that studies how physical forces and changes in mechanical properties affect the development of cells. Recent advances in the field suggest that changes in the extracellular matrix structure and mechanics can lead to the development of many diseases, including cancer and heart failure. As a result, it is extremely important to be able to apply known micro-forces to specific areas of the cell and study its development, as well as measuring mechanical properties of the extracellular matrix structure, such as its stiffness. Using MEMS-based micro-force sensors with embedded electronics can be expensive and hard to integrate into a standard biological test-bed for these applications. Therefore, this poster reports the design, fabrication, and testing of a wirelessly controlled mobile microrobot with an on-board real-time vision-based 2D micro-force sensor for biological applications. This robot can be used for force-guided manipulation tasks as well as stiffness measuring of micro-scale objects, such as cells and membranes. The micro-force sensing mobile microrobot (μ FSMM) uses compliant end-effectors with known stiffness and a computer vision algorithm to provide real-time micro-force feedback to the user while performing teleoperated or autonomous tasks. The entire hardware necessary to operate the μ FSMM is mobile and can be easily integrated into standard biological test-beds, such as an inverted optical microscope. This way, the μ FSMM can be used in mechanobiology studies. Additionally, the microrobot can also be used for force-guided micromanipulation and assembly tasks of biological cells and tissues, and for measuring the stiffness of micro-scale structures, such as fibers, cell membranes, and scaffolds. Furthermore, the microrobot can be adapted to mount on a probe to provide 3D vision-based force sensing information when used in conjunction with a micromanipulator, vision system, and optical microscope for similar applications.

Keywords: Microrobotics, Force Sensing, Micromanipulation, Computer Vision

Effects of spatial resolution and noise on 4D flow MRI velocity measurements

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Recent studies indicate that local hemodynamic factors, e.g. wall shear stress (WSS) and oscillatory shear index (OSI), relate to cerebral aneurysm progression. These hemodynamic metrics can be calculated from velocity measurements acquired in vivo with three-directional phase-contrast MRI (4D flow MRI). 4D flow measurements in cerebral vasculature can be affected by limited spatiotemporal resolution and noise present within the measurements. The noise in 4D flow MRI measurements is directly proportional to the velocity encoding parameter (venc). This study aims to investigate the effects of increased spatial resolution and measurement noise within 4D flow MRI by conducting in vitro flow measurements in a scaled cerebral aneurysm model.

In vivo measurements of an internal carotid artery aneurysm were provided by collaborators at Northwestern University. These measurements were used to 3D print an unscaled and a spatially-scaled in vitro model. The in vitro models were connected to a flow loop pumping a blood-mimicking fluid at steady flow rate and 4D flow MRI measurements were performed at several time points using multiple vencs. Two 4D flow techniques used in two models of different scale resulted in a total of four cases representing the same flow conditions. Error within the measurements was assessed based on the measured velocities' adherence to mass conservation. Results indicate that increasing the scale of the model by a factor of two reduces the error by approximately 75%. It was also observed that reducing the venc by 50% also led to a reduction in the error. This estimation of 4D flow MRI measurement error will allow for researchers to assess the accuracy of hemodynamic metrics.

Keywords: 4D flow MRI, Cardiovascular, Hemodynamics

Discovering optimal window length for dynamic functional connectivity

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Functional connectomes (FC) have been typically estimated using static descriptions of functional connectivity. While having more time points contributes to better estimations of FC between brain regions, static FC doesn't capture temporal features of connectivity. Analyzing whole-brain dynamical functional connectivity (dFC) may help to understand brain function, communication, and ultimately cognition. To date, the most common dFC approach is *sliding-window*, where data within a window of fixed length are used to estimate a "snapshot" FC and then the window is shifted to compute the next one. The choice of window length (WL) is critical as it might be individual- and/or task-sensitive and is usually chosen subjectively (typically 30-60s). If too short, the SNR of the estimated FC would be too low. If too long, potentially it could average distinct cognitive states. Here, we aim to find an optimal WL for a given subject and task in a data driven fashion. For any given window length, the *recurrence matrix* is obtained. It shows repeating patterns of functional connectivity at different time scales. A recurrence matrix of all ones would represent a static cognitive state while a diagonal recurrence matrix would represent a time series where cognitive states are never repeated; these two matrices represent the two opposite extremes of recurrence matrices. For each given subject and task, Jensen-Shannon distance between its recurrence matrix and the two extremes is computed for all WLs. The WL at which the average of these two distances peaks, represents the WL where the recurrent FC patterns are most diverse. This approach provides an automatic and data-driven way to find a WL for dFC that could be potentially applied for any fMRI task at the subject level.

Keywords: network neuroscience; brain connectomics; recurrence matrices; functional magnetic resonance imaging; dynamical functional connectivity

Characterizing dynamic hemoglobin changes after mild traumatic brain injury in rat brain

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Mild traumatic brain injury (mTBI) has gathered concerns for athletes involved with collision sports for its physiological and neurological damage. In this study, we try to measure blood flow changes in a rat model immediately before and after mTBI (caused by consecutive pressurized gas blasts to the head). Functional near infrared spectroscopy (fNIRS) is a non-invasive optical imaging technique, which can be easily adapted to study animal models. The relative hemoglobin changes in the brain and periphery (i.e., tail) were recorded in rats before and after an mTBI. Rats were subjected to three tests: consecutive (1-3) full brain blasts, consecutive (1-3) localized brain blasts, and non-blast plain anesthesia. Non-blasted rats under anesthesia were recorded to test for the effects of the anesthetics. The results showed that: 1) for full brain blasts, a decreased magnitude of low frequency oscillation (i.e., related to slow blood flow changes) was observed for most of the rats; 2) For localized blasting, no localized damage was observed, except for one rat (huge fluctuations hemoglobin in the first 5 min after the blasts); 3) drug effect is not dominant and not likely to be the reason for the observations above. In this pilot study, we successfully built the NIRS apparatus for rat, and showed the potential of measuring perfusion changed immediately before and after mTBI using NIRS

Keywords: mBTI, fNIRS

High frequency ultrasound imaging of tumbling magnetic microrobots

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Colorectal cancer is the second leading cause of cancer death in the world. This ailment causes fatigue, bloody diarrhea, weight loss, and abdominal pain. The best way to treat this devastating disease is early diagnosis with colonoscopies, however, due to the invasiveness of the procedure, patients often experience extreme discomfort and reluctance to undergo further examination. Non-invasive options such as bowel ultrasounds and quantitative fecal immunochemical tests exist, but only provide partial colon screening. Microrobots could offer an alternative as their diminutive size makes them advantageous for minimally invasive operations and precise, localized treatment. They have the potential to reduce patient discomfort and open new possibilities in disease diagnosis. In this study, we evaluate the abilities of a magnetic microrobot in a variety of conditions using a high frequency ultrasound system. Under the influence of an external rotating magnetic field, the microrobot tumbles end-over-end to propel itself forward. First, we quantified robot locomotion in an *ex vivo* porcine colon, testing the robot's constituent materials of polydimethylsiloxane (PDMS) vs. SU-8, tumbling lengthwise vs. sideways, and at three magnet rotation frequencies. Significance was found between materials and tumbling orientation, revealing that SU-8 lengthwise microrobots were the fastest with an average velocity of 2.12 ± 0.25 mm/s at a frequency of 1Hz. With this finding, the next tests were completed at 1Hz frequency with SU-8 lengthwise microrobots. We used *in vitro* agarose gels to maneuver the robot through a variety of trajectories, tested the microrobots *in situ* and *in vivo* murine colons as well. Average velocities were calculated for all tests with the *in vivo* murine colon tests finding an average velocity of 2.07 ± 0.05 mm/s. Overall, the microrobots maintained movement but found variation depending on terrain and solution. These findings suggest microrobots are promising for targeted drug delivery and other *in vivo* biomedical applications.

