Interdigitated Microelectrodes Biosensor for Thyroid Stimulating Hormone Detection

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***Abstract*—The detection of thyroid stimulating hormone (TSH) is critical for disease early intervention and the prevention of pandemics. However the low detection sensitivity and the high cost limit further application of TSH biosensor. In order to improve the sensitivity and reduce the cost, interdigitated microelectrodes (IDμE) biosensor was prepared for the sensitive and low cost detection of TSH. The IDμE used as the sensing structure of biosensor are fabricated by micro-electromechanical systems (MEMS) technology, which can be used in the large scale production. The IDμE biosensor made use of enzyme-linked immuno sorbent assays (ELISA) and enzymatic silver deposition reaction to get a high sensitivity. The biosensor based on IDμE was capable of detecting TSH as low as 0.014 mIU/L ranged from 0.02 mIU/L to 100 mIU/L and has a strong specificity to TSH. The proposed IDμE biosensor can also be used in the detection of other hormones, which are critical for disease early diagnosis.**

***Index Terms*—biosensor; interdigitated microelectrodes; thyroid stimulating hormone; hormone detection; silver deposition**

I. INTRODUCTION

The sensitive detection of thyroid stimulating hormone (TSH) has been widely used for disease early intervention and the prevention of pandemics [1]. A small change of hormone content in human body may reflect metabolism situation of one person, and has been a main basis for disease diagnosis. The concentration of thyroid stimulating hormone in the blood is the key index to realize the function of thyroid. It is very important for the clinical diagnosis and therapeutic evaluation. Therefore, in recent years, the sensitive detection of TSH becomes important in the research on clinical medicine. The research on high sensitive biosensors for TSH detection has been one of the key points [2-4].

Nowadays, most of TSH immunoassays use the "third" generation detection technology, that is, they have a sensitivity of ≤0.02 mIU/L, which is recommended by the National Academy of Clinical Biochemistry [5]. Most of these immunoassays used chemiluminescent or electrochemiluminescent detection method. Seungah Lee used gold-nanopatterned single-molecule sandwich immunoassay chip to perform wide-range quantification of human TSH, and got a detection limit of 360zM [6]. However, the techniques which can get a low detection limit have high cost, and the techniques which have low cost can't get a low detection limit. Therefore, TSH assay need a method comprising low cost and low detection limit.

Electrical conductivity measurement using microfabricated interdigitated electrodes is one of the electrochemical detection methods, and has advantages such as rapidity, simplicity and high sensitivity. Several researches have reported that some electrochemical biosensors based on interdigitated microelectrodes (IDμE) can be used to detect hormones [7,8]. For example, an impedimetric biosensor based on interdigitated microelectrodes was developed for the determination of atrazine residues, and the limit of detection could reach 0.19 μg/L [9]. Microgapped electrode array biosensor was used to detect prostate specific antigen, with detection limit of 0.9 pg/L [10]. With the development of microfabrication technology, the gap of interdigitated microelectrodes can be miniaturization and the biosensors based on interdigitated microelectrodes can be improved.

Therefore, we made the TSH biosensor based on IDμE in this paper. We take use of microfabrication techniques to make IDμE, modify the antibody on the gold fingers, and the biosensor was completed. TSH was captured by enzyme-linked immuno sorbent assays (ELISA) technique, and was detected by enzymatic silver deposition reaction. The TSH biosensor based on IDμE can satisfy the high sensitivity need of the TSH detection and have low cost.

II. EXPERIMENTAL

A. Reagent and Materials

TSH5E8 monoclonal antibody (mAb), TSH10C7 mAb, TSH antigen and EasyLink (EL) alkaline phosphatase (ALP) conjugation kit were obtained from Abcam (Hongkong, China). Glutaraldehyde, glycin and bovine serum albumin (BSA) were purchased from Dingguo Biological Products Company (Beijing, China). 2-mercaptoethanol and ascorbic acid 2-phosphatase (AAP) were acquired from Sigma-Aldrich (Shanghai, China). Millipore ultrapure water with specific resistance 18 MΩ•cm purified from a MilliQ purification

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system was used to prepare the solutions through the experiments. The reagents and chemicals in the experiments were all of analytical reagent grade. All the chemicals were used as received without any further purification.

