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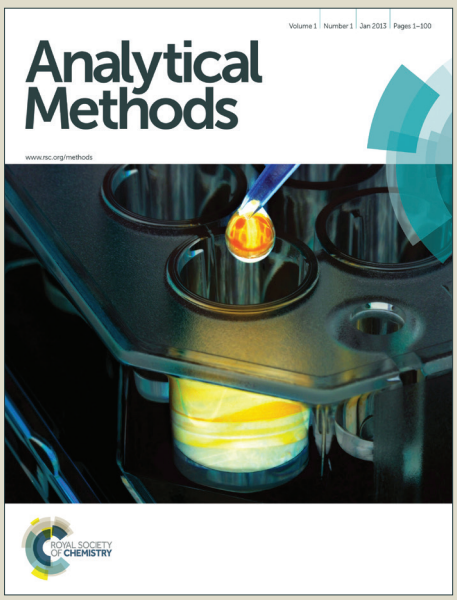

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A reusable and portable immunosensor using personal glucose meter as the transducer

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Abstract

In this research, a portable immunosensor was developed using the personal glucose meter (PGM) as the signal transducer. In this system, the *anti*-alpha-fetoprotein (*anti*-AFP) is immobilized on the screen-printed gold electrode (SPGE) surface. The presence of target brought in the fixation of *invertase*-*anti*-AFP on *anti*-AFP modified SPGE, and then *invertase* catalyzed sucrose to glucose, which is detected using the PGM. The reading of the PGM exhibited a linear relationship with AFP concentration. The detection limit is 0.18 ng mL^{-1}. After one test, this immunosenor can be reused at least 5 times by easy treatment. Moreover, the assay results of clinical serum samples are satisfactory.

Keywords: Personal glucose meter; Screen-printed gold electrode; Immunosensor; Alpha-fetoprotein.

Introduction

Immunoassays have good applications in clinical diagnoses, environmental control and biochemical studies because of their good sensitivity and selectivity [1-3]. Various immunoassays have been reported, such as radiological immunoassays [4], fluorescence immunoassays [5, 6], surface plasmon resonance immunoassays [7, 8], quartz crystal microbalance immunoassays [9], enzyme-linked immunosorbent assays (ELISA) [10], chemiluminescence immunoassays [11] and electrochemical immunoassays [12-14]. These immunsensors have been used successfully in the laboratory, which needs some bulky and expensive signal instruments for the measurement signals. And the results are output by the computers with professional software. Hence, it is difficult to apply online, even point-of-care due to their bulkiness and high cost.

The personal glucose meters (PGM), one of the most successful sensors, have been widely available in the personal family due to the portable "pocket" size, low cost, reliable quantitative results and simple operation [15]. Recently, Lu's group [16, 17] combined the PGM with immunoassay to quantify disease biomarkers on the magnetic beads. This design is to transform the binding event between targets and their antibodies into a PGM-detectable signal, which expanded the range of PGM-detectable targets. Su et al. has also used the PGM for point-of-care early cancer diagnosis [18]. These strategies [16-18] used magnetic beads to immobilize DNA and antibody, which is able to separate and rinse. Unfortunately, using these strategies it is difficult to be reused. It will be great significance if these sensors can be reused by some easy treatments from the point of view of time and chemical reagents saving.

Here, a reused portable immunesensor using the PGM as the transducer is first developed. In this system, the conventional sandwich format is applied, and alpha-

fetoprotein (AFP), an oncofetal glycoprotein [19, 20], is chosen as a model. The primary antibody (*anti*-AFP) is immobilized on a screen-printed gold electrode (SPGE) surface. In the presence of AFP, *invertase* (*In*) labeled secondary *anti*-AFP antibody (*In*-*anti*-AFP) would attach on the SPGE surface, and then *In* molecule would catalyze sucrose to generate glucose, which is detectable using the PGM with the detection limit of 0.18 ng mL^{-1}. Additionally, after the detection, this immunosenor can be reused by easy treatment, such as the interaction with the glycine-HCl buffer solution.

