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Author(s)	呉, 維東
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Description	Supervisor: 高村 禅, 先端科学技術研究科, 博士

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Abstract

Introduction

Owing to the scarcity of readily accessible diagnostics, the time-consuming nature of available diagnostic detection, and the delay in detecting emerging pathogens, severe infectious diseases often lead to high mortality rates, social instability, and economic losses worldwide. As such, the rapid detection of infectious diseases is of great significance to the containment of infectious diseases. In this study, we endeavor to employ the potential of high transconductance oxide Thin-Film Transistor (TFT) biosensors in tandem with the isothermal amplification Recombinase Polymerase Amplification (RPA), aiming to realize the rapid detection of nucleic acids for infectious diseases.

Experiments

First, we investigated the factors that cause the instability of Id variation in the blank measurements. High salt concentration buffer and low salt concentration buffer were used to do blank measurements. Next, we found that the water content(or moisture) of TFT biosensors may affect the Id-Vg curves measurement stability. Thus, 2 TFT biosensors were kept in the measurement buffer and desiccator, respectively. Then measured their Id-Vg curves variation. After that, we measured the DNA sample and compared the Id variation shift situation before and after incubation. In the same time, fluorescent labels were also used to analyze DNA hybridization on the TFT biosensor's surface. Finally, we optimized the RPA reaction system, employing TFT biosensors to detect the RPA products. This allowed us to determine the limit of detection (LOD) of TFT biosensors for *Leishmania* heat shock protein 70 (*HSP70*) RPA products.

Results and discussion

1.Buffer from low concentration (1mM PB+1mM NaCl) to high concentration (1mM PB+100mM NaCl) had been used for measurement. The TFT biosensors perform unstable in low concentrations, one of the main reasons is the reference electrode. According to Nernst-equation $E=E_0-RT/nF*\ln(a_{red}/a_{ox})$, the $\ln(a_{red}/a_{ox})$, value is small because the low concentration buffer contains less Cl^- ion. What's more, the Cl^- ion in the reference electrode will come out to buffer in measurement, which will cause the measured potential change. Therefore, high concentration buffer can improve the oxide TFT biosensors measurement stability.

2. After keeping the TFT biosensors in deionized water or measurement buffer, the Id-Vg curves measurement became stable. It indicates high water moisture can improve biosensor stability.

3. Compared to linker APTES with glutaraldehyde, another linker, GPTMS-treated TFT biosensors that without LaZrOx can successfully detect the target RPA products. It shows that the combination of the RPA and TFT biosensors is feasible, which provides the possibility for the POCT of DNA detection.

4. *Leishmania HSP70* was selected as the target nucleic acid for RPA. After optimizing the RPA reaction system, we found that the RPA products treated with proteinase K and SDS exhibited the greatest changes in their Id-Vg curves. Finally, we applied this method to process RPA products under different template DNA concentration, 0 copies/μL and 10^1 copies/μL-10^6 copies/μL, discovered that the TFT biosensors could detect at the lowest concentration of 10^1 copies/μL. Moreover, we designed a pair of universal probes for the TFT biosensors, namely the anti-probe and probe. The anti-probe is incorporated into the primer, ensuring that a majority of the RPA products carry the anti-probe sequence after amplification. The probe is pre-immobilized to the TFT biosensors' surface, allowing RPA products containing the anti-probe to specifically bind to the TFT biosensors' surface. This innovative approach significantly reduces the complexity and time of probe design, while offering the flexibility for these probes to be used with other target nucleic acids as well.

Keywords: Point-of-care testing, DNA detection, recombinase polymerase amplification, phase change materials, high transconductance oxide thin-film transistor biosensor