Keywords: high frequency ultrasound, imaging, colorectal cancer, microrobots, biomedical applications

Quantitative imaging in zebrafish traces noise propagation in BMP signaling

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Dorsoventral (DV) embryonic patterning relies on precisely controlled interpretation of morphogen signaling. In all vertebrates, DV axis specification is informed by gradients of Bone Morphogenetic Proteins (BMPs). Interestingly, the intrinsically stochastic production distribution of the BMP morphogen source is buffered and is integrated into precise and reproducible DV gradients of PSmad, a readout of BMP signaling. To quantify the inputs and outputs in this pathway, we developed a novel wavelet-based nuclei and object segmentation algorithm, that contains five main steps including 2D continuous wavelet transform, multi-scale object identification, 3D alignment, object division, and outlier removal. Using single-molecule mRNA quantification in zebrafish embryos, we determine the quantified *bmp2b* gene expression with single mRNA resolution throughout the embryo composed of between 8,000-10,000 cells. We applied similar wavelet-based segmentation algorithms for mature mRNA and nascent mRNA spot size. Next, we applied least-square algorithm to fit the intensity distribution of total fluorescence intensity for all mature mRNA spots and calculated individual mRNA molecule intensity. We evaluated the cell-to-cell and embryo-to-embryo levels of *bmp2b* mRNA and PSmad signaling in zebrafish. The results are consistent with a noisy morphogen source is averaged by extracellular diffusion to lead to greater precision in signaling output.

BOLD signal in deep anesthesia

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The blood-oxygen-level-dependent (BOLD) signal in functional magnetic resonance imaging (fMRI) indirectly measures the neuronal activations. However, previous studies found aperiodic, systemic low-frequency oscillations (0.01-0.1 Hz) in Global signal (GS) from the resting-state (RS) fMRI, which contain information from both neural activity [1-4] and non-neuronal sources such as cerebral perfusion [5]. During anesthetic-induced unconsciousness, the global reduction of neural activities and cerebral blood flow (CBF) were found in the brain [6, 7]. This study investigated the RS-fMRI derived GS in human brains during anesthetic-induced unconsciousness. Patients who were about to have a surgery were enrolled to be anesthetized with propofol. fMRI was used to record three conditions of RS brain activity, which includes: wakefulness, sedation, and deep anesthesia. In each scan, the GS was calculated by averaging BOLD signals in all the prescribed brain voxels by FSL. To visualize the propagations of low-frequency GS in the brain, a carpet plot was applied in each scan [8]. To show the propagations of low-frequency GS in the brain, the positions of brain voxels in each brain region, including gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) in the carpet plot were sorted based on the time shift between low-frequency BOLD signal in each voxel and GS. Our results show the SD of low-frequency (0.01-0.1Hz) GS in deep anesthesia is significantly smaller than that of in either wakefulness ($p < .005$) or sedation ($p < .05$), suggesting a global reduction of CBF and neural activities. In the carpet plot, low-frequency GS dominated the BOLD signals in the brain during wakefulness. However, the high-frequency GS (breathing signal, 0.16 Hz) dominated the BOLD signals in the brain during deep anesthesia. Our findings warrant more substantial investigations to dissociate CBF and neural activities from GS in anesthetic-induced unconsciousness.

Keywords: fMRI, BOLD, Anesthesia

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The dynamic pattern of low frequency oscillation in RS-fMRI using a carpetplot

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“Carpetplots,” an emerging tool for data denoising, have been used to assess the quality of functional magnetic resonance imaging (fMRI) scans. A carpetplot is a 2-dimensional plot of scaled fMRI voxel intensity values within a scan, with time on the x-axis and voxels stacked along the y-axis. It is a simple way to visualize the effect of motion artifacts on the intensity of the brain voxels over time, represented by sudden intensity value changes that are coherent across almost all voxels (i.e., a straight, vertical line). Systemic low frequency oscillations (sLFOs) are successfully identified from blood-oxygen-level-dependent (BOLD) fMRI and used to track cerebral blood flow (CBF) in the brain, the results of which have been cross-validated by comparison to dynamic susceptibility contrast (DSC) data. Here, a voxel-sorted carpetplot is constructed using sLFOs to investigate the propagation of sLFOs and dynamic patterns of CBF. A carpetplot is constructed by vertically concatenating scaled, demeaned sLFOs, with voxels sorted by the blood arrival time for each voxel. An algorithm was developed to automatically identify and calculate the slope of boundaries across which a sudden signal change was observed. Results show that multiple bands with sudden intensity change were observed and were successfully identified. The angle of a sudden intensity change due to the motion artifact is close to 90 degree, showing that the event was simultaneous in all voxels. The angles of other slopes ranged from 50-80 degrees—different from the angle of the slope due to the motion artifact. Various angles of identified slopes also imply the dynamic change of the CBF over time, with steeper lines implying faster blood flow.

Keywords: carpetplot, functional MRI, resting-state, low-frequency oscillation, cerebral blood flow

Dealing with metabolomics as a collection of functional groups through the MRM-profiling method

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Even though the word metabolomics is generally defined as the study of the set of metabolites present within an organism, cell, or tissue, it is estimated that 80% of the metabolome is “dark” since it is not structured or functionally characterized and organized in databases. For biomarker discovery, the use of a workflow where only chromatographic-selected, highly abundant and ionizable molecules are detected by full mass scan is limiting to the chemical information recovered. To add to that, the use of databases to filter out unknowns causes the loss of large percentages (typically 50-75%) of the MS data amplifies data loss. We proposed a strategy that shifts the definition of the metabolome from the complex molecule network to a collection of chemical functional groups. This new concept has a profound impact on how analytical methods are developed. To avoid loss of chemical information from the start, using flow injection is ideal. Applying neutral loss (NL) and precursor ion (Prec) scans allow the profiling of entire chemical groups in the samples, allowing diverse molecules that would not be detectable in full mass scan environment to show up. For fast and sensitive interrogation of individual samples, it is advantageous to transform the NL and Prec information into multiple reaction monitoring (MRM) scans. MRM data is analyzed as ion profiles. Structural analysis of informative MRMs is pursued only after statistical analysis. Usually so far unknown and relevant molecules for the disease diagnosis or a biological model understanding are found. We named this method MRM-profiling and have initially applied it to biomarker discovery in Parkinson’s disease cerebrospinal fluid. The method has been further applied to propose different diseases (coronary artery disease, atopic dermatitis, polycystic ovary syndrome), biological models (fat aging, fertility prediction, and mammalian developmental mechanisms), and even for food compliance.