Compared with the previous immunosensors, this proposed method has some advantages: (1) it is portable without expensive and large size equipment, even connection with PC; (2) it is the first reproducible immunosensor based on the PGM; (3) the experimental process is simple without any separation. It is envisioned that this method not only expends the PGM application, but also supplies the support for the design of other portable sensors.

Experimental

Materials and chemicals

AFP standards, *anti*-AFP (clone 1G7, buffered aqueous solution) antibodies, were purchased from Biocell Biotechnol. Co., Ltd. (Zhengzhou, PRC). *Invertase* (β-fructosidase, from baker's yeast) and carboxymethyl dextran were acquired from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals were of analytical grade and used without further purification. N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) and N-Hydroxysuccinimide (NHS) were received from Shanghai Medpep Co., Ltd. All solutions were prepared using Milli-Q reagent water (Milli-Q, Millipore, 18.2-MΩ

resistance). A sucrose solution (1 M) was prepared in the 40 mM Tris-HCl buffer solution (pH 7.6) and stored at 4^{o}C until used.

Instruments

The SPGE (L3.3 cm×W1.0 cm×H0.05 cm) was purchased from eDAQ Technology Corporation (Shanghai, China). It includes a gold working electrode (4 mm in diameter), a silver reference electrode and a gold counter electrode. SPGE was prepared and characterized using a CHI 660D electrochemical system (CH Instruments, Shanghai, China) at room temperature. In the detection section, a commercially available glucose meter (ACCU-CHECK Aviva) was used.

Synthesis of antibody-*invertase* conjugate

The *invertase*-labeled secondary antibody (*In-anti*-AFP) was prepared according to the previous report [21] with some minor modifications. Briefly, 50 uL of *anti*-AFP (1.28×10^{-3} M) was added into 40 mM Tris-HCl buffer solution (pH 7.6) containing invertase (33 mg/mL) and 5% glutaraldehyde. The mixture was incubated in 37 ^{o}C water bath for overnight, and then dialyzed against Tris-HCl buffer solution to obtain antibody-*invertase* conjugate Ab2.

Preparation of antibody-modified SPGEs

The immunosensor was prepared according to the previous report [22]. A clean electrode was covered with 10 μL of 50 mg/mL carboxymethyl dextran in distilled water overnight at room temperature, and then washed with distilled water. 10 μL EDC-NHS solution containing 0.4 M EDC and 0.1 M NHS was then placed on the electrode surface overnight. Next, 5 μL *anti*-AFP was dropped on the above gold working electrode surface

under 60% humidity for 2 h. After blocking the possible remaining active sites using 2.0 wt% BSA, the *anti*-AFP/SPGE was ready for target detection.

Detection of target

After dropping different concentrations of target antigen onto the *anti*-AFP/SPGE for 1 h (Ag/*anti*-AFP/SPGE), 0.5 mL of *In*-*anti*-AFP was applied to obtain a sandwich-type structure immunosensor (*In*-*anti*-AFP/Ag/*anti*-AFP/SPGE). Next, 0.1 mL of sucrose solution was delivered on the immunosensor surface carefully, and then incubated in 45 ^{o}C. 20 min later, 10 μL reacted sucrose solution was measured through a commercially available PGM, and the data from the PGM were collected for quantitative analysis.

Reusable experiment

After the assay, the immunosensor was immersed into 0.2 M glycine-HCl buffer solution (pH 2.8) for breaking the binding between antibody and antigen. And then, the regenerated immunosensor is used again.

Results and Discussion

Principle of this immunosensor

The principle of this immunosensor is shown in Figure 1. This sensor consists of target recognition and signal transducer, respectively. Target recognition is *anti*-AFP immobilized on the SPGE. Only in the presence of target, *In*-*anti*-AFP can attach on the electrode surface by antibody-antigen reaction to form the sandwich-type enzyme structure of *In*-*anti*-AFP/Ag/*anti*-AFP. With the assistance of *In*, sucrose would be catalyzed to generate glucose and fructose. Thus, glucose generated is detected using the PGM. Therefore, in this research the PGM can be used as signal transducer for the

monitor of target. Because of its small size and easy manipulation, the PGM is suitable to develop the portable sensor. It is one of significant advantages of this biosensor. After one test, the glycine-HCl buffer solution (pH 2.8) was covered on the SPGE surface for breaking the antibody-antigen interaction, and then the SPGE with *anti*-AFP is re-obtain again, which achieves the regeneration of the sensor. It is another significant advantage of this biosensor.

