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Nonenzymatic Glucose Biosensors: Latest Advances and Perspectives

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Abstract. Over the sixty years since glucose biosensor was introduced, development of glucose biosensor had experienced three generations. Considering the increasing demand of glucose biosensor, research and development has been carried out for more promising nonenzymatic glucose biosensor. Compared to the traditional enzyme-based glucose biosensor, nonenzymatic sensor is more capable to continuous glucose detection and has higher stability. Additionally, nonenzymatic glucose biosensor has a lower fabrication cost, which can be beneficial to the wide range of patients with the need for constant checking for blood glucose concentration. This paper introduces two proposed nonenzymatic glucose detection models: activated chemisorption and incipient hydrous oxide adatom mediator. As the developing trend of glucose biosensors is toward wearable and continuous glucose monitoring, two wearable sensors and working principles are introduced. This paper describes the application progress of different types of materials in enzyme-free blood glucose sensors, puts forward the existing problems in the application of enzyme-free blood glucose sensors, and looks forward to its future application prospects.

Keywords: Glucose Biosensor, Nonenzymatic Sensing, Nanomaterials.

1. Introduction

"The term diabetes mellitus describes a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both." A definition of diabetes mellitus from WHO [1]. With numerous long-term complications, including nonfatal or fatal heart attack and stroke resulting from large arteries and blindness and kidney disease caused by small arteries, diabetes mellitus has a detrimental effect on patients worldwide [2]. According to the 9th edition of the IDF Diabetes Atlas, by 2030, there will be 578.4 million people living with diabetes worldwide [3]. For all diabetes mellitus, monitoring their blood glucose level is crucial because it helps patients adjust their treatment [4]. The presence of glucose biosensors facilitates the monitoring, and there are mainly two types of glucose biosensors, namely enzymatic glucose biosensors and enzyme-free glucose biosensors.

The development of glucose biosensor can be traced back to the 1960s when Clark and Lyons introduced the first generation of enzymatic glucose biosensor, and over the following forty years, glucose biosensor based on enzymes has already gone through three generations [5]. Updike and

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Hicks improved in order to simplify the very first model. They immobilized the enzyme glucose oxidase (GOx) on an oxygen electrode, and the decrease in oxygen concentration was measured to obtain the glucose level. To tackle the drawbacks, the first generation was caused by the reliance on oxygen, and the second generation of glucose biosensors used a mediator to substitute oxygen. These redox mediators act as electron carriers, which are reduced at enzymes, picking up electrons and oxidizing at electrodes where they release electrons. Considering the toxicity of the mediator, the third generation of glucose biosensor with the removal of the mediator was introduced [6, 7]. Although enzymatic glucose biosensor has dominated the market of commercially accessible devices for glucose detection, it still has numerous defects that need to be improved. These disadvantages are mainly attributed to the utilization of enzymes, such as the intolerance of enzymes to extreme temperature and pH values, difficulties in controlling the amount of enzyme used and short shelf life [6, 8]. These mentioned drawbacks of enzyme-based glucose biosensors can lead to the biosensor's high fabrication price, which strains the diabetes treatment. More attention is currently drawn to developing enzyme-free glucose biosensors [6].

2. Working Mechanism Of Nonenzymatic Glucose Biosensor

Biosensors usually consist of three components: bioreactor, which recognizes and reacts with analyte; transducer, which converts biochemical signals to electrical signal and detector, which receive and amplify the transduced signal to provide visualized data [9]. The difference between enzyme-based and enzyme-free glucose biosensors is the bioreactor changing. Transformation is made by replacing enzymes such as glucose dehydrogenase (GDH) and glucose oxidase (GOx) using non-enzyme electrocatalysts, including metals, metal oxides, alloys, complexes and carbon as an oxidizing agents of glucose molecules [10]. In non-enzyme glucose biosensors, the atoms on the electrode surface directly oxidize the glucose. There are by far two models proposed to explain the yet uncertain process of how the oxidation of glucose molecules is catalyzed by the electrode surface [11].

