Glucose Biosensor: Basic Principles, Evolution and Practical Application

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Abstract. Glucose biosensors can detect the abnormal level of blood glucose levels, which plays an essential role for the initial phase of treatment in clinical practice. The association between fast plasma glucose (FPG) levels and presence of retinopathy to identify the threshold of glucose level with specific value which help to confirm whether or not getting the disease. Glucose biosensors measure the concentration of blood glucose by using the linear relationships between different molecules' consumption or formation. Glucose biosensors can still be altered by reducing used potentials, increasing durability and enhancing reproducibility. Some practical applications of glucose biosensors in day-to-day life include healthcare, pharmacy, food, agriculture and so on. In food products, the concentration of glucose can be an indicator of its quality and help control the amount of sugar consumed. In other areas, the measurement of changes in glucose levels when interacting with drugs will lead to different strategies used for treatments. This article contains the basic principles of glucose biosensor, some benefits and drawbacks of four generations and related applications.

Keywords: Biosensor; glucose biosensor; glucose detection.

1. Introduction

Diabetes mellitus (DM), which affects the body's normal function due to insufficient insulin secretion, ineffective insulin action, or both, can be brought on by hyperglycemia. This is because there are fewer cells in groups that respond to insulin as it goes by. The World Health Organization (WHO) estimates that 422 million people worldwide (or about 5% of the population) have diabetes. Diabetes is dangerous and is the seventh leading cause of mortality. Type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), gestational diabetes, and diabetes insipidus are common types of diabetes mellitus. Determining the specific class of DM may not be easy because it depends on certain conditions present in the diagnosis for patients.

Since the first generation's proposal, glucose biosensors have significantly advanced. Using biosensors has advantages such as high responsiveness, sensitivity, and stability. The glucose oxidase enzyme, which serves as a catalyst for the oxidation of glucose and the reduction of oxygen to produce hydrogen peroxide, is crucial to the operation of glucose biosensors. There is a linear relationship between increased hydrogen peroxide concentration and reduced oxygen concentration. The electrode detects the electron flow, which is directly proportional to the amount of glucose present, as a result of the redox reaction that occurs.

The purpose of this article is to introduce the concept of glucose biosensor, the mechanism based on the glucose oxidase to catalyze the reaction which involves the redox reaction, brief history of biosensors' developments and the practical applications of sensors in different fields.

2. Basic Principles of Glucose Biosensor

Biosensors can be defined as devices that integrate a sensitive biological recognition element in their analysts for the detection of specific chemicals or molecules. The biosensor is composed of several parts with various functions which can then meet to detect analysts. There are 3 main parts of biosensor, the first part is biologically recognizable elements that are critical to distinguish between various targeted molecules. The second part is a transducer which turns the signals of the recognition

elements into measurable signals and the third part is a processing device for translating measured signals into readable data that provides quantitative results to users. Figure 1 below is a clear illustration of three components.

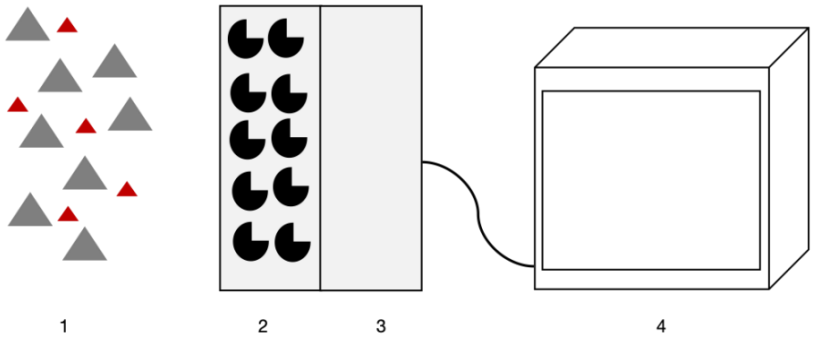

Fig. 1 Three main parts of biosensor: (1) targeted analytes represented by grey molecules while red ones represent the other unrelated molecules in the sample; (2) biological recognition elements with complementary active site to the grey molecules allows them to detect certain chemicals only which shows the specificity; (3) transducer which converts the signals; (4) displaying device which shows the numerical results.

