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Electrochemical Biosensor Based on Interdigitated Electrodes for Determination of Thyroid Stimulating Hormone

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Electrochemical biosensor based on interdigitated electrodes was prepared for the sensitive determination of thyroid stimulating hormone (TSH). The interdigitated electrodes used as the sensing structure of biosensor were fabricated by microelectromechanical systems (MEMS) technology. The TSH protein was immobilized to the capture and detection antibodies by enzyme linked immunosorbent assay (ELISA). The enzymatic silver deposition reaction occurred on the glass surface between the interdigitated electrodes. Then the deposition of silver was dispersed on the substrate to make the interdigitated electrodes connected electrically. The TSH concentration could be calculated according to the electrical conductance of interdigitated electrodes. TSH concentrations ranging from 0.02 mIU/L to 100 mIU/L can be readily determined by the electrochemical biosensor based on interdigitated electrodes. A detection limit of 0.012 mIU/L was achieved, which is below the guidelines recommended by the National Academy of Clinical Biochemistry. The proposed electrochemical biosensor can also be used in the detection of other hormones, which are critical for disease early diagnosis.

Keywords: Electrochemical biosensor, Interdigitated electrodes, Thyroid stimulating hormone, Enzyme linked immunosorbent assay**.**

1. INTRODUCTION

Thyroid stimulating hormone (TSH) is an important protein in the human serum, which plays a key role in the control of thyroid function [1]. The production of thyroid hormones is influenced by the

TSH concentration in the serum. As a rule, normal serum TSH concentrations range from 0.35–5.5 mIU/L [2]. The person with lower TSH levels may have some diseases, such as hypopituitarism and Graves' disease. However, the person with high TSH levels may have other diseases, such as congenital hypothyroidism, thyroid hormone resistance, benign tumor of the pituitary and Hashimoto's thyroiditis [3]. Therefore, the sensitive detection of TSH is critical for early intervention and prevention of thyroid disease [4]. In recent years, the research on sensitive and specific determination of TSH has been one of the key points in the research on life sciences and clinical medicine [5-7].

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 [8]. Most of these immunoassays such as radioimmunoassay [9], chemiluminescence immunoassay [10,11], electrochemiluminescent immunoassay [12], bioluminescent immunoassay [13], and fluorescent immunoassay [14,15] have been reported for the determination of TSH. Although many analytical methods are suitable for the determination of the hormone, the electrochemical detection method holds great promise for hormone owning to its advantages such as rapidity, simplicity, inexpensive cost and portability [16,17]. Several researches have reported that some electrochemical biosensors can be used to detect hormones [18,19]. Nevertheless, the sensitivities of the electrochemical biosensors remained insufficient compared with the biosensors with micro structures.

With the rapid development of the fabrication technology on microelectromechanical system (MEMS), some micro structures have been introduced to the electrochemical biosensors to get a low determination level [20,21]. For example, an impedimetric immunosensor based on interdigitated electrodes was developed for the detection of atrazine residues, and the limit of detection was 0.19 μg/L [22]. Microgapped electrode array immunosensor was used to sense the prostate specific antigen, and the detection limit could reach 0.9 pg/L [23].

The present study reports the biosensor based on interdigitated electrodes for the determination of TSH using electrochemical technique. The interdigitated electrodes, which are used as the substrate of the electrochemical biosensor, have been fabricated by the lift-off process in the MEMS techniques. The antibody was modified in the microgap between the interdigitated electrodes by silanization action, and the electrochemical biosensor was completed. The TSH was captured by enzyme linked immunosorbent assay (ELISA), and was detected by enzymatic silver deposition reaction. The electrochemical biosensor based on interdigitated electrodes can satisfy the sensitivity need of the TSH detection.

2. EXPERIMENTAL

2.1 Reagent and materials

TSH5E8 antibody, TSH10C7 antibody, TSH antigen and Easylink alkaline phosphatase (ALP) conjugation kit were acquired from Abcam (Hongkong, China). (3-aminopropyl) triethoxysilane (APTES), 4-methylumbelliferyl phosphate, ascorbic acid 2-phosphatase (AAP) and human serum were

products of Sigma-Aldrich (Shanghai, China). Glutaraldehyde, glycin and bovine serum albumin (BSA) were purchased from Dingguo Biological Products Company (Beijing, China). All the chemical reagents and chemicals in this experiment were all of analytical reagent grade. All solutions were prepared with ultrapure water of 18 MΩ•cm purified from a MilliQ purification system. 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). All the chemicals were used as received without any further purification.

