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Biosensor: An Emerging Analytical Tool

Vineetha Mohan* and Anand HV

Department of Biotechnology, Sapthagiri College of Engineering, Karnataka, India

Abstract

With increase in potential for bioterrorism, there is a great demand to detect the bio agents in the atmosphere in a quick, reliable and accurate method. Biosensor is an analytical device which uses enzymes, immunosystems, tissues that converts biological response into electrical, thermal or optical signals. Biosensor is an efficient and cost effective device which is most widely used for various day to day applications. Biosensor consists of two components: first the "sensing element" and second is the "transducers". Sensing element may be either enzymes, antibodies, DNA, tissues or whole cells which transduces the biochemical reaction into electrical signals. Basic advantage of biosensor is the use of nanomaterials, micro fluidics and transducer on a single chip. Biosensors have found its application in fermentation, food industry, diagnosis, imaging, DNA sequencing and biodefense. Development of nanotechnology leads to the development of macro and micro sensors which is small and sensitive.

Keywords: Biosensors; DNA biosensor; Glucose biosensor; Enzyme based biosensor

Introduction

Bio analysis is been performed by humans from time immemorial. Bio analysis is time consuming process when done manually, and then humans created devices which could reduce the human power for bio analysis. Biosensor was pioneered by Clark and Lyons in 1960 [1]. Enzyme based biosensor was reported by Updike and Hicks in 1967. Enzymes are immobilized on the sensing element which reacts with the analyte molecule; this biochemical energy is then transduced to electrical signals. Widely used enzymes are oxidoreductase, polyphenol oxidase, peroxidises and amino oxidases [2-4]. Microbe based biosensor was reported by Divies [5].

Biosensor is composed of biological element and sensing element. The biological element is highly sensitive to a particular type of analyte molecule. The biological element can be enzymes, antibody, tissues or living cells. Biological elements are immobilized onto the sensing elements by the membrane entrapment, physical adsorption, covalent bonding and matrix entrapment. Physical adsorption is a technique where the biological element is adsorbed onto the sensing element by Van der waals forces, hydrophobic forces and hydrogen bonding. In membrane entrapment method, the biological element is entrapped within the semi permeable matrix, and the analyte passes through this membrane and interacts with the bio elements [6-8].

Biosensors have become more popular with biochemistry and analytical chemistry. Biosensors are used to detect pollutants, microbial load, control parameters and metabolites. Leland C Clark is the father of biosensor who invented the glucose biosensor to determine the glucose level in the sample. Clark entrapped glucose oxidase in a dialysis membrane and placed within an oxygen electrode [6-8].

DNA sensor has been included in the family of biosensors which can be used for disease diagnosis. Biosensors are fabricated using nanotechnology, these devices helps use to analyze in a quick and accurately [9]. Nanobiosensors for DNA sequencing if cost effective, efficient, speed and ease of use are the key advantages. Biosensor is a powerful tool which adopts rapid technologies in field of clinical, diagnosis, food safety and environmental monitoring [9-11].

Types of biosensors

Resonant biosensor: The biosensor works based on the principle of change in the resonant frequency which is proportional to biochemical reaction. When the sample attaches specifically to the antigen immobilized on the sensing membrane. Due to the specific interaction of antigen and antibody, the mass of the membrane increases. This sensing element is coupled to an acoustic sound transducer, so due to change in mass there is a change in the sound frequency. This change is frequency is measured; this is then related to the concentration of analyte [11].

Optical detection biosensors: Biosensor is fabricated using photolithography. Initially the silicon wafer is prepared, on to which the antibody is added, and the photo mask is applied to active regions. Then the silicon wafer integrated with antibody is exposed to UV light, photo masked areas are active while unprotected areas become inactive. When the analyte molecules are loaded onto the sensor, the fluorescent tagged antigen specific to the immobilized antibody interacts. This antigen antibody complex is illuminated with the laser, the Ag-Ab complex absorbs light, so there is a change in voltage, and this transduced by the transducer and concentration of analyte is determined [11].

Thermal biosensors: Biosensor is equipped with sensing element, enzyme thermistor and transducer. This biosensor consists of the membrane immobilized with enzyme. So, when the analyte interacts with the enzymes, heat is liberated along with the product formation. So, the heat liberated is measured by the enzyme thermistor. This thermistor is coupled to transducer which transduces the temperature into set parameter. Enzyme thermistor is most widely used because of the advantage it is sensitive and accurate. Frequent calibration of temperature transducer is not required. The application of thermal biosensor is detection of pesticides [11].

Electrochemical sensor: This class of biosensor there is production or consumption of ions or electrons so there is a change in electrical parameters. This electrical parameter can be either of three types'

***Corresponding author:** Vineetha Mohan, Assistant Professor, Department of Biotechnology, Sapthagiri College of Engineering, Karnataka, India, Tel: +7204367799; E-mail: vinithareyansh@gmail.com

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conductimetric, potentiometric and amperometric. This electrical parameter is determined and transduced into the required parameter [11].

Glucose biosensor: Glucose biosensor was first developed by Leland C Clark. Glucose biosensor consists of the enzyme glucose oxidase (GOD) immobilized within dialysis membrane which is integrated inside a oxygen electrodes. GOD reacts with glucose present in the blood and produces gluconic acid, two electrons and two protons. After production of gluconic acid GOD is reduced. Then this GOD reduced reacts with oxygen, electron and proton to produce hydrogen peroxide and GOD oxidised. This GOD reacts with further glucose. Consumption of oxygen or production of hydrogen peroxide, higher the glucose content higher the oxygen demand while lower glucose level results in higher levels of hydrogen peroxide. Glucose biosensor is for detection of diabetes from the blood sample [12].

