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Biosensors: A novel innovation in medical science

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Abstract

The biosensor is an analytical device used to find biological analytes both in quantity and presence. After detection, it uses a transducer to transform the biological response into an electrical signal. Now a days, biosensors are used in a variety of fields, including the medical industry, the pharmaceutical industry (Drug design and development), the food industry, environmental monitoring, agriculture and the military. Glucometers utilizing the strategy of electrochemical detection of biosensors. Various biosensors ranging from nanomaterials, polymers to microbes have wider potential applications. It is quite important to integrate multifaceted approaches to design biosensors that have the potential for diverse usage. In light of this, this information provides an overview of different types of biosensors being used ranging from electrochemical, fluorescence tagged, nanomaterials, silica or quartz and microbes for various biomedical and environmental applications with future outlook of biosensor technology. Nanomaterials have an important part in efficiently sensing bioreceptors such as cells, enzymes and antibodies to develop biosensors with high selectivity, peculiarity and sensibility. It is virtually impossible in science and technology to perform any application without nanomaterials. Along with their use in other daily applications, biosensors can essentially be low-cost, extremely effective instruments.

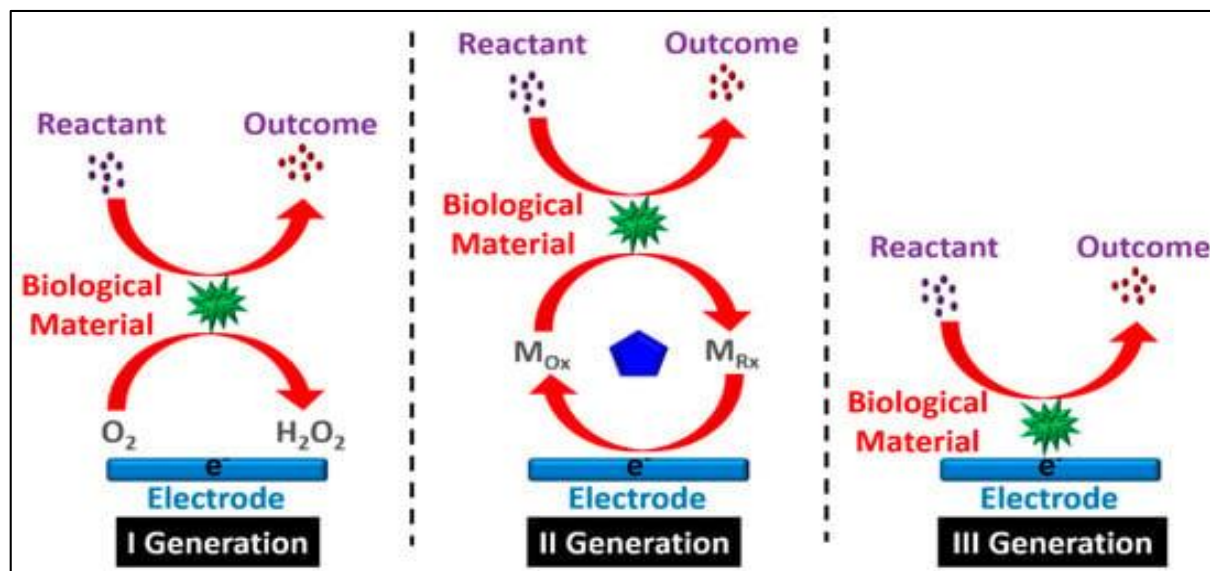
Keywords: Biosensors, microbial biosensor, transducer, pathogen detection, nanomaterials, fluorescence-tag, bioelectronics, polymer

Introduction

A sensor is a device that measures a physical parameter and converts it into a signal that can be interpreted by either a human observer or a device. A biosensor is a specialized analytical tool that translates a biological response into an electrical signal. It identifies, records, and transmits information related to changes or processes occurring in the physiological state. A biosensor has two main parts: the physical component, which includes the transducer and amplifier, and the biological component, which consists of a bioelement sensitive to the analyte. The development of biosensors began in 1962 when Leland C. Clark, known as the "father of biosensors," invented the Clark Oxygen Electrode. This groundbreaking device enabled real-time monitoring of a patient's blood oxygen levels, making surgeries safer and more successful for millions worldwide (Darsanaki *et al.*, 2013) ^[11]. A biosensor integrates biological materials such as enzymes, antibodies, nucleic acids, or whole cells that are immobilized to detect, monitor, and communicate information about physical changes. It also measures the presence and concentration of specific substances in test samples. In today's technologically advanced world, biosensors are widely applied across various industries such as biotechnology, electronics (e.g., smartwatches), physics, mechanical engineering, environmental monitoring, and medicine. For example, glucometers help diabetic patients track blood sugar levels, and pregnancy tests detect the presence of the HCG protein in urine (Sawant *et al.*, 2022) ^[38].

