GLUCOSE FACTOR IN THE TEST OF ISCHEMIA HEART DISEASE FOR DIABETIC PATIENTS USING MOLECULARLY IMPRINTED POLYMER /METHYLENE BLUE SENSING ELECTRODES

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ABSTRACT

The abstract introduces the impact of glucose on the sensing efficacy of the molecularly imprinted polymer (MIP)-based electrode employing methylene blue (MB) as the probing reagent for the first time. Experimental findings reveal that the sensing ability of the MIP/MB/Au electrode for cTnT detection was compromised, leading to an increase in the limit of detection (LoD) from 24.27 to 36.14 pg/ml in the presence of glucose. This suggests that such sensing technology could lead to diagnostic errors with harmful consequences for diabetic patients with AMI disease.

KEYWORDS

Molecular Imprinted Polymer, Methylene Blue, Cardiac Troponin T, Glucose, Blue Bottle.

INTRODUCTION

Recent investigations have revealed a higher mortality risk among in-hospital diabetic patients with acute myocardial infarction (AMI), which can be mitigated by prolonged surveillance and strict control of other risk factors [1,2]. Cardiac troponin T (cTnT) is the preferred serologic test for diagnosing AMI [3]. Therefore, for diabetic patients in this context, an efficient sensor capable of monitoring their cardiovascular health in a cost-effective manner could assist physicians in implementing appropriate treatment measures to reduce mortality risks. Several point-of-care sensing technologies have been developed to achieve this goal. Among them, the molecular imprinting polymer (MIP)-based sensor has emerged as a leading technology in medical handheld devices due to its inherent advantages, including extended storage stability, heightened specificity, and resilience to harsh environmental conditions compared to conventional enzyme sensors [4].

Previously, we have developed MIP-based sensing electrodes for cTnT and NT-proBNP, aiding in cardiovascular disease prediction. In this study, we integrated an electropolymerized methylene blue (MB) layer into the MIP for direct biomarker concentration detection in serum or urine tests, enhancing its utility for cardiovascular disease prediction. However, this convenience may lead to diagnostic errors, particularly harmful for diabetic patients with AMI disease. This paper investigates and discloses the potential sensing reliability issues associated with the widely adopted MB layer in MIP sensor design.

PLAUSIBLE CAUSE AND EXPERIMENTAL

Fig. 1 illustrates a scenario demonstrating the reliability issue associated with the utilization of MB in MIP sensors. In the well-known "blue bottle experiment", MB functions as a redox reagent whose oxidized form is progressively reduced by glucose, (Fig. 1(a)), resulting in a colorless solution. Glucose weight is approximately 180.16 kDa, which is larger than that of cTnT, i.e., ~35 kDa. If glucose passes through the three-dimensional pores left behind by cTnTs after elution (Fig. 1(b)), it could react with the underlying MB film, leading to a decrease in peak current. Consequently, as long as the MIP/MB/Au sensing electrode comes into contact with glucose, the electrode's functionality could not only deteriorate gradually but also exhibit a lower peak oxidation current. Fig. 2 shows the decrease in peak oxidation current resulting from the oxidation current reduction of MB when the MB/Au electrode is immersed in a PBS with the addition of 3 mg/ml glucose.

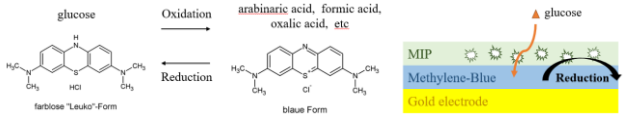

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Figure 1:(a) blue bottle experiment and (b) glucose passes through the three-dimensional pores of the MIP film and reacts with the MB film below.

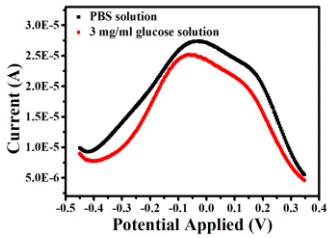

Figure 2: Comparison of the DPV measurement of the MB/Au sensing electrode immersed in the PBS solution with/without the addition of glucose.

The MIP/MB/Au electrodes for cTnT detection are fabricated to verify the glucose effect and the corresponding fabrication can be referred to our prior arts [6,7]. The fabrication comprise four major processes as follows: 1) Deposition and photo-patterning of a Ti (20nm)/Au (60nm) layer on a silicon substrate to define the electrode. 2) electrochemical polymerization of a MB film onto the Au electrode in a PBS buffer solution (0.1 M, pH=7.4) using cyclic voltammetry from -0.4 to 1.2V, 3) electrochemical polymerization of a MIP film onto the MB film in an PBS solution using cyclic voltammetry from -0.4 to 0.8V, and 4) The elution of embedded cTnT molecules from the MIP in a 0.5 M HCl solution, yielding the cTnT sensing electrode.

During the electrochemical polymerization, the voltage-induced excitation and binding of molecules will cause peak elevation and shifts, manifesting the MB or MIP film on the electrode. In Fig. 3,

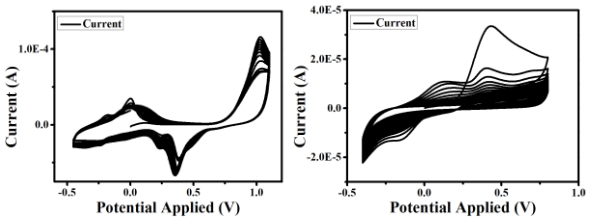

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Figure 3:(a) The C-V curve of electropolymerized MB film and (b) The C-V curve of electropolymerized MIP film.

