Extended Abstracts of the 2013 International Conference on Solid State Devices and Materials, Fukuoka, 2013, pp358-359

A Label-Free and Rapid Molecular Biosensor Based on the Combination of the Extended Gate field Effect Transistor and AC Electrokinetics

E-Ling Huang[1], I-Fang Cheng[2*], Wei-Chou Hsu[1*], Tzu-Ying Chen[2]
[1] Institute of Microelectronics, Department of Electrical Engineering,
and Advanced optoelectronic Technology Center,
National Cheng Kung University,
No. 1 University Road, Tainan, 70101, Taiwan
Phone: +886-6-2757575#62350, E-mail: wchsu@eembox.ncku.edu.tw
[2] National Nano Device Laboratories, National Applied Research Laboratories,
No. 1 University Road, Tainan, 70101, Taiwan
Phone: +886-6-2082408#8711, E-mail：ifcheng@ndl.narl.org.tw

Abstract

In this study, we present a label-free and rapid molecular biosensor based on the combination of the extended gate field effect transistors (EGFET) and AC electroosmosis (ACEO). The ACEO significantly promotes the sensitivity and reaction rate of EGFET-based molecular biosensor that can achieve 0.5 ng/ml sensitivity and minute-level detection time. The results show that ACEO-EGFET not only significantly reduces the detection time of antibody-antigen binding from hours to a few minutes, but also effectively lowers detection limit by 100 times to reach sub-ng level sensitivity, which is compared to the conventional incubation method. This rapid, portable and highly sensitive platform is very applicable to be used for field-use detection.

1. Introduction

In recent years, immunoassay plays an important role in the prevention of infectious disease spread and pathologies. Therefore, researches had been more interesting in development of immunosensors for detection of sub-nM concentratioresns of biomoleculars in a few mines [1, 2]. In order to realize highly sensitive and rapid of immunosensor, enzyme-linked immunosorbent assay (ELISA) integrated with real-time transducer had been the most widely adopted technique because it can achieve high sensitivity, but it still requires a much longer analysis time due to the antibody-antigen binding is limited by the diffusion-dominated kinetics. For example, Chen et al [3] demonstrated a GaN nanowire based EGFET for DNA detection, which has wide detection range, 10^{-19}-10^{-6} M, but DNA-DNA docking still needs over 30 min. While, substantial studies have been performed pico-Molar sensitivity by improving the performance of transducers. For a very dilute molecular solution, the low diffusion coefficient and long diffusion length are still major factors that cause the hour-level detection time. Therefore, rapid concentration of analyte techniques were integrated into immunosensors has developed and attracted much attention in many biochemical applications [4,5]. AC electroosmosis (ACEO) is generated by the electric field-induced ion migrations drag the fluid to form a bulk flow over the electrodes. The evident ACEO occurs at frequencies below the charge relaxation frequency of the analyte solution. The field-induced fluid stirring would accelerate the molecules binding on the detection surface in a short time.

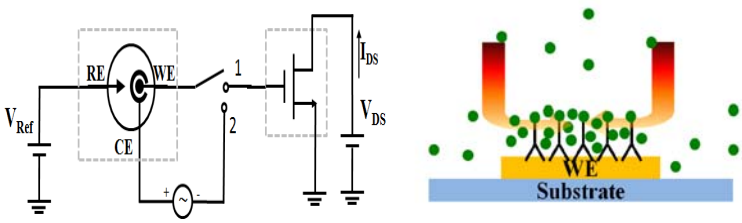
In this investigation, utilizing the combination of EGFET and ACEO to develop a minute-level detection time and sub-ng/ml sensitivity for application on protein A detection. Moreover, comparing incubation method, the EGFET with ACEO concentration not only significantly reduces detection time by 100 times but also effectively improves the sensitivity to reach 0.5 ng/ml. .

