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Biomarkers and Diagnostics

Session Moderator: Paul Yager, Bioengineering
MGH 389
3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Characterization of Quantum Dot Toxicity for Potential Use as a Biomarker in Brain Injury

Kate Brianna Hildahl, Senior, Chemical Engineering
Mary Gates Scholar, UW Honors Program
Mentor: Elizabeth Nance, Chemical Engineering
Mentor: Mengying Zhang, Molecular Engineering and Science

Fluorescent nanocrystal quantum dots (QDs) show promise for biomedical application, but are often negatively associated with cellular toxicity. To investigate the potential of QDs as a biomarker in the brain, a systematic evaluation of potential toxicity is necessary. In this study, we evaluated QDs with various surface functionalities and assessed toxicity as a function of concentration and exposure time. We utilized organotypic brain slices obtained from healthy postnatal day 14 (P14) rat pups. Four cadmium selenide (CdSe)-core QD conjugates were evaluated: mercaptopropionic acid (MPA), hydroxyl-polyethylene glycol (PEG-OH), amine-PEG (PEG-NH2), and methoxy-PEG (PEG-MeO) at 0.01, 0.1, and 1.0 μM concentration. Cell toxicity was primarily quantified by measuring lactate-dehydrogenase (LDH) production, which is an indicator of cell death, over a five-day period. Results were supplemented with confocal microscopy analysis of two imaging-based assays: propidium iodide, a stain of the nuclei of dying cells, and fluoro-jade C, a stain of degenerating neurons. QD-MPA treated slices had 7-8% greater toxicity than the non-treated (NT) control. All other functionalities were comparable to the NT control except QD-PEG-OMe, which had 3% lower cytotoxicity suggesting a possible neuroprotective effect. Alternatively, certain functionalities (MPA, NH2) may show lower than expected toxicity due to aggregation before cellular uptake. Compared to 0.1 μM concentrations, 0.01 μM QD treated groups had around 4% lower toxicity. Similarly, 1-3% greater toxicity was observed in extended QD exposure conditions (24h) versus shorter exposures (1h). Results show that toxicity is dependent on surface chemistry, concentration, and exposure time. This is useful in identifying QD conjugates with low cytotoxicity in the developing brain. Understanding QD toxicity can lead to rational design of QDs for site and cell-specific uptake in the brain as a biomarker of neurological disease severity, improving the selectivity of current imaging techniques and providing a powerful diagnostic with regards to diseased cell fate.

Red Blood Cell Derived Exosome: A Promising Biomarker to Track Parkinson's Disease

Sabrina Xie, Junior, Biology
Mary Gates Scholar
Matthew Shane (Matt) Bercow, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Jing Zhang, Pathology
Mentor: Tessandra Stewart, Pathology, Pathology

Parkinson's Disease (PD), a neurodegenerative disorder, features accumulation and transmission of toxic species of the protein a-synuclein in specific brain regions. Because examination of such proteins in the brain is invasive and expensive, a robust diagnostic or predictive blood biomarker for PD is strongly required. Previous studies have observed that oligomeric a-synuclein was present in the red blood cells (RBCs) of PD patients, but it did not work well as a biomarker. Because toxic forms of proteins are secreted from cells to the extracellular space in membrane-bounded vesicles called exosomes, therefore we believe that the RBC derived exosomes could be an potential biomarker for PD diagnosis. Also, isolation of specific type of exosome could be equivalent for testing the originated cells. However, there are all types of exosomes in blood released not only by the RBC but also by most cell types, particularly large fenestrated organs such as liver and kidney. To test these vesicles, we have developed the technique of immunoprecipitation to purify distinct kinds of exosomes by targeting its novel unique markers, such as the RBC-specific membrane protein CD235a, and quantifying the amount and size by nanoparticle tracking analysis. My preliminary data have shown that the antibody of CD235a can successfully target the membrane proteins on RBC mem-

brane and its derived exosomes and can collect pure extract of exosomes by immunoprecipitation. In sum, we think the RBC derived exosomes is a promising candidate for PD diagnosis that is worth further investigation. However, the ratio of CD235a positive exosome in whole plasma is still unknown and further tests need to be done on the amount and size distribution of RBC derived exosome in a PD cohort.

