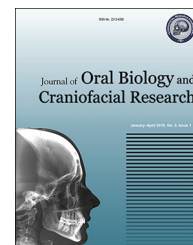


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Review Article

Biosensors and their applications – A review

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ABSTRACT

The various types of biosensors such as enzyme-based, tissue-based, immunosensors, DNA biosensors, thermal and piezoelectric biosensors have been deliberated here to highlight their indispensable applications in multitudinous fields.

Some of the popular fields implementing the use of biosensors are food industry to keep a check on its quality and safety, to help distinguish between the natural and artificial; in the fermentation industry and in the saccharification process to detect precise glucose concentrations; in metabolic engineering to enable in vivo monitoring of cellular metabolism. Biosensors and their role in medical science including early stage detection of human interleukin-10 causing heart diseases, rapid detection of human papilloma virus, etc. are important aspects. Fluorescent biosensors play a vital role in drug discovery and in cancer. Biosensor applications are prevalent in the plant biology sector to find out the missing links required in metabolic processes. Other applications are involved in defence, clinical sector, and for marine applications.

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1. Introduction

Biosensors are analytical devices that convert a biological response into an electrical signal. Quintessentially biosensors must be highly specific, independent of physical parameters such as pH and temperature and should be reusable. The term “biosensor” was coined by Cammann,¹ and its definition was introduced by IUPAC.^{2–4}

Fabrication of biosensors, its materials, transducing devices, and immobilization methods requires multidisciplinary research in chemistry, biology, and engineering. The materials used in biosensors are categorized into three groups based on their mechanisms: biocatalytic group comprising enzymes, bioaffinity group including antibodies and nucleic acids, and microbe based containing microorganisms.

2. Material and methods

The term “biosensors” was searched in Pubmed, Sciencedirect, and google, and all articles based on its applications were selected.

3. Review of literature

3.1. Types of biosensors

Biosensors started in the 1960s by the pioneers Clark and Lyons. Various types of biosensors being used are enzyme-based, tissue-based, immunosensors, DNA biosensors, and thermal and piezoelectric biosensors.

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The first enzyme-based sensor was reported by Updike and Hicks in 1967. Enzyme biosensors have been devised on immobilization methods, i.e. adsorption of enzymes by van der Waals forces, ionic bonding or covalent bonding. The commonly used enzymes for this purpose are oxidoreductases, polyphenol oxidases, peroxidases, and aminooxidases.⁵⁻⁷

The first microbe-based or cell-based sensor was actualized by Diviès.⁸ The tissues for tissue-based sensors arise from plant and animal sources. The analyte of interest can be an inhibitor or a substrate of these processes. Rechnitz⁹ developed the first tissue based sensor for the determination of amino acid arginine. Organelle-based sensors were made using membranes, chloroplasts, mitochondria, and microsomes. However, for this type of biosensor, the stability was high, but the detection time was longer, and the specificity was reduced.

Immunosensors were established on the fact that antibodies have high affinity towards their respective antigens, i.e. the antibodies specifically bind to pathogens or toxins, or interact with components of the host's immune system.

The DNA biosensors were devised on the property that single-strand nucleic acid molecule is able to recognize and bind to its complementary strand in a sample. The interaction is due to the formation of stable hydrogen bonds between the two nucleic acid strands.¹⁰

Magnetic biosensors: miniaturized biosensors detecting magnetic micro- and nanoparticles in microfluidic channels using the magnetoresistance effect have great potential in terms of sensitivity and size.¹¹

Thermal biosensors or calorimetric biosensors are developed by assimilating biosensor materials as mentioned before into a physical transducer.

Piezoelectric biosensors are of two types: the quartz crystal microbalance and the surface acoustic wave device. They are based on the measurement of changes in resonance frequency of a piezoelectric crystal due to mass changes on the crystal structure.

Optical biosensors consist of a light source, as well as numerous optical components to generate a light beam with specific characteristics and to beeline this light to a modulating agent, a modified sensing head along with a photodetector.¹²

Green fluorescent protein and the subsequent autofluorescent protein (AFP) variants and genetic fusion reporters have aided the development of genetically-encoded biosensors.¹³⁻²⁰ This type of biosensor is user-friendly, easy to engineer, manipulate and transfer into cells. Single-chain FRET biosensor is another example. They consist of a pair of AFPs, which are able to transfer fluorescence resonance energy between them when brought close together. Different methods may be used to regulate changes in Förster resonance energy transfer (FRET) signals based on intensity, ratio or lifetime of AFPs. Peptide and protein biosensors are easily manufactured through synthetic chemistry followed by enzymatic labelling with synthetic fluorophores. Due to their independence of genetically-encoded AFPs, they are readily utilized to control target activity and constitute attractive alternatives and have an added advantage of being able to enhance signal-to-noise ratio and sensitivity of response through introduction of chemical quenchers and photoactivatable groups.