2.1. Activated Chemisorption Model

Pletcher proposed a model called the activated chemisorption model [12]. This model suggests that the oxidation process of glucose is initiated when the surface of the electrode surface absorbs glucose molecules. At this stage, bonds are formed between absorbate, glucose, and the metallic electrocatalysts with d-electrons and d-orbitals. After that, a hydrogen atom originally attached to the hemiacetal carbon is abstracted and then bonds to the electrode surface. As the removal of the hydrogen atom, the oxidation state of glucose changes, leading to a change in glucose-metal interaction; hence the bond strength of the glucose-metal bond decreases, and glucose desorption occurs. The bond between glucose and metal on the electrode surface is formed and broken later in the process, which means that ideal intermediate bond strength is essential for the continuity of oxidation. During the catalytic process, the abstraction of hydrogen molecules is the rate-determining step. Pletcher suggested that this abstraction happens at the same time as the chemisorption of glucose,

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meaning that the adjacent metal active sites would be occupied by only one absorbate each time [10,11,13].

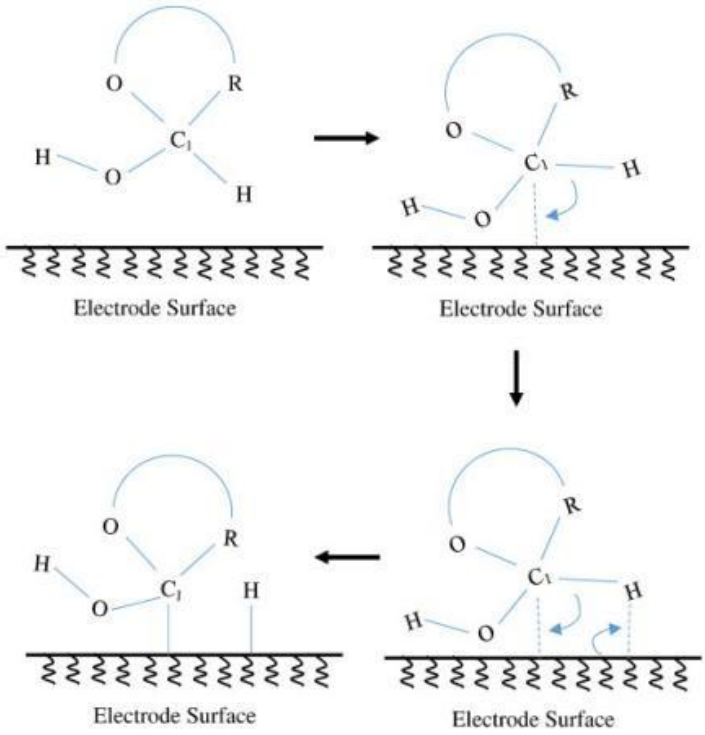


Figure 1. The illustration of the mechanism of concentric absorption theory [13].

2.2. Incipient Hydrous Oxide Adatom Mediator

The second model, Incipient Hydrous Oxide Adatom Mediator (IHOAM), was proposed by Burke. This proposal is suggested according to the observed pre-monolayer oxidation of active metal atoms on the surface of the electrode, which forms an incipient hydrous oxide, OH_{ads}. In this model, the process initiates the formation of hydrous oxide, followed by the chemisorption of glucose molecules. The hydrous pre-monolayer then mediates the electrooxidation of the absorbed glucose molecules and regenerates the metal surface. The abovementioned "active" metals have low lattice coordination values and stabilization energy. Their insufficient stability leads to high reactivity and pre-monolayer oxidation at lower potentials.

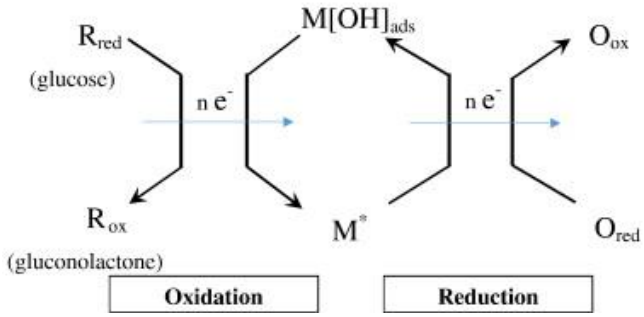


Figure 2. An illustration of the Incipient Hydrous Oxide Adatom Mediator model [13].