Keywords: Metabolomics, Lipidomics, Exploratory Analysis, Multiple Reaction Monitoring Scan, Flow Injection

A tumbling magnetic microrobot system for biomedical applications

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A microrobot system comprised of an untethered tumbling magnetic micro-robot, a two degree of freedom rotating permanent magnet, and an ultrasound imaging system has been developed for *in vitro* and *in vivo* biomedical applications. The microrobot tumbles end-over-end in a net forward motion due to applied magnetic torque from the rotating magnet. By turning the rotational axis of the magnet, two-dimensional directional control is possible and the microrobot was steered along various trajectories, including a circular path and P-shaped path. The microrobot is capable of moving over the unstructured terrain within a murine colon in *in vitro*, *in situ*, and *in vivo* conditions, as well as a porcine colon in *ex vivo* conditions. High frequency ultrasound imaging allows for real-time determination of the microrobot's position while it is optically occluded by animal tissue. When coated with a fluorescein payload, the microrobot was shown to release the majority of the payload over a one-hour time period in phosphate-buffered saline. Cytotoxicity tests demonstrated that the microrobot's constituent materials, SU-8 and polydimethylsiloxane, were nontoxic to murine fibroblasts, even when the materials were doped with magnetic neodymium microparticles. The microrobot system's capabilities make it promising for targeted drug delivery and other *in vivo* biomedical applications.

Keywords: targeted drug delivery, precision medicine, magnetic microrobotics

A workflow for modeling and validating parallel transmit array coils

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Purpose: The parallel transmission (pTx) technique uses multiple transmit coils driven by independent radiofrequency (RF) pulses to tailor the spatial distribution of the transmit magnetic field (B_1) and excite a region of interest. The scattering matrix of the pTx coil is an important bench measurement for characterizing the coil performance. A general workflow to optimize the S parameter matrix is demonstrated in this work.

Method: We propose a workflow to model a transmit array coil with co-simulation, validate with bench measurements and ADS circuit simulation on a subset of the transceiver array, optimize the scattering matrix of the co-simulation model, and quantify the Specific Absorption Rate (SAR) with Virtual Observation Points (VOPs). Our workflow is suitable for any transmit array coil with inductive decoupling circuits¹, matching circuits and lumped capacitors. Three constrained optimization solvers are explored: the self-organizing migrating algorithm (SOMA), the generic algorithm (GA) and *fmincon* from the MATLAB toolbox. The 10-g averaged SAR for the pTx all-transmitting case are quantified. The 10-g SAR are compressed to VOPs, and the corresponding spatial locations are plotted on the 10-g SAR maps.

Results: We model a 16-channel transceiver coil at 7 T in XFDTD v7.7.0.5 (Remcom Inc., State College, PA, USA). In total, we have 118 variables including capacitors and inductors for optimization. The optimized S parameters have good decoupling ($S_{12} < -12$ dB) and matching ($S_{11} < -30$ dB) behaviors at 298 MHz. To validate our optimization model, we measure the input impedance of a subset of array elements and model it in the ADS circuit simulation. The ADS circuit simulation facilitates determining the suitable ranges of inductors and capacitors value for the constrained optimization. The optimization solvers' performances. Finally, we validate the optimization results with *in vivo* images. In the direct simulation ($S_{ii} = -50$ dB, $S_{ij} = -0.1$ dB), results are directly obtained from XFDTD, without the presence of inductive decoupling circuits. The direct simulated coils have less $|B_1|$ compared to the *in vivo* data, but the phase maps are very similar to the *in vivo* data. In the optimized co-simulation ($S_{ii} = -30$ dB, $S_{ij} = -12$ dB), the $|B_1|$ maps are similar to the *in vivo* data, but the phase maps notably have disagreements. Compressed VOPs, and their spatial locations are overlaid on the 10-g SAR spatial.

Discussion: The proposed optimization solvers suffer from instability, because for wider constraint bounds the convergence time is longer, and the optimization stops before reaching the minimized S parameter. Future work is to derive the closed form cost function and apply Lagrangian Multipliers to solve for the optimal variables.

Conclusion: The proposed workflow can facilitate RF coil design and validation with RF safety standards.

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Region-based T1-weighted MRI morphometric changes in high school collision sport athletes

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Quantification of brain volume changes is a critical morphometric task when individuals may be at risk of longitudinal exposure to neurotrauma. Current automated tools (e.g., SIENA-FSL) work only on a whole-brain basis, whereas we here present a novel approach to characterize region-specific volume changes using existing atlases. The new approach exhibits high sensitivity in detecting changes in athletes who experience repetitive subconcussive trauma coupled with a good replication of whole-brain findings from automated tools. The approach also facilitates group-level comparisons for (normalized) region-specific tissue volumes, allowing visualization of brain volume changes on a standard template/atlas. Performance was assessed against SIENA-FSL on a pooled dataset of high school (ages 14-18) collision sport athletes experiencing repeated subconcussive events (CSA: 17 female soccer; 21 male football), and age-matched non-collision athletes (NCA: 14 male; 14 female). CSA (soccer and football) underwent five MRI sessions keyed to the date of onset of collision activities (Pre=before; In1=1-6 weeks after onset; In2=5-9 weeks; Post1=15-20 weeks; Post2=26-29 weeks). NCA were imaged twice, 4-6 weeks apart, once before (Test) and once after (ReTest) training and competition onset. At each session, high-resolution anatomical imaging (1mm isotropic T1-weighted FSPGR; 16-channel Nova Medical Inc. brain array) was performed on a General Electric 3T Signa HDx. Each anatomical scan was registered via affine and high-dimensional nonlinear transformations to MNI152 standard space, and segmented into three tissue classes: gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF). GM, WM volumes were sub-divided in each subject-session using parcellations of 278 and 20 regions-of-interest (ROIs), respectively. For both CSA genders, volumes of brain tissues (WM; GM; CSF) exhibited significant deviations during the season that largely recovered to pre-participation levels at Post2 (4-5 months post-season). Volumetric changes in brain tissues indicated a decrease in both GM and WM, with an increase in CSF. These changes varied longitudinally during continued participation in collision-based activities. All of these within-season changes are consistent with prior studies of acute dehydration, but are here observed in a more chronic form. Concern remains for the well-being of CSA given the relatively long duration of the observed changes in brain volume.

Efficacy of a home-based treatment paradigm, EMST and SpeechVive, to improve communication in Parkinson's Disease

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Background: Nearly 90% of individuals with Parkinson's disease (PD) develop hypokinetic dysarthria – a speech disorder characterized by reduced loudness, dysprosody, and a breathy voice^{2,5}. Despite the high occurrence of speech deficits secondary to PD, only 3-4% of individuals with PD receive therapy from speech-language pathologists. Travel to/from medical centers and time commitments required by common treatment programs are likely obstacles. Thus, there is a strong need to develop accessible speech treatment paradigms for individuals with PD. The SpeechVive device and Expiratory Muscle Strength Trainers (EMST) are evidence-based speech treatment devices that may be used at home. Previous studies have reported improvements in loudness, speech rate, and respiratory support for speech after treatment with the SpeechVive^{8,11}. Similarly, EMST effectively increases muscle strength for increased respiratory drive and loudness^{1,3,10}. The purpose of this study is to investigate the impact of a combined home-based treatment modality, SpeechVive and EMST, on speech, respiratory, and quality of life outcomes for people with PD and their caregivers.

