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Review Article

Dentistry Section

Biosensors in the Field of Dentistry

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ABSTRACT

Biosensors are small, integrated, self-contained and self-analysing scientific devices that are used to identify and measure topics of interest. Biological detection components (e.g., enzymes, antibodies and nucleic acids) are closely related to transducers (e.g., optical, electrochemical, piezoelectric) that makes the concept of biodegradation more complex and quantitative. As a general rule, the strength of the output signal corresponds to a group of analysers. Finally, the results are created using applied gadgets and the programming framework that are involved. These provide an easier and advanced visualisation that can be handled even by a non expert. In simpler words biosensor can be termed as an 'easy-to-use' tool for diagnostic purposes, that are developed to help in the early diagnosis and treatment of disease. Early diagnosis is the key to successful treatment of many diseases. Biosensors utilise the unique properties of biological and physical materials to recognise a target molecule and effect transduction of an electronic signal. The key advantages of biosensors are fast responsiveness and high sensitivity. Also, the basic advantage for 'Point of Care' (POC) devices such as biosensors would include integration of nano materials, microfluidics, automatic samplers and transduction devices on a single chip. Biosensors are also being used as new analytical tools to study medicine related diagnostic aspects. This paper reviews the significance of biosensors for clinical diagnosis and therapeutic applications in the field of dentistry and its application during the Coronavirus Disease 2019 (COVID-19) pandemic era. It provides a comprehensive account of progress in biosensors for dental applications.

Keywords: Application, Biorecognition, Diagnostics, Microfluidics

INTRODUCTION

Scientists have developed new approaches in the field of chemical analysis that often involves a large selection of biological recognition systems using bioreceptors. Biosensors are now being used by research scientists and medical societies to test food and water toxins, manage human biologic processes, determine precise health diagnosis and in several other fields. Researchers and medical practitioners require safe and cost effective methods for conducting research, guaranteeing public safety and providing patients with personalised health options. Biosensors can be used to quickly implement one such approach. Biomedical diagnostic researches are becoming increasingly important in the modern medical professional field. Screening of infectious illness, early detection of pathologies, chronic disease therapy, health management and well-being tracking are some major applications of the sophisticated technology by biosensors. Advanced biosensor technology allows for the detection of disease and the monitoring of the body's reaction to treatment and medicines. In addition to various transduction methods, these biological recognition factors have aided in the rapidly evolving fields of bioanalysis and related technologies with the application of biosensors and biochips [1].

Definition of Biosensors

According to the International Union of Pure and Applied Chemistry (IUPAC), "a biosensor is a device that uses specific isolated enzymes-mediated biochemical reactions to detect chemical compounds either by thermal, electrical or optical signals" [2].

History of Biosensors

Clark LC (1918-2005) who is also known as the father of the biosensor developed a large number of early biosensors in the mid 1960s using "enzyme electrodes" to estimate glucose concentration with an enzyme called Glucose Oxidase (GOD). The integrated multi-analyser sensor has progressed after achieving a single analytical sensor capable of conducting more comprehensive research, such as a device that detects glucose, lactate and potassium. Technological improvements were conducted in the manufacture of more powerful and small integrated biosensors to determine glucose, lactate and urea in microscopic samples of whole blood or plasma. Minimisation also allowed additional diagnostic tools in the form of biosensors such as detectors used in the process of chromatography or the detection of capillary form of electrophoresis. The rise of new generation of biosensors included a high-throughput capable small mustimeter analysis immunosensor gadget and 1000 uniquely speaking electrodes per square centimetre. These tools can detect analyses even within the ATP mol range [3].

Components of Biosensor

Biosensor consists of three basic components:

