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Research Article

Biosensor Technology as Diagnostic Tool for Detection of Pathogen

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ABSTRACT

The detection of pathogenic microorganisms is the key in the prevention and identification of problems related to health and safety. Legislation is particularly tough in areas such as the food industry, where failure to detect an infection may have terrible consequences. Traditional and standard pathogen detection methods may take up to 7 or 8 days to yield an answer. This is clearly insufficient. Many researchers have recently geared their efforts towards the development of rapid diagnostic methods. The advents of new technologies, namely biosensors, have brought in new and promising approaches. The biosensor is an analytical device, which detect pathogens with a help of a bio-recognition receptor and convert the result into a measurable signal with the help of a transducer. It has vital application in areas such as clinical diagnosis, food industry, environmental monitoring and in other fields, where rapid and reliable analysis are needed. Starting with the discussion of various sensing techniques commonly used in microbial biosensing, this paper offers a description of recent developments in pathogen detection, identification and quantification, with an emphasis on biosensors.

Keywords: Biosensor; Food; Pathogen; Receptor; Technology

INTRODUCTION

Pathogens are disease-causing microorganisms. They are ubiquitous in nature. Many studies have large impacts on host physiology, population dynamics, and community composition [1-3]. Today, many medical advances were made to safeguard against infection by pathogens, with vaccination, antibiotics and fungicide, still pathogens continue to threaten living things. However, the obstacle is how we can detect this dangerous pathogen [4].

To quickly determine the presence of a pathogen, researchers need reliable and accurate tools, which can cater to the increasing need of quick and accurate analytical techniques for discovering agents/pathogens [5].

Conventional methods for the detection and identification of microorganisms mainly rely on specific microbiological and biochemical identification. These methods can be sensitive, inexpensive and give both qualitative and quantitative information on the number and the nature of the microorganisms, but these methods have major drawbacks like labor intensive and time consuming as it takes 2-3 days for confirmation [6].

Although a complex series of tests is often required before confirming any identification. The results of such tests are often difficult to interpret and not available on the time scale desired in the clinical laboratory. Some new technologies such as, Polymerase Chain Reaction (PCR) method is extremely sensitive but requires pure samples and hours of processing and expertise in molecular biology [7,8].

Over the last decades, a great deal of research has centered on the development of biosensors for the detection of microorganisms, allowing rapid and "real-time" identification of pathogens [6]. Two commonly cited definitions for biosensor are, "a biosensor is a chemical sensing device in which a biologically derived recognition entity is coupled to a transducer, to allow the quantitative development of some complex biochemical parameter" or "biosensor is an analytical device incorporating a deliberate and intimate combination of specific biological element (that creates a recognition event) and physical element (that transduces a recognition event)" [9].

Biosensors are usually classified into various groups either by the type of transducer employed (electrochemical, optical and piezoelectric) or by the kind of bio- recognition element utilized (antibody, enzyme, nucleic acids, and whole cells). Both components of the biosensor, namely, bio-recognition element (referred as a receptor) and transduction platform (referred as transducer) play an important role in the construction of a sensitive and a specific device for the analyte of interest (referred as a target). [10].

Biosensors can have a variety of biomedical, industrial and

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military applications. Biosensors have tremendous potential for commercialization in other fields of applications such as biosensor-based instruments in food and beverage production, environmental sampling, and noninvasive instruments for clinical analysis. However, commercial adoption has been slow because of several technological difficulties. For example, due to the presence of biomolecules along with semiconductor materials and biosensors contaminations are the major issues [9] (Figure 1).

The aim of this paper is to provide information on this newly emerging technology in relation to basic known functional principles of bio recognition elements and transducers.

HISTORY

Professor Leland C Clark Jr. is the father of the biosensor concept. In 1956, Clark published his definitive paper on the oxygen electrode. Based on this experience and addressing his desire to expand the range of analytes that could be measured in the body, he made a landmark address in 1962 at a New York Academy of Sciences symposium in which he described how "to make electrochemical sensors (pH, polarographic, potentiometric or conductometric) more intelligent" by adding "enzyme transducers as membrane enclosed sandwiches" [11] (Table 1).

