Letter of Intent

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Technology to be used and expertise required to do a technical review: Field effect enzymatic detection; antibiotics susceptibility test

Dear Sir/Madam,

We are interested in submitting an application to the Stage 1 of "Antimicrobial Resistance Rapid, Point-of-Care Diagnostic Test Challenge Competition". We will demonstrate a platform for rapid diagnosis of infectious diseases and antibiotics susceptibility testing for point-of-care (POC) use. The platform and its intended use are described below. Thank you for your attention.

Sincerely,

Siu-Tung Yau (Cleveland State University)

J. Scott VanEpps (University of Michigan)

The assay system

The platform is based on *field effect enzymatic detection* (FEED), an ultrasensitive biological-electrochemical detection method invented by the PI. FEED features an intrinsic signal amplification, which is controlled by an external voltage V_G. FEED has been used to detect molecular analytes at the pico-molar (10^{-12}M) level. The mechanism of FEED has been studied in detail. The platform integrates FEED with the immunosensing scheme to detect a range of bacteria.

The FEED-based immuno-detection system consists of a conventional three-electrode electrochemical cell modified with additional gating electrodes for applying an external gating voltage V_G to the sensing electrode. A redox enzyme (the sensing element) is immobilized on the sensing electrode via a sandwich antibody-bacteria-antibody immune complex, wherein the enzyme is conjugated to the detection antibody. V_G induces an electric field at the electrode-solution interface so that the field penetrates across the enzyme and the complex. The field modulates the height of the complex tunnel barrier between the active site of the enzyme and the electrode and, therefore, regulates the quantum mechanical tunneling of electrons (interfacial electron transfer), resulting in voltage-controlled amplification of the signal current. V_G has been shown to facilitate the

direct electron transfer through the immune complex. The immune-complex will be formed on screen printed electrodes (SPEs), which costs ~$2/piece. Multi-organism detection will be realized by preparing a set of SPEs, which are individually immobilized with the capture antibody specific to a particular organism. Each SPE operated using a hand-held meter (~$200) will detect a particular organism in a sample that contains multiple organisms due to the specificity of the antibody-organism immune reaction. Alternatively, such SPEs will be integrated to form a panel for simultaneous detection.

The intrinsic amplification of the platform has allowed us to detect *E. coli* with a detection limit of less than 10 CFU/mL directly in samples without performing culture. We were able to monitor the changes in the concentrations of wild type and resistant *E. coli* with and without the presence of antibiotics in 1 hour. Our observations indicate the feasibility of the platform for bacteria detection, identification and antibiotics susceptibility testing.

Intended use

Our ultimate goal for the platform is to implement a significantly more effective methodology for the diagnosis and treatment of infectious diseases. The new approach will be drastic improvement from the current culture-based approach.

Technically, the unique feature and most important advantage of the proposed detection platform is the intrinsic amplification of the detection signal, which is solely provided and controlled by an external voltage. The amplification facilitates the direct detection of bacteria in ultra-low concentration samples without requiring culture, thus eliminating the time- and labor-consuming culture process. A high degree of detection selectivity is provided by the bacteria-antibody immunological reaction. The POC, inexpensive and ease-of-use nature of the platform will allow it to be used in a wide range of applications.

A diagnostic kit will consist of a set of detection electrodes and a hand-held meter. Each electrode will be used to detect one of the most pathogenic bacteria that are typically found in a certain type of sample, e.g. blood, urine or sputum. The current assay time for the detection of one bacterium is 72 min. Initially, for the purpose of demonstration, three bacteria, namely, *E. coli*, *K. pneumoniae* and *S. aureus*, which include gram negative and positive organisms, will be detected as the target bacteria. Conducting the detection in a serial manner will result in the detection and identification of the bacteria in 79 min. This assay time is to be compared to 48-72 hours for the culture-based detection-identification process. Copies of the electrodes showing the presence of certain bacteria will be used to monitor the growth of the detected bacteria in one hour in the presence and absence of different antibiotics. The results will indicate which antibiotics are most effective against the identified pathogen.