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2.3. Mechanism Related to Specific Materials (Metal) of Electrode

The next subsection lists some of the highly investigated metals used for non-enzyme glucose biosensor and their detailed mechanisms.

2.3.1. Platinum Electrode. The process of oxidation of glucose molecules based on platinum includes three steps. Chemisorption and dehydrogenation of the glucose molecule take place first. Then, the hydroxide radicals are produced by decomposing water molecules, and the absorbed hydroxide anions subsequently oxidize glucose. The final step is the oxidation of glucose by platinum oxide. The experiments support the activated chemisorption model and IHOAM on platinum [11,13].

2.3.2. Gold Electrode. Gold shows a higher electroactivity to glucose oxidation when compared to platinum, and so it is also highly investigated for the utilization of a non-enzyme glucose biosensor. The process catalyzed by gold includes dissociating water to form hydroxide anions, and the hydrous gold oxide is formed when the gold surface absorbs hydroxides to oxidize glucose [11].

2.3.3. Nickel Electrode. The mechanism of nickel-based oxidation of glucose differs from that of platinum and gold. The nickel surface is oxidized to oxyhydroxide, whereas the other two metals' surface forms a hydrous pre-monolayer. The glucose oxidation based on nickel is believed to be finished by the redox couple of Ni (II) and Ni (III). At first, $Ni(OH)_2$ is oxidized to NiOOH. Hydrogen is abstracted from glucose at the metal surface, forming radical intermediate and $Ni(OH)_2$. Finally, the radical intermediate is oxidized to gluconolactone by hydroxyl [13].

3. Classification

According to the different chemical detection methods, enzyme-free glucose sensors can be divided into three types: potential, current, and voltammetry. The current type of enzyme-free glucose transducers is the most widely studied type of transducers. It is used to measure the concentration of glucose concerning the oxidation current produced by directly promoting the oxidation of glucose on the electrode surface [14].

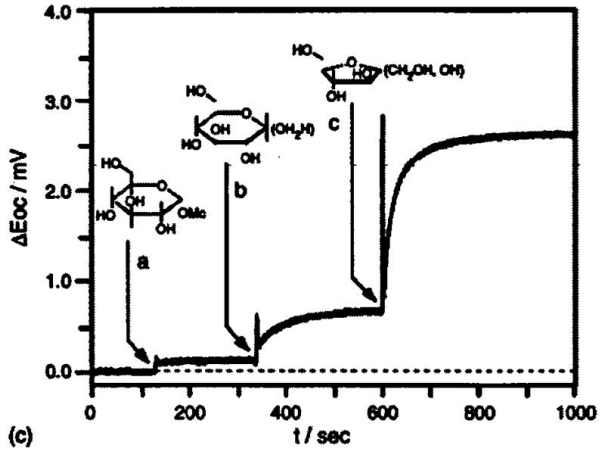


Figure 3. The potential nonenzymatic glucose sensor based on polyaniline boric acid [16].

The response mechanism of the potential glucose sensor is to measure the glucose concentration by the potential change caused by the concentration difference of electrolytes on both sides of the ion-selective membrane due to the reaction between glucose and the recognition molecule. This type of glucose sensor is suitable for the detection of high concentrations of glucose because the direct potential analysis of glucose produces a relatively small potential signal. However, the potential sensor can be combined with the multi-channel array sensor to realize the sensor multifunctionalities, and the

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operation of the potential sensor is simple, low cost, has good reproducibility, and is suitable for industrial mass production [15].

The voltammetric enzyme-free glucose sensor indirectly detects glucose concentration in a solution by the characteristics of the voltammetric current produced by the energy transfer molecule [15].

