DOI 10.5162/EUROSENSORSXXXVI/PT6.115

Electrochemical Biosensor Platform for Leptospirosis Diagnosis in Urine Samples

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Summary:

We developed a sensitive genosensor to detect DNA *Leptospira interrogan* bacteria in urine sample that is non-invasive patient sample collection. Diazonium salt was electrodeposited onto the gold working electrode, showing external carboxylic acid from the surface, and the specific Loa22-probe to leptospires was immobilized. After that, targeted DNA testing showed the EIS change in the range of 0.5×10^{-5} - 0.016 pg/ml with a correlation R^2 of 0.9726. The high sensitivity was indicated 0.5×10^{-5} pg/ml. In addition, the developed sensor was highly specific with other urine-contaminated bacteria.

Keywords: Genosensor, CMA, Screen-printed electrode, Leptospirosis

Background

Leptospirosis is the pathogenic *Leptospira* bacteria of infection. This can cause the disease in both humans and animals. The clinical manifestations are mild to moderate and not specific leading to 90% of fever-developed patients are undifferentiated febrile illnesses [1]. Hence, laboratory testing with high accuracy is required. Recently, the electrochemical sensor has been remarkable in medical device development for easy-to-use portable and point-of-care testing [2, 3]. Therefore, DNA sensors are alternative way for early stage of the disease detection with high sensitivity, accuracy, and stability[4, 5].

In this study, we developed the genosensor to detect DNA of leptospires bacteria in the urine sample. The gold (Au) working electrode was deposited by a 4-carboxymethyl aryl diazonium (CMA) molecule before immobilizing a Loa22-specific single-stranded DNA (ssDNA) probe. The specificity with other contaminated bacteria in the urine was tested.

Methods

The Au electrode was electrodeposited by CMA solution 15 times on ice, showing a carboxyl group (-COOH). Carbodiimide crosslinking stimulated -COOH group by using 0.4 M of 1-ethyl- 3-(3-dimethyl aminopropyl) carbodiimide (EDC) and 0.2 M N-hydroxysuccinimide (NHS) in a ratio of 1:1 v/v with the amine at 5' end of *Loa22* oligonucleotide probe. Then, the surface was blocked with 5% bovine serum albumin for 30 min. The DNA sample was denatured at 95 °C for 5 min to hybridize leptospires DNA with modified working electrode for 25 min at RT. The surface was characterized by cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS).

Results

The Nyquist plots of electrode modified were fitted by Randles equivalent circuit model (see Fig. 1c). The impedance result of DNA in different concentrations from 0 to 0.0016 pg/ml (see Fig. 1a). The impedance was decreased when increased DNA target concentration. This pattern causes the polyanionic nature of the DNA, the negative charges of a phosphate group are accumulated leading to another charge transfer partway [6].

To analyze the R_{ct} between each concentration, the DNA at 0 pg/ml was normalized with other DNA concentrations. This can be demonstrated by the sensitivity of the work. A calibration curve is demonstrated in the percentage of $\Delta\%R_{ct} = [R_{ct}(\text{initial}) - R_{ct}(\text{sample})] / R_{ct}(\text{initial}) \times$

100 to each concentration of DNA leptospires from 0 to 0.016 pg/ml with an R^2 value of 0.9726 with showing linear fitting regression equation as %ΔRct = 5.4941(ln DNA concentration) + 99.477 (see Fig. 1b). The result demonstrated a low detection limit of 5 fg/µl or 5 ag/ml.

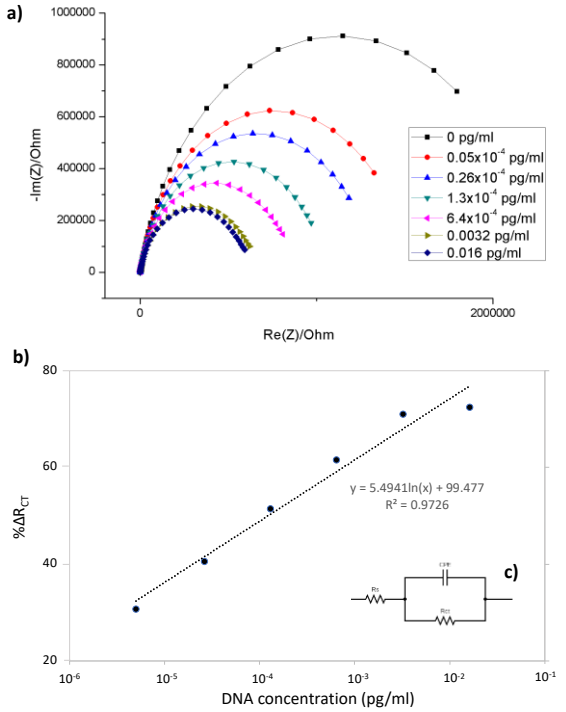

Fig. 1. a) EIS impedance after leptospires DNA various concentrations from 0 to 0.016 pg/ml in the solution of $[Fe(CN)_6]^{3-/4-}$ 5 mM in PBS. b) linear relationship between 0 to 0.016 pg/ml.

The specificity was examined with other bacteria that can be contaminated in urine sample e.g., *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi* and non-pathogenic leptospires *L. biflexa* serovar Patoc. The impedance result was calculated to $\Delta\%R_{ct}$ for specificity testing. These results of the pathogenic *Leptospira* serovar *Sherman*i demonstrated highest impedance response when compare with other bacteria. This result showed high specific of the genosensor to detect leptospires bacteria (see Fig 2).

The developed assay genosensor was advantageous in leptospirosis detection with the highest sensitivity and specificity. This could be examined the leptospira in urine and environment samples. This platform showed various advantages e.g., rapid analysis and low sample volume, which could screen leptospirosis disease in the early-stage infection.

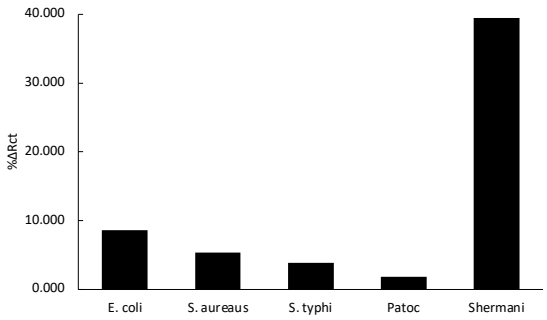

Fig. 2. Specificity testing of other bacteria e.g., E. coli, S. aureus, S. typhi, non-pathogenic leptospires, leptospires serovar Patoc and leptospires serovar Shermani.

Conclusion

The Fabrication genosensor for leptospires DNA detection in urine sample was successfully designed, demonstrating a sensitive and selective genosensor. Diazonium salt was electrodeposited onto the gold working electrode, showing external carboxylic acid from the surface, and the specific Loa22-probe to leptospires was immobilized. To verify the specificity of genosensors against other bacteria that may contaminate urine, e.g., *E. coli, S. aureus*, and *S. typhi* and non-pathogenic leptospires *L. biflexa* serovar Patoc the genosensor demonstrated excellent specific against pathogenic leptospires.

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