Feasibility

Control experiments have been done to certify the feasibility of the proposed principle. After incubation with 1 M sucrose solution for 20 min, the resulted solutions on different SPGEs are detected by the PGM. As shown in Figure 2, for *anti*-AFP/SPGE (histogram a), the PGM records "0 mM", showing that there is no glucose generated; for Ag/*anti*-AFP/SPGE (histogram b) and *In*-*anti*-AFP/*anti*-AFP/SPGE (histogram c), the records are still "0 mM", showing that in the presence of target antigen, no glucose is generated due to the lack of enzyme. While for *In*-*anti*-AFP/Ag/*anti*-AFP/SPGE (histogram d), the PGM records "7.5 mM", displaying that 7.5 mM glucose is generated with the assistance of *In*. This result also indicates that *In* labeled on the antibody still sustains its catalytic activity. Additionally, the result of PGM can be estimated by the color indictor consisting of a round colored control window on the back of the test strip itself. In the presence of glucose, the color changes from yellow to green. In above three control experiments, the color does not change (strip a, b and c in inset), proving that there no glucose generated. While the formation of the sandwich-type enzyme structure of *In*-*anti*-AFP/Ag/*anti*-AFP would catalyze sucrose into glucose, resulting in the color change (strip d in inset). Therefore, it is feasible to use the PGM for the immunoassay.

Optimized condition

In order to obtain better results, some experimental conditions had been optimized. Firstly, the temperature and time of *invertase*-catalyzed sucrose had been studied. With the change of incubation temperature from 25 to 50 ^{o}C at the incubation time of 20 min, the signal of the PGM increases greatly and reaches a maximum at 45 ^{o}C (Figure 3A). The reason lies in that 45 ^{o}C is a suitable temperature for *invertase*. On the other hand, increasing the incubation time, the signal of the PGM enhances slowly (Figure 3B). Hence, 45 ^{o}C and 20 min are chosen as the optimal temperature and time for the reaction.

Assay of target

To study the relationship between the concentration of target and the signal of the PGM, targets with different concentrations are assayed using this proposed immunosensor, and the signals of the PGM recorded for the quantitative analysis. As shown in Figure 4A, with increasing target concentration, the signal of the PGM gradually increases. When target concentration exceeds 50 ng/mL, the PGM signal deviates from linearity and reaches a plateau at the max 33.3 mM, which is the upper limit of the PGM. The signal of the PGM has a linear relationship with the concentrations of target in the range of 0.5~50 ng mL^{-1} (See the inset in Figure 4A), and the regression equation is

$$Y=0.3624+6.222X \quad R=0.9996$$

where X is the concentration of AFP, Y is the signal of the PGM (mM), R is the regression coefficient. The detection limit is 0.18 ng mL^{-1} based on 3σ/slope (σ is the standard deviation of blank sample for 5 times), which performed better than those of electrochemical immunoassays [23, 24, 25] piezoelectric immunoassay [26], fluoroimmunoassay [27], microfluidic reflectometric interference spectroscopy [28].

Besides the record of the PGM for monitoring the target concentration, the colors from the round colored control window are also related to the concentration of AFP (See Figure 4B).

Selectivity

Herein, three proteins are used as interferents for the specific experiment, CEA, BSA and lysozyme, respectively. The concentration of target is 20 ng mL^{-1}, while that of each interferent is 200 ng mL^{-1}. As shown in Figure 5, in the presence of interferents, the PGM displays in the range of "0 mM"~"0.5 mM". While only in the presence of target, the PGM records "14.1 mM". This result shows that this method has good selectivity, and interference from some interferents can be ignored.