4. Various advanced materials for enzyme-free glucose biosensor

4.1. Precious Metals

Single metal electrodes have high metal activity, and Pt, Pd, Au, Ag, etc., are often used as modified electrodes to construct sensors. The preparation process of this kind of sensor is relatively simple, and it also has a good catalytic effect on glucose. Pt is one of the first metal elements applied to the enzyme-free sensing of glucose, and Pt-based modified electrodes show a good response to glucose in alkaline and neutral solutions. Guo et al. used 3 mmol/L chloroplatinic solutions as the electrolyte, and the constant potential deposition method was used to prepare Pt nanoflower electrode and construct a glucose sensor in an ultrasound environment. The electron microscopy results showed that the surface of the Pt nanoflower electrode was rough and spherical with screws. Electrochemical tests showed that the Pt nanoflower electrode had a good electrocatalytic oxidation activity for glucose, and it has a potential application in the field of the blood glucose sensor [18]. The Au-based modified electrode has better electrochemical activity than the Pt-based modified electrode. According to the principle of surface plasmon resonance, Kurniawan et al. used layer-by-layer deposition to deposit Au nanoparticles on Au electrodes and constructed sensors with a three-electrode system. The voltammetric characteristics of the sensor show that the Au NPS coated Au electrode has a larger glucose response current than the bare gold electrode, and its characteristics, such as low detection limit and high sensitivity, make it suitable for blood glucose detection [19].

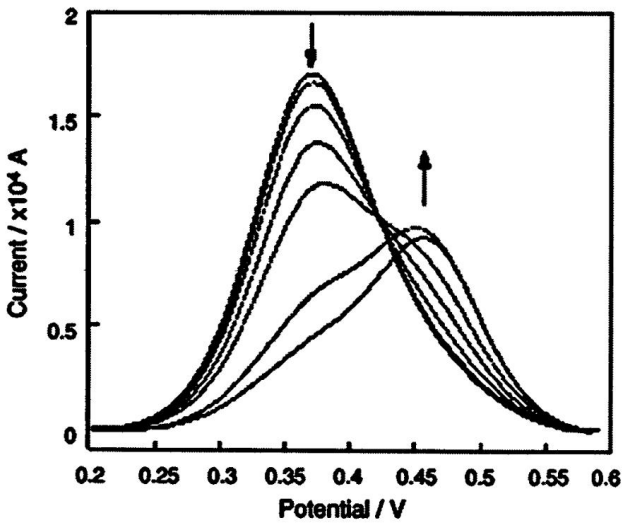


Figure 4. The voltammetric nonenzymatic glucose sensor based on boric acid derivatives [17].

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4.2. Metal-Free Nanomaterials

The surface effect of nanometer materials and small size effect make it has a large specific surface area, chemical properties and lively, the surface atoms of nanomaterials, especially at the edges and has high chemical activity, the atoms of the Angle of the atoms is the active site, nanometer materials have much due to the characteristics of active surface centers and high reactivity, Can catalytic glucose and another small molecule organic matter, which is beneficial to improve the sensitivity of detection, the performance was applied to chemical sensor research, improve the electrical signal response of the sensor sensitivity, in addition, nanometer materials modified electrodes or functional nanomaterials can increase the selectivity of the electrode, on the electrode surface because glucose catalytic oxidation reaction occurs, The involvement of its nanomaterials can make the working potential lower than the potential that interferes with the oxidation of the substance. Nanomaterials are mainly used in electrochemical sensors in four aspects: (1) accelerating electron transport, (2) increasing the electrochemical reaction rate, (3) immobilization of biomolecules, and (4) labeling biomolecules. Many literatures have documented the achievements and challenges of nanomaterials applied to enzyme-free glucose sensors. It has been reported that nanomaterials can improve the performance of detection systems, resulting in faster response time, improved stability and selectivity of sensors. Some researchers used Pd nanoparticles and graphene-treated carbon nanotubes to synthesize Pd-G multi-walled carbon nanotubes, and some scholars modified carbon nanotubes with Ni nanoarrays to prepare CNT-Ni nanostructured electrodes. These carbon nanotubes constructed sensors could realize the measurement of glucose concentration. TiO2 nanotubes, which have the advantages of being highly ordered and controllable in size, have also been confirmed to be useful for enzyme-free glucose sensing [20].

Table 1. The comparison of detection results of graphene and carbon nanotube materials in nonenzymatic glucose sensors [21].