Evolution of Biosensors

The concept of biosensors was first introduced by Leland Charles Clark Jr. in 1962, who proposed the integration of a bioreceptor with a transducer device to create a functional biosensor (Naresh *et al.*, 2012) ^[23]. Figure 1 illustrates the three stages in the progression of biosensor development, and Table 1 provides an overview of the evolution timeline.



Applications of biosensor

Biosensor devices consist of two primary components: a biological element and a physicochemical detector, with the primary purpose of detecting specific analytes. As a result, biosensors are extensively used across various sectors, including the pharmaceutical industry, healthcare, industrial applications, food processing, environmental monitoring, agriculture, and military fields. In recent years, these sensors have gained significant popularity due to their compact size, affordable cost, ease of use, rapid results, enhanced stability, and high sensitivity and specificity (Mehrotra, 2016) ^[19].

Pharmaceutical and medical field

Ion selective field effect transistors (ISFETs) are used to develop potentiometric biosensors for a variety of applications, including the detection of chiral amine salts, acetylcholine esterase enzyme inhibitors, pharmaceutical preparations like lidocaine, procaine, and tetracaine, and ionic surfactants like sodium dodecyl sulfate (SDS) and dodecyl trimethyl ammonium bromide. Fluorescent biosensors are employed in drug discovery to identify compounds using high content and high throughput screening methods. Early biomarker identification in molecular and clinical diagnosis is accomplished using fluorescent biosensors. In addition to confirming processes including signal transduction, transcription, cell cycle, and death, they are employed in the detection of gene expression and protein localization. This biosensor is used to detect cancer, viral infections, metastases, inflammatory illnesses, cardiovascular diseases, and neurological diseases.

The uses of biosensors in the medical field are growing quickly. The primary application of biosensors is the quantitative measurement of bodily fluids such as urea, cholesterol, and glucose. Glucometers are electrochemical biosensors that are frequently used in clinical settings to diagnose diabetes mellitus and aid in accurate blood glucose regulation. 85% of the global market is made up of people who use glucose meters at home. Biosensors are used to identify pathogens and diagnose infectious disorders, primarily urinary tract infections. An innovative biosensor is used to detect human interleukin-10 early. Gold-immobilized antibodies are employed as a biosensor to detect the Human Papilloma Virus (HPV). hCG in urine is detected using a pregnancy kit. Recently, biosensors have been utilized to identify the specific SARS-Co-2 virus.

Industrial applications

A common industrial process utilized in dairy, alcohol, and other products is fermentation. Process safety and product quality are critical in fermentation businesses. Biosensors help regulate the fermentation process by monitoring fermentation products, biomass, enzymes, and the estimation of different ions.

Food industries

The food sector uses biosensors to monitor the quality of its products. It examines the amount of carbohydrates, amino acids, alcohol, gasses, and other substances and is used to determine the nutritional value, freshness, and odor of food. Biosensors are used to detect artificial sweeteners, one of the most common food additives. Because artificial sweeteners contribute to the development of unwanted conditions such as type 2 diabetes, obesity, cardiovascular disease, and dental cavities.

Environmental monitoring

In addition to being effective for monitoring pollutants, chemical residues, pesticides, poisons, or microbes in rivers, reservoirs, and sea water, biosensors are also very beneficial for pollution control and environmental monitoring. One kind of electrochemical biosensor utilized for pesticide detection is the amperometric biosensor. Biochemical oxygen demand (BOD) biosensors are used to identify biological molecules that pollute water sources and cause fatal illnesses.

Agriculture industries

The amount of heavy metals, pesticides, and herbicides in soil and ground water is measured using biosensors. To use biosensors for biological soil diagnosis in order to stop soil disease contamination at an early stage. Nitrate biosensors are used to measure the quantity of nitrate present in soil.