3.2. Applications of biosensors

Biosensors have been applied in many fields namely food industry, medical field, marine sector etc., and they provide better stability and sensitivity as compared with the traditional methods.

3.2.1. In food processing, monitoring, food authenticity, quality and safety)

An arduous quandary in food processing industry is of quality and safety, maintenance of food products and processing. Traditional techniques performing chemical experiments and spectroscopy have shortcomings due to human fatigue, are expensive and time consuming. Alternatives for food authentication and monitoring with objective and consistent measurement of food products, in a cost effective manner, are desirable for the food industry. Thus development of biosensors in response to the demand for simple, real-time, selective and inexpensive techniques is seemingly propitious.²¹

Ghasemi-Varnamkhasti et al.²² worked on the monitoring of ageing of beer using enzymatic biosensors, based on cobalt phthalocyanine. These biosensors evinced a good capability to monitor the ageing of beer during storage.

Biosensors are used for the detection of pathogens in food. Presence of *Escherichia coli* in vegetables, is a bioindicator of faecal contamination in food.^{23,24} *E. coli* has been measured by detecting variation in pH caused by ammonia (produced by urease-*E. coli* antibody conjugate) using potentiometric alternating biosensing systems. Washing the vegetables such as sliced carrots and lettuce with peptone water provides us with the liquid phase. It is then separated by amalgamating it in a sonicator, to disaffiliate bacterial cells from food items.²⁵

Enzymatic biosensors are also employed in the dairy industry. A biosensor, based on a screen-printed carbon electrode, was integrated into a flow cell.²⁶ Enzymes were immobilized on electrodes by engulfment in a photocrosslinkable polymer. The automated flow-based biosensor could quantify the three organophosphate pesticides in milk.

One of the popular food additives extensively used today are sweeteners, which are adversely causing undesirable diseases including dental caries, cardiovascular diseases, obesity and type-2 diabetes. It is believed that artificial sweeteners are addictive and coax us to eat more high-energy food unconsciously, inadvertently causing weight gain. Thus their detection and quantification are of prime importance. Traditional methods to distinguish the two types of sweeteners are ion chromatographic methods, which are complicated and laborious.

A more efficacious method, which combined lipid films with electrochemical techniques as biosensors for speedy and sensitive screening of sweeteners has been explored by multi-channel biosensor, which detect the electrophysiological activities of the taste epithelium. The signals are analyzed using spatiotemporal techniques, on MATLAB, where glucose and sucrose represent natural sugars while saccharin and cyclamate comprise artificial sweeteners. Since all sweeteners are mediated by heterodimeric G-protein coupled receptors in Type-II cells in the bud, they have a plurality of binding sites to identify sweet stimuli of different structures respectively. Studies suggest two types of sweet stimuli: cyclic adenosine

monophosphate pathway which utilizes natural sugars such as sucrose, and the second is, inositol triphosphate and diacylglycerol pathway exploited by artificial sweeteners for purpose of signal transduction. The response to artificial sweeteners greatly depends on residues in the amino terminal domains of taste receptors as ligand binding sites. The signal responses of taste receptor cells towards natural and artificial sweeteners are discrete. The taste epithelium biosensor delivered sparse signals with positive waveforms, when glucose was applied whereas sucrose sustained signals with negative spikes. The taste epithelium responded to artificial sweeteners with more intensive signals, showing that the responses to artificial sweeteners were quite different from those of natural sugars, in both time and frequency domains.

3.2.2. In fermentation processes

In fermentation industries, process safety and product quality are crucial. Thus effective monitoring of the fermentation process is imperative to develop, optimize and maintain biological reactors at maximum efficacy. Biosensors can be utilized to monitor the presence of products, biomass, enzyme, antibody or by-products of the process to indirectly measure the process conditions. Biosensors precisely control the fermentation industry and produce reproducible results due to their simple instrumentation, formidable selectivity, low prices and easy automation. Nowadays, several kinds of commercial biosensors are accessible; capable of detecting biochemical parameters (glucose, lactate, lysine, ethanol etc.) and are widely used in China, occupying about 90% of its market.

