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Titania nanotubes decorated with gold nanoparticles for electrochemiluminescent biosensing of glycosylated hemoglobin

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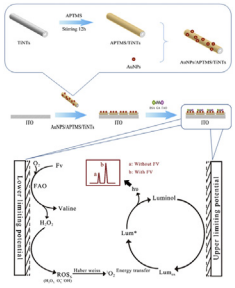
HIGHLIGHTS

- The enhanced electrochemiluminescence of luminol by AuNPs/TiNTs.
- An ECL biosensor for HbA1c assay with ultra-high sensitivity.
- A promising disposable device for diabetic diagnosis and treatment even for POCT.
- The excellent regression of detected results with gold-standard method.

GRAPHICAL ABSTRACT



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ABSTRACT

A glycated hemoglobin (HbA1c) biosensor with high performance has been constructed in this work. Here the fructosyl amino acid oxidase was immobilized onto a pre-functionalized indium tin oxide glass with titania nanotubes decorated with gold nanoparticles. The property of nanocomposite was characterized by transmission electromicroscopy, scanning electron microscopy, electrochemistry and spectroscopy. Under the optimum conditions, fructosyl valine was detected by this biosensor. It exhibited a linear detection range from 4.0×10^{-9} M to 7.2×10^{-7} M, and a limit of detection for 3.8×10^{-9} M at the signal-to-noise ratio of 3. Thus the HbA1c level in whole blood samples of healthy individuals or diabetic patients were evaluated with designed biosensor after pre-treatment of hydrolysis. The results of our detection were closely consistent with that of the standard method. At the same time, our biosensor has some advantages including high sensitivity, disposable usage and low cost, which implies its great promising application in point-of-care testing of HbA1c.

1. Introduction

The 2014 report of International Diabetes Federation (IDF) has pointed out that 8.2% (about 387 millions) of adults (aged from 20 to 79) were suffered from diabetes mellitus worldwide, and still in ever-increasing rapidly [1]. Diabetes mellitus is a chronic metabolic disease characterized by high blood glucose. Diabetes itself does not affirmatively result in severe harm to patients, but a chronic high blood glucose level will lead to dangerous syndromes for heart, brain, blood vessels, eyes, kidneys, feet, peripheral nerves, teeth and so on. According to the report of WHO, there are more than 100 kinds of complications of diabetes [2–4]. Also the diabetic patients have higher risk of infections from bacteria or virus.

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Conclusively, diabetes mellitus has already been proved to be a global public health issue. In a representative sample, the prevalence of diabetes of Chinese adults has exceeded 10%, which shares in the greatest portion of diabetic patients in the world [5–7].

The blood glucose index [8] reflects an immediate fluctuating glucose level, but it is easily disturbed by daily diet thus requires frequent measurements. Another more useful diagnostic marker, glycated hemoglobin (HbA1c), an irreversible coalition of hemoglobin with D-glucose via the N-terminal group of its β-chain, is accepted as a months-long standard of diabetic condition [9–14]. Not only related to the concentration of blood glucose, the HbA1c index reflects the average blood glucose level during past 2–3 months without fluctuation since it has a half-life time of 7–8 weeks [15]. This is the most beneficial feature of HbA1c assay for diabetes diagnosis and treatment.

Currently, the methods include high-performance liquid chromatography (HPLC), electrophoresis, electroendosmosis, ion exchange chromatography, boronate affinity chromatography, isoelectric focusing method, immunoassay, liquid chromatography-tandem mass spectroscopy, fluorometry, colorimetry and so on have been applied for clinical HbA1c assay [12]. Among them, HPLC is generally accepted as the gold standard method. But these methods require expensive equipments, complex operation, consuming time, high cost and trained persons to operate. And there are multiple controversies due to the discrepancy on precision and accuracy between various commercial methods [16]. The development of portable HbA1c testing device, with high accuracy, simple/convenient operation and low cost, is still in broad promising application and also has high commercial value.