2. Results and Discussion

The diagram of AC electroosmosis enhanced Extend Gate field effect Transistors (ACEO-EGFET) shown in Fig. 1(a), which was includes two modes: One is measurement mode, when working electrode (WE) was connected to gate of commercial n type metal-oxide-semiconductor field-effect-transistor (n-MOSFET; CD4007) as an extended gate. All of characteristic of I_{DS}-V_{Ref} were obtained in measurement mode with 0.001×PBS solutions, where V_{DS} was fixed at 0.1 V and swept voltages from -1.5 V to 3V was applied on reference electrode (RE), Ag/AgCl (Sat. NaCl). Another is concentration mode (switching connected to”2”) that is used for concentrating and trapping protein A on WE by electokinetic forces, In Fig. 1(b). The IgG immobilization on WE has six functionalization steps, shown in Fig. 2(a) and listed as follow: (1) Cleaning WE-surface by piranha solution; (2) Self-assenbled monolayer of 11-MUA; (3) Functionalization by EDC/NHS; (4) IgG immobilization; (5) blocking of the reactive immobilization sits by BSA; (6) detection of protein A. The functionalization steps were detected by EGFET, shown in Fig. 2 (b).

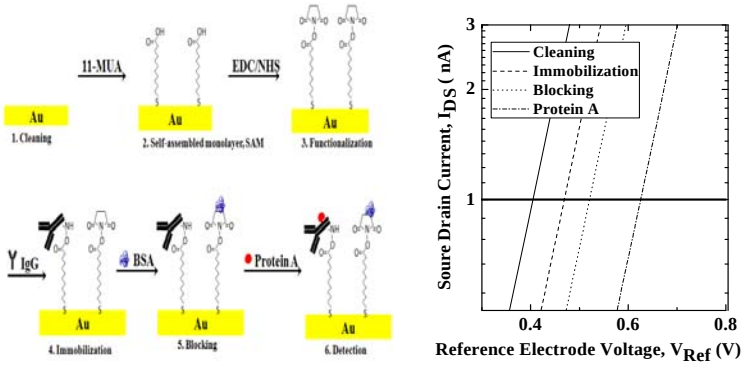
To compare the characteristic of ACEO and conventional ELSA method, in the detection steps, the WE is immersed in PBS with various concentrations of protein A to form incubation-EGFET. The response time of ACEOC-EGFET compared to incubation were shown in Fig. 3. The ACEO-EGFET in 5 ng/ml protein A has response at 5min and near saturated at 8 min, which is much faster than incubation-EGFET, 60min and near saturated at 90min. In Fig. 4, the ACEO-EGFET and incubation in var-

ious concentrations of protein A was further discussed. The sensitivity of ACEO-EGFET is 57.1 mV/(ng/ml) and 30.9 mV/(ng/ml) in detection range of 0.5-5 ng/ml and 5-10 ng/ml, respectively. The performance of ACEO-EGFET is greater than incubation-EGFET, 0.4 mV/(ng/ml) range of 50-200 ng/ml. The results are demonstrated that the ACEO-EGFET not only significantly reduce detection time, from many hours to couple of few minutes, but also effectively improves 100 times of sensitivity and realizes sub-ng level sensing region.



(a) (b)

Fig. 1 (a) The structure of ACEO-EGFET. RE is reference electrode; WE is working electrode; CE is concentration electrode. V_{DS} is voltage supplied to drain, I_{DS} is the current of drain-source, and V_{Ref} is swept voltage supplied to RE. (b) Protein A are carried to the WE by ACEO.



(a) (b)

Fig. 2 (a) Schematic illustration of the IgG immobilization on WE-surface. (b)The shift of characteristic I_{DS}-V_{Ref} in each immobilization steps (cleaning, immobilization, blocking, and detection).

