UROP Proposal

Title of Proposal

Optimization Of Tuberculosis Biomarker Detection In Breath Condensate Via Engineered Electroactive Solution

Problem/Topic of Research or Creative Work

The World Health Organization has cited tuberculosis (TB) as a global health emergency. As this is a disease that mainly affects those in developing countries, it is important to provide a rapid and affordable means of diagnosis. Emerging work has shown breath biopsy to be a promising resource for diagnosing a variety of diseases, but is particularly promising for TB, as it negates the need for sputum collection that can cause many problems in young or ill patients and can provide results at point of care.

The breath of a patient diagnosed with TB contain volatile organic biomarkers (VOBs) that are given off by the bacteria that cause the disease. Detection of VOBs via functionalized titanium dioxide sensors has been successful in very sick patients, however it is limited in its ability to detect low analyte levels and has unknown specificity in a complex human breath matrix. Preliminary results indicate that the use of an engineered electroactive solution (EAS), a liquid-phase complex which utilizes a functional metal in solution, can improve the current sensing platform by simplifying the electrode configuration and allowing the use of more complex electrochemical techniques (in this case square wave voltammetry (SWV)). Because the margins of detection can be quite small, successful optimization of SWV parameters is vital. The proposed project will explore a means of optimizing these parameters by collecting a variety of sample data in order to determine how the electrochemical activity of the EAS is altered when biomarkers are introduced.

Figure 1 shows a graphic summary of the proposed sensing platform.

Relevant Background/Literature Review

According to the World Health Organization, TB is one of the top ten causes of death worldwide, killing 1.6 million people in 2017 [1]. A means for early detection is important to treating the disease. Traditional culture testing is heavily dependent on the ability of a patient to produce a sufficient sputum sample, providing a barrier for those who are ill or too young [7]. A culture can have an incubation time of days or weeks and is reliant on expensive equipment and trained technicians. In the majority of populations where TB is common, individuals are from impoverished communities making the need for travel and cost of these tests to be minimal [2].

Another method of detection, Sputum smear microscopy, is most commonly used in these areas as it provides quicker results and a lower cost. It is, however, still reliant on a sputum sample as the name implies, and has a sensitivity that can be variable in the presence of other diseases, making it unreliable [2].

The utilization of volatile organic biomarkers for disease diagnosis has proven to be very successful in a variety of diseases [3][4]. There are two VOBs that have been specifically correlated to TB that are not present in other diseases, methyl nicotinate and methyl p-anisate [6]. Previous work in our group [5] has shown cobalt functionalized titanium dioxide nanotube arrays (Co-TNA) to be viable in sensing these VOBs in the gas phase. Similar to traditional metal oxide sensors, it uses a simple two electrode setup and uses chronoamperometry, a method in which a single bias voltage is applied which stimulates a reaction between the biomarker of interest and the functionalized metal, causing a change in current. The Co- TNA sensor does, however, have

limitations namely lapses in sensitivity and specificity, as well as a complicated TNA fabrication procedure.
Because methyl nicotinate and methyl p-anisate are only semi-volatile and soluble in water in relatively high amounts, liquid breath biopsy has been determined to be a potential solution to these limitations. Liquid breath biopsy provides more flexibility, providing the ability to effectively concentrate VOBs as breath condensate. It can also simplify the sample matrix by removing more reactive volatile compounds which won't dissolve in water and theoretically concentrate 10-20 L worth of breath into a small aqueous sample resulting in higher VOB concentrations. In addition, isolating the biomarkers to an aqueous solution allows us to use a more stable three-electrode setup, allowing for the use of more complex electrochemical techniques which we can tailor to our specific application.

In this new sensing platform, an EAS containing a functional metal of known reactivity is mixed with the liquid breath sample, allowing us to observe the interaction in solution. Unlike the metal oxide sensor p reviously used, the functional metal is contained in the analyte solution rather than adhered to the electrode, making it easier to modify as needed. Copper was chosen for this application, as previous modeling studies [8] suggest it may interact with the TB biomarkers at low potentials. The use of a liquid electrolyte and three electrode system means that potential-scanning techniques can be used to better understand the metal-VOB interactions.
Pulsed voltammetry is a commonly used method in traditional liquid electrochemistry that delivers potential in pulses, with current measured just before each change in potential. This is useful when compared to other voltammetric methods, such as cyclic voltammetry, because it effectively negates the charging current, leaving a clearer signal resulting in a lower limit of detection (LOD). In particular, square wave voltammetry (SWV), which delivers equal pulses in the forward and reverse direction, will be used.
A known biomarker solution will be mixed with an engineered copper EAS, then deposited as a droplet on a simple, unmodified carbon screen printed electrode, and SWV will be used to display the different oxidation peaks of the metal in the EAS. Each peak represents a different redox reaction of copper, and a change in peak height or peak potential is correlated to that particular oxidation state reacting with the analyte solution. The oxidation peaks of the EAS with and without methyl nicotinate (one of the TB biomarkers) present can be seen in figure 2, demonstrating the interaction of the biomarker with the copper in solution. I hypothesize that by optimizing the parameters of the SWV technique currently used (potential range, frequency, amplitude and potential step), the signal change when biomarker is present vs the baseline EAS can be improved, lowering the LOD and better distinguishing the biomarker signal from possible confounders. The parameters to be studied are illustrated in figure 3, which shows the general potential-step diagram for square wave voltammetry.