Reusability and repeatability

After the assay, the used electrode is immersed into the glycine-HCl buffer solution (pH 2.8) for 1 hr to break the antibody-antigen linkage, and then carried out the repeated assay step. After 5 repeated experiments, the reading of the PGM retains 80 % of the original signal, and the RSD of 5 experiments is 5 %. This result shows that this method has good reusability. In addition, 3 gold electrodes were cleaned for the same experiments as above as parallel experiments. The similar results are achieved with RSD of 6.5%, indicating that this method has good repeatability.

Analysis of clinical serum samples

To validate this method in the real-life samples, three clinical serum samples (provided available by Fujian Provincial Hospital, China) are assayed. The results are shown in Table 1. The concentrations of AFP in the serum samples are 2.4 and 4.5 ng mL^{-1}, respectively, which is similar with the reference results from the hospital, and the

recoveries of this proposed method are between 90%~105%, showing that it has a satisfactory accuracy.

Conclusion

In summary, a reused portable immunosensor using the PGM as the transducer was demonstrated. The recognition event between antibody and antigen can be monitored using the PGM, and it is found that the record of the PGM has a relationship with the concentration of target AFP with the detection limit of 0.18 ng/mL (S/N=3). In addition, this proposed sensor has good reusability, and is followed by assays of AFP in serum samples with the satisfactory results. More importantly, because the transducer is small and portable, hence, it is possible for public to do diagnosis and detections in personal family.

Acknowledgement

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Figure Captions

Figure 1 Sensing principle and regeneration of the portable immunosensor using the PGM.

Figure 2 The signals of the PGM under different condition. a: *anti*-AFP/SPGE; b: Ag/*anti*-AFP/SPGE; c. *In-anti*-AFP/*anti*-AFP/SPGE; d. *In-anti*-AFP/Ag/*anti*-AFP/SPGE. Inset: the corresponding color changes of the round control window on the back of the test strip upon above conditions.

Figure 3 (A) Effect of the catalyze temperature on the readout of PGM. (B) The optimization of incubation time between invertase and sucrose. The concentration of sucrose is 1 M.

Figure 4 (A) Calibration curve between AFP concentrations and the signals of the PGM, from l to 8, the concentrations of AFP are 0.5, 1.0, 3.0, 8.0, 20.0, 30.0, 50.0 and 100.0 ng mL^{-1}. (B) The corresponding color changes of the round control window on the back of the test strip with the increasing the concentration of AFP.

Figure 5 Selectivity of the portable immunosensor toward other proteins, containing CEA, BSA, and Lysozyme. Their concentrations are 200 ng mL^{-1}, while target concentration is 20 ng mL^{-1}.

Table 1 Evaluation of this portable immunosensor in clinical serum specimen

Sample	Detected Result ($ng\ mL^{-1}$)	Reference Result ($ng\ mL^{-1}$)	Add AFP ($ng\ mL^{-1}$)	Detection after Addition ($ng\ mL^{-1}$)	Recovery (%)
1	2.4	2.6	2	4.2	90
			4	6.3	97.5
			8	10.5	101.3
2	4.5	4.1	2	6.6	105
			4	8.8	91
			8	12.2	96.3
3	5.4	5.0	2	7.5	105
			4	9.4	100
			8	12.9	93.8
4	3.3	3.7	2	5.4	105
			4	7.4	102.5
			8	11.2	98.8