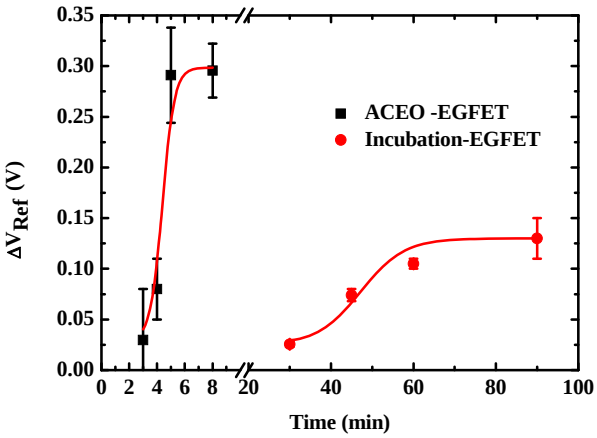


Fig. 3 Response time of ACEO- EGFET and Incubation-EGFET.

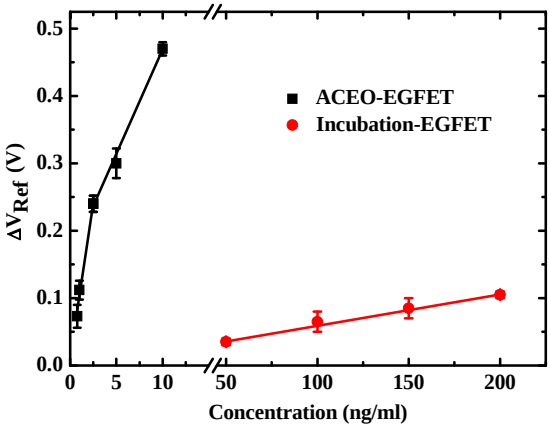


Fig. 4 ACEO-EGFET and Incubation-EGFET in various concentrations of protine A.

3. Conclusions

An ACEO-enhanced EGFET biosensor has been developed and well demonstrated in this study. The enhancement of the detection time and sensitivity were also investigated by comparing ACEO-EGFET with the conventional incubation EGFET. The result shows that ACEO can accelerate the reaction rate to allow molecules binding on the detection surface of EGFET rapidly and finish the detection in only 5 min. The sensitivity is as high as 57.1 mV/(ng/ml) and 30.9 mV/(ng/ml) in detection range of 0.5-5 ng/ml and 5-10 ng/ml, respectively. The detection limit was reduced 100 times and the detection time was reduced from 90 min to 5 min. Thus, ACEO-EGFET not only significantly reduces detection time but also effectively improves the sensitivity and sensing region. This rapid and portable positive–negative diagnostic platform would be a preliminary screening step for many applications such as urethral irritation and environmental monitoring in field.

Acknowledgements

This work was supported by the National Science Council of Republic of China under the contract No.NSC 100-2221-E-006-041-MY3, NSC 00-2221-E-006-039-MY3, NSC 101-2218-E-492-002- and NSC 101-3113-E-006-014-. Also, we would like to thank the Bureau of Energy, Ministry of Economic Affairs of R.O.C. for financial support for this research under contract No. 101-D0204-6 and the LED Lighting and Research Center, NCKU for the assistance regarding measurements.

References

[1] F. Riccia, G. Adornetto, G. Palleschia, Electrochimica Acta, **84** (2012) 74.

[2] J.L. Arlett, E.B. Myers, M.L. Roukes, Nat. Nanotechnol., **6** (2011), 203.

[3] C.-Pei Chen, A. Ganguly, C. Y. Lu, T. Y. Chen, C. C. Kuo, R. S. Chen,W. H. Tu, W. B. Fischer, K. H. Chen, L. C. Chen, Anal. Chem., **83** (2011) 1938.

[4] I-F. Cheng, H.-W. Han, H.-C. Chang, Biosens. Bioelectron., **33** (2012) 36.

[5] P. K. Wong, C. Y Chen, T. H. Wang, C. M. Ho, Anal Chem, **76** (2004) 6908.