2024 Multidisciplinary Research Program in Medicine Project*: Towards data-rich, accurate diagnostics at the point of need: biofunctionalization of multiplexed silicon photonic biosensors*

Hypothesis or Research Question(s): How do the performance characteristics (e.g., usability, sensitivity, specificity, limits of detection, limits of quantification, quantification uncertainty) of a data-rich, portable, low-cost biosensor using silicon photonics technology compare with a state-of-the art laboratory-grade assay after piezoelectric inkjet-based covalent functionalization and interdisciplinary system optimization?

PROJECT BACKGROUND & SUMMARY

The COVID-19 pandemic illuminated the need for portable, accurate, and widely available diagnostics. One unmet need for quantitative at-home diagnostics is in tracking hormone levels relevant to women's health and menopause, where hormonal changes during the menstrual cycle can necessitate daily quantitative measurements. Our silicon photonic (SiP) biosensor technology leverages semiconductor manufacturing economies of scale for integration onto a portable device that brings data-rich diagnostics to the point-of-need. Specificity is afforded by functionalization with bioreceptors that capture diagnostic biomarkers. Multiplexed measurement of several biomarkers from the same sample is becoming increasingly important for diagnosis of conditions for which levels of a single biomarker are insufficient. Multiplexed measurements can be achieved on SiP sensors when functionalized with multiple bioreceptors in a spatially localized manner. This project will build on our team's preliminary work developing assays and designing a multi-nozzle piezoelectric inkjet system to facilitate multiplexed functionalization. This will be combined with research in the areas of immobilization chemistries and microfluidics to develop and analytically validate multiplexed assays toward point-of-care diagnostics. The students (TS) will complete the design and integration of the inkjet system's electrical, mechanical, and software components. TS will characterize and optimize the system's dispensing performance by printing fluorescently labeled antibodies onto SiP sensors and using imaging to quantify accuracy, resolution, and consistency. TS will perform analytical validation of our sensors by running immunoassay experiments. Assay reagents/samples are typically delivered to SiP sensors using microfluidics. TS will design, fabricate, and use microfluidic devices using different materials and fabrication processes (e.g., PDMS and laminated pressure-sensitive adhesives) to characterize sensor performance in 1-plex and multiplexed assays. TS will use antibodies targeting hormone markers of interest for women's health (pregnanediol (PdG) and follicle-stimulating hormone (FSH)). TS will evaluate detection performance separately for each biomarker, then use inkjet printing to functionalize sensors for simultaneous detection of both. Using lab-scale testing, TS will quantify the sensors' lower and upper limits of detection and quantification. TS will use automated data processing to extract analyte concentrations from binding dynamics profiles. TS will optimize functionalization parameters and flow regimes to improve detection limits. Cross-reactivity will be assessed using multi-analyte solutions and by spiking in negative control proteins.

Anticipated outcomes: (1) optimized bioreceptor dispensing, multiplexed functionalization, microfluidics fabrication, and assay and data analysis workflows, and (2) proof-of-principle analytical validation data, benchmarking our sensor technology against ELISA for detection of PdG and FSH.

BENEFIT TO THE STUDENTS

Through this multidisciplinary project at the convergence of engineering, medicine, and chemistry, TS will gain hands-on experience and new skills while working with samples and equipment that are not commonly encountered in classes.

TS1 will work with graduate students in Biomedical Engineering (BME) and Electrical and Computer Engineering (ECE) to complete the inkjet system prototype, including designing and implementing new system components and finalizing existing ones. The project includes (a) optimizing dispense waveforms for various bioreceptor "inks", (b) implementing stroboscopic imaging for droplet visualization, (c) implementing environmental control, (d) finalizing a GUI to control key functionalities of the system, (e) operating the inkjet system to deposit antibodies on sensors, and (f) performing fluorescence microscopy and image analysis to quantify antibody printing performance. TS1 will gain skills in surface chemistry, piezoelectric inkjet printing, microscopy, image processing, firmware development and testing, computer-aided design (CAD), and Python software development.

TS2 will work with a postdoctoral fellow (PDF) using a microfluidic system to test SiP sensors by running immunoassay measurements on fluid samples. The project will include (a) designing (CAD) and fabricating microfluidic devices, (b) integrating microfluidic devices with SiP sensors, (c) employing an automated fluid control system (Fluigent) to deliver assay reagents/samples to sensors to perform immunoassays, and (d) collecting and analyzing the sensor data using custom Python software. TS2 will gain skills in microfluidic device design and fabrication, operation of the Fluigent and SiP systems, troubleshooting, and analytical validation.

TS3 (Med 1 student) will work with a PDF to demonstrate analytical validation of the system for proof-of-principle 1-plex biosensing assays: detection of PdG and FSH. TS3 will quantify the sensor performance for both biomarkers in artificial urine, benchmarking against ELISA. TS3 will characterize our sensors with known concentrations across the clinically relevant range, first in a simple buffer solution, then in artificial urine. TS3 will acquire data from 6 or more on-chip sensors/experiment and run at least 3 replicate experiments/condition for each diagnostic target, gaining skills in sensor development, analytical validation, microfluidics, and optical sensing.

TS will collaborate with one-another and their mentors to optimize the full multiplexed functionalization workflow. Sensor surfaces will be chemically modified to facilitate covalent antibody attachment, followed by inkjet-based functionalization of the sensors with both PdG and FSH capture antibodies. Binding assays will be performed with buffer solutions containing known concentrations of both antigens and spiked with negative control proteins. Biosensing data will be analyzed to quantify cross reactivity and compare detection performance to the 1-plex assays.

TS will have access to state-of-the-art instruments (e.g., optical and electrical probe stations and test equipment) and microfluidics fabrication tools. Microfabrication, surface functionalization, sensor characterization, and data analysis protocols established by our team will be available to TS. TS will learn from hands-on training and written protocols, and consult members of our team in 1:1 and small-group meetings to understand our current technologies and approaches. Direct feedback from various lab members will be available to TS through frequent small-group meetings. Troubleshooting will be done with assistance from the graduate student and PDF supervisors. TS will attend biweekly full-team meetings to provide progress updates. TS will interact with a diverse group of researchers, including trainees in BME, ECE, and the Life Sciences Institute. TS will develop time and project management and interdisciplinary communication skills, while fostering critical thinking through planning and troubleshooting experiments. These practical skills will be valuable to their future careers, in industry, medicine, or in preparation for graduate studies. By the end of the summer, TS will give presentations to the two collaborating labs detailing inkjet system performance, microfluidics design and fabrication, and

biosensing assay results. Upon project completion, TS will work with the graduate students and PDF to draft journal manuscripts relevant to their summer projects.