Methods: Twenty-four individuals with PD and their caregivers participated. PD participants utilized a combined treatment modality, EMST and SpeechVive, in the comfort of their homes for 8 weeks. Data was obtained at 3 time points (pre, mid, post). Participants attended weekly telehealth sessions to adjust device settings. Speech acoustics, respiratory kinematics, and respiratory strength metrics were obtained. Additionally, participants and their caregivers completed a series of quality of life surveys pre- and post-treatment.

Results/Conclusions: The use of a combined home-based treatment modality, the EMST and SpeechVive, is an effective, accessible treatment source for communication impairments secondary to PD. Preliminary data shows increased loudness from pre- to post-treatment and with the SpeechVive on. Utterance length and respiratory strength improved from pre- to post-treatment. Pause patterns, speech rates, and maximum inspiratory pressures did not change.

Keywords: Parkinson's disease, dysarthria, SpeechVive, Expiratory Muscle Strength Training, telehealth

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Edible cryptographic primitives for anti-counterfeiting of medicines

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Counterfeit medicines are a fundamental security problem. Counterfeiting medication poses a tremendous threat to patient safety, public health, and economy in developed as well as less developed countries. The current solutions for pharmaceutical authentication and anti-counterfeiting rely on surface marking, printing, taggants, and radio-frequency identification, all of which are often vulnerable due to the limited security levels. We propose that the highest protection against counterfeit medicines would be a combination of a physically unclonable function (PUF) with on-dose authentication. As originally developed for hardware security, a PUF can provide a ‘digital fingerprint’ with multiple pairs of input challenges and output responses, which are virtually impossible to be cloned. As a form of oral consumption, on-dose (or in-dose) authentication can verify every individual pill or dose without removing the identification object or tag. Here we report on-dose PUFs that can be directly attached to the surface of medicines, be swallowed, and digested. Fluorescent proteins and silk proteins serve as edible photonic biomaterials, capable of facile processing and scalable production. The photoluminescent properties of fluorescent protein-expressed silk are used to achieve parametric support of challenge-response pairs for reliable PUFs. After von Neumann debiasing, cryptographic keys generated by edible PUFs support general satisfactory PUF performance, including bit randomness, encoding capacity, device uniqueness, readout reproducibility, and low false rates. Edible cryptographic primitives of all protein-based PUFs can play an important role in on-dose authentication, pharmaceutical anti-counterfeiting, and other security applications where immediate destruction or vanishing features are required.

Keywords: Cryptographic primitive, physically unclonable function, anti-counterfeit, on-dose authentication, fluorescent silk

Using Cortical growth map patterns to observe relations to epilepsy

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Background: Intractable epilepsy is a chronic disorder in which neuronal brain activity is disrupted, leading to seizures that fail to come under control with treatment. Long-term effects of uncontrolled epilepsy include cognitive abnormalities and brain tissue damage. While cortical damage is known to occur, clinical MRI scans often fail to show the precise location and degree of cortical loss.

Hypothesis: The goal of this study is to determine the feasibility of novel MRI analysis methods to map cortical loss over disease progression. We hypothesize that our method will highlight areas of clinically-confirmed cortical damage. Individualized maps highlighting cortical loss or, in the case of the developing brain, reductions in cortical growth, may serve as a valuable research and diagnostic tool.

Methods: In this exploratory study, we considered four pediatric subjects with intractable epilepsy. Two T1-weighted volumetric scans were obtained per subject at Riley Children’s Hospital. Baseline scans were acquired at a variety of ages (1.5-11 years) and follow-up scans were acquired 0.5-2 years later. Using the segmentation software FreeSurfer, MRI scans were reconstructed into three-dimensional surfaces, including representations of the white matter, midthickness, and pial brain surfaces. The segmentations of the early and later time points were processed through aMSM (anatomically-constrained Multimodal Surface Matching), a software that maps local surface deformations (growth or shrinkage of the cortex) between younger and older surfaces based on mechanical strain energy minimization.

Results and Discussion: Preliminary results suggest that aMSM may generate useful maps highlighting specific regions of cortical damage in cases of focal epilepsy. However, results depend on accurate reconstructions of the cortical surface, which could not be obtained in all subjects with current methods. Future work will explore alternative segmentation algorithms, including those designed for MRI data from subjects with brain lesions.

Inhibition of alpha-synuclein aggregation and prion-like propagation as intervention strategies to slow the progression of Parkinson's Disease

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A practical therapeutic goal for Parkinson's disease (PD) should be slow the progression of the pathology by (i) preventing the formation of new aggregates in existing live dopaminergic neuronal cells; and (ii) inhibiting propagation of the disease by interfering with cell-to-cell spreading of aggregated alpha-synuclein (aSyn), which is thought to be the major toxic species. aSyn has been shown to bind to anionic phospholipid vesicles, and recent studies by our lab and other groups suggest that aSyn-membrane interactions can catalyze the protein's aggregation at the membrane surface, a process that leads to vesicle permeabilization. We have identified four heptapeptides that interact with membrane-bound aSyn and inhibit membrane-induced aggregation and vesicle permeabilization. These peptides have been shown to exhibit neuroprotection in a primary midbrain neuronal culture model of PD. We have also identified an adenylyltransferase, called HYPE, which can interact with aSyn and decrease its aggregation and membrane permeabilization propensity through adenylation of the protein.

In parallel, to understand molecular mechanisms behind the prion-like progression of aSyn aggregates, we have developed cellular and in vivo models that enables us to examine the internalization of aSyn PFFs and the fate of the fibrillar seed in the recipient cell. With the use of a pH-dependent fluorophore conjugated aSyn which fluoresce only in the acidic environment, we have been able to monitor the dynamics of sonicated fibril uptake and in the endo-lysosomal compartment. This prion-like model is also being used to study morphologically different fibrils to answer the questions associated with patient-specific disease progression and symptoms.

Together, these studies will yield insights into the molecular underpinnings of aSyn neuropathology in PD and other synucleinopathy disorders and set the stage for developing therapeutic strategies to slow disease progression.

Keywords: Parkinson's Disease, alpha-synuclein, prion-like, HYPE, aggregation.

Non-invasive telemetry system for detection of dysautonomia after spinal cord injury using machine learning

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Dysautonomia is the dysfunction of the Autonomic Nervous System (ANS) which often gives rise to impaired control of cardiovascular activity, thermoregulation, gastrointestinal function, and bladder function. Current methods of detection of ANS function are difficult and time consuming, requiring a great deal of precision and experience. We propose developing a non-invasive, multi-parametric system to quantify the cardiovascular and neural changes due to dysautonomia in spinal cord injuries (SCI). We will focus on detection of autonomic dysreflexia (AD) which can have severe consequences if it is not properly managed. There is a need for a wearable tool which can detect the onset of AD in real time and identify the trigger to allow better management. To address this need we will develop a system which combines novel sensing and machine learning techniques to determine unique signatures of AD caused by various triggers. Through the development of the system we can create a tool which can help newly injured individuals to manage their AD better.

