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Online Proceedings

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New Diagnostic Tools for Seeing and Sensing Disease

Session Moderator: Benjamin Freedman, Medicine/Nephrology

JHN 175

12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Quantitative Reverse Transcriptase PCR Detection of Organisms from Ribosomal RNA

Kevin Zhou, Senior, Microbiology
Joshua Y. Lee, Senior, Microbiology
Ruoyun (Brian) Gao, Senior, Microbiology
Mentor: Sean Murphy, Laboratory Medicine & Pathology

Pathogenic microorganisms cause a large burden of disease globally. Rapid and accurate detection of these pathogens is essential for treatment. The goal of our project is to evaluate the analytical and clinical sensitivity of Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR) for detecting such microorganisms compared to conventional qPCR. qPCR targets and amplifies specific DNA sequences and is widely used in laboratories to detect these organisms. However, when there is only a single organism in a diagnostic sample, the DNA amplified from such a limited number of template copies may not be detectable by qPCR. RT-qPCR overcomes this by targeting ribosomal RNA (rRNA), which is in greater abundance compared to the rRNA-encoding DNA. rRNA-targeted assays can provide more sensitive detection because the target is more abundant. In addition, rRNA is relatively resistant to degradation, unlike more labile mRNA targets. To evaluate RT-qPCR, we selected important human pathogens, designed reagents for DNA dye based (LCGreen) or probe-based amplification/detection assays, and isolated total nucleic acids from samples by using a silica-based extraction instrument (bioMérieux). Nucleic acids from extracted samples were tested on a real-time PCR machine using with and without RT (+RT/-RT). We evaluated the relative increase in rRNA compared to coding DNA by comparing the +RT and -RT standard curves' cycle thresholds (Ct). In all experiments, RT-qPCR detected the target organism earlier than qPCR although the relative enrichment of rRNA varied by organism. For instance, in *Toxoplasma gondii*, 18S rRNA was up to 500 times more abundant than the coding DNA. In contrast, in *Cryptosporidium parvum* rRNA was only modestly enriched at 50-fold increase compared to coding DNA. Our work will provide a survey of relative enrichment of rRNA and will evaluate whether this target should be widely pursued as an improved approach for pathogen detection.

Development of a Fluorescence-Based Immunoassay for Rapid, Highly Sensitive, and Low-Cost Point-of-Care Influenza Diagnosis

Philip Meng En (Philip) Lee, Senior, Bioengineering
Mary Gates Scholar, UW Honors Program
Mentor: Paul Yager, Bioengineering
Mentor: Koji Abe, Bioengineering

The World Health Organization reports that seasonal influenza (flu) epidemics result in 3 to 5 million cases of severe illness with upwards of 500,000 deaths. In the United States, 200,000 hospitalizations and 36,000 deaths occur annually with an economic burden of $87 billion. Hence, there is an urgent need to develop highly-sensitive, low-cost diagnostics for use at the point-of-care (POC) to facilitate immediate treatment at the early stages of infection. POC diagnostics can be implemented in paper-based, lateral flow assays to capture influenza nucleoproteins (NPs) with antibodies. Despite their utility, these assays suffer from moderately poor sensitivity. The highest sensitivity measured in a study evaluating six common colloidal gold colorimetric flu tests was 71%. To improve assay sensitivity, fluorescence detection has been demonstrated to detect analyte concentrations 1000-fold lower than with colloidal gold in lateral flow assays. My project aims to develop a fluorescence-based immunoassay for influenza A NPs to achieve improved sensitivities over those of colorimetric tests. The assay is carried out on lateral flow strips, making it readily translatable to point-of-care use. Upon capture of NP by immobilized antibodies, a biotinylated immunoglobulin G (IgG) binds to the NP at a different epitope. Subsequently, streptavidin-bound horseradish peroxidase (HRP) binds to the IgG. HRP oxidizes dihydrorhodamine 6G, a non-fluorescent substrate, into the

fluorophore rhodamine 6G (R6G). Hence, an observable fluorescent signal is generated in the presence of NP. In preliminary experiments, R6G adsorbed to the membrane; R6G accumulates near HRP, increasing the signal-to-background ratio. An immunoassay for NP shows observable fluorescence signals at the capture and control regions for their respective positive and negative sample conditions. The remainder of the project aims to optimize reagent concentrations to further improve the assay's sensitivity. Ultimately, the fluorescence-based assay developed would serve as a translatable platform for highly-sensitive detection of other viral analytes.