2.2 Instrumentation

The electrochemical measurements were performed on an Autolab PGSTAT100 electrochemical workstation (Metrohm, Switzerland). The electrochemical biosensor was placed in air at room temperature (ca. 25 °C). The electrochemical workstation was connected to a computer and the two poles were connected to the each side of the interdigitated electrodes, respectively.

2.3 Fabrication of interdigitated electrodes substrate

The fabrication process of interdigitated electrodes substrate is schematically shown in Fig 1. The interdigitated electrodes were fabricated by lift-off process, which belonged to MEMS technology. All the steps need to be completed under clean room ambient conditions.

First, photolithography was done on the surface of glass to make electrodes pattern. As shown in Fig. 1A, the place which should be plated by gold film was left without any photoresist on it, and other place was all covered by photoresist. Next, as illustrated in Fig. 1B, for the lift-off procedure, 100 nm gold (Au) flim was evaporated onto the surface of the glass and the photoresist on glass. Last, as depicted in Fig. 1C, after the evaporation, photoresist was stripped by acetone and the gold film on the photoresist was gone with the stripping process. The lift-off was done and the gold interdigitated electrodes were left on the glass. The fabrication of interdigitated electrodes was completed.

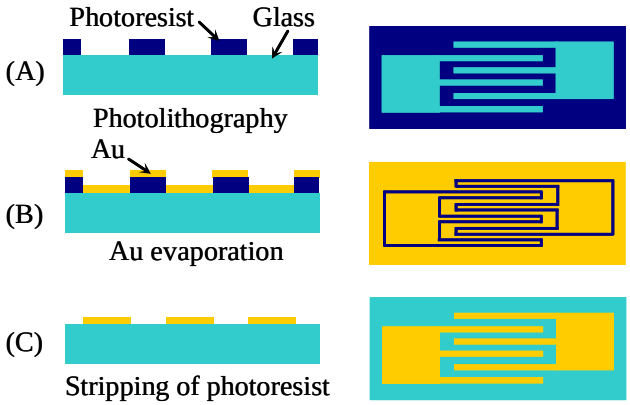

Figure 1. Fabrication process of interdigitated electrodes substrate. (A) Patterning of photoresist on glass to form the interdigitated electrodes. (B) Gold evaporation on the photoresist and the glass. (C) Stripping of photoresist to get the interdigitated electrodes substrate.

2.4 Preparation of electrochemical biosensor

As shown in Fig. 2A, in order to immobilize biomolecules in the gap of interdigitated electrodes, the substrate was sanitized with acetone, absolute ethanol, piranha solution, NaOH solution and DI H_2O respectively. The cleaned substrate was immersed in ethanol solution containing 2.5% APTES and 1% H_2O for 24 h. After rinsed with ethanol, the substrate was dried by nitrogen flow and stored at 4 °C for 1 h.

The TSH10C7 antibody was used as the capture antibody. The immobilization of capture antibody was performed on the substrate as depicted in Fig. 2B. The substrate was immersed in 5.0% glutaraldehyde solution for 1 h, rinsed with water. Next, the substrate was incubated in PBS containing capture antibody at 37 °C for 1 h, and then washed with PBS buffer to remove 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 antibody-modified substrate was rinsed with water and could be stored at 4 °C for future use.

2.5 Analytical protocol of electrochemical biosensor

The TSH5E8 antibody was used as the detection antibody. The preparation of detection antibody-ALP conjugate was performed according to an Easylink fabrication method from Abcam. After the capture antibody was connected in the inter-electrode gap as a catcher, the TSH and ALP-detection antibody were added on the biosensor in Fig. 2C, and 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 1 mmol/L AAP and 5 mmol/L $AgNO_3$, pH 9.0) was added in Fig. 2D, and incubated at 37 °C for 10 min in the dark. The substrate was then rinsed with water and dried under nitrogen flow.

Electrical measurements were performed at room temperature (ca. 25 °C) using an electrochemical workstation. The linear sweep voltammetry (LSV) was adopted within a potential range from 0 to 50 mV with a scan rate of 1 mV/s and an interval of 1 mV. The curve of current vs. potential was recorded, so the electrical conductance between two interdigitated electrodes was calculated as the quantitative analysis of TSH concentration.