Specific Activities to be Undertaken and Timeframe for Each Activity

It has been arranged that I will work in lab 10-12 hours per week, Monday through Friday.
January:

Begin initial sample preparation and collection of control data based on previous data collected. This will include testing the EAS with no biomarker present as well as basic tests to see what potential ranges (fig.2) the EAS redox peaks is most visible.

Estimated hours: 25

February:

Once a range has been established for where the reaction can be detected, work will begin on varying frequency (fig.2). This will likely occur initially in orders of magnitude until a range

can be narrowed down and focused on for a total of 3-5 trials in order to get a comprehensive data. Each of the two biomarkers will be added at an established base concentration and tested for comparison against the blank sample.
Estimated hours: 35

March:

A variance in the amplitude and potential step of the SWV will also potentially have an effect on the sharpness and clarity of the peaks. Amplitude (fig.2) will be varied initially in increments of 10 mV. Next, a change in potential step (fig.2) with differences of 5 mV will be tested. This will again be run with each of the biomarkers and compared.
Estimated hours: 30

April:

Once a comprehensive optimization of parameters has occurred and optimal conditions have been found, concentration of each biomarker in solution will be varied under these conditions to attempt for the lowest possible range. Concentrations will vary in half an order of magnitude until the current is insignificant. Data will be assembled and if needed a few additional tests may be run for further comparison data. Preparation of data and poster for UROP Presentation will be done late march/early april with conclusive results collected.
Estimated hours: 30

Project Products:
Abstract submitted for AICHE Presentation
Data Collected for manuscripts and potential co-author on a publication UROP Presentation

Relationship of the Proposed Work to the Expertise of the Faculty Mentor

Dr. [redacted] has conducted research in the areas of renewable energy and biofuels, nanotechnology, and global health diagnostics. Some of his most recent and impactful work has been in the area of biosensors. For the past five years, his lab has been focused primarily on the implementation of a TiO_2 breath sensor for tuberculosis. With continual contact and annual trips to areas such as Uganda where his research is making the most impact, my mentor is able to ensure his work is able to better the lives of those who need it most. He is incredibly successful as mentor, having been previously in the top 15% of instructors for the College of Engineering, and successfully hosting many undergraduates in his lab. Dr. [redacted] and I will meet bi-weekly to discuss my progress through the project, and weekly meetings will be held with a supervising graduate student who will provide any additional training or assistance needed. As Safety is a top priority, I will be working under the direct supervision of the attending graduate student and have completed chemical hygiene safety training.

Relationship of the Proposed Work to Student's Future Goals

I am currently a sophomore majoring in Chemical Engineering. This research builds off of my previous research experience and interest in electrochemistry while also familiarizing me with the rapidly growing field of nanotechnology and biosensors. I plan to attend graduate school and pursue research at the intersection of these areas, as I feel this is where I will be able to make the most impact. I will finish with a better knowledge of electrochemical techniques, practice in experiment design, and presentation experience. Research experience with UROP will enable me to better complete these goals as well as ensure I gain the skills and knowledge to find success in

any profession.

References

[1] World Health Organization, "Tuberculosis Fact Sheet," 2018. [Online]. Available: https://www.who.int/en/news-room/fact-sheets/detail/tuberculosis.

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[3] K. D. van de Kant, L. J. van der Sande, Q. Jöbsis, O. C. van Schayck, and E. Dompeling, "Clinical use of exhaled volatile organic compounds in pulmonary diseases: a systematic review," Respir. Res., vol. 13, no. 1, p. 117, 2012.

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[5] D. Bhattacharyya, Y. R. Smith, M. Misra, and S. K. Mohanty, "Electrochemical detection of methyl nicotinate biomarker using functionalized anodized titania nanotube arrays," Mater. Res. Express, vol. 2, no. 2, p. 025002, Jan. 2015.

[6] D. Bhattacharyya, Y. R. Smith, S. K. Mohanty, and M. Misra, "Titania Nanotube Array Sensor for Electrochemical Detection of Four Predominate Tuberculosis Volatile Biomarkers," J. Electrochem. Soc., vol. 163, no. 6, pp. B206-B214, Mar. 2016.

[7] A. Moeller, P. Franklin, G. L. Hall, F. Horak, J. H. Wildhaber, and S. M. Stick, "Measuring Exhaled Breath Condensates in Infants," Pediatr. Pulmonol., vol. 41, no. 2, pp. 184-187, Feb. 26.

[8] Ray R, Sarma B, Mohanty S, Prisbrey K, Misra M. "Assessment of metals in detection of TB biomarkers: Novel computational approach." Journal of Materials Chemistry and Physics. 161 (2015) 1-8