Developing the Detection and Treatment of Fetal and Newborn Infections via HRV Monitoring and Vagus Nerve Stimulation

Yael Leah (Leah) Frank, Sophomore, Pre-Major
Victoria Ann Dahl, Junior, Pre-Sciences
Mentor: Martin Frasch, Obstetrics and Gynecology

Neonatal sepsis, a blood infection in newborns, is one of the top five mortality causes in the NICU and can result from undetected fetal infections, which can also increase chances of serious injuries post-birth. Currently, it is difficult to detect such infections early in order to intervene therapeutically. In two animal models exposed to lipopolysaccharide (LPS) to trigger inflammation, we aimed to derive a method in detecting fetal infection early on and applied it to newborns exposed to an infectious stimulus to further monitor inflammation and test whether stimulating the cervical vagus nerve (VNS) can reduce the inflammation. Fetal sheep modeled the fetal infection, and newborn piglets modeled the neonatal sepsis. From the fetal ECG, the measure of the heart's electrical activity, we developed a heart rate variability (HRV) – the variation in time between heartbeats – index that tracks fetal inflammation. When applied to the piglet model, we found that HRV reliably tracks inflammation in the newborn piglets both when only LPS was given and also when LPS + VNS were given. We also collected vagus electroneurogram (VENG) signals at various experiments in both the fetal sheep and the piglets: the vagus nerve transmits complex afferent and efferent signals and we look to use the VENG data to improve the understanding of the vagus nerve's "code" of communications and their mathematical properties in order to find the optimal VNS method to reduce inflammation and refine HRV's detection of it. From this, we hope to create a more systematic approach of using the HRV index in detecting inflammation, and thus improve early detection of fetal infection.

The Mechanism of Interference of Hemoglobin and Bilirubin in Heparin Monitoring by Anti-Xa Assay

Alicia Bui, Senior, Medical Laboratory Science
Mentor: Wayne Chandler, Laboratory Medicine, Seattle Children's Hospital

Heparin is given to patients on Extracorporeal Membrane Oxygenation (ECMO) to prevent clot formation in the ECMO circuit. The anti-Xa assay is a chromogenic assay used to monitor heparin therapy by measuring the absorbance of a colored product generated from the reaction between reagent Factor Xa (FXa) and a chromogenic substrate to represent the heparin activity in plasma. Hemolysis and hyperbilirubinemia are mechanical complications of ECMO that interfere with heparin monitoring by anti-Xa assay by reporting falsely lower heparin activity measurements. There are three possible mechanisms for the apparent decrease in heparin activity due to hemoglobin and bilirubin: increased FXa activity, heparin neutralization, or spectrophotometer interference. The objective of this study is to investigate the mechanisms by which hemoglobin and bilirubin interfere with heparin monitoring by anti-Xa assay. The slope of absorbance change, heparin activity, and absorbance values reported between the measurement window were determined on the Stago STA Compact®analyzer for plasma samples spiked with heparin and varying dilutions of hemoglobin or bilirubin. The addition of hemoglobin and bilirubin to the assay resulted in an apparent decrease in the measured heparin activity that was proportional to the amount of hemoglobin and bilirubin added to the sample at 0, 0.3 and 0.6 U/mL heparin levels. The rate of the reaction increased proportionally to the amount of hemoglobin or bilirubin added and the relative increase in apparent reaction rate was independent of the heparin concentration in the reaction. Changes in absorbance values were linear between the measurement window for samples with a range of increasing initial absorbances. We concluded that the apparent decrease in heparin activity was a result of increased FXa activity caused by hemolysis and bilirubin, not heparin neutralization or spectrophotometer interference. This study improves the understanding of interferences in heparin monitoring by the anti-Xa assay in clinical laboratories.

Detecting Diffuse Liver Disease through Tissue Harmonic Ultrasound Imaging

Joanna Gloria (Joanna) Sun, Senior, Bioengineering
UW Honors Program
Mentor: Michalakis Averkiou, Bioengineering

Parenchymal disease of the liver, known as diffuse liver disease, led to 1.4% of the total deaths in the United States in 2013. It causes impaired liver functioning and may lead to portal hypertension, encephalopathy, or hepatocellular carcinoma. Current methods of diagnosing diffuse liver disease are often invasive, subject to heterogeneous variation, and fail to distinguish between intermediate disease stages. Ultrasound imaging has long been used to monitor changes in tissue structure, and it is known that sound attenuation changes with disease progression. Tissue is acoustically nonlinear, meaning that propagating sound waves are distorted due to tissue properties and generate higher order harmonics. Since

fatty deposits and fibrosis change with disease progression, we hypothesize that associated attenuation changes may be detected by changes in the nonlinear signal distortion and can be quantified as a marker of disease. We have designed a methodology based in Tissue Harmonic Imaging to use a quantified marker of attenuation to specifically detect diffuse liver disease. This project comprises of (1) a theoretical and experimental investigation of the influence of sound attenuation on harmonic content in tissue, (2) clinical patient data collection and development of MATLAB analysis tools utilizing the disease marker developed in Phase I, and (3) development of new imaging sequences capable of grading diffuse liver disease when coupled with the analysis tools. The noninvasive, easy to use, sensitive, and clinically usable methodology developed in this project has the potential for aiding in diagnosis of diffuse liver disease and enhancing quality of patient care.