Characterizing the Immunophenotype of Leukemic Blasts and its Impact on Patients with AML

Mike Quan Huynh, Senior, Biology (Bothell Campus)
Mentor: Derek Stirewalt
Mentor: Era Pogosova-Agadjanyan
Mentor: Brooke Willborg

Acute myeloid leukemia (AML) is cancer that affects immature blood called myeloblasts. These abnormal cells continuously multiply and build up in the bone marrow and blood. Despite aggressive and timely treatments most patients relapse with a five-year survival rate of 27%. More research is needed to improve the prognostic models for patients with AML. Current risk models are inadequate for predicting responses and additional prognostic biomarkers may help identify patients who will benefit from the standard treatments and those who will not and should consider more experimental treatments and transplants. The Stirewalt Lab at the Fred Hutchinson Cancer Research Center is focusing its efforts on the discovery and validation of novel prognostic biomarkers in the homogenous populations of myeloblasts from AML patients to determine if we can develop improved risk-assessment models. Most previous biomarker studies have been performed in cryopreserved samples, which contain leukemic myeloblasts, dead and dying cells and non-leukemic cells. Evaluation of enriched populations of leukemic myeloblasts may remove the contaminating signals from the dead/dying and non-leukemic cells. Fluorescence Activated Cell Sorting (FACS) was used to enrich mononuclear cells (MNCs) for viable myeloblasts. Hematopoietic cell surface markers CD34 and CD117, along with DAPI, CD38, and CD45 were used to identify variable myeloblast populations and to differentiate between more and less mature myeloblasts. DNA and RNA were extracted from unsorted MNCs and enriched myeloblasts. Genomic and transcriptional biomarkers were evaluated in both. Analyses are underway to determine the clinical significance of the mutations with the goal of developing a refined risk-assessment model that is more predictive than the current models to predict clinical outcome in patients. This model will be validated in an independent population of patients. I will present the data that characterizes the heterogeneity of the leukemic blast immunophenotypes.

Predicting Tooth Decay with a Non-Contact pH Measurement

Jasmine Yu Graham, Senior, Bioengineering
Levinson Emerging Scholar, Mary Gates Scholar
Mentor: Eric Seibel, Mechanical Engineering

A pH measurement of oral biofilms is helpful for monitoring the impact of acidogenic bacteria in the caries process. Demineralization of dental enamel is closely related to the time dependent pH of human plaque. Therefore, providing a means to easily measure the local pH of biofilms is a useful clinical diagnostic in the arsenal of caries prevention tools. Optical measurement methods of plaque metabolism can use intrinsic fluorescence or extrinsic fluorescence from added dyes. Autofluorescence spectral features of human oral biofilms at green (500 nm) and red (634 nm) fluorescence wavelengths using 405 nm excitation did not demonstrate a spectral or intensity shift between neutral and acidic conditions. Chlorin e6, an ingredient in chlorophyllin food supplement, exhibited a spectral and intensity shift of fluorescence emission in buffered solutions, but this quantitative pH-dependence was not transferable to a human plaque environment. Finally, a ratiometric quantitative pH measure was achieved by exciting (405 nm laser) a mixture of two dyes, fluorescein and rhodamine B. This two-dye mixture produced two strong fluorescent bands centered at 515 nm (fluorescein) and 580 nm (rhodamine B), where the 515 nm band was pH sensitive and the 580 nm band served as a pH insensitive reference. This dual-dye fluorescence ratio exhibited a linear response over pH 7 to 5 in human oral biofilms during a sugar challenge. We have explored methods to use non-contact, optical measures of local acidity levels in difficult to access dental locations such as occlusal fissures using various pH sensitive fluorescent dye systems.

Refining Oral Swabs as a Non-Invasive Tuberculosis Diagnostic

Rita Noor Olson, Senior, Microbiology
Mentor: Gerard Cangelosi, Environmental and Occupational Health Sciences
Mentor: Rachel Wood, Department of Environmental & Occupational Health Sciences

Conventional diagnostics for tuberculosis use the patient's sputum, mucus from the lower airways, as the primary sample type. Collecting sputum can be difficult and hazardous and for some patients, especially pediatric cases, sputum must be induced through an invasive procedure. An alternative to sputum samples is oral swabs, which have been used to detect the presence of Mycobacterium tuberculosis DNA in clinical trials. However, the current threshold of oral swabs is not yet sufficient for widespread implementation. In a new phase of assessing the viability of oral swab samples, nine different swab types were compared to improve DNA yield and subsequent qPCR readouts. The comparison was based on

qPCR Cq values after spiking the swabs with mTB DNA, and analysis of each swab's suitability for culturing. Earlier results suggest that oral swabs are a feasible alternative to sputum samples when using swabs that are heavily "flocked", meaning that they have many small fiber particles deposited across a surface. Moreover, samples performed with dissolvable swabs may be better suited for further molecular and microbiological analysis.