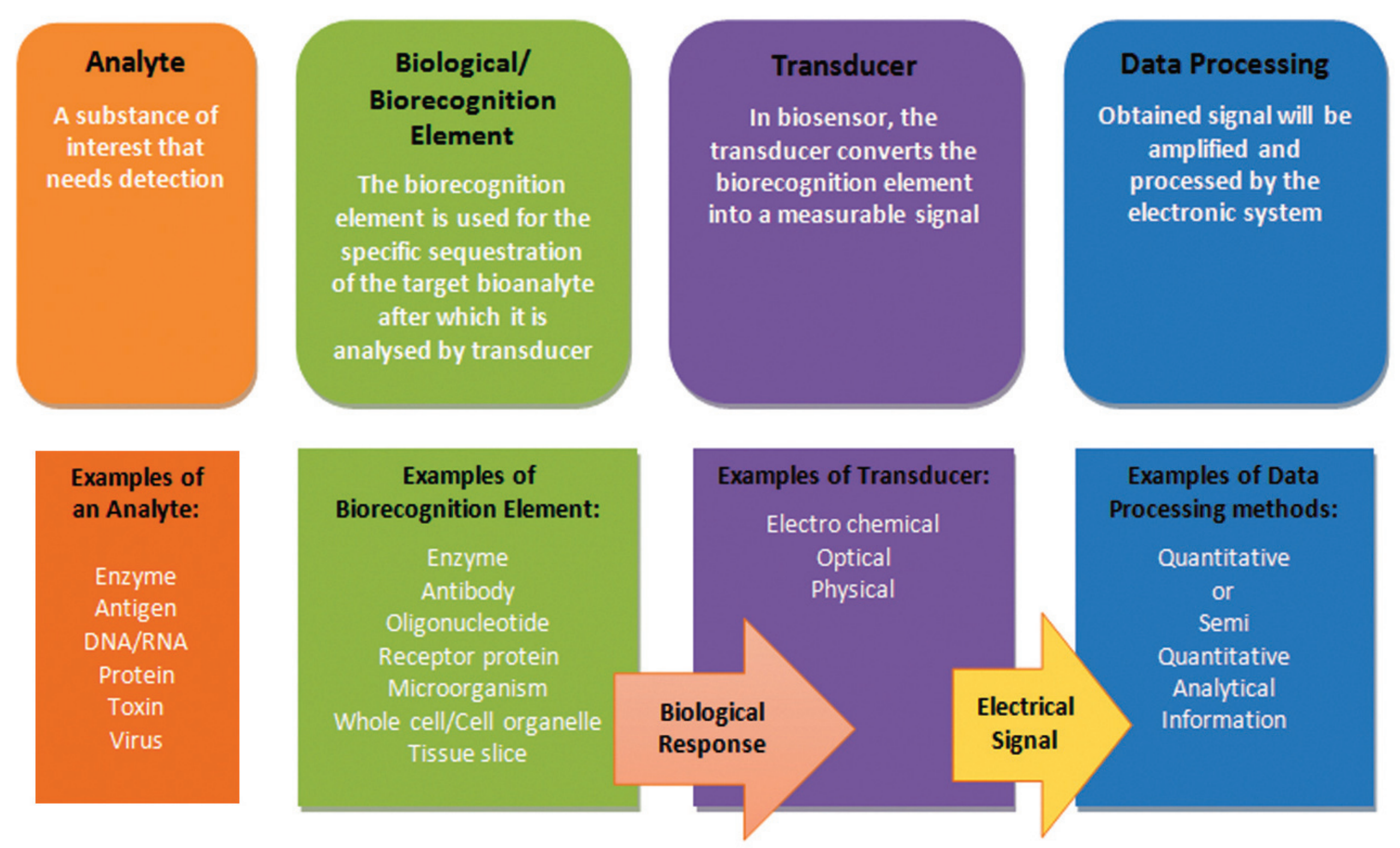
(i) A detector to detect the biomolecule and generate stimulus;

(ii) A transducer to convert the stimulus to output signal; and

(iii) A signal processing system to process the output and present it in an appropriate form [4].

WORKING PRINCIPLE OF BIOSENSORS

Biosensor is a bioanalytical device that consists of a biosensitive layer which is attached to the device framework that helps in the process of signal detection. The biosensitive layer works by stabilising the biological receptor component (catalyst, neutraliser, oligonucleotide, receptor protein, microorganism or whole cell) on the biosensor membrane. The desired biological material is usually in the form of an enzyme. By a process known as an electro-enzymatic approach which is a chemical process of converting the enzymes into corresponding electrical signals (usually current) with the help of a transducer. One of the commonly used biological responses is the oxidation of the enzyme. Oxidation acts as a catalyst and alters the pH of the biological material. The change in pH will directly affect the current carrying capacity of the enzyme, which is once again in direct relation to the enzyme being measured. Output of the transducer i.e., the current is a direct representation of the enzyme being measured [Table/Fig-1]. The current is generally converted into voltage so that it can be properly analysed and represented [5].