3. RESULTS AND DISCUSSION

3.1 Analytical principle of electrochemical biosensor

The electrochemical biosensor based on interdigitated electrodes was founded on an enzyme linked sandwiched immunoassay format with enzymatic silver deposition for electrical detection of TSH. The analytical principle of the electrochemical biosensor was illustrated in Fig. 2. The capture antibody was covalently immobilized on the glass between the gold fingers of interdigitated electrodes through APTES for capturing the TSH protein. The addition of TSH protein together with detection antibody-ALP conjugate resulted in the formation of a sandwiched complex, which was composed of

TSH protein, capture antibody and detection antibody conjugated with ALP due to the specific immunoreactions. Then, ALP was immobilized on the glass in the microgaps of gold electrodes, and could catalyze the hydrolysis of AAP to produce a reductive agent, ascorbic acid (AA). The AA could reduce silver ions (Ag^+) in the silver deposition solution to metallic silver (Ag) over the microgaps. The silver deposition allowed the microgapped interdigitated electrodes to be electrically connected. The increase in electrical conductance of interdigitated electrodes can be used for quantitative analysis of TSH concentration. According to Ohm's law, the current through two poles of interdigitated electrodes is proportional to the voltage applied on both sides of interdigitated electrodes, with a slope equal to the electrical conductance. Thus, the electrical conductance of interdigitated electrodes could be calculated immediately from the I–V curve obtained in LSV measurements from Autolab electrochemical workstation. It was observed that the proposed electrochemical detection method based on interdigitated electrodes has the safe and inexpensive operation compared with radioimmunoassay [9].

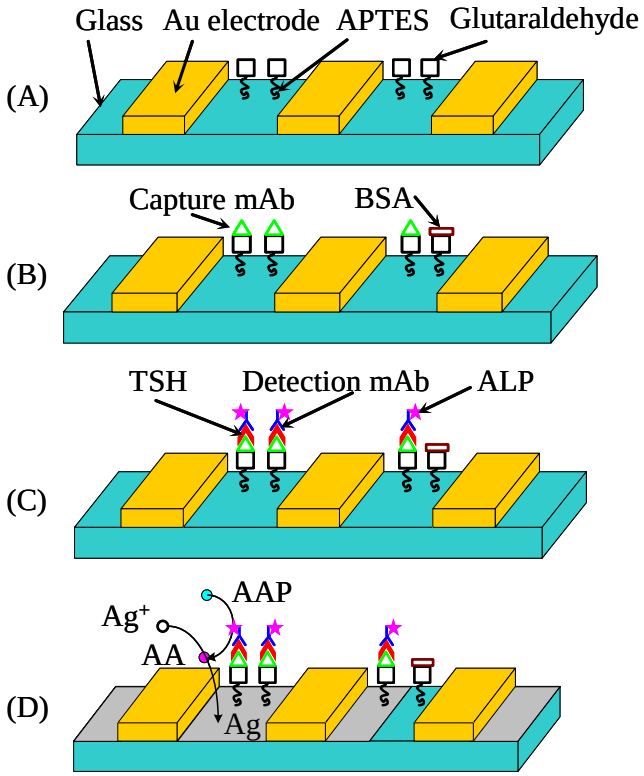

Figure 2. Schematic representation of the preparation process and the detection strategy for TSH on electrochemical biosensor.

3.2 Determination of TSH on electrochemical biosensor

Many biology materials including capture antibody, detection antibody, and TSH protein were used in the electrochemical experiments of TSH detection. Several control experiments were performed to measure the I–V curves of the interdigitated electrodes 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 electrochemical biosensor in response to 20 mIU/L TSH displayed linearity in the detection potential range, which showed that the interdigitated electrodes 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 BSA LSV curve obtained was observed to have a low slope compared with the TSH curve. When the detection antibody-ALP conjugate was replaced by ALP without detection antibody in control experiment, the LSV curve obtained was observed to have a low slope compared with the curve of the biosensor modified with detection antibody-ALP conjugate. While the capture antibody was not modified in the microgaps between interdigitated electrodes in control experiment, the LSV curve obtained showed a low slope compared with the curve of the biosensor modified with capture antibody. The electrical conductance of the electrochemical biosensor was low, which meant that the ALP was not immobilized on the biosensor. It implied that the sandwiched complex composed of TSH protein, capture antibody and detection antibody could be not formed without anyone of them.