Rosiglitazone Improves CLA-Mediated Insulin Resistance

Kate Turk, Senior, Biology (Physiology)
UW Honors Program
Mentor: Laura den Hartigh, Medicine

Conjugated linoleic acid (CLA), the only naturally-occurring trans-fat, is a fatty acid that is known to promote weight loss and increase energy expenditure. While it is considered a potential treatment for those struggling with weight-induced illness, previous research has shown its association with impaired glucose metabolism and inflammation. Using qRT-PCR to analyze expression of genes important for glucose metabolism and inflammation, we studied whether rosiglitazone, an insulin-sensitizing agent, improves CLA-mediated insulin resistance and adipose tissue inflammation in obese mice. We have previously examined epididymal and inguinal white adipose tissues and found that rosiglitazone increased expression of genes important for insulin responsiveness (such as adiponectin and PPARG), but decreased genes associated with inflammation (such as Mac2). In this study, we assessed the same gene expression profiles in mesenteric and retroperitoneal white adipose tissues, two adipose tissue depots that are understudied, but that could be important sites of CLA and/or rosiglitazone metabolism. Rosiglitazone effectively increased insulin sensitivity in mice with CLA-mediated insulin resistance, as made apparent by improved glucose and insulin tolerance tests. Further, rosiglitazone improved CLA-mediated decreases in the insulin sensitizing hormone adiponectin, measured in plasma and in both mesenteric and retroperitoneal white adipose tissues. Rosiglitazone had no effect on adipose tissue inflammation, however. This study demonstrates possible methods to improve CLA-induced weight loss by increasing insulin sensitivity with rosiglitazone treatment, and provides a possible intervention for those struggling with weight-related disease.

Investigating the Effect of Physiological Strain Cues on the Differentiation and Maturation Capacity of Muman Skeletal Muscle Myoblasts *in Vitro*

Rahil Hudda, Senior, Biology (Physiology)
Mentor: David Mack, Rehabilitation Medicine & Bioengineering, Institute for Stem Cell and Regenerative Medicine

The pharmaceutical industry's "one-size-fits-all" approach to drug discovery, coupled with the use of animal models that do not maximize resources or accurately represent human disease, is a significant obstacle to the development of more effective treatments with the capacity to significantly improve patient wellbeing. The concept of personalized medication needs to be prioritized within the drug development process to advance the identification of drug candidates with the greatest potential to ameliorate patient-specific symptomatic progression. This is especially true in neuromuscular diseases like Duchenne Muscular Dystrophy and X-Linked Myotubular Myopathy, which show wide variability in severity and age of onset. Myogenesis is the formation of muscular tissue, specifically during embryonic development. The involvement of chemical cues to promote the fusion of myoblasts into multinucleated structures called myotubes is fairly well understood. However, the specific mechanical forces associated are yet to be characterized due to the complex cellular machinery at play. Recent evidence suggests that exposing muscle cells in culture to rehabilitation techniques, such as mechanical and electrical stimulation that mimic resistance exercise, has profound functional consequences. Therefore, I hypothesize that subjecting human skeletal muscle myoblasts (HSMM) to mechanical stretch will improve maturation in vitro and manifest a phenotype that mimics muscle tissue. To test this hypothesis, experiments are currently underway using the STREX Cell Stretching System to examine the various parameters that invoke differentiation of these HSMM cells. Varying cyclical strain frequencies and ratios along with durations of stretch and rest that mimic static/dynamic stretch cues from rehabilitation therapy are being used to determine the ideal stretch criteria for muscle cell growth and differentiation. This stretch protocol will eventually provide guidance toward the production of skeletal tissue engineered products *in vitro* to be used for drug development to promote the recovery of muscle function after injury and possibly forestall disease progression.

Design of Activatable Recognition Proteins for Improved *In Vitro* Diagnostics

William Andrew (Bill) Koski, Senior, Bioengineering
UW Honors Program
Mentor: Wendy Thomas, Bioengineering

Biological recognition proteins are widely used for a variety of biomedical applications, including *in vitro* diagnostics (IVD). IVD tests that use antibodies as recognition pro-

teins, such as rapid antigen tests, are one of the fastest methods for providing point-of-care diagnosis of infectious diseases. However, many current applications of antibody-based molecular diagnostics are restricted by poor sensitivity in a sample with low target antigen concentration. One method which has shown promise for improving the sensitivity of rapid antigen tests is to pre-concentrate a target sample. We are using selection from a random library of *Escherichia coli* bacteria expressing the adhesion protein, FimH, to develop an activatable recognition protein for capture-and-release preconcentration of a target antigen. FimH is a two-domain bacterial adhesion protein that exhibits conformation-dependent binding to mannose. We predict that mutations in the CDR2 and CDR3 loops of the FimH binding pocket can be used to design a protein with binding specificity for a diagnostic target while maintaining conformation-dependent affinity. In previous work, random mutations of the amino acid residues in the FimH binding pocket produced a library of FimH variants with variable binding specificity. Our results suggest that using a biomagnetic selection assay to screen this library of FimH mutants against the small molecule targets digoxigenin and cortisol enriches FimH variants with target binding specificity. Currently, we are designing and optimizing an assay, iterating on the wash conditions, conjugation strategy, and other parameters, to screen the library against protein targets such as the viral antigens influenza hemagglutinin and dengue NS1. In future work, we seek to demonstrate that a variant with binding specificity for a diagnostic target has distinct conformation-dependent affinity which can be actively regulated to capture and release a target antigen.