In fermentation process, saccharification was monitored by traditional Fehling's method. Since this method involves titration of reducing sugar, its outcomes were inaccurate. However, since the launch of glucose biosensor commercially in 1975, the fermentation industries have been benefited. Now the factories successfully use glucose biosensors to control production in the saccharification and fermentation workshop and utilize the bioenzymatic method to produce glucose.

Biosensors are also employed in ion exchange retrieval, where detection of change of biochemical composition is carried out. For instance, glutamate biosensor has been used to conduct experiments on ion exchange retrieval of an isoelectric liquor supernatant of glutamate. The fermentation process is a byzantine process with multiple pivotal variables, most of which are laborious to measure in real-time. On-line monitoring of critical metabolites is essential to facilitate quick optimization and to control biological processes. In past years, biosensors have attracted a lot of attention in online monitoring in fermentation process due to its simplicity and quick response.²⁷

3.2.3. Biosensing technology for sustainable food safety

The term food quality refers to the appearance, taste, smell, nutritional value, freshness, flavour, texture and chemicals.¹¹ Smart monitoring of nutrients and fast screening of biological and chemical contaminants are of paramount importance, when it comes to food quality and safety. Material science, nanotechnology, electromechanical and microfluidic systems are striding in to make sensing technology imminent for use in market. Efforts are being made for developing control systems

ensuring food quality and safety and, as a consequence, human health.

Glucose monitoring becomes indispensable as during storage the food content and composition may get altered.²⁸ German²⁹ studied the electrochemistry of glucose oxidase immobilized on a graphite rod, altered by gold nanoparticles (AuNPs), which improved its sensitivity.

Glutamine is the nitty-gritty of crucial functions such as (signalling, transport and precursor in biosynthesis of nucleic acids, amino sugars and proteins). Patients deficient in glutamine suffer from pathologies such as malabsorptive disorders and have to be supplemented, to improve immune functions, preserve intestinal functionality and lessen bacterial translocation.³⁰ Glutaminase-based microfluidic biosensor chip with a flow-injection analysis for electrochemical detection has been used for detection in fermentation process.³¹

Biosensors are being employed to perceive general toxicity and specific toxic metals, due to their capability to react with only the hazardous fractions of metal ions.³² Pesticides pose grave threats to the environment. The common pesticides used are organophosphates and carbamic insecticide species. Immunosensors have proved their merit as sensitive, high-speed agrifood and environmental monitoring. AChE and butyrylcholinesterase biosensors have been devised for aldicarb, carbaryl, paraoxon, chlorpyrifosmethyl etc. Oxon utilizing screen-printed electrodes was developed by Arduini and colleagues.³³ A similar type of biosensor is used to detect pesticides in wine and orange juice.^{5,34,35} Arsenic can be measured with the help of bacteria-based bioassays.³⁶

3.2.4. In medical field

In the discipline of medical science, the applications of biosensors are growing rapidly. Glucose biosensors are widely used in clinical applications for diagnosis of diabetes mellitus, which requires precise control over blood-glucose levels.³⁷ Blood-glucose biosensors usage at home accounts for 85% of the gigantic world market.³⁸

Biosensors are being used pervasively in the medical field to diagnose infectious diseases. A promising biosensor technology for urinary tract infection (UTI) diagnosis along with pathogen identification and anti-microbial susceptibility is under study.

Identifying end-stage heart failure patients, prone to adverse outcomes during the early phase of left ventricular assisted device implantation, is important. A novel biosensor, based on hafnium oxide (HfO₂), has been used for early stage detection of human interleukin (IL)-10.³⁹ Interaction between recombinant human IL-10 with corresponding monoclonal antibody is studied for early cytokine detection after device implantation. Fluorescence patterns and electromechanical impedance spectroscopy characterize the interaction between the antibody-antigen and bio-recognition of the protein is achieved by fluorescence pattern. Chen et al. applied HfO₂ as a greatly sensitive bio-field-effect transistor.⁴⁰ HfO₂ biosensor has been functionalized for antibody deposition with detection of a human antigen by electrochemical impedance spectroscopy.

The biggest dilemma faced today is of heart failure with about one million people suffering from it. Techniques for detection of cardiovascular diseases include immunoaffinity

column assay, fluorometric, and enzyme-linked immunosorbent assay.⁴¹⁻⁴⁵ These are laborious, require qualified personnel and are time consuming. Biosensors established on electric measurement employ biochemical molecular recognition for desired selectivity with a particular biomarker of interest.