Biosensor in cancer research

Biosensor measurement techniques classify detection methods into label-free and label-based categories. In label-free detection, analyte molecules are directly captured by the bio-recognition element without modification, while label-based methods (such as amperometric and fluorescent) rely on attaching labels like fluorophores, nanoparticles, or enzymes to analytes to produce detectable signals. Metal

nanoparticles are frequently used in cancer research due to their strong affinity for cancer cells, but the labeling process can alter the properties of the molecules, potentially causing nonspecific binding or disrupting cell metabolism. Consequently, label-free techniques like quartz crystal microbalance (QCM) and surface plasmon resonance (SPR) are becoming more popular for real-time analysis of molecular interactions in terms of kinetics and thermodynamics. Biosensors have wide-ranging applications in health monitoring, pathology, environmental and food safety, and even criminology. In cancer diagnostics, biosensors can help identify biomarkers for early detection and assess treatment effectiveness, ultimately improving survival rates and patients' quality of life. Cancer encompasses more than 200 types, influenced by both genetic and environmental factors. Advancements in biosensor technologies are vital for diagnosing and monitoring cancers, especially those diagnosed at more advanced stages (Bohunicky *et al.*, 2010) ^[4].

Biosensors and pathogen detection

Microbial diseases are a leading cause of death in developing nations (Syam *et al.*, 2012) ^[43], making pathogen detection crucial for ensuring health and safety. The most widely used techniques for pathogen identification include polymerase chain reaction (PCR), culture and colony counting, and immunology-based methods. These approaches involve analyzing DNA, counting bacteria, and observing antigen-antibody interactions, respectively. Although these methods can be time-consuming or complex, they remain areas where further advancements are possible. Recently, biosensors have been described as analytical devices that incorporate a biological material closely connected with or integrated into a physicochemical transducer or microsystem. These transducers can be optical, electrochemical, thermometric, piezoelectric, magnetic, or micromechanical. The three primary classes of biological recognition elements used in biosensors are enzymes, antibodies, and nucleic acids. In detecting pathogenic bacteria, enzymes are often used as labels rather than actual recognition elements. These enzymes can label antibodies or DNA probes, similar to their use in ELISA assays. In amperometric biosensors, enzyme labels play a vital role. More advanced techniques, such as surface plasmon resonance (SPR), piezoelectric, or impedimetric biosensors, can function without labeling the recognition element. Currently, antibodies are more commonly used in biosensor applications than DNA probes, and much of the focus is on antibody-based biosensors. The figure illustrates the most frequent methods for antibody immobilization. Various types of biosensors are being employed for detecting pathogenic microbes. For example, piezoelectric immunosensors have been developed for detecting *Listeria monocytogenes* and members of the Enterobacteriaceae family (Plomer *et al.*, 1992) ^[29]. In the immunogravimetric microbial assay, a PZ crystal coated with anti-*C. albicans* antibody was used to detect *C. albicans* concentrations ranging from 106 to 108 cells/ml. Another study demonstrated the indirect detection of *Escherichia coli* O157:H7 using a fluorescent-labeled antibody method (Pyle *et al.*, 1995). Amperometric biosensors have been developed for the indirect detection of *E. coli*, as well as for *Salmonella* detection (Nakamura *et al.*, 1991) ^[22]. Light-addressable potentiometric sensor arrays have been

developed for detecting *Neisseria meningitidis* and *Brucella melitensis*. Nucleic acid hybridization-based biosensors are also being designed for pathogens like *E. coli* and *Mycobacterium tuberculosis*. In addition, bioluminescence systems have been employed for detecting a wide range of microorganisms (Syam *et al.*, 2012) ^[43].

