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Ion-Sensitive Field Effect Transistor (ISFET)-Based DNA Detection for Enterotoxigenic *E. coli* (ETEC)

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Summary:

In this study, we demonstrate novel bacterial detection using DNA hybridization-based electrochemical biosensors for enterotoxigenic *Escherichia coli* (ETEC). The ion-sensitive field-effect transducer (ISFET) was immobilized with LT-probe, which is specifically for the heat-labile gene of ETEC. Potentiometric measurement and Electrochemical impedance spectroscopy (EIS) are performed to improve the successful development of the detection of ETEC, which represents a promising technique for rapid and sensitive bacterial detection.

Keywords: ISFET biosensors, DNA-based biosensors, ETEC, Bacterial detection, DNA hybridization

Background

Enterotoxigenic Escherichia coli (ETEC) is foodborne pathogenic bacteria, that causes cholerae-like diarrhea in children younger than 5 years old. Due to the fecal-route infection, ETEC can be transmitted via contaminated food and beverage. The detection of ETEC is commonly identified by molecular-based detection, such as polymerase chain reaction (PCR) and real-time PCR (qPCR), which use a heat-labile toxin (LT) gene as a target. Although these methods are highly sensitive and specific to ETEC, they require expensive equipment and well-trained staff. Moreover, they may require an enrichment process, which is time-consuming [1], [2], [3]. Thus, the development of rapid, easy, and point-of-care (POC) detection is a challenge for bacterial detection. In recent years, ion-sensitive field effect transducer (ISFET)-based biosensors have gained interest in the fields of detection, due to miniaturization, cost-effectiveness, and high sensitivity [4], [5]. In this study, we developed a more rapid, highly sensitive, and highly specific DNA-based ISFET biosensor for the detection of ETEC contaminated in water.

Methods

The ISFET's surface was activated by UV/Ozone procleaner to modify hydroxyl groups on the surface and then were silanized with 11-(triethoxysilyl) undecanal (TESUD) by the vapor-phase method. After functionalization, the ISFET was placed in an oven at 100°C for 1h. The aminated-LT-probe was dropped and incubated overnight at 4°C. After that, the immobilized ISFET was rinsed with TE buffer pH 8 and dried with N_2. 1% Ethanolamine was dropped on the ISFET and incubated for 1 h at RT to block unspecific binding molecules. The DNA sample, extracted from ETEC, was heated for 5 min to denaturation of double-stranded DNA structure and incubated with the immobilized ISFET for 10 min. The ISFET was rinsed with PBS buffer pH 7.4 and dried with N_2. The potentiometry and electrochemical impedance spectroscopy (EIS) of the ISFET were determined using PBS buffer pH 7.4

Results

LT-probe immobilized ISFET was investigated by analyzing various concentrations of extracted DNA from ETEC ($1.9x10^{-5}$ to $1.9x10^{-1}$ µg/mL). Filtrate PBS was used as a negative control to provide the reference signal of the ISFET sensor. Potentiometry and EIS were measured in PBS solution pH 7.4. Fig. 1 demonstrates the responsible ISFET to various DNA samples, measured by drain current (I_{DS}) and transconductance (G_m) vs. gate-to-source voltage (V_{GS}) at drain-to-source voltage (V_{DS}) = 0.5 V. I_{DS} and G_m vs. V_{GS} have slightly shifted to the negative value when increasing the concentration of ETEC's DNA. The threshold voltage (V_T), extracted from I_{DS} and G_m vs. V_{GS}, demonstrated the decrease of V_T when increasing the bacterial concentration.

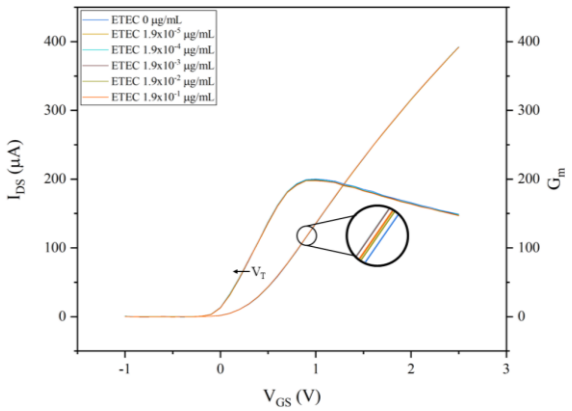

Fig. 1. I_{DS} and G_m vs V_{GS} obtained by analyzing ETEC's DNA samples in TE buffer ($1.9x10^{-5}$ to $1.9x10^{-1}$ µg/mL) at V_{DS} = 0.5 V.

Due to the narrow change of the potentiometric signal, EIS measurement was used to reach the sensitivity of the detection. The Nyquist plot of EIS at different concentrations of DNA samples ($1.9x10^{-5}$ to $1.9x10^{-1}$ µg/mL) decreased the charge transfer resistance on the modified ISFET surface, compared with the reference, ETEC 0 µg/mL, as shown in Fig 2.

These results improve the successful development of a label-free DNA-based ISFET sensor for the detection of ETEC's DNA using LT-probe.

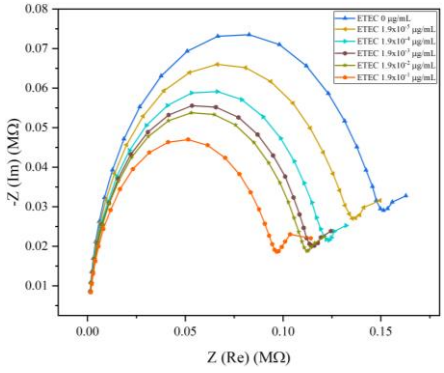

Fig. 2. Nyquist plots obtained by analyzing ETEC's DNA samples in TE buffer ($1.9x10^{-5}$ to $1.9x10^{-1}$ µg/mL). EIS frequency was ranged from 100 Hz to 200 KHz, with E_{ac} 100 mV and E_{dc} 0 V.

Conclusion

This work has proven the successful development of a DNA-based ISFET biosensor for the detection of heat-labile toxin gene which is specifically for ETEC. Potentiometry and EIS measurements were used to determine the efficiency of the ISFET sensor, which provided a decreasing trend when increasing the concentration of the DNA samples. It can represent the rapid detection of bacterial detection, which is a promising technique for point-of-care detection.

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