The various other biosensors applications include: quantitative measurement of cardiac markers in undiluted serum, microfluidic impedance assay for controlling endothelin-induced cardiac hypertrophy, immunosensor array for clinical immunophenotyping of acute leukemias, effect of oxazaborolidines on immobilized fructosyltransferase in dental diseases; histone deacetylase (HDAC) inhibitor assay from resonance energy transfer, biochip for a quick and accurate detection of multiple cancer markers and neurochemical detection by diamond microneedle electrodes.

3.2.5. Fluorescent biosensors

Fluorescent biosensors are imaging agents, for use in cancer and drug discovery. They have enabled insights into the role and regulation of enzymes at cellular level. GFP-based and genetically encoded FRET biosensors play a vital role.

Fluorescent biosensors are small scaffolds onto which one or several fluorescent probes are mounted (enzymatically, chemically or genetically) through a receptor. The receptor identifies a specific analyte or target, thereby transducing a fluorescent signal which can be readily detected and measured.^{46,47} Fluorescent biosensors can probe ions, metabolites, and protein biomarkers with great sensitivity and can also report the presence, activity or status of the target (serum, cell extracts) in complex solution. They are employed in probing gene expression, protein localization, and conformation in fields such as signal transduction, transcription, cell cycle and apoptosis. Indication of arthritis, inflammatory diseases, cardiovascular and neurodegenerative diseases, viral infection, cancer and metastasis is done using these sensors.

Fluorescent biosensors are used in drug discovery programmes for the identification of drugs by high throughput, high content screening approaches, for postscreening analysis of hits and optimization of leads. These are considered potent tools for preclinical evaluation and clinical validation of therapeutic potential, biodistribution and pharmacokinetics of candidate drugs.⁴⁸⁻⁵² Fluorescent biosensors are effectively employed for early detection of biomarkers in molecular and clinical diagnostics, for monitoring disease progression and response to treatment/therapeutics, for intravital imaging and image guided surgery.⁵³

A genetically-encoded FRET biosensor developed for detection of Bcr-Abl kinase activity was used on cancer patient cells to assess Bcr-Abl kinase activity and to establish an interrelation with the disease status in chronic myeloid leukaemia. This probe was further employed to regulate response to therapy, and to observe the onset of drug-resistant cells, permitting prediction for alternative therapeutics.⁵⁴⁻⁵⁶

3.2.6. Biodefense biosensing applications

Biosensors can be used for military purposes at times of biological attacks. The main motive of such biosensors is to sensitively and selectively identify organisms posing threat in virtually real time called biowarfare agents (BWAs) namely, bacteria (vegetative and spores), toxins and viruses. Several

attempts to device such biosensors has been done using molecular techniques which are able to recognize the chemical markers of BWAs.

Nucleic acid-based sensing systems are more sensitive than antibody-based detection methods as they provide gene-based specificity, without utilizing amplification steps to attain detection sensitivity to the required levels.

The human papilloma virus HPV (double stranded DNA virus) has been categorized into two types: HPV 16 and 18; and is related to invasive cervical cancer. HPVs can be rapidly detected using a novel leaky surface acoustic wave peptide nucleic acid biosensor with double two-port resonators. This probe directly detects HPV genomic DNA without polymerase chain reaction amplification, and can also bind to the target DNA sequences with a lot of efficacy and precision.

3.2.7. In metabolic engineering

Environmental concerns and lack of sustainability of petroleum-derived products are gradually exhorting need for development of microbial cell factories for synthesis of chemicals. Researchers view metabolic engineering as the enabling technology for a sustainable bioeconomy.⁵⁷ They have also envisioned that a substantial fraction of fuels, commodity chemicals and pharmaceuticals will be produced from renewable feedstocks by exploiting microorganisms rather than relying on petroleum refining or extraction from plants. The high capacity for diversity generation also requires efficient screening methods to select the individuals carrying the desired phenotype. The earlier methods were spectroscopy-based enzymatic assay analytics however they had limited throughput. To circumvent this obstacle genetically encoded biosensors that enable in vivo monitoring of cellular metabolism were developed which offered potential for high-throughput screening and selection using fluorescence-activated cell sorting (FACS) and cell survival, respectively.