[Table/Fig-1]: Biosensor assay

TYPES OF BIOSENSORS

Electrochemical biosensors are integrated devices that provide specific or semi quantitative analytical information using a biological detection element attached to an electrochemical transduction element.

There are six main types of biosensors:

- Potentiometric biosensors that use ion selective electrodes to determine changes in the concentration of chosen ions. Examples of potentiometric biosensors are membrane-based Ion-Selective Electrodes (ISE), Screen-printed electrodes, Ion-Selective Field Effect Transistors (ISFET).
- Amperometric biosensors that measure the electric current associated with electron flow resulting from redox reactions. Example of amperometric biosensors are those biosensors which are used for the detection of creatine, urea, lactate and pyruvate determination.
- Conductometric biosensors that measure changes in the conductivity of a medium as a result of enzyme reactions that change its ionic composition. Example of conductometric biosensors is bi-enzymatic conductometric biosensor that detects heavy metal ions and pesticides in water.
- Piezoelectric biosensors that rely on alternating capacitance and produce a waveform at a specific frequency in the crystal. This frequency is highly sensitive to the surface properties of the crystal. If the crystal is coated with a biological detection element, capturing the target analysis with the receiver changes the resonance frequency. Examples of the piezoelectric biosensors are the biosensors used in microphones, amplified guitars and medical imaging equipment.
- Thermometric biosensors are made by combining enzymes with a temperature sensor. When the analyser is exposed to the enzyme, the heat of the enzymatic reaction is measured and calibrated against the analyte concentration. Example of thermometric biosensors is the biosensors used in the analysis of blood metabolites in ICU patients.
- Optical biosensors detect changes in absorption, Resistance1 (R1), Photoluminescence (PL) or fluorescence. Example is the advanced Evanescent-wave optical biosensors that are used for the detection of nucleic acids [6].

Oral Fluid Based Biosensors

They are the biosensors that use Gingival Crevicular Fluid (GCF) and saliva as the biomedia.

Gingival Crevicular Fluid (GCF) based biosensors: GCF is a serum transudate or inflammatory exudate collected at the gingival margin or within the gingival crevice. Various types and amounts of biomarkers used for the diagnosis and prognosis of periodontal diseases are present in the GCF. Hence, detecting these biomarkers with the help of suitable biosensors will definitely guide the clinicians for the purpose of risk assessment and decision making with respect to the treatment planning of the associated periodontal conditions [7].

Saliva based biosensors: The determination of blood biomarkers is a common clinical diagnosis method. However, it is an intrusive technique that may be too aggressive for certain people. Saliva sample is simpler to collect, less technique sensitive and it contains a variety of disease signaling indicators, hence it can accurately reflect normal and disease states in humans. Saliva has become a very popular diagnostic fluid, with a rising number of assay improvements and technical advancements for the detection of various salivary biomarkers. Salivary biomarkers have been discovered that may be beneficial in the clinical diagnosis and prognosis of a wide range of malignancies (oral, pancreatic, lung, breast and liver) [8].

According to the biosensing targets, the salivary based sensors can be divided into three main categories: Deoxyribonucleic Acid (DNA) biosensors, Ribonucleic Acid (RNA) biosensors and protein biosensors [9].

i) DNA biosensor: Target DNA extracted from human bodily fluid had to be centrifuged and purified in most situations. By altering the working electrode with complementary sequences, DNA biosensors detect the target DNA. Working electrodes for electrochemical setups are typically gold electrodes, screen-printed carbon electrodes and glassy carbon electrodes, though Indium Tin Oxide (ITO) can also be employed. By combining exonuclease III-assisted amplification with dual signal ratiometric output mode, a ratiometric electrochemical DNA sensor was developed by Tan Y et al., to directly analyse target DNA in simulated saliva specimens. As a result, it is possible to identify biomarkers at lower concentrations [10].