In contrast, the LSV curve of the biosensor in response to 20 mIU/L TSH showed a large slope with an electrical conductance about 3100 pS, which was much greater than the approximate 200 pS for the control experiments. Just like other interdigitated electrodes sensors [23], the electrochemical biosensor based on interdigitated electrodes showed a low limit of detection with good signal to background ratio.

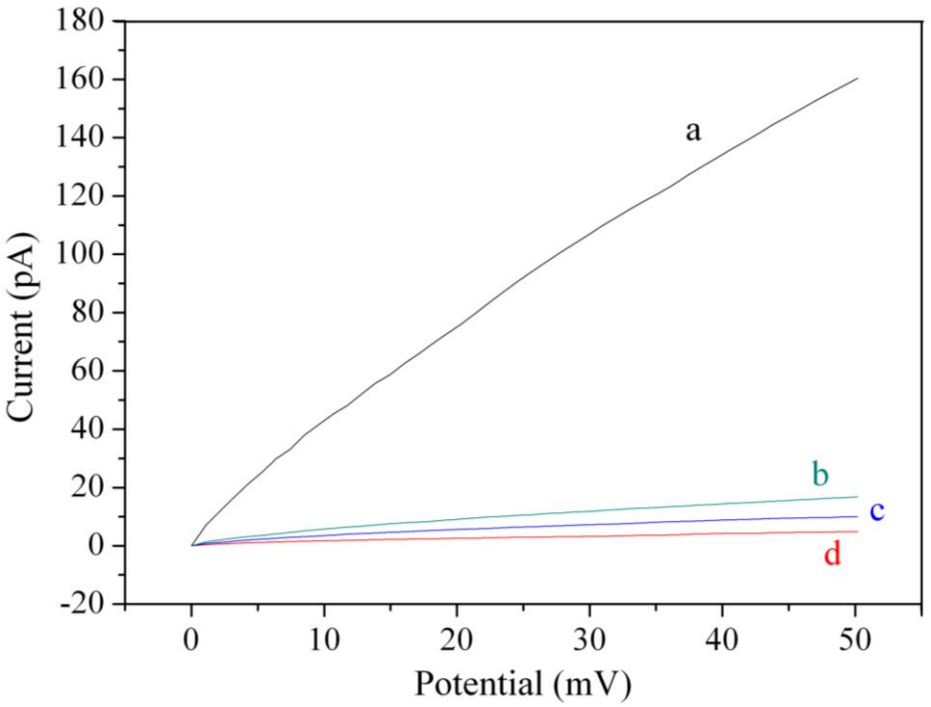

Figure. 3. LSV curves of the electrochemical biosensor in response to (a) 20 mIU/L TSH, (b) 1% BSA, and in the control experiments (c) with ALP instead of detection antibody-ALP conjugate, (d) without capture antibody modification.

3.3 Analytical performance of the electrochemical biosensor

The sensitivity and dynamic range of the electrochemical biosensor were monitored by LSV measurements from Autolab electrochemical workstation. Fig. 4 displays the LSV curves for the electrochemical biosensor based on interdigitated electrodes in response to TSH proteins of varying concentrations in the range from 0.02 to 100 mIU/L. Linear volt-ampere characteristic curves were obtained for the TSH samples, which indicated behavior for the electrochemical biosensor 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.

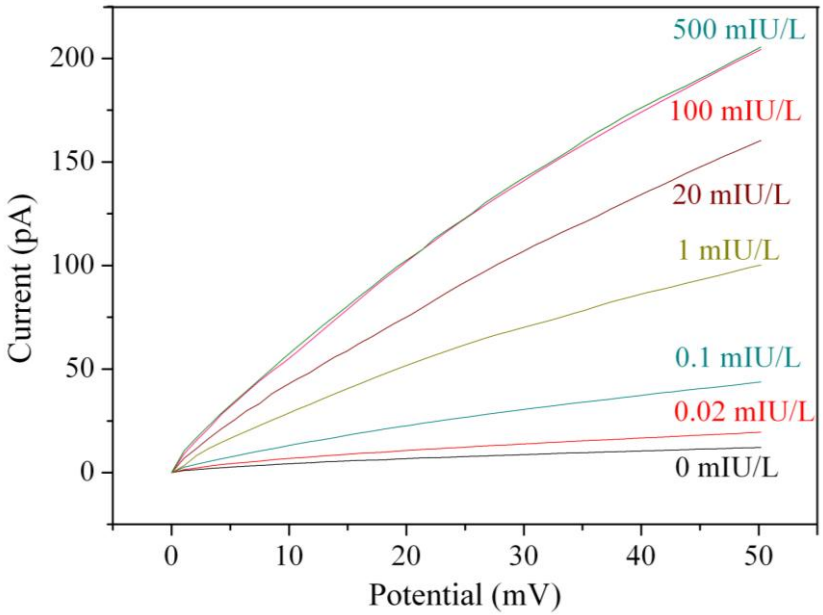

Figure 4. LSV curves of the electrochemical biosensor in detecting TSH of varying concentrations.