FRET sensors comprised a pair of donor and acceptor fluorophores, and a ligand-binding peptide was sandwiched between the two. When it was bound by a ligand of interest the peptide underwent a conformational change thereby a FRET change.^{58,59} Though they had high orthogonality, temporal resolution, and ease of construction, FRET sensors were merely able to report the copiousness of metabolites concerned and were unable to exert downstream regulation to the signal.⁶⁰

Transcription factors are natural sensory proteins evolved to regulate gene expression in response to changes in environment for high throughput screening.⁶¹ It is accomplished by hacking into host transcription system and employing a synthetic condition specific promoter to drive the expression of a reporter gene. These exhibit poor orthogonality and background noise.^{62,63}

The third class of biosensors comprises riboswitches; the regulatory domain of a mRNA that can selectively bind to a ligand and thereupon change its own structure, consequently regulating transcription of its encoded protein. As opposed to TF based biosensors, they are comparatively faster as the RNA has already been transcribed, also they do not rely on protein-protein or protein-metabolite interactions. In the recent decades ribosomes have been extensively engineered in bacterial systems.^{64,65}

3.2.8. Biosensors in plant biology

Revolutionary new technologies in the areas of DNA sequencing and molecular imaging, have lead to advancements in plant science. Traditional methods of mass spectroscopy for gauging insights into cellular and subcellular localization, and measure of ion and metabolite levels had unprecedented precision but lacked the key information regarding location and dynamics of enzyme substrates, receptors and transporters. However, this information can be easily successfully tapped using biosensors. To measure a dynamic process under physiological conditions, we need to device tactics to visualize the actual process, for instance, the conversion of one metabolite into another or triggering of signalling events. This visualization can be done by sensors which respond dynamically.

Roger Tsien's lab was the first to develop protein prototype sensors to measure caspase activity and control levels of calcium in live cells.⁶⁶ These sensors were based on FRET (FRET) between two spectral variants of GFP.^{67,68} In vivo application of biosensors involves high temporal resolution imaging of calcium oscillations using cameleon sensors.

Biosensors can be utilized to identify missing components pertinent to metabolism, regulation, or transport of the analyte. FRET sensor for sucrose, responsible for the identification of proteins, performs a transport step in phloem loading-sucrose efflux from the mesophyll. Fluorimeter-based assays with FRET sugar sensors successfully recognize sugar transporters that can function immediately after exposure of starved yeast cells to glucose.^{69,70} Similar assays identify genes that affect cytosolic or vacuolar pH in yeast,^{71,72} and justify that biosensors can be applied in genetic screens provided imaging technologies of suitable throughput are available.⁷³

4. Future scope

Cell and tissue-based biosensors consist of genetically engineered proteins that are infused into cells ex vivo or in vivo. They allow the researcher to sense levels of hormones, drugs, or toxins, continuously and noninvasively, using biophotonics or other physical principles. The scope in this regard could be of value in ageing research.

Biosensors are used for marine applications for detection of eutrophication using nitrite and nitrate sensors. Various sensors based on nucleic acid hybridization detection have been developed for organism detection; "Environmental Sample Processor" is under process at the Monterey Bay Aquarium Research Institute whose goal is the automated detection of toxic algae in situ from moorings using ribosomal RNA probes is a promising development in this field. Also detection of pollutants, heavy metal and pesticides through biosensors is one of the prime goals.

Applications of nanomaterials in biosensors provide opportunities for building up a new generation of biosensor technologies. Nanomaterials improve mechanical, electrochemical, optical and magnetic properties of biosensors and are developing towards single molecule biosensors with high throughput biosensor arrays. Biological molecules possess special structures and functions, and determining how to

fully use the structure and function of nanomaterials and biomolecules to fabricate single molecule multifunctional nanocomposites, nanofilms, and nanoelectrodes, is still a great challenge. The processing, characterization, interface problems, availability of high quality nanomaterials, tailoring of nanomaterials, and the mechanisms governing the behaviour of these nanoscale composites on the surface of electrodes are also great challenges for the presently existing techniques. Ways to enhance the signal to 116 noise ratio, how to enhance transduction and amplification of the signals, are also major impediments.

Future work should focus on clarifying the mechanism of interaction between nanomaterials and biomolecules on the surface of electrodes or nanofilms and using novel properties to fabricate a new generation of biosensors. Nevertheless, nanomaterial-based biosensors show great attractive prospects, which will be broadly applied in clinical diagnosis, food analysis, process control, and environmental monitoring in the near future.

Conflicts of interest

The author has none to declare.

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