DNA Biosensor: DNA biosensors are used for detecting DNA and known as bio detectors. DNA sensors are used to isolate and detect the strength of single DNA-DNA interaction. DNA sequencing is very important for drug discovery and diagnosis of genetic disorders [13]. Due to mutations at single nucleotide (SNP) there is mismatch of sequence which leads to particular disease this can be detected using DNA sensor. Electrochemical sensors were developed for detection of bio molecules for clinical diagnostics [14]. Electrochemical sensors require small amount of sample, highly specific and sensitive and relatively fast that is works within few seconds to minutes. Due to these key advantages of electrochemical biosensor, it is been widely used for DNA diagnostics.

Nanofabrication helps in development of miniaturized sensors, which increases the sensitivity, efficiency and reduces the sample volume for analysis. Goal of development of DNA electrochemical sensor is it could be used for various application for disease diagnosis, pre-treatment, drug delivery and detection. DNA biosensor is equipped with a electrode, capture probe, reporter probe and DNA target. Capture probe hybridizes with the target DNA and usually it is integrated on the surface of electrode. Reporter probe generates electrochemical signals based on the biochemical reaction. Capture probe and reported probe bind with high specificity to target DNA. Probes are usually immobilized with ssDNA, peptides and proteins. Due to miniaturized sensors it has high surface to volume ratio which increases the strength of signal and hybridization of DNA based on sequence recognition by the probes [15].

Electrochemical DNA biosensors employ hybridization based detection utilizes probe and target nucleotides. When the target DNA or nucleotide is complementary then there is hybridization of target with the probe, this leads to change in voltage this is transduced into biological parameter. Second detection is enzyme based DNA detection. DNA enzymes are immobilized on the sensors which catalyses the conversion of substrate into product. Key advantage of enzyme biosensor is its high sensitivity and specificity [15].

Advantages of Biosensors

DNA biosensors are most widely used due to key advantages; they are highly specific, efficient, accurate and fast detection techniques. EC biosensors utilize low power. DNA biosensors depend on the hybridization of nucleotide during detection of the analyte; hybridization depends upon the electrostatic charge and strong hydrogen bonding. DNA is composed of sugar, phosphate backbone and sugar. Due to the negative charge of phosphate group hybridization of nucleotides faces a strong repulsive force. In order to hybridize the DNA with target DNA they overcome the repulsive forces.

DNA biosensor is used due to their simplicity in manipulation of sample molecules, which is carried has electric current generated by the electrodes. Through precise manipulation of sample leads to high efficient detection target DNA. EC sensor can generate high intensity electric field at a short time interval.

Detection speed

There are several stages in DNA detection which includes DNA recognition, sample manipulation and signal reading. Application of DNA sensor can detect the DNA within several seconds to few minutes. Series of process occurring at the time of detection are sample preparation, sample uptake and signal reading can be accomplished within few minutes. Rapid detection is important for several disease diagnoses.

Sensitivity

Biosensors require small amount of sample in terms of microliters.

Specificity

Biosensors are highly specific. They are designed in way that they can recognise the DNA and leads to single base matching with probe nucleotide sequence.

Convenience

Development of nanotechnology helps in engineering biosensors on a single platform accurately. Thus biosensors can be used for on-site analysis of several samples.

Industrial Application of Sensor

Glucose biosensors

Enzyme biosensor is a emerging analytical tool used for various applications for the detection of glucose concentration by utilizing the enzyme glucose oxidase. Qualitative and quantitative analysis of sugars levels such as fructose, sucrose, glucose, galactose and starch. Estimation of sugar levels is important to determine the shelf life, storage methods, quality, nutrition, safety and efficacy of the products. Glucose levels are estimated usually in food industry because higher levels of glucose in food lead to browning phenomena due to dehydration of foods during storage. Determination of blood glucose level is a important to know the level of glucose. Higher the levels of glucose in blood lead to diabetes.

Diabetes Mellitus is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action or both. Diabetes is a condition characterized by the body's inability to regulate glucose levels in blood. Diabetes is due to either the pancreas unable producing enough insulin or the cells of the body not able to respond properly to the insulin produced. There are two types of diabetes. Type 1- Insulin dependent diabetes mellitus (IDDM) and Type 2 which is non-insulin depended diabetes mellitus (NIDDM) [16].

Enzyme based biosensors are most widely used for glucose determination in food industry and to determine the blood glucose level. Modern techniques uses two approaches first is by using biosensors and second non enzymatic technique such as HPLC. Glucose exists in two conformations one is α-Glucose and other β-Glucose. When equilibrating the glucose solution at 25°C and pH 7, 63% of glucose adopts the β–Glucopyranose structure and 37% exists in α- Glucopyranose structure [17]. Glucose oxidase is a enzyme of class oxidoreductase which catalyses the conversion of substrate β-Glucosse into product β-D glucolactone in PPP [17].

Conclusion

Biosensors composed of genetically engineered proteins infused into cells ex vivo or in vivo. This facilitate researcher to sense levels of hormones, toxins, drugs, continuously and noninvasively, using physical principles. The scope of biosensor is in regards with the value in ageing research.

Basic advantage of biosensor is the use of nanomaterials, micro fluidics and transducer on a single chip. Biosensors have found its application in fermentation, food industry, diagnosis, imaging, DNA sequencing and biodefense. Development of nanotechnology leads to the development of macro and micro sensors which is small and sensitive.

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