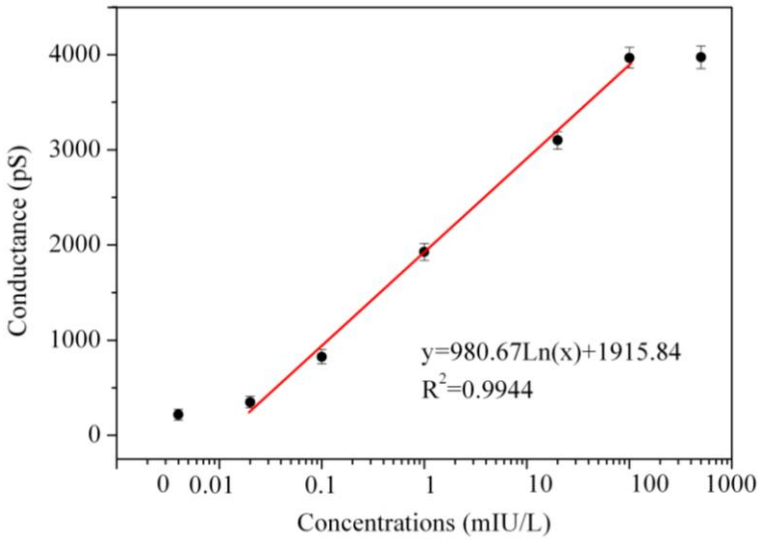

Figure 5. Calibration curve of conductance vs. TSH concentration for the electrochemical biosensor.

The dependence of the conductance of the electrochemical biosensor on the TSH concentration is depicted in Fig. 5. The electrical conductance of the biosensor exhibited a dynamic increase with increasing TSH concentration. High response sensitivity between the conductance and the logarithm of TSH concentration was obtained over a linear range from 0.02 to 100 mIU/L. The detection limit for TSH by electrochemical biosensor was determined to be 0.012 mIU/L, which was comparable with chemiluminescence immunoassay (0.010 mIU/L) [24] and commercially available immunoassay (0.03 mIU/L) [25]. Furthermore, the electrochemical biosensor could also completely meet the requirement of clinical diagnosis due to the normal TSH values of 0.35–5.5 mIU/L in normal human serum [2]. These results clearly indicated that the electrochemical biosensor electrochemical immunoassay possessed acceptable precision and stability.

3.4 Selectivity coefficient

To evaluate the selectivity of the technique, the electrochemical biosensor based on interdigitated electrodes was investigated for the detection of possible coexisting or matrix proteins, e.g. thyrotropin-releasing hormone (TRH) and thyroxine. Fig. 6 depicts the conductance signals of the electrochemical biosensor in response to different proteins. As seen from Fig. 6, higher conductance was acquired with the TSH analyte than those of other analytes. For a fixed 20 mIU/L concentration of TSH in PBS solution of BSA and mixed solutions of TRH and thyroxine, the conductance responses exhibited only small variations, which suggested that the 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. Furthermore, the TSH5E8 and TSH10C7 antibodies were confirmed to have satisfactory specificity due to no cross-reactivity with other hormones [15,26]. These observations supported a highly specific interaction between TSH and its antibodies and that enzymatic silver deposition was a highly specific reaction mediated by ALP.