The biological recognition elements are usually the enzyme which binds with complementary substrates to catalyze the reaction. Glucose biosensors typically use several enzymes, including hexokinase, glucose oxidase (GOx) and glucose-1-dehydrogenase (GDH). In each biosensor, only one enzyme is required to perform the function of measuring glucose. Of these enzymes, GOx is the most commonly used enzyme for the glucose biosensor. A coenzyme (a non-protein compound that is necessary to help to carry out the function of enzymes) flavin adenine dinucleotide (FAD) is needed as an electron acceptor to FADH, and then donates the electrons to form hydrogen peroxide. GOx catalyses the oxidation of glucose to produce glucose lactone and hydrogen peroxide [1]:

$$\text{glucose} + \text{oxygen} \rightarrow \text{glucose lactone} + \text{hydrogen peroxide} \quad (1)$$

Followed by this reaction, the concentration of hydrogen peroxide increases and decreases in oxygen concentration. The hydrogen peroxide is then oxidized at platinum (Pt) anode where redox reaction happened [1]. These changes are both proportionate to the level of glucose and this proportionality provides quantitative results.

3. First-Generation of Glucose Biosensors

By using electrochemical measurements of oxygen consumption or hydrogen peroxide generation, Clark and Lyons suggested the notion of using glucose biosensors to assess blood glucose levels in 1962 [2]. This is the first-generation of Glucose Biosensors. Before the biosensor construct is proposed, immobilized enzymes are discovered to develop many applications in biosensors or other mobilization techniques. The first generation of enzymatic glucose biosensors rely on enzyme to identify oxygen consumption or hydrogen peroxide production during redox reactions that take place when FAD serves as a cofactor and GOx is present [3]. Future glucose sensors will be built on the fundamental principles of the first generation while being improved. Eq.1 can be used to describe the first-generation glucose biosensor's mechanism.

Nonetheless, the first generation still has a number of drawbacks. This biosensor highly depends on oxygen extracts from nature which requires reduction of oxygen or oxidation of hydrogen peroxide. As a result, the glucose biosensor requires a high potential for measuring oxidized hydrogen peroxide that could be hazardous. Furthermore, the high reliance on oxygen supplies makes it notable for the accuracy of the glucose biosensor. Oxygen extraction from air should be modified by a two-dimensional cylinder electrode to increase the oxygen/glucose permeability ratio developed by Gough

et al. [4]. A number of other methods are also available to reduce fluctuations in the supply of oxygen to the air in order to reduce the oxygen deficit.

4. Second-Generation of Glucose Biosensors

Although first-generation glucose biosensors work to measure the concentration of glucose in the blood, this is still not an effective system because the mediator is oxygen. In second-generation of glucose biosensor, further improvements have taken to address the oxygen limitation as a mediator to redox active molecules which enable a reversible, rapid redox reaction to take place which is more efficient than using oxygen [5]. The reaction is also catalyzed by GOx and cofactor FAD. The function of this biosensor can be explained below:

$$\text{Glucose} + \text{FAD} \rightarrow \text{gluconic acid} + \text{FADH}_2 \quad (2)$$

$$\text{FADH}_2 + 2\text{Mediator(ox)} + 2\text{electron} \rightarrow \text{FAD} + 2\text{Mediator(re)} + 2\text{hydrogen ion} \quad (3)$$

In the above reactions, the mediator changes its oxidized state to a reduced state by receiving the electrons given by FADH, then the mediator reduces loses two electrons to the oxidized state. All the redox reaction is happening on the electrode surface.

Second-generation is more frequently used than the first-generation not only because of its high sensitivity and reliability, but also low potential it requires to run the whole process which ensures the home-use biosensors' safety. Contrary to the first generation in which the mediator is oxygen extracted from nature, the second generation uses the redox active molecules, therefore less potential necessary for a high selectivity of the biosensor.