MAIN AREA REQUIRING PATHOGEN DETECTION

Pathogen detection is of the utmost importance primarily for health and safety reasons. The three areas of application account for over two thirds of all research in the field of pathogen detection. These are the food industry [6,12], water and environmental quality control [13-15] and clinical diagnosis [16]. The remaining efforts go into fundamental studies [17,18], method performance studies [19,20] or development of new applied methods [21,22]. Amongst the growing areas of interest, the use of rapid methods for defense applications stands out [23,24].

One of the major driving forces for the development of biosensors is biomedical diagnosis. The most popular example is glucose oxidase-based sensor used by individuals suffering from diabetes, to monitor glucose levels in blood. Biosensors have found also potential applications in the agricultural and food industries. However, very few biosensors have been commercialized [25].

Clinical diagnosis

Although biosensor development made a huge progress in recent years, their application in clinical diagnosis is not very common, except for glucose biosensors representing about 90 % of the global biosensor market. Interferences with undesired molecules during measurements with real samples and also high selectivity and accuracy are still serious issues. This is very important, since treatment is often dependent on individual levels of clinical markers. Most of the described biosensors are based on amperometric techniques [26]. Glucose concentration is one of the most monitored indicators in many diseases, such as diabetes

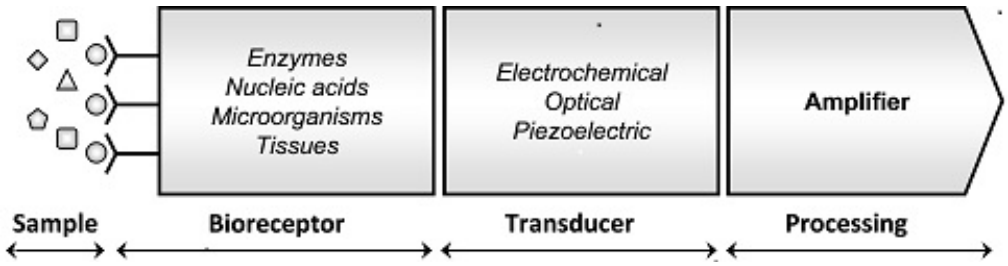

Figure 1: Representation of a typical biosensor, including the different constituent parts.

Table 1: Defining events in the history of biosensor development.

1916	First report on the immobilization of proteins; adsorption of invertase on activated charcoal.
1922	First glass pH electrode.
1956	Invention of the oxygen electrode (Clark).
1962	First description of a biosensor ; an amperometric enzyme electrode for glucose (Clark)
1969	First potentiometric biosensor; urease immobilized on an ammonia electrode to detect urea.
1970	Invention of the Ion Selective Field –Effect transistor (ISFET) (Bergveld).
1972/5	First commercial biosensor; Yellow Springs Instruments Glucose Biosensor.
1975	First microbe – based biosensor, First immunosensor; ovalbumin on a platinum wire invention of the pO2/ pCO2optode.
1976	First bedside artificial pancreas (Miles).
1980	First fiber optic PH sensor for in vivo blood gases (Peterson).
1982	First fiber optic based biosensor for glucose.
1983	First surface Plasmon resonance (SPR) immunosensor.
1984	First mediated amperometric biosensor; ferrocene used with glucose oxidase for the detection of glucose.
1987	Launch of the MedisenseExacTechTM blood glucose biosensor.
1990	Launch of the Pharmacia BiacoreSpr-based biosensor system.
1992	I – STAT launches hand-held blood analyser.
1996	Glucocard launched., Abbott acquires medisense 867$.
1998	Launch of life scans Fast take blood glucose biosensor.
1999-current	-Bio NMES, Quantum dots, Nano particles, Nano-cantilever,Nano-wire and Nanotube.

and other endocrine metabolic disorders. Blood glucose is also the most common analyte measured after electrolytes and blood gases [27].