Two kinds of buffers were used in the electrochemical experiments: 0.01 mol/L PBS (0.1 mol/L NaCl, pH 7.4) and 0.1 mol/L glycin-NaOH buffer (pH 9.0). The preparation of detect mAb-ALP conjugate was performed according to an EL fabrication method from Abcam. The solution of detect mAb-ALP conjugate can be used after preparation or stored at 4 °C before use.

B. Instrumentation

All electrochemical measurements were performed at room temperature (ca. 25 °C) with an Autolab PGSTAT100 Electrochemical Workstation (Metrohm, Switzerland). The IDμE biosensor was placed in air, and the two poles were connected to the both sides of the IDμE.

C. Fabrication of IDμE

The fabrication process of IDμE substrate is shown in Fig 1. They are all MEMS technology, and can be used in the large scale production. All the steps above need to be completed under clean-room ambient conditions. First, 200 nm thick Au layer was evaporated onto the glasses. After evaporation, photolithography was done to make electrodes pattern on the surface of Au film. With the protection of photoresist mask, Au layer was wet etched by Au etchant (mixture solutions of I_2 and KI). Finally, the photoresist on the top of Au electrodes was stripped and the fabrication of IDμE substrate was completed.

D. Preparation of IDμE Biosensor

First, in order to immobilize the biomolecules on the gold fingers of IDμE arrays, the substrate was washed down with acetone, absolute ethanol, piranha solution (7:3 mixture of H_2SO_4 and H_2O_2), NaOH solution and de-ionized (DI) water respectively. The cleaned substrate was immersed in water solution containing 5% 2-mercaptoethanol solution for 12 h. After sanitized with DI water, the substrate was dried under nitrogen flow and stored at 4 °C for 1 h. Then the substrate was immersed in 5.0% glutaraldehyde solution for 1 h, rinsed with DI water (Fig. 2a).

Next, the immobilization of capture mAb on the substrate was performed, and the biosensor was completed (Fig. 2b). The substrate was incubated in PBS containing capture mAb at 37 °C for 1 h, and then washed with PBS buffer to remove the excess and weakly protein. Then, the substrate was incubated in 1% BSA at 37 °C for 1 h to block nonspecific adsorption sites on the substrate. The capture mAb modified substrate was rinsed with water and could be stored at 4 °C for use in future.

E. Analytical Protocol of IDμE Biosensor

After the capture mAb was connected on the gold fingers as a catcher, the TSH protein and detect mAb-ALP conjugate was added on the surface of biosensor, and then incubated at 37 °C for 30 min. After washing the biosensor thoroughly with PBS buffer, silver deposition solution (0.1 mol/L glycin-NaOH containing 0.001 mol/L AAP and 0.005 mol/L $AgNO_3$, pH 9.0) was added and incubated at 37 °C for 10 min in the dark. The substrate was then rinsed with DI water and dried by nitrogen flow.

The electrical measurements of the IDμE biosensors were performed with the electrochemical system at room temperature (ca. 25 °C). The linear sweep voltammetry (LSV) was adopted within a potential range from 0 to 50 mV with an interval of 1 mV and a scan rate of 1 mV/s. The curve of current vs. potential was recorded, so the electrical

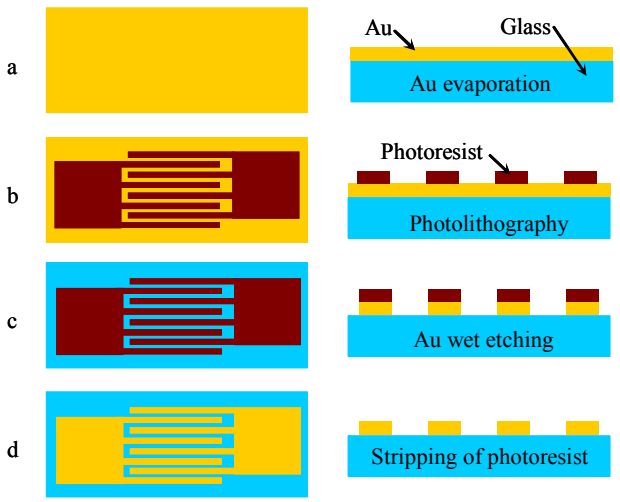

Fig. 1. Schematic fabrication process of IDμE. (a) Au evaporation, (b) lithography, (c) Au wet etching, (d) stripping of photoresist.