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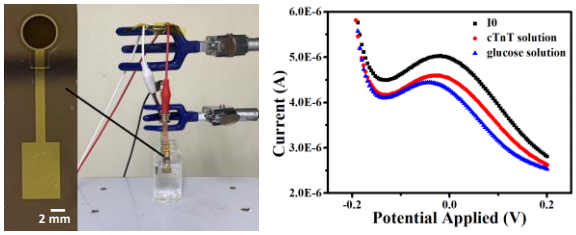

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Figure 4: (a)Measurement set up and (b) DPV measurement of the MIP/PMB/Au sensing electrode in the PBS solution added with cTnT molecules and with/without the incorporation of glucose before and after the elution of cTnT molecules in the MIP film.

the peak at +1V rises, the peak at 0V shifts right, and the peak at +0.5V shifts left, indicating the polymerization of MB molecules on the electrode (Fig. 3(a)). A prominent peak at 0.5V resulting from numerous bonding reactions during the initial formation of o-phenylenediamine (o-PD) will gradually decrease due to the obstruction of ionic flow. Fig. 4 shows the measurement set up and current response of the cTnT sensing electrode where Differential Pulse Voltammetry (DPV) measurement is performed via a three-electrode configuration in the sample. The peak currents of the electrode with/without the elution of cTnT molecules in MIP film in the PBS solutions with/without the addition of 3 mg/ml glucose indicating the glucose indeed affect the peak current response.

RESULTS AND DISCUSSION

A patient exhibiting a blood sugar level exceeding 2 mg/mL two hours after a meal can be diagnosed with diabetes. Fig. 5 depicts the normalized current response of the MIP/MB/Au electrode with respect to various glucose and cTnT concentrations ranging from 0.6 to 2 mg/ml and 20 to 600 pg/ml, respectively, in PBS solutions. Figure 5(a) demonstrates that the cTnT sensing electrode exhibits a sensitivity of 3.19×10^{-2} $(mg/ml)^{-1}$ in PBS solutions without the addition of cTnT molecules, suggesting a greater extent of oxidation current reduction of the MB involved in the reaction with glucose. Fig 5 (b) shows the correlation between cTnT concentration and

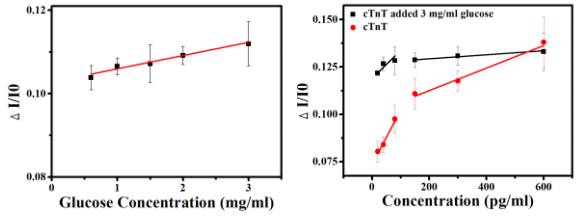

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Figure 5:(a) Sensitivity measurement of the sensing electrode in the PBS with different concentrations of the glucose and (b) Sensitivity measurement of the sensing electrode in the PBS with different cTnT concentrations and with/without adding glucose.

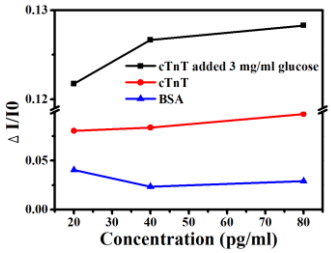

Figure 6: Sensitivity measurement of the sensing electrode in the samples with different cTnT concentrations< 80 pg/ml.

normalized current change as well as the one detected with additional glucose, i.e., 3 mg/ml. The sensing electrode exhibits two linear responses in the PBS solutions only added with cTnT molecules where R^2=0.92 and 0.96 for low and high (> 80 pg/ml) concentration regimes, respectively. The corresponding sensitivities $((pg/ml)^{-1})$ and limit of detection (LoD) are 2.87×10^{-4}, 5.94×10^{-5}, and 24.27 pg/ml, respectively. Meanwhile, the sensing electrode exhibits two linear responses in the PBS solution added with cTnT molecules and 3 mg/ml glucose where R^2=0.67 and 0.91 for the low and high concentration regimes, respectively. The corresponding sensitivities and LoD become 1.48×10^{-4}, 1.07×10^{-5}, and 36.14 pg/ml, respectively.

Bovine serum albumin (BSA) was typically used as an interference agent for cTnT detection using the MIP sensing electrode due to its smaller molecular size (only 5–6 nm) [6,7]. Fig. 6 illustrates that the normalized current response of the sensing electrode remains unaffected by solutions with BSA but significantly increases when immersed in a PBS solution containing both cTnT and glucose molecules, suggesting the oxidation current reduction of MB by the glucose dominates the sensor response.

CONCLUSION

The MIP/MB/Au sensing electrodes designed for cTnT detection exhibit sensitivities to both cTnT and glucose levels. The reduction in oxidation current of the MB due to the presence of glucose, intended for cTnT detection, has the potential to affect the sensor's accuracy. Notably, the sensor's sensitivity to both low and high concentrations of cTnT was compromised and reduced in the presence of elevated glucose concentrations. Therefore, caution should be exercised while using the sensor for diagnosing diabetic patients with AMI disease.

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