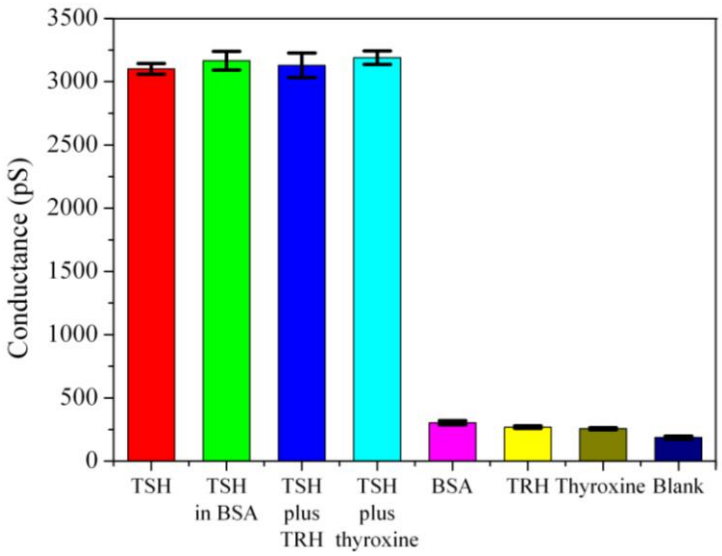

Figure 6. Conductance responses of the electrochemical biosensor to possible coexisting proteins.

3.5 TSH Samples detection

In order to prove the ability of the electrochemical biosensor based on interdigitated electrodes for the real TSH samples, the measurements of TSH in human serum samples were carried out. The human serum without TSH, human serums added with 0.2 mIU/L TSH and 3.5 mIU/L TSH were test respectively. The black line curve in Fig. 7 is the LSV cure obtained for the human serum without TSH. The slope is close to the background level (Fig. 3) and no TSH was found in the sample according to the calibration curve (Fig. 5). Then, the human serums added with different concentrations of TSH were analyzed. The concentrations of TSH were calculated as 0.207 mIU/L (red line) and 3.390 mIU/L (blue line) by the calibration curve in Fig. 5, which were closed to the added contents of 0.2 mIU/L and 3.5 mIU/L. Then, the relative standard deviations for the TSH samples detection were calculated as 2.4% and 2.2%, which were close to the result of other TSH immunoassay [27].

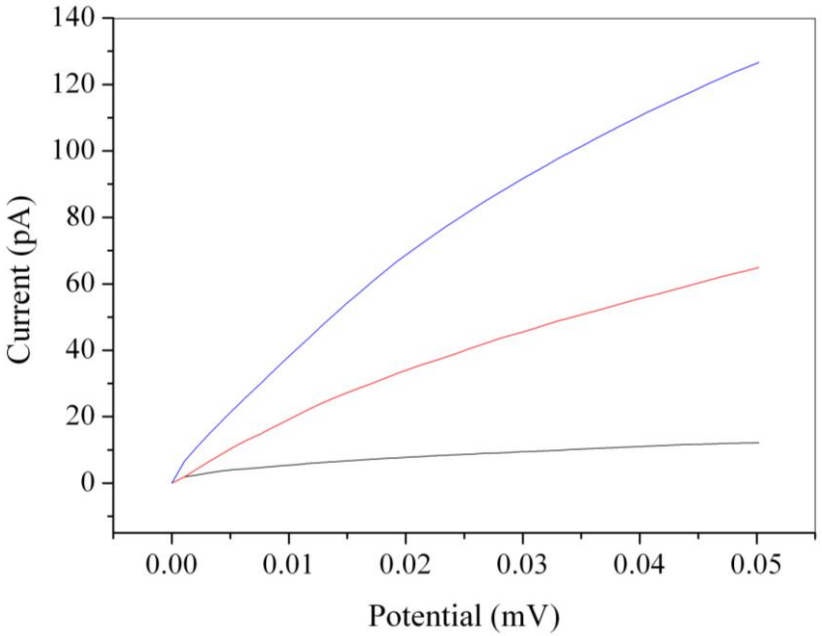

Figure 7. LSV curves depicting the detection of TSH in human serum samples.

4. CONCLUSION

In this work, the MEMS fabrication technology and electrochemical detection method were integrated for the sensitive determination of TSH. The interdigitated electrodes of the electrochemical biosensor were fabricated by lift-off process from the MEMS technology. TSH protein was immobilized on the glass surface between the interdigitated electrodes as sandwich-type by ELISA. Silver deposition solution was used to translate biochemical signal to electrical signal. The dynamic range, detection level and selectivity of the electrochemical biosensor were evaluated through the analysis of different measurements by electrochemical workstation. It was proved that the proposed

electrochemical biosensor based on interdigitated electrodes could achieve the sensitive and selective detection of TSH. The proposed electrochemical biosensor was critical for thyroid disease intervention strategies, and could also be used in the detection of other hormones.

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