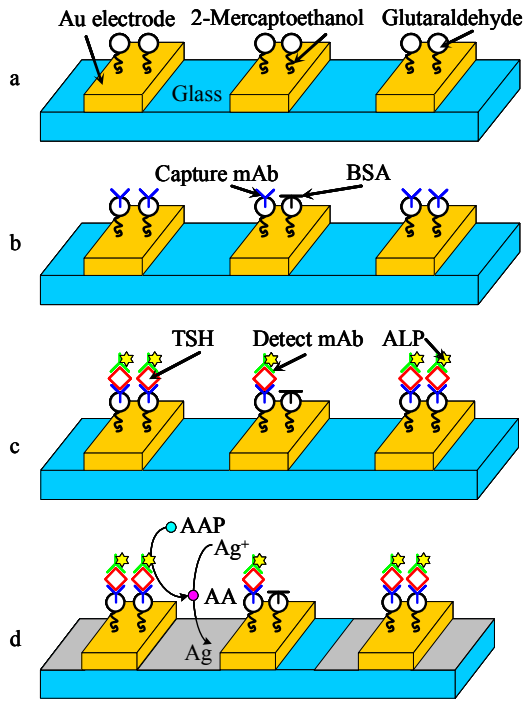

Fig. 2. Preparation and assay of TSH biosensor. (a) 2-mercaptoethanol performance and glutaraldehyde conjugate, (b) capture antibody immobilization and BSA block nonspecific adsorption sites, (c) capture antibody/TSH/ALP-Detection antibody sandwich structure composition, (d) silver precipitate by AA produced from AAP.

conductance between two electrodes could be calculated for the quantitative analysis of TSH.

III. Results and Discussion

A. Analytical Principle of IDμE Biosensor

The IDμE biosensor was based on an enzyme-linked sandwiched immunoassay format with enzymatic silver deposition for electrical detection of TSH. The analytical principle of the IDμE biosensor is illustrated in Fig. 2. TSH10C7 mAb was covalently immobilized on the Au fingers of IDμE through a sulfhydryl bond for capturing the TSH protein. The introduction of a TSH sample together with TSH5E8 mAb-ALP conjugate resulted in the formation of a sandwiched complex of TSH with the capture mAb and the detect mAb conjugated with ALP on the surface of fingers due to the specific immunoreaction (Fig. 2c). Then, ALP on the surface of Au electrodes catalyzed the hydrolysis of AAP and produced a reductive agent, ascorbic acid (AA), which can reduce Ag ions in the silver deposition solution to metallic silver over the microgaps (Fig. 2d). The Ag deposition allowed the microgapped IDμE to be electrically connected, and an increase in electrical conductance of IDμE can be used for quantitative analysis the TSH concentration. According to Ohm's law, the current through two poles of IDμE is proportional to the voltage applied on both sides of IDμE, with a slope equal to the electrical conductance. Thus, the electrical conductance of IDμE can be calculated immediately from the I–V curve obtained in LSV measurements from Autolab electrochemical workstation.

B. Determination of IDμE Biosensor

Many biology materials including TSH5E8 mAb, TSH10C7 mAb, and TSH protein were used in the experiments of TSH detection. Several control experiments were performed to measure the I–V curves of the IDμE so as to study the function of the biology reagents mentioned above. The LSV curves of the biosensor over a potential range from 0 to 50 mV in the detection of TSH sample in control experiments were depicted in Fig. 3. The LSV curves of the IDμE biosensor in response to 20 mIU/L TSH displayed linearity in the detection potential range, which showed that the IDμE filled with silver deposition acted as a physical resistor measured by the electrochemical workstation.

While the TSH sample was replaced by 1% BSA solution, the detect mAb-ALP conjugate was replaced by ALP without detect mAb, or the capture mAb was not added in control experiment, the LSV curve obtained was observed to have a low slope compared with the normal TSH test curve. The electrical conductance of the IDμE biosensor was low, implying that the ALP can not be immobilized on the Au electrodes. TSH5E8 mAb, TSH10C7 mAb, and TSH protein were the necessary conditions to immobilize the ALP. If one of them was not added in the reaction, there would not be the capture mAb/TSH/detect mAb-ALP sandwich structure.

In contrast, the LSV curve of the biosensor in response to 20 mIU/L TSH showed a large slope with an electrical conductance about 2700 pS, which was much greater than the approximate 40 pS for the control experiments. The IDμE based biosensor showed a low limit of detection with good signal to background ratio.