Electrode matrix	Sensitivity (μA mM-1 cm-2)	LOD (μM)	Linear range	Applied Potential (V)
Cu NPs/N-rGO	48.13	1.3	0.004-4.5 mM	0.5
Pt-CuO NPs/rGO	3577	0.01	0-12 mM	0.35
CuO/rGO	1065	-	1 μM-8 mM	0.6
Ni NPs/rGO	865	1.85	5 μM-550 μM	0.5
Pt nanoflowers/rGO	1.26	2	2 μM-20.3 mM	0.47
NiO-Pt NPs/rGO	668.2	0.2	0.05-5.66 mM	0.6
Pt/MWCNTs	11.83	50	1 mM-23 mM	0.45
CuO/SWCNTs	1610	50	0.05-1800 μM	0.5

4.3. Transition Metals

Transition metals with their REDOX properties are often used as electrode materials for enzyme-free glucose sensors due to empty D-orbitals that can act as electron donors or acceptors. Transition metals mainly include copper, nickel, manganese, cobalt and their metal oxides, which have low cost and good electrical conductivity and have strong electrocatalytic oxidation performance of glucose in alkaline conditions [22]. Enzyme-free glucose sensing based on Ni-modified electrodes. The device's operating principle is as follows: under alkaline conditions, $Ni(OH)_2$ loses electron NiOOH and uses NiOOH catalytic oxidation to realize enzyme-free glucose sensing detection.

Manganese-based materials are considered one of the most attractive transition metals due to their low toxicity, abundant raw materials and low price, and are widely used in electrochemistry, soft

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magnetism and catalysis. However, due to the poor conductivity of the material, limited charge seepage behavior occurs, and the electrochemical process occurs at the interface between the electrode and the electrolyte, which leads to its low sensitivity to glucose and poor selectivity when used as the electrode material of the glucose sensor. There are few studies on using it as an electrode modification material alone. Therefore, composite materials with large surface area and high conductivity are needed to improve ion mobility. Among transition metals, CO-based nanomaterials have low cost, good electronic, optical and electrocatalytic properties, and low bandgap characteristics, making them widely used in supercapacitors, catalysts, electrochemical sensors, and lithium-ion batteries.

4.4. Foam Metals

3D porous metallic **foams** have a good specific surface area and porous structure, increasing the active area and improving the catalytic performance. Porous copper and nickel foam have been widely used as working electrode materials in preparing working electrodes for glucose sensors [23].

5. Application

For glucose monitoring, invasive pinprick is the most commonly used. Although blood is a very suitable body fluid for monitoring the level of various types of chemicals, this invasive and painful method is expected to be replaced by other noninvasive detection [24]. Plus, patients with diabetes need to keep track of their glucose levels consistently. At this stage, the frequent sample from the fingertips can cause potential problems such as wound infection [25]. Therefore, more research is carried out on noninvasive and continuous glucose monitoring devices. The development in glucose detecting techniques also have the trend to overcome drawbacks owing to the intrinsic properties of enzymes, so in this section, several examples of noninvasive glucose monitoring techniques using nonenzymatic glucose biosensor are introduced.

5.1. A Wearable Sensor Detecting Perspiration Glucose

This technique uses an integrated wristband to detect glucose in perspiration, and the result of detection is uploaded to an application on a smartphone using Bluetooth (Figure 3). The working electrode in the glucose biosensor used is made of gold. An alkaline environment is prepared for the high electrocatalytic activity of Au in oxidizing glucose and is achieved by setting a high negative potential (-2.0 V) to trigger the reduction of protons. Meanwhile, the chronopotentiometry technique is applied to measure the pH value. After the preparation, the potential is altered to 0.2 V for glucose oxidation. A positive potential is applied to clean the electrode due to the possible effects on the electrode caused by the products of glucose oxidation. Considering the interference from other chemicals in perspiration, the Au electrode is covered by a layer of Nafion and Kel-F. The Nafion selectively prevents anions from attaching to the surface of the electrode, whereas the Kel-F repels substances carrying charges [26].

