A DNA HYBRIDIZATION ASSAY USING NANOGOLD-STREPTAVIDIN, ELECTRO-MICROCHIP AND SILVER ENHANCEMENT TO ANALYZE IMPEDANCE SIGNALS

Chia-Hsien Yeh[1], Yu-Huai Chang[2], Hong-Ping Lin[3], Tsung-Chain Chang[4], and Yu-Cheng Lin[1, 2]

[1]Department of Engineering Science, National Cheng Kung University, Taiwan
[2]Institute of Nanotechnology and Microsystems Engineering, National Cheng Kung University, Taiwan
[3]Department of Chemistry, National Cheng Kung University, Taiwan
[4]Department of Medical Laboratory Science and Biotechnology, National Cheng Kung University, Taiwan

ABSTRACT

This paper reports a novel DNA hybridization detection method using gold-streptavidin conjugates as a reporter, the silver enhancement reaction to magnify the detection signal, a commercial LCR meter to detect the hybridization signal. The relationships between sample concentration and detection signal are discussed and the detection limit for the DNA sample is 0.825 ng/mL. Moreover, we discuss the probe specific test for different species of bacteria, for same species but different strains, and for same genus but different species.

KEYWORDS: Gold-streptavidin, Silver enhancement reaction, LCR meter

INTRODUCTION

The DNA hybridization method is based on the materials of labeling, which can be classified as chemiluminescence staining, fluorescent staining, and so on [1]. Recently, the immunogold silver staining (IGSS) technique was found to have the capability to increase the sensitivity of sequence-selective DNA detection [2]. Moreover, we combined surface chemistry modification, which used (3-glycidoxypropyl) trimethoxysilane (3-GPS) for creating epoxy-silane group, to increase biomolecule binding capability with amine group on glass slide surface [3].

Based on the superior performance of the DNA assay proposed by Mirkin group [4], we utilized it in this work for new method of DNA hybridization. In this study, our strategy is based on using the gold-streptavidin probe coupled with silver enhancement for the DNA hybridization, and using a conventional LCR meter for the signal measurement. Gold-streptavidin and DNA sample (modified biotin) conjugates were used as the detection probe to catalyze the reduction of silver ion to silver metal, resulting in a particle-size enlargement and impedance differentiation. The electrical signal produced from the binding of nanoparticle-labeled streptavidin could then be observed by the LCR meter while the silver enhancement solution was introduced to cause the silver amplification effect.

MATERIAL AND METHOD

The principle of the proposed method is illustrated in Fig. 1. The framework of this study is based on the DNA hybridization detection which is designed for qualitative and quantitative analysis. These silver precipitations enables the electrons to pass between the two electrodes of the electro-microchip, and so further decrease the impedance. The measurement system includes an LCR meter and an electro-microchip.

978-0-9798064-2-1/μTAS2009/$20

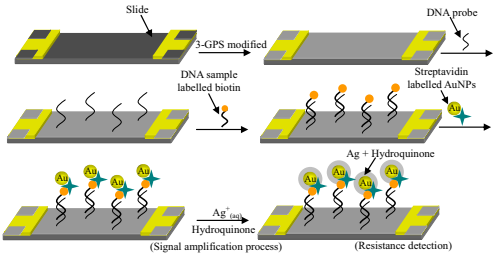
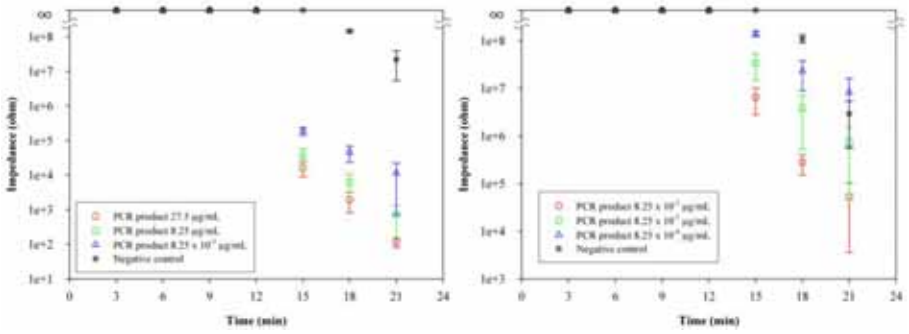


Figure 1: The principle of the proposed method for DNA hybridization, in which silver ions are reduced to silver metals by hydroquinone, inducing the silver particles to cover on the gold nanoparticle surfaces.