C. Analytical Performance of IDμE Biosensor

According to the analytical protocol of IDμE biosensor, several TSH samples in different concentrations were tested with the IDμE biosensor, and the LSV curves for the IDμE based biosensor in response to TSH proteins of varying concentrations were displayed in Fig. 4. Linear volt-ampere characteristic curves were obtained for the TSH samples indicating ideal behavior for the biosensor based on IDμE as an electrical resistor. The slope of the curves, i.e., the electrical conductance of the biosensor, grew bigger with increasing concentration of TSH samples.

The dependence of the conductance of the biosensor on the TSH concentration is depicted in Fig. 4. The electrical conductance of the biosensor exhibited a dynamic increase with increasing TSH concentration in the range from 0.02 mIU/L to 100 mIU/L. High response sensitivity was obtained over a linear range from 0.02 mIU/L to 100 mIU/L. The detection limit for TSH by IDμE biosensor was determined to

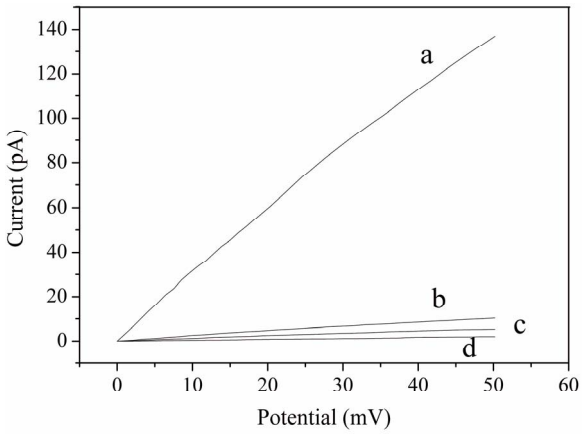

Fig. 3. LSV curves of the IDμE biosensor in response to (a) 20 mIU/L TSH, (b) 1% BSA, and in a control experiment (c) with ALP instead of detect mAb-ALP conjugate, (d) without capture mAb modification.

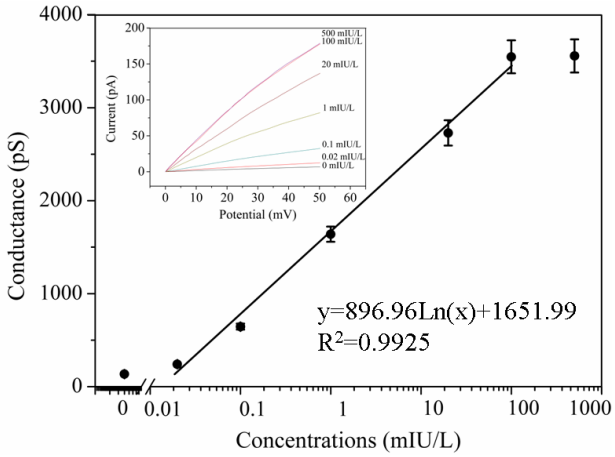

Fig. 4. LSV curves and calibration curve of conductance in detecting TSH samples of varying concentrations for the IDμE biosensor.

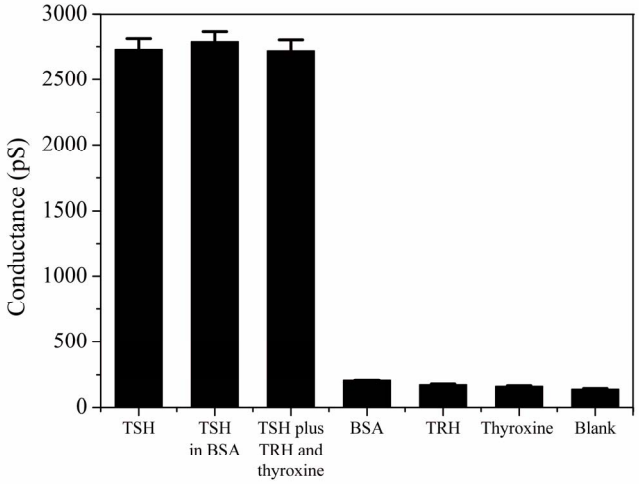

Fig. 5. Conductance responses of the IDμE biosensor to possible coexisting proteins or matrices.

be 0.014 mIU/L, which was sufficiently low for the detection of the TSH concentration range of human serum. These results clearly indicated that the IDμE biosensor electrochemical immunoassay possessed acceptable precision and stability.