Computational Model of Asymmetric Amplification to Enable HIV Drug Resistance Testing in Low-Resource Settings

Annapurni Parameswar Sriram, Senior, Bioengineering
Mary Gates Scholar, UW Honors Program
Mentor: Barry Lutz, Bioengineering
Mentor: Nuttada Panpradist, Global Health

The Oligonucleotide Ligation Assay (OLA) specifically detects point mutations in the HIV polymerase gene conferring drug resistance. Screening patients for drug-resistant HIV is a necessary step in determining the proper treatment. However, the traditional OLA is complex and lengthy, making it non-ideal for low-resource settings. We are exploiting a novel approach to adapt the OLA into a point-of-care, paper-based format. To enable downstream isothermal ligation and paper-based detection steps, the amplification step of the OLA must be designed to preferably generate many copies of single-stranded HIV DNA (ssDNA). Typically, this can be achieved using a Linear-After-The-Exponential Polymerase Chain Reaction (LATE-PCR), which uses an uneven ratio of forward and reverse primers. Though the LATE-PCR is useful for producing large amounts of ssDNA, it involves a plethora of parameters whose contributions to the reaction output are not well understood. Thus, the LATE-PCR is often optimized via trial and error and may not yield the most desirable outcome. In order to minimize wasted resources from unnecessary trials and achieve a high-efficiency amplification, we aim to understand the behavior, predict the outcome, and develop a platform for LATE-PCR optimization. We are using PCR theory and mass-action kinetics to predict exponential amplification of symmetric OLA PCR reactions. This forms the basis for a LATE-PCR model. We will incorporate the effects of key reaction parameters in this model by observing the behavior of LATE-PCR reactions when altering the number of cycles run, primer concentration ratios, enzyme concentrations, enzyme rates, salt concentrations, and primer sequences. Analysis of the model results will elucidate the effects of thermodynamics and kinetics on the LATE-PCR. Future research will focus on expanding the model of the LATE-PCR to other instrumented-free isothermal amplification approaches for a simplified point-of-care OLA.

Cell Phone-Imaged Quantum Dot Lateral Flow Immunoassay for Improved Influenza Diagnostics at the Point of Care

Vidhi Singh, Junior, Bioengineering
Mary Gates Scholar
Mentor: Kamal Shah, Bioengineering
Mentor: Paul Yager, Bioengineering

Influenza is a prevalent infectious disease that claims the lives of 800,000 people globally. Current diagnostic methods include cell culturing, antibody staining, and nucleic acid amplification tests that take from 4 hours to 3 days. The need for rapid, cost-effective and accurate influenza detection tests can be met by lateral flow immunoassays (LFA), which are paper-based test strips similar to pregnancy tests. Most LFAs use colored particles such as gold nanoparticles to indicate the presence or absence of a biomarker by producing a line when the biomarker is present. However, influenza LFAs require over 10 fmol of influenza nucleoproteins to produce a readable signal, which is impractical for influenza because it is present at lower levels at earlier stages of infection. There is a need for a more sensitive influenza LFA that has an improved limit of detection. Here, we investigated how replacing gold nanoparticles with quantum dots, a fluorescent label, improves assay performance when imaged with a mobile phone. We optimized our LFA by varying the concentration and volume of the reagents needed to produce a visible signal. We excited 605 nm quantum dots with a UV LED and used an iPhone SE or Nexus 5X to image the LFAs. Results showed that quantum dot-labeled LFAs imaged with cell phones had a 10x lower-limit of detection than gold nanoparticle-labeled LFAs. Limits of detection with the mobile phones of 1.5-2.6 fmol were comparable to that on a lab-based fluorescence reader, a gel imager (limit of detection of 1.9 fmol). These results suggest that cell phone imaging and fluorescent labels can be combined to make cost-effective LFAs that can be used for rapid and efficient detection in point-of-care settings.

A Ubiquitous Screening Technology for Sleep Apnea

Parker Scott (Parker) Ruth, Sophomore, Bioengineering, Computer Engineering
Mary Gates Scholar, UW Honors Program
Mentor: Shwetak Patel, Computer Science & Engineering
Mentor: Edward J. Wang

Obstructive sleep apnea (OSA) is a condition estimated to affect 5% to 15% of the population, in which individuals stop breathing for extended periods while asleep. Treatment is usually successful when provided; however, the vast majority of OSA patients go undiagnosed, in part because the superficial symptoms of OSA in a wakeful state are varied and nonspecific, including drowsiness, hypertension, heart disease, diabetes, and depression. The current diagnostic gold standard is an overnight sleep study, which is expensive and requires access to a specialized sleep medicine facility. This

motivates a need for a convenient and accurate technology to screen for sleep apnea in homes and clinics. A ubiquitous screening solution must be both sensitive to slight variations, and specific to apnea in spite of many interfering physiological factors; these challenges are further complicated by the constraints of low-cost sensing devices. Our approach is to detect persistent changes in the sympathetic nervous system that are caused by frequent apnea events; although these changes cannot be measured directly, they manifest in discernible changes to heart signals. To test our system we record cardiac and respiratory signals while participants execute a series of breathing maneuvers, such as breath holding and breath rate control. We record in parallel with (1) a smartphone running a custom application and (2) a commercially available wireless biomedical recorder. Using digital signal processing, we extract informative features from the locations and amplitudes of peaks and troughs in the PPG, SCG, and ECG signals. Equipped with these extracted features and the ground truth diagnoses provided for each patient by the Harborview Sleep Medicine Center, we use existing machine learning libraries to train a predictive model for apnea. We expect results to demonstrate a correlation between cardiac regulation and sleep apnea severity.