Keywords: dysautonomia, spinal cord injury, machine learning, unique signatures

Virtual hyperspectral imaging for noninvasive blood hemoglobin measurements – *mHematology*

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Blood hemoglobin (Hgb) testing is a routine procedure in a variety of clinical situations. However, noninvasive, continuous, and real-time Hgb measurements are still challenging, deviating from clinical laboratory Hgb values. Although portable (in-house) or point-of-care blood analyzers are currently available, these still remain unaffordable and inadequate in low-income country and home settings. Recently, digital image-based noninvasive and clinical examinations have also received attention, but these techniques still do not have sensitivity and specificity enough to replace conventional blood tests. We have developed a new class of computational spectroscopy – virtual hyperspectral imaging that virtually transforms a built-in camera in the smartphone into a hyperspectral imager of computing blood hemoglobin content. Our mobile version of virtual hyperspectral imaging combines imaging of peripheral tissue (conjunctiva) and spectroscopic quantification of total Hgb without a priori personalized calibration. As a data-driven technology, virtual hyperspectral imaging completely simplifies the hardware complexity and avoids the use of additional smartphone attachments. The key features include mobility, simplicity, and affordability (no additional cost for hardware) for rapid field adaptation in homecare settings. Thus, our smartphone-based virtual hyperspectral imaging has the potential for measuring Hgb content in grams per deciliter (i.e. g dL⁻¹) without a blood draw

Keywords: virtual hyperspectral imaging, conjunctiva, hemoglobin, mHealth, computational spectroscopy

Characterizing cardiac disease kinematics in mice using four-dimensional ultrasound

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Cardiac disease remains the number one cause for all mortality in the United States, prompting a continued effort to understand the various factors that exacerbate disease. In this endeavor, mouse models of cardiac disease have served a crucial role by allowing for both investigation of disease factors and longitudinal tracking of cardiac function. Routine assessment of cardiac function in mice can be acquired using high-frequency ultrasound; however, conventional techniques must rely on idealized cardiac geometries to calculate function metrics, as images are only from a single-plane view. Aiming to overcome these limitations, our group has recently developed and validated a high frequency four-dimensional ultrasound (4DUS) technique that provides full volumetric information of the mouse heart synced over one representative cardiac cycle. Analysis of this 4DUS data can provide region-specific wall kinematic information, in contrast to global metrics such as ejection fraction and stroke volume. Quantification of wall kinematics with spatial specificity can not only confirm the presence of cardiac disease, but also help uncover which regions of the heart most strongly correlate to disease progression. Preliminary applications of our technique have demonstrated abnormal left-ventricular contractile patterns in mouse models of cardiac hypertrophy, as well as ventricular remodeling in models of myocardial infarction. These initial efforts suggest that widespread adoption of 4DUS has the potential to help increase the quality of information obtained while studying mouse models of cardiac disease.

Keywords: cardiac disease, hypertrophy, ultrasound, kinematics, strain

Low cost, rapid detection and analysis of EVs

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There are over 20,000 papers published in the last dozen or so years focusing on extracellular vesicles (EVs). 70% have been published within the past 6 years. What is clear in the literature is the difficulty researchers face in dealing with analyzing these EV suspensions. While there are several technologies used, none are particularly easy, some are very time consuming and some are very expensive. There seems to be little doubt in the scientific community that EVs are vitally important and that our knowledge base must increase if we are to understand the many different types of EVs and their impact. In the area of cancer, numerous papers outline the potential roles of EVs in terms of transport, communication or even as potential diagnostic indicators particularly with regard to solid tumors.

Many companies have recognized the demand for reagents to facilitate EV isolation and there are now almost 20 different EV isolation kits available on the market. Not a single kit provides any details of what the “concentrated EVs” are that they produce. What is needed is an easy to use, inexpensive technology that will provide quantitative data on numbers, size and most importantly phenotype of the EVs present. This can be achieved using currently available monoclonal antibodies that are mostly used in the field of flow cytometry. We present a small bench top instrument not much larger than a typical pH meter or lab balance, but with the capacity to provide the above details within a few minutes on just a few microliters of sample. This technology is focused on the well-known Blu-ray technology, which when adapted for liquid samples can achieve the challenging demand of accurate analysis from 20-200nm and with the addition of a fluorescence detector, measure the phenotype based on addition of fluorescence conjugated antibodies.

Keywords: Extracellular Vesicles, Detection, Cancer Biomarker

Drug adsorption in endovascular magnetic filtration device

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Drugs used in cancer chemotherapy target specific sites of the disease, where higher drug concentrations are desirable; however, the increase dosing can cause side effects. We collaborate with the University of California San Francisco on developing catheter-based devices to selectively remove specific drugs from the blood stream in order to reduce systemic toxicities. This work is focused on a magnetic filter device which can capture chemotherapy agents bound to the Ferumoxytol, i.e. FDA-approved superparamagnetic iron-oxide nanoparticles (SPIONs). After the drugs injected in the supplying arteries have had an effect on the tumor, their excessive amount can be filtered by an arrangement of cylindrical magnets deployed in the draining veins during the procedure.

The motion of the SPIONs is governed by both magnetic and hydrodynamic forces and thus depends on the magnetic field gradient and fluid velocity. In this study, alternative configurations of the magnetic filter were examined to optimize SPIONs capture. Multi-physics CFD simulations were conducted for cylindrical magnets with diametrical and axial magnetization. Magnetic devices with different spacing between the magnets were simulated and the numerical results were compared to in vitro experiments. Furthermore, the magnets deployed in series and in parallel were evaluated. Preliminary results show that placing the magnets in the near-wall regions where velocity is decreased can improve particle capture as compared to the original design with the magnets deployed along the centerline of the vessel. Filtering devices with various configurations and magnetic parameters will be further analyzed by coupling hemodynamics, electromagnetism, and particle dynamics, in order to optimize filtering performance. The ultimate goal is to develop a device filtering up to 90% of the toxins in the first pass, without causing flow stagnation and thrombosis.