RESULT AND DISCUSSIONS

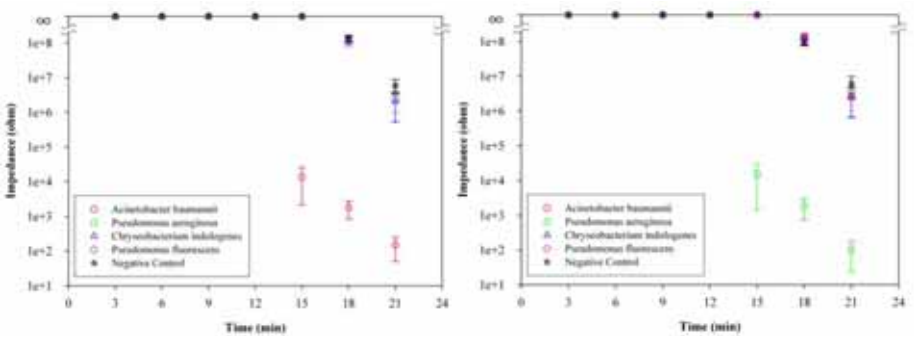
Figure 2 shows the dynamic ranges of different concentrations of DNA samples could be determined from impedance of the silver enhancement solution. The impedance values as table 1 show that the reaction could be observed by LCR meter even at DNA samples concentration of 8.25×10^{-4} μg/mL. For the highest concentration, the gray level values reached the saturation point promptly after about 15 minutes, indicating that all silver ions appeared to be reduced to the silver metal. Obviously, the time of the whole detection (within 15 minutes) was shorter than the conventional enzyme staining (approximately 60 minutes). In experiments, the concentration of probe was 5 μg/mL, and the dilute multiple of gold-streptavidin was 10^{-3} μM.



(a) (b)

Figure 2. The impedance spectrum of different concentrations of DNA samples (Unit: μg/mL). The detection limit of DNA concentration is 8.25×10^{-4} μg/mL.

Figure 3 shows the electrical impedance spectrum of probe specific test for different species of bacteria. Our experiment results are proved to be accurate by using various probes of different species bacteria. The results show that the hybridization of different species of bacteria DNA sample only had reaction with it own specific probe. Therefore, we could prove that the hybridization of different species of bacteria provided specificity and had no cross-reaction with other species bacteria. Further, we proved the correctness and feasibility of electrical DNA hybridization.



(a) (b)

Figure 3. The impedance spectrum of probe specific test for different species of bacteria (Unit: μg/mL).

Figure 4 shows the result of the electrical impedance value of DNA hybridization which used *Acinetobacter baumannii* as the specific probe. There was no influence of impedance for the result of same genus but different species. Therefore, we could prove that the hybridization of same genus but different species of bacteria provided specificity and had no cross-reaction with other species bacteria.

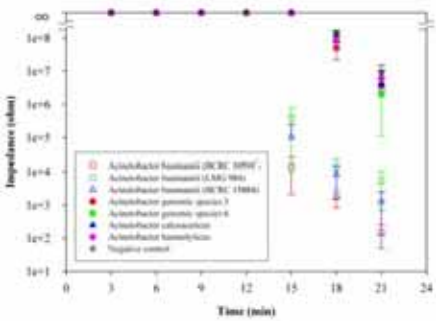


Figure 4. The impedance spectrum of same species but different strains, and same genus but different species.

CONCLUSIONS

We successfully developed a novel DNA hybridization based on an electrical hybridization chip to detect the DNA hybridization signal. Experimental data show that the detection limit of DNA samples concentration is 8.25×10^{-4} μg/mL. The hybridization of different species of bacteria provided specificity and had no cross-reaction with other species bacteria. And the hybridization of same genus but different species of bacteria provided specificity and had no cross-reaction with other species bacteria. Further, we proved the correctness and feasibility of electrical DNA hybridization.

REFERENCES

[1] S. K. Chiu, M. Hsu, W. C. Ku, C. Y. Tu, Y. T. Tseng, W. K. Lau, R. Y. Yan, J. T. Ma and C. M. Tzeng, "Synergistic effects of epoxy- and amine-silanes on microarray DNA immobilization and hybridization," *Biochem. J.*, Vol. 374, pp.

625-632, (2003).

[2] T. A. Taton, C. A. Mirkin, R. L. Letsinger, “Scanometric DNA Array Detection with Nanoparticle Probes,” *Science*, Vol. 289, pp. 1757-1760, (2000).

[3] Y. Nam, D. W. Branch, B. C. Wheeler, “Epoxy-silane linking of biomolecules is simple and effective for patterning neuronal cultures,” *Biosens. Bioelectron.* , Vol. 22, pp. 589-597, (2006).

[4] T. A. Taton, C. A. Mirkin and R. L. Letsinger, “Scanometric DNA array detection with nanoparticle probes,” *Science*, Vol. 289, pp. 1757-1760, (2000).