Biosensors for COVID-19

The rapid emergence and widespread transmission of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes COVID-19, presented significant challenges for societies worldwide. A critical need has arisen for effective medical diagnosis and smooth clinical management, which rely on innovative diagnostic methods and technologies. Recently, molecular diagnostic tools based on nucleic acid amplification have become the gold standard for diagnosing COVID-19. Despite their effectiveness, there remains a pressing demand for widespread testing solutions that provide fast and efficient results in various epidemiological contexts to combat the pandemic. Biosensors have already demonstrated potential for affordable and accessible diagnostics, especially in situations where traditional laboratory methods may not be feasible. In particular, paper- and cellulose-based biosensors are particularly suitable during a pandemic due to their renewability, ability for mass production using sustainable practices, and environmentally safe disposal. This review highlights and explores paper-based diagnostic devices designed to target SARS-CoV-2, offering a quick and cost-effective way to conduct point-of-care (PoC) diagnosis. These devices are useful for detecting viral genomic material, viral antigens, and conducting serological antibody tests. Additionally, devices designed to monitor inflammatory markers critical to COVID-19 are examined, offering fast and reliable tools for bedside diagnostics to aid in patient treatment and follow-up (Pinheiro *et al.*, 2020) ^[28].

Oestrus detection

In the cattle breeding industry, where artificial insemination is practiced, accurately predicting the onset of estrus can result in significant cost savings for herd management. Visual observation is the most common method for detecting estrus, but it is not always aligned with ovulation and can occur in pregnant animals as well. Monitoring progesterone levels in milk provides a more reliable method not only for predicting ovulation but also for identifying pregnancy and fertility issues. Optimal fertility rates are typically achieved when insemination occurs three days after progesterone levels drop below 5 ng/ml in whole milk. While ELISA test kits can detect estrus with 98% specificity, these methods require time and expertise that are not always available on farms. The creation of a real-time milk progesterone biosensor would offer a highly valuable tool for fertility tracking. Researchers have developed various methods to measure progesterone levels in milk, such as a disposable screen-printed amperometric progesterone biosensor operating in a competitive immunoassay format (Pemberton *et al.*, 1998) ^[27]. This biosensor works by decreasing the binding of alkaline phosphatase-labelled progesterone to the sensor surface in the presence of natural milk progesterone. The enzyme substrate used is naphthyl phosphate, and the 1-naphthol generated is electrochemically oxidized, creating a signal that is inversely related to the concentration of unlabelled

progesterone in the milk. The use of screen-printing technology enables the mass production of transducer elements, allowing for the creation of cost-effective, disposable sensors. This technology has been used to develop an automated progesterone detection system in fresh whole milk, connected to a herd management database (Mottram *et al.*, 2000) [21]. The prototype system can detect progesterone concentrations between 3 and 30 ng/ml in fresh milk, which are typical physiological levels, and it shows a strong regression correlation ($R^2 = 0.965$).

Veterinary drug residue screening

The use of antibiotics and chemotherapeutic agents in livestock farming has resulted in the presence of veterinary drug residues in animal-derived food products. Conventional microbial screening techniques lack the necessary sensitivity to comply with new regulations, while traditional physicochemical methods such as chromatography and mass spectrometry are often impractical due to the required expertise, skills, and high costs. As a result, immunological techniques have gained popularity for monitoring therapeutic substance levels. Sulphonamides, a group of chemotherapeutic drugs, are commonly used to treat and prevent animal diseases. In mastitis treatment, sulphonamides are typically administered when infections are caused by Gram-negative bacteria like *E. coli*. Toxicological studies have shown that sulphonamides can affect thyroid function in both animals and humans. In Europe, the maximum residue limit for total sulphonamides in milk is set at 0.1 mg/kg. A surface plasmon resonance (SPR) biosensor was compared with existing methods like microbial inhibitor assays, microbial receptor assays, ELISA, and HPLC for detecting sulfamethazine (SMZ) residues in milk (Mellgren *et al.*, 1996) [20]. The Pharmacia BIAcore system, a commercial SPR tool, was able to detect SMZ levels as low as 0.9 µg per kg of milk (below HPLC's detection threshold) and provided several advantages, including no sample preparation, high sensitivity, rapid results, and real-time analysis, making it a viable alternative for monitoring residue levels in food. Another study was the first to explore the possibility of on-site drug screening at an abattoir using an immunobiosensor. The biosensor successfully detected SMZ in pig bile samples at a set threshold of 0.4 mg/L, accurately indicating whether the tissue samples exceeded the maximum residue limit. All positive control pigs were correctly identified, with a false positive rate of just 0.3% and no false negatives.