Keywords: Cardiovascular Disease, Drug Adsorption, Magnetic Filtration, SPIONs, Hemodynamics

Longitudinal ultrasound assessment of a murine model for cerebral aneurysms

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Approximately 30,000 people in the United States suffer a brain aneurysm rupture each year, resulting in death and higher incidence of neurological complications. Current murine models are suboptimal for simulating human brain aneurysms. This study presents a novel murine model for saccular aneurysms that allows for control of diameter and thrombus formation. Female Apolipoprotein E (apoE) deficient mice (n=8) were anesthetized using 2% isoflurane, and an aseptic incision was made to isolate the right common carotid artery (RCA). A suture was placed 2-5mm distal from the clavicle to completely ligate the RCA. Topical porcine pancreatic elastase (10mg/mL) was applied to the artery for 5 minutes followed by a triple saline rinse, and .2% β -aminopropionitrile monofumarate (BAPN) drinking water was administered throughout the study. Small animal ultrasound (Vevo3100, FUJIFILM VisualSonics) was used to image the right and left carotid artery on days 0, 1, 4, 7, 21, 35, and 49. We observed rightward expansion of the RCA in all eight mice which lead to asymmetrical aneurysm formation with three aneurysms that appeared more saccular in shape. An increase in maximal aneurysm diameter coincided with increases in length of suture placement distal to the clavicle ($p < 0.05$). Average maximum aneurysm diameter at day 49 was 1.01mm with 0.48mm average growth from baseline. A 13.5 percent dilation of the left carotid artery was observed at day 49. This is likely a hemodynamic response to counteract RCA ligation. In this study, we produced an elastase-BAPN induced carotid aneurysm model and have quantified geometrical differences in aneurysm development over a 49-day period. Future work will investigate the effects of arterial outflow on aneurysm geometry, as well as histological analysis.

Key Words: Murine, Carotid Artery, Aneurysm, Ultrasound Imaging

Impact of FITC conjugation to fibrinogen on fibrin clot formation and digestion

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Thrombosis is the pathological formation of a blood clot in the body that blocks blood circulation, leading to high morbidity and mortality rates worldwide. Fibrin is formed following the cleavage of fibrinogen by thrombin and is primarily responsible for clot formation and thrombosis. Fibrin clots in the human body are dissolved by plasmin, a serine protease. Characterizing the dynamic process of physiologically relevant fibrin digestion is necessary to study thrombosis and therapeutics. Existing ways to monitor clot lysis *in vitro* either requires the use of specialized equipment like Thromboelastography or is highly dependent on the clear absorbance of the sample at certain wavelengths like clot turbidimetry. FITC labeling is widely adopted in research labs to track conjugated proteins quantitatively via absorbance and fluorescence intensity. Thus, forming a fibrin clot with FITC labeled fibrinogen and tracking the absorbance in the clot supernatant make it possible to compute a clot digestion rate. This study sought to explore the impact of FITC-fibrinogen conjugation on fibrin clot formation and clot fibrinolytic properties by plasmin. Clot samples were formed by mixing thrombin with untagged, 4, 6, and 10 FITC tagged fibrinogen. Clot properties were compared using turbidity (550 nm via Spectrometer), clot strength (Maximum Amplitude via Thromboelastography) and plasmin digestion rate (280 nm of clot supernatant). We observed an increase in turbidity and a decrease in clot strength of fibrin at increasing FITC conjugations per fibrinogen. Higher turbidity and lower clot strength at a fixed fibrinogen concentration is indicative of thicker fibrin fiber formation. The results suggest that the conjugation of FITC to primary amines of lysine residues on fibrinogen is interfering with fibrin fiber formation by sterically blocking packing sites. The thicker fibers formed by 10-FITC per fibrinogen also showed a 2.5-fold slower digestion rate by plasmin compared to untagged fibrinogen.

Keywords: Fibrin, Clot, FITC, Turbidimetry, Thromboelastography

Closing the loop on gastric electrical stimulation: Multichannel, non-invasive recording and analysis of vagal nerve activity for improving gastroparesis symptom management

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Gastroparesis (GP) is a chronic condition characterized by a slowed transit of food from the stomach without any visible obstruction. When dietary modification and medications fail, gastroparetic patients may become candidates for gastric electrical stimulation (GES) therapy, a potentially life changing neurostimulation therapy for intractable forms of nausea and vomiting associated with diabetic or idiopathic GP.

Despite common reports of symptom improvement from open-label studies using the Medtronic Enterra GES device, the time-to-efficacy and degree of symptom relief are unpredictable. Variable reports of efficacy may stem from improper stimulating electrode placement, the open-loop, “one-size-fits-all” stimulus parameter tuning protocol, an incomplete understanding of the mechanisms-of-action, or a general lack of unified knowledge of the underlying anatomy and functions of the vagal-gut connectome.

Prior Indiana CTSI-funded research has identified vagal activity associated with the efficacy of GES and has led to a comprehensive, noninvasive investigation of vagal activity for clinical applications. Specifically, novel nerve response analysis techniques (Autonomous Neural Control, or ANC) and machine learning techniques were used to associate specific vagal activity and efficacy of GES. Preliminary results correlated specific vagal activity patterns with alleviation of specific symptoms.

Here we describe development and use of a new multichannel recording platform for sensitive and high-resolution measurement of underlying nerve activity. This system is being tuned towards enhanced characterization of vagal nerve activity. Ultimately, the investigators hope that clinicians may use this technology during GES device implantation and optimization to better determine device placement and stimulation parameters.

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Computational modeling of drug transport and mixing in the chemofilter device

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Liver cancer is among the leading causes of cancer-related mortality worldwide. The preferred treatment for unresectable cases of primary liver cancer is intra-arterial chemotherapy (IAC), in which chemotherapeutics are delivered into blood vessels directly supplying a tumor. The effectiveness of IAC is limited by the large fraction of drugs, e.g. Doxorubicin, that pass into systemic circulation and cause cardiac toxicity. These excessive drugs can be captured by the Chemofilter (CF) – a 3D-printable, catheter-based device deployed in a vein downstream of the liver during IAC. The CF chemically adsorbs Doxorubicin via ion-exchange with a surface-coated resin, thereby reducing systemic concentrations. In this study, alternative configurations of the Chemofilter device were compared by evaluating their hemodynamic and filtration performance through multiphysics computational fluid dynamics simulations. Two designs were evaluated, a honeycomb-like assembly of parallel hexagonal channels (honeycomb Chemofilter) and a cubic lattice of struts (strutted Chemofilter). Device design was optimized using a sensitivity analysis of flow and geometry parameters. The electrochemical binding was modeled based on concentrated solution theory, where diffusion and ion migration were incorporated into an effective diffusivity term. The Navier-Stokes and Advection-Diffusion-Reaction equations were coupled using ANSYS Fluent. The computationally optimized CF design contained three honeycomb stages, each twisted, perforated, and aligned with the flow direction to enhance mixing and drug binding. These multiphysics simulations predicted an overall 66.8% decrease in concentration with a 2.9 mm-Hg pressure drop across the optimized device compared to a 50% concentration decrease observed during in-vivo experiments with the strutted Chemofilter. These simulations demonstrate the utility of the CF in removing excessive drugs from circulation while minimizing pressure drop and eliminating flow stagnation regions prone to thrombosis. Simultaneously, these results prove the importance of the multiphysics modeling strategy in device optimization and experimental burden reduction.