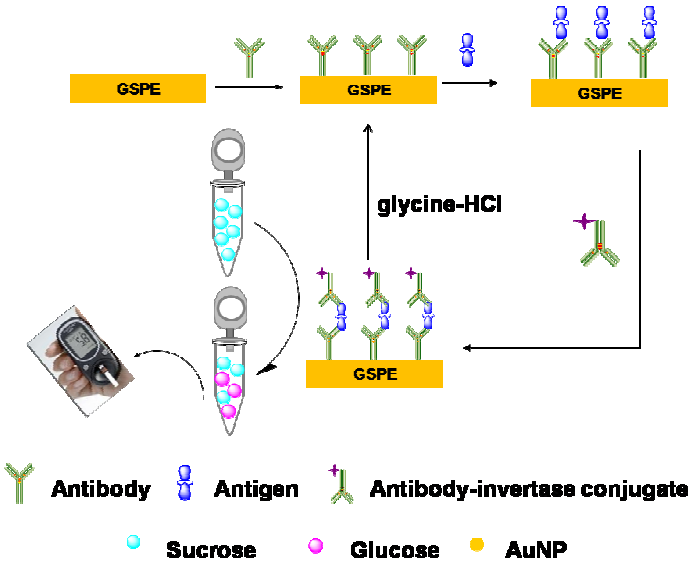

Figure 1

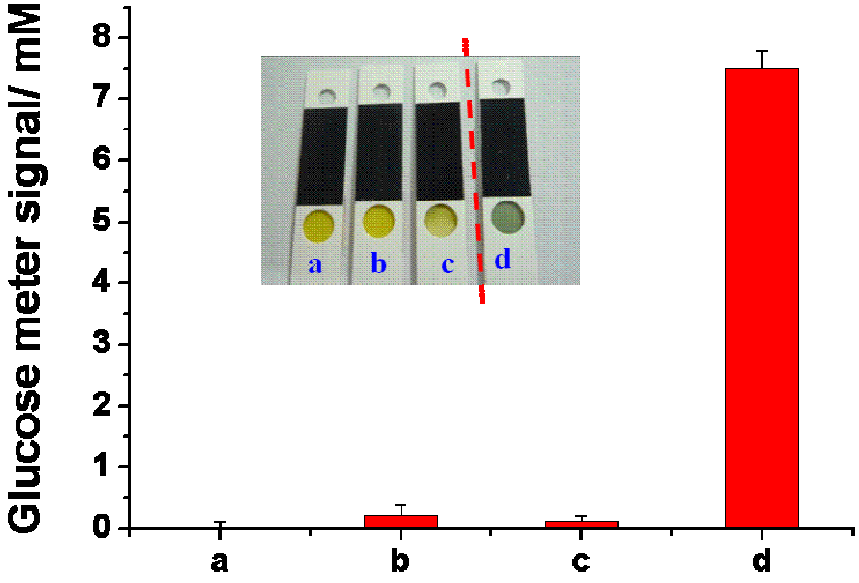

Figure 2

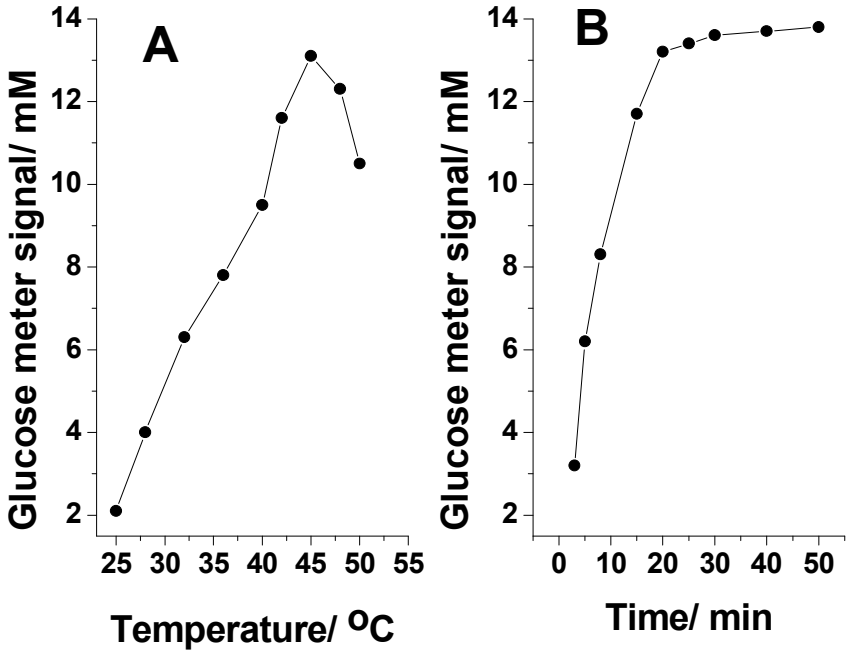