ii) RNA biosensor: The RNA biosensor works by the principle of recognition event using sequence specific hybridisation between nucleic acids. A solid substrate can be immobilised with an immobilised probe that has a complementary sequence to the RNA strand we want to detect. The sample solution is then added and if the complimentary strand is present, hybridisation of the probe with it proceeds. Hybridisation can be detected in a variety of ways, including electrochemical, optical and mass change based methods. The junction technique is frequently used to integrate RNA biosensors with magnetic beads to ensure that the biosensors acquire increased sensitivity and distinguishing ability [11].

iii) Protein biosensor: For POC and clinical analysis, electrochemical biosensors for detecting protein cancer biomarkers provide a sensitive, fast, and low cost diagnosis framework. The surface of the electrodes in these biosensors is frequently enhanced with receptors like antibodies or aptamers. Aptamer based biosensors also have label-free, high sensitivity for electrochemical detection when compared to conventional biosensors [12].

ROLE OF ORAL FLUID-BASED BIOSENSORS IN DENTISTRY

Dental Caries

Despite technological advancements, the most extensively used and reliable diagnostic approach for dental caries is still clinical examination. Tactile investigation with tools and the dentist's visual perception are used in the oral examination method. These extremely subjective procedures have the potential of errors including false positives causing patient discomfort. Dental caries detecting biosensors have been introduced in which the formed extracellular polysaccharide in the carious lesions is used and the overall effect is observed spectroscopically [6]. The study conducted by Lynge Pedersen AM and Belstrøm D concluded that saliva absorption decreased with increased bacterial activity. Therefore, this test can monitor *Streptococcus mutans* in saliva because its level is related to tooth decay [13].

Saliva α-Amylase (SAA), which is one of the components of human saliva, binds to a high association with a select group of oral streptococci. In addition, this enzyme is also found in the obtained enamel pellicles, indicating its role in α-amylase-binding bacterial synthesis. The SAA biosensor was developed on a colourimetric assay platform. The colour intensity of the reaction product is measured photometrically to determine the concentration of SAA [6].

Periodontitis

Recent studies have shown that periodontal infection is a major risk factor for heart and cerebrovascular sickness. Various clinical parameters such as pocket depth, bleeding, clinical attachment level and radiographic assessment of bone destruction signify the development of periodontitis [14-16]. The role of biomarkers to assess periodontal diseases at the atomic, cellular, tissue and clinical levels is very crucial. Several biomarkers that are associated with inflammation, soft tissue and bone destruction have been found in saliva and GCF. However, no single marker is sufficient for a reliable diagnosis [10].

Interleukin (IL)-1β, Matrix metalloproteinase (MMP)-8, Tumour Necrosis Factor (TNF)-α, IL-6, and C-Reactive Protein (CRP) are some of the biomarkers that are associated with periodontitis. Saliva based biosensors were developed at the University of Texas at Austin on the basis of the salivary biomarkers seen in periodontitis. It was a Lab-on-a-Chip (LOC) system that combined microfluidics and fluorescence based optical systems. In this sensor, sandwich immunoassay was performed on chemically sensitive beads [17].

Oral Cancer

In the world, oral cancer is the eighth most leading cancer among men and the fourteenth most leading cancer among women. Oral cancer is one of the most common cause of death and illness in developing countries [18]. Therefore, various biomarkers have been developed for early detection and risk assessment of oral cancer. For the detection of oral cancer, various salivary proteins such as IL-8, TNF-α, Epidermal Growth Factor Receptor (EGFR), and microRNA (miRNA) such as salivary transferorin and genome can act as potential biomarkers [19].

In tumour angiogenesis as well as in metastasis, IL-8 which is an anti-inflammatory chemokine plays a very significant role. A surface-stable optical protein sensor was developed for the detection of IL-8 protein cancer markers. In this biosensor, the surface stabilisation analysis was performed with the capture probe which accurately responded with the biotinylated monoclonal antibodies. The emitter from the fluorophore-conjugated light with the reporter probe detected the signal and the optical noise reduction was carried out by confocal optics [20].

Potential biomarkers for cancer can be tested and detected with the help of a saliva based biosensor that functions by exfoliation of cells in the oral cavity. Levels of anxiety and discomfort among the patients are reduced greatly when compared to regular biopsy procedures. Weigum SE et al., developed a novel biosensor that initiated Nano-Bio-chip Cellular (NBC) analysis for the classification of malignant and premalignant lesions and was also used for the assessment of Estimated Glomerular Filtration Rate (EGFR) and cytomorphometry in the models of exfoliative cytology. Quantification and detection of morphological alterations in nucleus and EGFR expression was carried out using NBC sensor assay, which gave indication that they diagnosed the cellular changes in tumour tissue [21].