Glucose biosensors

Amperometric glucose biosensors are among the most widely used and successful commercial biosensors, available in various forms such as glucose pens and large display devices. This section aims to provide a detailed analysis of several glucose biosensors. The foundation of glucose biosensors traces back to the pioneering work of Leland C. Clark (Fraser, 1994) [9], who conducted the first key experiment in this field. He utilized platinum (Pt) electrodes to detect oxygen levels, placing the enzyme glucose oxidase (GOD) near the electrode surface by trapping it with a dialysis membrane. The activity of the enzyme was influenced by the oxygen concentration in its vicinity. The reaction catalyzed by GOD involves glucose interacting with the enzyme to form gluconic acid while simultaneously generating two electrons and two protons,

which reduce the GOD. The reduced form of GOD, along with oxygen, electrons, and protons, then reacts to form hydrogen peroxide (H_2O_2) and the oxidized form of GOD. This oxidized enzyme can catalyze further reactions with glucose. Higher glucose concentrations lead to increased oxygen consumption and a corresponding decrease in oxygen detection. Conversely, increased glucose levels also result in higher H_2O_2 production. Therefore, either the consumption of oxygen or the production of H_2O_2 can be measured using platinum electrodes, serving as indicators for glucose concentration.

Future prospects

Bio-sensors based on nano-materials:

Various nanoparticles, such as gold, silicon, silver, copper, and carbon-based materials like graphite, graphene, and carbon nanotubes, are commonly employed in biosensor immobilization (Sang *et al.*, 2016) [37]. These nanoparticles are known for their high sensitivity and specificity, which contributes to the enhanced performance of biosensors developed using nanomaterials. Among them, gold nanoparticles are particularly valued for their stability against oxidation and lack of internal toxicity (Su *et al.*, 2011) [42], in contrast to silver, which can oxidize and pose toxicity risks when used internally (Nie *et al.*, 2007) [24]. Despite these differences, nanomaterials are widely regarded as effective for improving the sensitivity and detection capabilities of biosensors, including those designed for single-molecule detection (Turner *et al.*, 2013) [47]. For instance, platinum nanoparticles have been utilized for detecting low concentrations of DNA (Li *et al.*, 2011) [18], while quantum dots and iron oxide nanoparticles are effective in targeting tumor antigens (Kunzelmann *et al.*, 2014) [16].

Synthetic fluorescent / genetically encoded biosensors

Fluorescent and genetically encoded biosensors offer valuable insights into cellular biological processes and molecular pathways (Randriamampita *et al.*, 2014) [35]. The initial development of fluorescently labeled antibodies for imaging fixed cells paved the way for the creation of sensors that utilize second messengers, small molecules that bind to analytes, and biological proteins (Randriamampita *et al.*, 2014) [35]. Today, fluorescent biosensors are employed to analyze motor proteins using single-molecule detection (Oldach *et al.*, 2014) [25], though this remains a challenging task despite the advantages. Genetically encoded biosensors targeting components involved in energy production, such as reactive oxygen species and cAMP, provide a deeper understanding of mitochondrial function (Johnson *et al.*, 2014) [15]. Additionally, FRET-based biosensors have been developed to monitor cAMP, cGMP, and calcium levels in cells, with cGMP being a key signaling molecule in the cardiovascular system (Park *et al.*, 2013) [26]. Through process optimization, these methods have facilitated the creation of biosensors targeting specific microbial and cellular organelles (Nie *et al.*, 2007) [24]. While electrochemical, electromechanical, and optical biosensors offer superior detection compared to molecular techniques (Gutiérrez *et al.*, 2015) [13], the field is now shifting toward the development of optical-based genetic biosensors capable of analyzing whole genomes. It is recognized that this objective can be achieved through a combination of small molecules, nanomaterials, and fluorescence-based optical

biosensors.

Conclusion

In conclusion, sensitivity, specificity, non-toxicity, and small molecules are the main factors that influence the development of biosensors. This study provides a quick overview of the biosensor concept, kinds, benefits, applications, and most recent developments. A recently created analytical tool called a biosensor is beneficial for diagnosing diseases as well as detecting different chemical reactions. This analysis examines every facet of the biosensor in light of recent developments. These techniques have found applications for a wide range of sample matrixes, including muscle, urine, honey, and prawns. Taking into account these traits will finally solve essential requirements as well as the issue of significant biosensor technological limits. New types of biosensors are produced as a result of some electrochemical sensor advancements combined with nanomaterials.

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