Figure 3

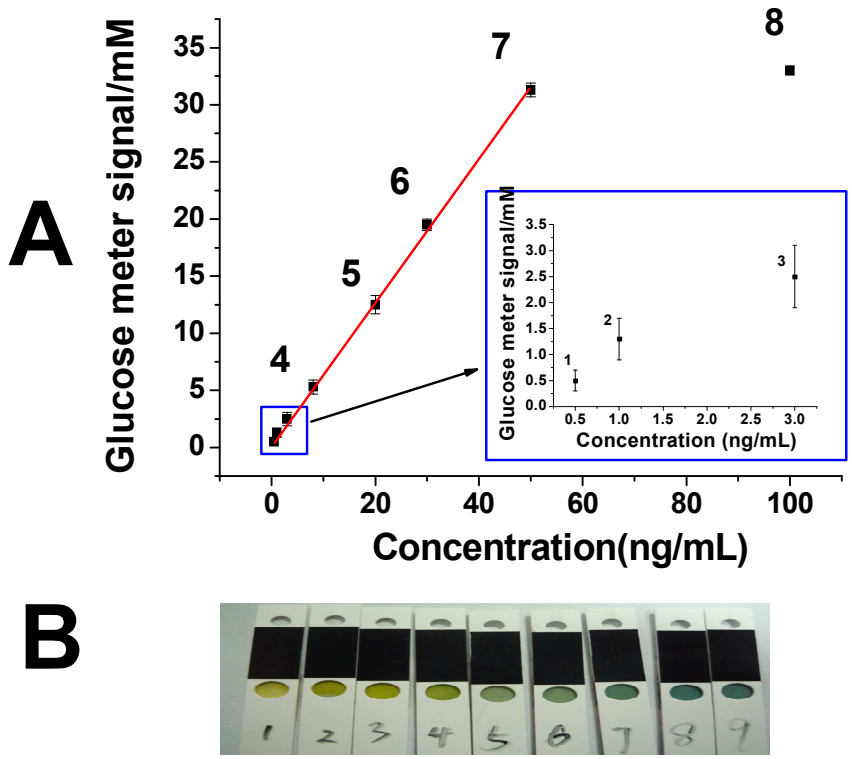

Figure 4

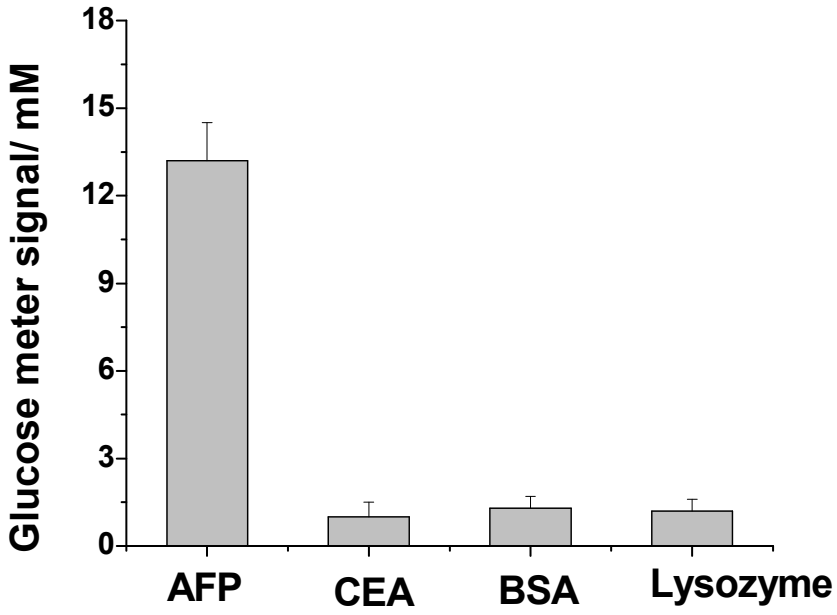

Figure 5