D. Specificity of the IDμE Biosensor

To evaluate the selectivity of the technique, the biosensor based on IDμE was investigated for the detection of possible coexisting or matrix proteins. Fig. 5 depicts the conductance signals of the biosensor in response to different proteins. Fig. 5 shows that, for a fixed 20 mIU/L concentration of TSH in PBS solution of BSA and mixed solutions of thyrotropin-releasing hormone (TRH) and thyroxine, the conductance responses exhibit only small variations, suggesting that complicated matrix such as BSA has little interference with the determination of TSH using this strategy. Also, in the absence of TSH, the presence of a high concentration (1 g/L) of other proteins or matrices such as BSA, TRH, thyroxine did not show significant conductance responses. These observations support a highly specific interaction between TSH and its monoclonal antibodies and that enzymatic silver deposition was a highly specific reaction mediated by ALP.

IV. Conclusion

In this work, IDμE made by MEMS technology is used in the sensitive electrochemical determination of TSH, which shows a new immunoassay protocol combining low cost and high sensitivity. The IDμE of the TSH biosensor is made by lithography process, and can be large scale fabricated with low cost. TSH protein is immobilized on IDμE as sandwich-type by ELISA, therefore, the biosensor have a good specificity because of the antigen–antibody interaction. Silver deposition solution is used to translate biochemical signal to electrical signal, and it can improve the sensitivity of the biosensors. The proposed IDμE biosensor was simple, sensitive, cheap and efficient. The biosensor based on IDμE can achieve the sensitive and cheap detection of TSH, and is critical for disease intervention strategies. The IDμE biosensor can also be used in the detection of other hormones.

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References

[1] Giorgio Iervasi, Antonio Bottoni and Elena Filidei, “Is recombinant human TSH as effective as thyroid hormone withdrawal in the detection and treatment of metastatic differentiated thyroid cancer?”, Expert Rev. Endocrinol. Metab., vol. 7, pp. 633–635, June 2012.

[2] David J.You, TuSanPark, and Jeong-YeolYoon, “Cell-phone-based measurement of TSH using Mie scatter optimized lateral flow assays”, Biosensors and Bioelectronics, vol. 40 pp.180–185, July 2013.

[3] Yulin Zhou, Xiaohu Xia, Ye Xu, Wei Ke, Wei Yang, Qingge Li, “Application of europium(III) chelates-bonded silica nanoparticle in time-resolved immunofluorometric detection assay for human thyroid stimulating hormone”, Analytica Chimica Acta, vol. 722, pp. 95– 99, Feberary 2012.

[4] Haoxu Wang, Peitao Dong, Di Di, Chaoguang Wang, Yanzhe Liu, Jian Chen, and Xuezhong Wu. “Interdigitated microelectrodes biosensor with nanodot arrays for thyroid-stimulating hormone detection”, Micro & Nano Letters, vol. 8(1), pp. 11–14, January 2013.

[5] Z.Baloch, P.Carayon, B.Conte-Devolx, “Laboratory medicine practice guidelines”, Thyroid, vol. 13, pp. 3, January 2003.

[6] Seungah Lee, Seong Ho Kang, “Wide-range quantification of human thyroid-stimulating hormone using gold-nanopatterned single-molecule sandwich immunoassay chip”, Talanta, vol. 99, pp. 1030–1034, August 2012.

[7] S. Rajaraman, S.-O Choi, M. A. McClain, J. D. Ross, M. C. Laplaca, and M. G. Allen, “Metal-transfer-micromolded three-dimensional microelectrode arrays for in-vitro brain-Slice recordings,” Journal of Microelectromechanical Systems, vol. 20, pp. 396-409, April 2011.

[8] C.-T. Kuo, and C.-H. Liu,”A bubble-Free AC electrokinetic micropump using the asymmetric capacitance-modulated microelectrode array for microfluidic flow control”. Journal of Microelectromechanical Systems, vol. 18, pp. 38-51, Feberary 2009.

[9] J. Ramon-Azcon, E. Valera, A. Rodriguez, A. Barranco, B. Alfaro, F. Sanchez-Baeza, and M.-P. Marcoa, “An impedimetric immunosensor based on interdigitated microelectrodes (IDμE) for the determination of atrazine residues in food samples”, Biosensors and Bioelectronics, vol. 23, pp. 1367-1373, December 2008

[10] Y. Huang, T.-H. Wang, J. Jiang, G.-L. Shen, and R.-Q. Yu, “Prostate specific antigen detection using microgapped electrode array immunosensor with enzymatic silver deposition,” Clinical Chemistry, vol. 55, pp. 964–971, January 2009.