MicroRNAs are a class of small, endogenous RNAs of 21-25 nucleotides (nts) in length. They play an important regulatory role in animals and plants by targeting specific mRNAs for degradation or translation repression. miRNAs are short non coding RNAs that are encoded throughout the gene. Early detection of changes in the genetic factors of miRNA can be observed that will aid in the diagnosis and advanced treatment of oral cancer. An electrochemical biosensor method has been developed to detect oral cancer related miRNA at the molecular level to detect miRNA using magnetically controlled gold electrodes. The purpose of this biosensor is enzymatic catalytic amplification based on magnetic beads that enhance the sensitivity of the biosensor [22].

Dental Fluorosis

Optical biosensors are the most sensitive gadgets for identification and evaluation. It has a wide range of applications in biomedical exploration, medical services, medicine, natural surveillance and battlefield. A biosensor contains a biological element called an enzyme, antibody or nucleic acid interacting with analyte and producing an electronically predictable signal. Various substances can be used as biological elements like nucleic acids, proteins and complex substances. For example, the amount of fluoride in drinking water groundwater directly affects the nature of drinking water. It has been demonstrated that a 2-dimensional photonic crystal based biosensor with line defects can detect various fluorides in water. There is a slight change in frequency from band formation to small changes in resistor I, acting as a sensor. This suggests that it is exceptionally sensitive to resistor I change. Induction and probe technology has been implemented for the successful diagnostics for CaF, CsF, KF, LiF and SrF2 using the procedure a peak in the fluoride content was observed. This serves as an important factor in the detection of dental fluorosis caused by presence of fluoride content in water. The Finite-difference Time-domain (FDTD) technique was used for testing [23].

APPLICATION OF BIOSENSORS IN COVID-19 PANDEMIC

Nowadays, there is indeed a lot of interest in creating efficient, reliable, and sensitive novel biosensors for COVID-19 diagnostics, which would be a one-step identification or sensing technique that would minimise separation (nucleic acid extraction), incubation, and the need of any signal reporting agents. Biosensors for COVID-19 are based primarily on surface nucleoproteins which bind to the host Angiotensin-Converting Enzyme-2 (ACE-2) receptor and the internal genetic material, according to Liu Z et al., [24].

According to Ponti G et al., in 2020, the detection of biomarkers from human hosts other than antibodies or immunoglobulins could be a strategy for building novel COVID-19 biosensors [25]. Several host biomarkers have been detected that are potentially capable in COVID-19 diagnostics. Some of the biomarkers used for such diagnostic purpose are based on lymphocyte count, neutrophil count, Neutrophil–Lymphocyte Ratio (NLR), CRP, Erythrocyte Sedimentation Rate (ESR), Procalcitonin (PCT), IL-6 and biochemical that includes Creatine Kinase (CK), troponin, D-dimer and aspartate aminotransferase. By the detailed review of multiple case studies it has been found out that several novel biomarkers that potentially play a pivotal role can be detected such as homocysteine and angiotensin II. Nanomaterials like gold and carbon have generated a huge interest in sensor technology systems in recent years there has been development of several sophisticated devices with the purpose of sensing or biorecognising the virus and its biomolecules. Certain nanomaterials, particularly associated with an analyte such as a complementary single stranded nucleic acid aptamer, might provide a revolutionary approach to detect Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in clinical specimens [26].

CONCLUSION(S)

Lately, chair side/bedside checking tests have acquired significance over routine lab tests as they are simpler and quicker to perform without requiring skilled faculty. In addition, biosensors allude to POC gadgets created to help early diagnosis, periodic monitoring and treatment of illness. These gadgets use organic responses for distinguishing and estimating a specific substance (analyte) of interest. Until this point, blood has been the highest quality level symptomatic liquid for different illnesses. Be that as it may, oral fluids like saliva and gingival crevicular liquid offer benefits like the non intrusive sample collection, smaller sample aliquots, simple storage and transportation continued examining for checking after some time, and more prominent affectability, making them an optional clinical apparatus over serum and tissues for some biomedical diagnostic measures.

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