Engineering DNA-based electrochemical diagnostics for translational applications

by

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Low-resource settings are disproportionally burdened by infectious diseases due to limited access to early and accurate disease detection. Current gold standard methods, while effective, have high turnaround times and require costly infrastructure that is often impractical in these environments. Electrochemical biosensors are a promising alternative to due to their sensitivity, selectivity, low-cost, portability, and rapid response time. Among various biorecognition elements, DNA is particularly advantageous due to its versatility, comparable stability, and low production cost. However, there is a significant gap between laboratory proof-of-concept biosensors and commercially viable biosensors. The main challenges associated with commercializing DNA-based sensors include ineffective surface chemistries, limited target range, poor long-term stability, and high-cost, non-scalable manufacturing processes. **In my thesis, I resolve these specific challenges by engineering DNA-based electrochemical biosensors systems to support their translation into commercially-viable products.**

First, I address ineffective surface chemistries for modifying screen-printed carbon electrodes, which are widely used for bioelectrochemical systems due to their low production cost and scalable manufacturing. However, effective modification with biomolecules remains a challenge as the main methods are either non-specific, require harsh reagents, or form weak monolayers. In this project, we develop a new facile, bio-orthogonal, and biocompatible surface chemistry for modifying screen-printed carbon electrodes. This approach enables the modification of electrode surfaces with DNA, whole cells, and proteins while maintaining bioactivity, supporting applications in both biosensing and clean energy.

Next, I expand the range of targets for nucleic acid electrochemical detection. Electrochemical hybridization assays are sensitive and specific but are limited to very short nucleic acids. To resolves this, we develop a restriction enzyme-assisted electrochemical hybridization assay for improved nucleic acid detection. By incorporating target-specific restriction enzymes, I detect long nucleic acids, with performance dependent on the location of the cut site relative to the electrode surface. Thus, I establish guidelines for assay design to serve as a generalizable platform for robust electrochemical detection of long nucleic acids.

Subsequently, I solve the challenge of long-term storage of sensors. Commercialization of DNA-based electrochemical biosensors is challenged by the stability and shelf-life of the DNA monolayer. There is no technology that allows storage of these sensors long term at room temperature at dry conditions. Here, we report a novel method to preserve DNA-based biosensor through a protective coating of polyvinyl alcohol. We show that the coating significantly improves the shelf life at both room temperature and elevated temperatures. We further demonstrate that the DNA is viable for downstream sensing. Our finding allows facilitates the commercialization of DNA-based biosensors as viable products.

Finally, I design and fabricate a multiplexed electrochemical diagnostic device for respiratory viruses. While electrochemical biosensors are a major research area of diagnostics that can be utilized in low resource settings, effectively integrating assays into a seamless, inexpensive fluidic device is difficult. In this project, we first develop an assay to detect three types of respiratory viruses with sensitivity comparable to PCR. We then integrate the workflow into a shelf-stable diagnostic utilizing low-cost materials and a scalable manufacturing process. Our device offers a practical solution for device integration and future disease control for vulnerable populations.

Overall, the methods developed in this work have the potential to support the transition of DNA-based electrochemical biosensor from academic research to commercially-viable products, paving the way for more FDA-approved diagnostics for early disease detection and advancing health equity in vulnerable populations.

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