5. Third-Generation of Glucose Biosensors

Third-generation glucose biosensors utilize direct transfer of energy (DET) between enzyme and electrode to solve issues in the first and second generations of glucose biosensors by removing the presence of mediator [1]. The electrode can carry electrons directly by means of organically conductive materials. The underlying mechanism can be described as below:

$$\text{Glucose} + \text{FAD} \rightarrow \text{gluconic acid} + \text{FADH}_2 \quad (4)$$

$$\text{FADH}_2 \rightarrow \text{FAD} + 2\text{hydrogen ion} + 2\text{electron} \quad (5)$$

Without the mediator, the potential is significantly reduced. The cost and incubation time of enzymes become lower than in past and the enzyme's sensitivity to glucose increases. As a result, this change has made the use of the third generation more effective and has improved quality.

However, elimination of the role of mediator leads to high dependence on glucose oxidase quality which can be affected by external environment such as the extreme increase or decrease of pH and high temperature, which adjust the binding site of biological catalyst thus reducing the specificity of enzyme to substrate.

6. Fourth generation of glucose biosensors

In the fourth generation, this is a free enzymatic mechanism that is unaffected by the nature of proteins. Hence, the working mechanism would be the direct detection of glucose which does not need another mediator or other molecules present as a product to show a linear relationship. This sensor can evaluate 10M or higher glucose concentration which is above the normal blood glucose level, and can detect abnormal level of glucose to help to prevent any further negative development of disease [6].

The fourth generation detection mechanism is based on the change in copper oxide status during the oxidation reaction. In the process, Cu^{2+} in the copper oxide is oxidized into Cu^{3+} which is present in the compound of CuOOH [7]. Then glucose lactone is produced, followed by Cu^{3+} reduced to Cu^{2+}

to form CuO [7]. Due to the presence of the redox reaction, there are electrons transferring close to the electrode surface, and the electron flux causes the generated current [7]. Biosensors then receive it and measure the amount of glucose present in the blood sample. Simple and affordable chemistry can be used to develop a non-enzymatic glucose sensor.

The overall mechanism of four generations of glucose biosensors can be described in Figure 2 below:

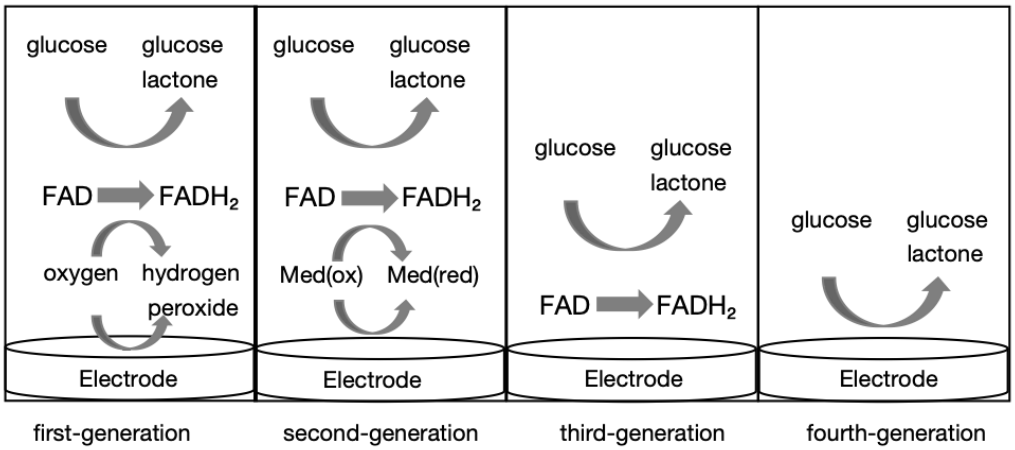

Fig. 2 All of the reactions happen near the electrode surface. In the reaction, glucose converts into glucose lactone. While the first-generation is indirect glucose sensing and the second to fourth generation perform direct glucose sensing.