Nowadays, the electrochemical biosensors have been proved to be a kind of widespread, multitudinous and gradually commercialized devices. The matrix of electrochemical biosensors always include a biological recognition element to ensure its unique identification and high selectivity towards target molecule. They are widely used in chemistry, biology, food safety, environmental monitoring, pharmaceutical or clinical laboratories and so on [11]. Recently, considering the possibility to overcome those mentioned limitations of the methods for HbA1c assay, the electrochemical biosensor profiles a bright future. The prominent advantages of electrochemical biosensor for HbA1c generally including miniaturized size, portability and convenient usage make it most likely succeeding in clinical point-of-care testing (POCT) [16–18]. But, only few reports have focused on it until now.

There are two categories of electrochemical HbA1c biosensor known as the enzyme sensor [19–24] (based on detection of fructosyl valine (FV) under the catalysis of fructosyl amino-acid oxidase, FAO) or immunosensor [25–28]. The advantages of enzymatic method include good reliability, high sensitivity and relatively fast response. The principle for HbA1c assay is supposed to consist of following steps:

$$\underset{\text{(N-terminal residue of b-chain in HbA1c)}}{\text{HbA1c}}\xrightarrow{\text{endoproteinase Glu.C}}\text{Fru-Val-His-Leu-Thr-Pro-Glu-Glu-Lys-ser}\ldots \quad (1)$$

$$\underset{\text{(N-terminal residue of b-chain in HbA1c)}}{\text{Fru-Val-His-Leu-Thr-Pro-Glu-Glu-Lys-ser}\ldots}\xrightarrow{\text{Protease}}\underset{\text{(FV)}}{\text{Fructosyl valine}}+\underset{\text{(His, Leu, Thr)}}{(\text{amino acid})_{n-1}} \quad (2)$$

$$\text{FV}+\text{O}_2+\text{H}_2\text{O}\xrightarrow{\text{FAO}}\text{Valine}+\text{D-glucose}+\text{H}_2\text{O}_2 \quad (3)$$

The electrochemiluminescent (ECL) analysis is an attractive method owing to its high sensitivity, good controllability, simplicity, rapidity, and low cost [29–31]. Because of the non-toxicity, cheapness, and high light-emitting quantum yield, luminol is one of the most significant luminophore and has been used in ECL detection of some biomarkers [32–35]. The HbA1c in blood samples will be transformed into FV during the enzymolysis, and thereafter to be oxidized under the catalysis of FAO. As one of the reactive oxygen species (ROSs), the co-produced H_2O_2 of this reaction will greatly intensify the ECL of luminol [36,37]. But, as far as we know, no related work about ECL enzyme sensor for HbA1c has been reported yet.

Series of specially structured nanosized titania, as nanotubes, arrayed nanotubes, nanobelts, nanowires or nano-rods have been prepared and applied in a lot of important researches because of their outstanding performance [38–41]. We have also synthesized and intensively studied the application of hollowed titania nanoshell (HTNSs) [42] and titania nanotubes (TiNTs) [43] in ECL analysis with luminol as a luminophore. These two materials can promote the redox of hydrogen peroxide, to further increase the ECL of luminol and greatly promote the sensitivity of H_2O_2 detection. Also as we all know, AuNPs have a large specific surface area, excellent electrical conductivity and good biocompatibility, it also can promote the ECL of luminol [44]. In recent years, we have also reported some modified electrodes of TiO_2 and AuNPs or AuAg alloy nanocomposite for enhancement of the luminol ECL [45,46].

In this work, we successfully prepared an Au/TiNTs nanocomposites by the means of physical/chemical functions of (3-aminopropyl)trimethoxysilane (APTMS). Then it was covered on an indium tin oxide (ITO) glass together with cross-linked FAO to acts as a sensing matrix for ECL detection of FV in pretreated blood sample. It is known that two FV molecules would be released during the protease digestion from one HbA1c molecule, thus a quantitative relation toward HbA1c is established and therefore the HbA1c level (HbA1c %) can be calibrated as the ratio of glycated hemoglobin to total hemoglobin. Compare with other current testing methods, including electrochemical methods, the fabricated ECL sensor showed a higher sensitivity, lower detection limit, good reliability and faster implementation. The design of this new sensor is not only to simply assemble those materials and signaling technic together, but also to deeply exploit the potentiality of synergic effect between those nano-materials themselves, the ROSs from enzymatic reaction and the electrolysis induced redox to trigger stronger light-emission of luminol which proportionally related to the concentration of substrate. This is a sole report about the ECL biosensor for HbA1c with highest sensitivity but equally simple preparation/operation.