5.2. Improved Wearable Sensor for Perspiration Glucose Detection

One year after the article introducing the noninvasive, painless glucose detecting device, researchers from the School of Biological Science and Medical Engineering in Southeast University, China, proposed an improved model (Figure 4). Despite some similarities with the former version of the sensor, this biosensor with water splitting-assisted electrocatalytic property and metal-organic framework has made considerable progress and managed to overcome some defects. The introduced metal-organic framework has several advantages: it can serve as an electrocatalyst for the water-splitting reaction by lowering the activation energy. It can also decide the reactions involved to prevent the production of unwanted substances which might cause interference. Unlike the previous version with an Au working electrode, this newly proposed sensor uses a "Pd nanoparticle encapsulated in a Co-based zeolite imidazolate framework" as the working electrode. The alteration is made to tackle a problem due to the Au electrode. For the high electrocatalytic ability of Au, an alkaline environment is required. However, adding a reagent to provide an alkaline condition disables

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the sensor from being wearable. Plus, the evolution of hydrogen bubbles from the reduction of protons influences detection sensitivity.

A multipotential process is used on the working electrode. First, a high negative potential is applied for an alkaline condition for water splitting. Water molecules are split into hydroxide ions and hydrogen molecules, which decompose into hydrogen atoms by Pd nanoparticles on the electrode. Secondly, the Co couple catalyzes glucose oxidation. In the final stage, the same electrode cleaning process occurs [27].

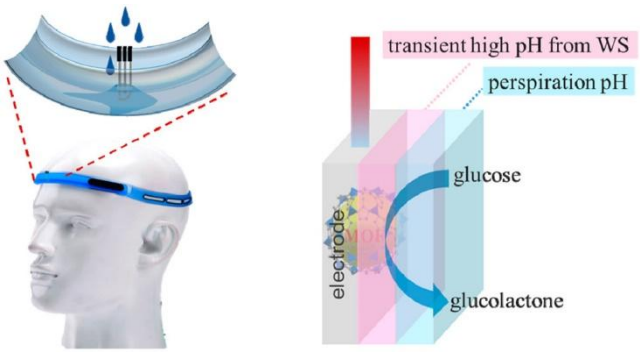


Figure 5. Improved wearable glucose sensor [17].

6. Conclusion

Nanomaterials' special microstructure, which shows some is better than that of traditional materials, scholars of precious metals, transition metal materials, carbon nanotubes, bubbles of different types of materials such as metal materials, building a new type of enzyme glucose sensor, application in blood samples with the larger breakthrough. However, there are also some problems in the current research on enzyme-free glucose sensors.

First of all, the preparation process for blood glucose sensors is complicated. Although noble metal nanomaterials Pt and Au electrodes have good metal activity, they are expensive, difficult to obtain, and expensive to prepare. During the catalytic oxidation of glucose, intermediates are easily attached to the electrode, leading to electrode poisoning. Metal catalysts are generally used in the preparation of carbon nanotubes, and some impurities will remain in the product, which needs to be treated with concentrated acid, and the process is relatively complicated. Excessive metal nanomaterials are inexpensive, and the toxicity potential of the electrode is relatively low, but the electrode preparation needs to go through multiple steps. The preparation process is tedious. However, considering the hydrogen production rate, the three-dimensional porous metal foam modified electrode makes the electrode pores uniform, and the actual preparation is difficult.

Secondly, the performance of all aspects of the glucose sensor cannot be effectively guaranteed, and the anti-interference and selection are relatively poor. Accuracy, stability, anti-interference, reproducibility, sensitivity and linear range are important indicators to measure the performance of blood glucose sensors, and sensors constructed with different materials have their advantages and disadvantages.

Thirdly, the toxicity of the sensor still exists, which limits the long-term use of the sensor. In the serum, known as the human body, is extremely strong adsorption ability; no enzyme in the process of actual test blood sugar glucose sensor, serotonin and other organic matter will increase adsorption gradually on the surface of the working electrode that sensor, the sensor poisoning occur, response signal will occur obvious attenuation to disappear entirely, affect the service life of the electrode activity and limit the long-term application of sensor in the clinic. In order to achieve the popularization and application of enzyme-free blood glucose sensors, the following aspects should be done: first, continue to develop appropriate electrode materials, prepare working electrodes with high stability, high sensitivity and wide detection range, and improve the performance of the sensor in all

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aspects; The second is to explore the catalytic oxidation mechanism of glucose further, analyze the oxidation potential of glucose and interfering substances, improve the selectivity and anti-interference of the sensor, and reduce the toxicity. This will be a future struggle for biosensor researchers.

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