Keywords: Chemofilter, device, chemotherapy, computational fluid dynamics, multiphysics

Pulse wave interactions at the Circle of Willis

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In the 17th century, Thomas Willis proposed a theory of collateral flow to characterize anastomosis in the human brain. Later dubbed the circle of Willis (CW), this structure is commonly thought to protect the brain from ischemic injury. However, analogous structures in Animalia (unlikely to experience stroke) and evolutionary contradictions (disease of the elderly does not drive selection) raise the question: what would have triggered the formation of such a structure? We hypothesize that communicating arteries (CoA) of the cerebral circulation allow for passive filtering of the cardiac pulse wave. Delayed arrival of the pressure wave at the CoA junctions may reduce rate of pressure change, peak pressure, and pulse pressure. We developed a transmission line model of the arterial system to examine the effects of anatomy on pressure propagation through the systemic circulation. In order to distinguish unappreciated functions of the CW, we considered two states of cardiac function: heavy exercise and flight-or-fight response. As purely collateral vessels, absent or abnormal CoA have minimum impact on patient health; however, they may play an important role in protecting the brain from hemorrhage due to an abrupt pressure variations that rupture thin-walled cerebral arteries.

Keywords: pressure, pulse wave, cardiovascular, circle of Willis, biomechanics

Benzamidine-to-Benzamidine proximity dependent inhibition of human plasmin

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Plasmin and its derivatives are being explored for dissolving blood clots in diseases like myocardial infarction and venous thromboembolism. Plasmin is a serine protease which causes clot digestion by fibrin degradation. Benzamidine is a reversible inhibitor of plasmin which binds directly to its active site. Our group's previous study compared plasmin inhibition by monovalent benzamidine derivatives and pentamidine (bivalent benzamidine separated by 0.7 nm, FDA approved for infection treatment). These molecules demonstrated a wide range of inhibition constants (K_i), varying from 2.2-1,074 μM (lower K_i is indicative of stronger plasmin inhibition). Pentamidine inhibited plasmin 15-fold better than the best monovalent inhibitor. In our current study, we sought to understand the effect of valency and proximity of benzamidine-to-benzamidine on plasmin inhibition. Four bivalent molecules were synthesized with benzamidines separated by 1.6, 2.6, 5.5 and 9.8 nm using 4-aminomethyl benzamidine and monodisperse PEG linkers. These inhibitors were purified using RP-HPLC and confirmed via mass spectrometry. Pentamidine and monovalent 4-aminomethyl benzamidine were also tested. Inhibition assays were carried out at a fixed concentration of human plasmin with multiple inhibitor (0-300 μM) and chromogenic substrate S-2251 (100-500 μM) concentrations to determine K_i using Dixon plots. K_i for the monovalent molecule was 1,395 μM whereas K_i values for bivalent molecules ranged from 1.7-326.3 μM and were linearly correlated to the distance between benzamidines. Pentamidine, the shortest bivalent molecule had the smallest K_i, exhibiting 192-fold greater inhibition than the longest bivalent molecule tested. Moreover, even the longest bivalent molecule with the highest K_i is a 4-fold better inhibitor than the monovalent molecule. Further studies with higher order valency molecules (trivalent, tetravalent, etc.) and varying proximities of benzamidine-to-benzamidine will be carried out to expand on these inhibition correlations.

Keywords: benzamidine, proximity, valency, plasmin, inhibition

Selective collection of exhaled breath condensate (EBC) for non-invasive glucose detection

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Two thirds of patients with diabetes avoid regularly monitoring their blood glucose levels because of the painful and invasive nature of current blood glucose detection. As an alternative to blood sample collection, exhaled breath condensate (EBC) has emerged as a promising non-invasive sample from which to monitor glucose levels. However, the inconsistency in the methods used to collect EBC significantly impacts the reliability of reported analyte concentrations in EBC. For example, current EBC glucose measurements have resulted in dilution factors ranging from 1/1000 to 1/50000. Thus, there is a need for a systematic and selective EBC collection method to ensure accurate analyte detection and quantification. Herein, we develop and characterize a low-cost, portable condenser which selectively collects exhaled breath that has been exchanged with lung fluid in a temperature-based manner. We demonstrate that for ~15 L of exhaled air, our device can condense reproducible volumes of EBC (>130 μ L) in under 3 minutes ($p > 0.05$, $n = 3$). Our results indicate that a higher concentration of glucose can be detected in the collected sample with selective valve opening ($p < 0.05$, $n = 3$). Furthermore, EBC and blood glucose samples measured from both normoglycemic and type 2 diabetic subjects over the course of a three-hour oral glucose tolerance test (OGTT) indicate higher dilution factors in diabetic patients. This device enables a robust, non-invasive collection method that can evaluate the correlation of glucose concentrations between EBC and blood. Ultimately, this work aims to develop a non-invasive tool for glycemic control and distinguish key differences in regulation of glucose from plasma to respiratory fluid for normoglycemic and diabetic individuals.

Keywords: diabetes, glucose, non-invasive, detection, respiratory

Longitudinal ultrasound assessment of murine aneurysm expansion and intraluminal thrombus formation

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Abdominal Aortic Aneurysms (AAA) are focal dilations of the infrarenal aorta. Determining risk of aneurysm rupture and efficacy of novel treatments is challenging, creating the need for animal models that mimic the human condition. In this study, we use in vivo ultrasound to characterize longitudinally a mouse AAA model that develops large aneurysms with intraluminal thrombus. To induce aneurysms, 5 μ L of elastase (heat inactivated, 2.5 mg/mL, 5 mg/mL, or 10 mg/mL; $n = 5-8$ /group) was topically applied to the infrarenal aorta of male C57BL/6 mice. Mice were also given 0.2% beta-aminopropionitrile (BAPN) dissolved in drinking water beginning 2 days prior to surgery. BAPN binds to lysyl oxidase, inhibiting cross-linking of collagen and elastin, and results in continuously growing aneurysms. Over a 56-day period, mice were imaged weekly by ultrasound to assess aneurysm growth. Short-axis images were used to estimate maximum diameter and M-Mode was used to calculate the in vivo circumferential Green-Lagrange strain. All animals in the 2.5 mg/mL, 5 mg/mL, and 10 mg/mL groups developed an aneurysm, as defined by a 50% increase in diameter, by day 14. These AAA continued to grow over the 56-day period. At the end of the study, the highest elastase concentration, 10 mg/mL, yielded an average maximum diameter increase of ~600% compared to baseline ($p < 0.001$). Additionally, 63% of mice developed thrombus, with some animals showing layered thrombus. Aneurysm formation was preceded by a significant decrease in circumferential strain by day 2 compared to baseline ($p < 0.001$) that remained below 5% throughout the study ($p < 0.001$). In contrast, at the lowest elastase concentration, 2.5 mg/mL, the average maximum diameter increase was ~250% compared to baseline with no intraluminal thrombus. These results suggest that treatment with varying concentrations of elastase can be used to induce a range of aneurysm sizes, including large aneurysms with intraluminal thrombus.