7. Related Practical Applications

7.1. Health Care

Diabetes mellitus (DM) is diagnosed based on the blood glucose level, which is also the most typical use for glucose biosensors. Blood glucose is used as a reference to quantify any aberrant levels of molecules. Determine the glucose threshold based on the correlation between FPG levels and the presence of retinopathy [8]. The early stages of treatment can be helped by using this strategy to regulate blood glucose. As a result, the following DM diagnostic criteria can be measured:

- Long standing diagnostic 2-h PG value of ≥ 200mg/dl (11.1mmol/l)
- Fasting plasma glucose of ≥ 126mg/dl (7.0 mmol/l)
- 2-h values in the oral glucose tolerance test (OGTT 2-h value) in venous plasma ≥ 200mg/dl (11.1mmol/l)[9]

In the real case scenario, the results of the test should be repeated in order to get confirmation due to lack of laboratory environment which leads to lots of factors can affect the final result. The reproducibility of the test will confirm the patients have DM. This is also the reason why some patients who have blood glucose level near the threshold doing the test repetitively for confirmation. This removes the error caused by devices that are outside of environmental conditions.

7.2. Pharmacy

Drug treatments can also be a way to affect blood glucose, which leads to hyperglycemia or hypoglycemia. The use of glucose biosensors can help determine how drugs are used to treat severely sick patients affect blood glucose measurements. In the experiment, the detected concentration values of blood glucose without the presence of drugs are used as a control sample to compare the concentrations of blood glucose with drugs as the test sample [8]. With medication interference that might hide the fact that abnormal levels of blood glucose are detected in patients' bodies [8]. As a result, it can be the key to appropriate treatments, or otherwise inappropriate doses of drugs will lead

to the appearance of other diseases such as inappropriate insulin therapy. Measuring accurate data about changes in blood sugar levels becomes crucial in the initial stage of treatment.

7.3. Food Industry

Glucose levels are also critical for food in glass jars, metal cans and beverages. For instance, honey contains a large amount of sugar, such as glucose, fructose and sucrose, which can turn into an energy source in the body. Foods with interior plastic wrap, such as fruit, pudding and metal cans, are used as experimental samples [10]. Bisphenol A (BPA) increases in proportion to increases in expiry date, glucose concentration and sodium chloride [10].

Controlling the amount of glucose intake may prevent suffering from illness. Too much glucose intake from foods in the diet will raise the blood glucose content causing hyperglycemia. In addition, excess sugar attaches to proteins or fat in bloodstream creates advanced glycation end products (AGEs) which are harmful molecules in the body. AGEs can also form in foods which have been exposed to high temperature. The compound causes damage to the collagen in the skin.

7.4. Agriculture

Maintaining the quality of food from farms, industries and fields is a critical way to achieve the goal of universal nutrition. Fruits and vegetables are rich in vitamin A, vitamin C, fiber and potassium. As a result, fruits and vegetables are also the ingredient contained in daily diet, but they do not have long-shelf life which are highly perishable. The harvest of these fruits must be selected before the right ripening period in order to move them from one place to another. Though the proper period of harvesting should still depend on different situations as non-climacteric, the fruits will not continue to grow after picking, while climacteric fruits can continue to become ripen during the transportation process. The concentration of glucose, organic acid, and other components in the fruits fluctuate as the fruit ripens, changing the balance. According to research on the creation of a needle type biosensor used to measure glucose concentration, the sensor's response time to 100 mg/L of glucose is roughly 40 seconds [11]. When the temperature rises from 27 to 55 degrees Celsius, the sensor's response also rises, and this linear variation lasts up to 200 mg/L of glucose [11]. Using glucose biosensors, it is possible to identify the fruit's glucose concentration and the harvesting stage.

8. Conclusion

In conclusion, comparing the four generations of glucose biosensors, it becomes more efficient to use which obtained the results with high reliability and low potentials to use which ensure its safety. However, there are still several drawbacks need to be overcome such as the invasive methods to obtain the sample and passive relation between healthcare system and patients to get proper treatments. Apart from usage of sensors, the enzymatic base of sensing methods is limited by the nature of GOx which cannot suffer from extreme conditions and show low stability. Therefore, the fourth generation of glucose biosensor is a start to change dependence on enzyme. The evolution of glucose biosensors is still continuing to improve the quality of sensors and increase its ability to detect the higher concentration of glucose.

In the future, biosensors have great potential to become commercially successful which would be portable without the invasive process to detect the level of glucose for patients. Reducing the complex procedures required for carrying out the test is also essential to improve.

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