Keywords: aneurysm, thrombus, ultrasound, murine

Engineering design and simulation of mechano-instructive collagen scaffolds for treatment of difficult-to-heal wounds

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Aim: Difficult-to-heal wounds of the skin are among the most common and costly medical problems experienced. Wounds lead to displeasing cosmetic outcomes and also carry a high burden of loss-of-function, scarring, contracture, or amputation due to nonhealing. There exists a need for regenerative dermal replacement strategies that adapt and grow with the individual, but a continuing challenge is identification of optimal scaffold parameters for healing. We present a new computational model for prioritization of oligomer scaffold design parameters for dermal regeneration. **Methods:** In previous animal experiments, we evaluated dermal replacement scaffolds custom-fabricated from fibril-forming collagen oligomer with controlled fibril density (4 - 40mg/cm³) and spatial gradients in rat excisional wounds. Wound contraction and cellularization were monitored by gross and histological image analysis for comparison with model outcomes. We now parameterize the scaffold parameters using nonlinear curve fitting for use in the computational model of wound healing. A preliminary chemo-bio-mechanical finite element model including collagen, cells, and representative cytokine signaling was adapted to simulate wound healing results. **Results:** Collagen scaffold microstructure was quantified from scanning electron micrographs. A constitutive law for collagen mechanics was fit to experimental uniaxial tensile tests to quantify scaffold stiffness parameters. Using this information, we conducted preliminary three-dimensional finite element model simulations for validation against experimental wound contraction, recellularization, and collagen remodeling data collected from various prototype scaffolds. We will iteratively inform the model by comparing computational model predictions with actual experimental outcomes. **Conclusions:** This work represents the first step towards a computational model of wounds treated with collagen scaffold dermal replacements. In turn, the model will be used to explore cell-scaffold interactions for purposes of prediction and optimization of tissue regeneration outcomes. We anticipate future work to further improve the model of mechanotransduction, cytokine signaling, and recellularization.

Keywords: collagen, biomechanics, mechanobiology, wound healing, regeneration

Twenty-four-hour urinary sodium excretion from a spot urine sample may be used as an indicator of intake in CKD patients

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Sodium (Na) intake can elevate blood pressure, which is a factor in developing chronic kidney disease (CKD). Twenty-four-hour urinary sodium (24hUNa) is the gold standard for assessing Na intake but is burdensome. Validated equations estimate 24hUNa (e24hUNa) from a spot urine sample, but these estimations are not validated against known Na intake in CKD. The current study is a secondary analysis of a controlled feeding study in moderate CKD patients matched to healthy adults. Only CKD patients were used for the current analyses (n=8). Participants consumed a controlled diet for 9 days, providing ~2400 mg Na/d. On days 7 and 8, participants collected all urine in an inpatient setting, beginning with a fasting sample on day 7. Urine sample mineral analyses were performed by inductively coupled plasma optical emission spectroscopy and urinary creatinine by the Jaffe reaction. The day 7 fasting urine sample was used to calculate e24hUNa using 6 published equations. Log-transformed Na intake, measured 24hUNa, and e24hUNa were compared by repeated-measures ANOVA. Fifty percent of the CKD patients (n=4) were female; 63% (n=5) were white, and 37% (n=3) were black. On average, participants were aged 56.6±13.8 y with a BMI of 31.7±9.4 kg/m² and eGFR of 40.7±7.9 mL/min. Based on compliance data, average Na intake on day 7 was 2024±388 mg. Average measured 24hUNa was 2529±1334 mg. The main ANOVA was significant (p=0.02). Planned contrasts found that e24hUNa from the SALTED cohort, an equation developed for CKD patients, was significantly higher than both Na intake (p<0.001) and measured 24hUNa (p=0.007). For the remaining 5 equations, e24hUNa was not significantly different from measured 24hUNa nor Na intake. Our results suggest e24hUNa calculated using most published equations may provide a reliable and low-burden method of assessing Na intake in moderate CKD patients. These findings should be confirmed in larger samples.

Keywords: Sodium, chronic kidney disease, twenty-four-hour urinary sodium excretion, spot urine

Murine atherosclerosis characterization using cross-sectional lipid-specific photoacoustic and longitudinal 4D ultrasound imaging

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Dual-modality photoacoustic tomography (PAT) and 4D ultrasound (4DUS) imaging have recently shown utility in studying atherosclerosis progression in small animals. PAT uses pulsed laser light to induce acoustic waves and reconstruct lipid-specific compositional images of tissue. 4DUS captures dynamic volumetric information and can be used to estimate 3D Green-Lagrange strain using a direct deformation estimation method. Here we hypothesized that PAT/4DUS can be used to assess changes in arterial strain and hemodynamics, as well as quantify lipid volume and localization in animals that have undergone partial carotid ligation (PCL) induced-atherosclerosis. A 40 MHz transducer (Vevo2100, VisualSonics) and a ND:YAG pulsed laser (Surelite EX, Continuum) were used to image five apolipoprotein-E deficient mice that underwent PCL of the left carotid artery while being fed a Western diet [4]. Animals were imaged using 4DUS at days 0, 1, 4, 7, 10, and 14 to obtain pulsed-wave Doppler for hemodynamic characterization and 4DUS images for strain mapping. At day 14 all animals were euthanized and 3D *in situ* PAT images of the left carotid artery were acquired using 1210nm light. Lipid volume and localization was quantified using 1210nm signal-to-noise (SNR) ratio. The results show that atherosclerotic lesions can be characterized via PAT to localize both lipid accumulation and volume. On the other hand, 4DUS can be used to assess dynamic changes in carotid artery strain and hemodynamics during plaque progression.

Keywords: Murine, atherosclerosis, photoacoustic, 4D, ultrasound

Pentagalloyl glucose effects on murine aortic strain and diameter

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Abdominal aortic aneurysms are a dilation of the largest artery in the body and are accompanied by significant risk of mortality due to potential rupture. Current treatments are surgical in nature and carry additional risks and complications. As such, there is a need for a minimally invasive method to stabilize and preserve the mechanical properties of the aorta. This study evaluated the potential of pentagalloyl glucose (PGG) to accomplish this using two models of aortic injury. The first model used elastase (5.0mg/mL) applied topically to the aorta to induce aneurysms (n=17). Prior to elastase application, animals received 0.30% PGG (n=8) or saline (n=9) treatment administered by soaking gauze in the solution and applying it to the aorta. High frequency ultrasound imaging to assess aortic diameter and strain was performed prior to surgery, three days following surgery, and weekly thereafter for 2 weeks. The second model used calcium chloride (CaCl₂, n=11) to injure the aorta. Gauze was soaked in a 0.5M CaCl₂ solution and applied to the adventitial surface of the aorta. Topical treatment of 0.30% PGG (n=6) or a saline control (n=5) was administered prior to CaCl₂ by the aforementioned method. High frequency ultrasound imaging was performed prior to surgery and weekly thereafter for 4 weeks. No significant difference in diameter between treatments was seen for either model, and there was no significant difference in circumferential strain in the topical elastase model ($p=0.50$). However, there was a significant difference in circumferential strain between treatment groups in the CaCl₂ model from day 14 through day 28 ($p<0.05$). At day 28, PGG treated animals had $6.7\pm 1.4\%$ strain, while saline treated animals had $4.0\pm 0.8\%$ strain. These results highlight model specific differences and suggest PGG preserves vessel pulsatility in the CaCl₂ model but not in the elastase model.

Keywords: abdominal aortic aneurysm, ultrasound, vascular imaging