Food industry

Biosensors for the measurement of carbohydrates, alcohols, and acids are commercially available. These instruments are mostly used in quality assurance laboratories or at best, on-line coupled to the processing line through a flow injection analysis system. Their implementation in-line is limited by the need of sterility, frequent calibration, analyte dilution, etc [25].

Potential applications of enzyme-based biosensors in food quality control include measurement of amino acids, amines, amides, heterocyclic compounds, carbohydrates, carboxylic acids, gases, cofactors, inorganic ions, alcohols, and phenols. Biosensors can be used in industries such as wine, beer, yogurt, and soft drinks producers. Immunosensors have important potential in ensuring food safety by detecting pathogenic organisms in fresh meat, poultry, or fish.

Environmental screening

In the environmental pollution, monitoring chemical analysis by itself may not provide sufficient information to assess the ecological risk of polluted waters and wastewaters [28].

Due to this, a lot of bioassays and biosensors for toxicity evaluation were developed in recent years. For example, the toxicity assays Microtox® (Azure, Bucks, UK), is based on the use of luminescent bacteria, Vibriofischeri, to measure toxicity from environmental samples. Another example is the Cellsense®, which is an amperometric sensor that incorporates Escherichia coli bacterial cells for rapid ecotoxicity analysis. It uses ferricyanide to divert electrons from the respiratory system of the immobilized bacteria of a suitable carbon electrode. The resulting current is proportional to a bacterial respiratory activity [29].

BIOSENSORS IN PATHOGEN DETECTION

Biosensing methods for pathogen detection are centered on four basic physiological or genetic properties of microorganisms: metabolic patterns of substrate utilization, phenotypic expression analysis of signature molecules by antibodies, nucleic acid analysis and the analysis of the interaction of pathogens with eukaryotic cells [30].

Many of today's popular commercially available rapid methods use culture-based methods coupled with automated or semi-automated nucleic acids, antibody, or substrate utilization-based methods to obtain results in 24-72 h. Interestingly, many of the modern day biosensor based methods are developed, utilizing one of the above four principles or combinations of some sort. However, antibody-based methods are the most popular because of their versatility, convenience and relative ease in interpretation of the data. It is interesting to note that a majority of biosensors use antibody for capture and detection of the target analyte [31]. The advantage of the biosensor is that it offers higher specific and sensitive results in quick time. Further, it can be used for a broad spectrum of analytes and in complex sample matrices (like blood, serum, urine or food) with minimum sample pre-treatment [32].

Biosensors for bacterial detection generally involve a biological recognition component such as receptors, nucleic acids, or antibodies in intimate contact with an appropriate transducer. Depending on the method of signal transduction, biosensors may be divided into three basic groups, namely optical, mass, and electrochemical (Figure 2).

CLASSFICATION OF BIOSENSORS

Bio-receptors

Bio-receptors or the biological recognition elements are the key to specificity for biosensor technologies. A bio-receptor is a molecular species that utilizes a biochemical mechanism for recognition. They are responsible for binding the analyte of interest to the sensor for the measurement. Bio-receptors are classified into five different major categories. These categories include antibody/antigen, enzymes, nucleic acids/DNA, cellular structures/cells, bio-mimetic and bacteriophage (phage). The enzymes, antibodies and nucleic acids are the main classes of bio-receptors, which are widely used in biosensor applications [33].

Antibody Bioreceptors

An antibody is a complex biomolecule, made up of hundreds of individual amino acids arranged in a highly ordered sequence. Antibodies are biological molecules that exhibit very specific binding capabilities for specific structures. The way in which an antigen and its antigen-specific antibody interact may be understood as analogous to a lock and key fit, by which specific geometrical configurations of a unique key enables it to open a lock. In the same way, an antigen specific antibody "fits" its unique antigen in a highly specific manner. This unique property of antibodies is the key to their usefulness in immunosensors [34].

Enzyme Bioreceptors

The biosensors that use enzymes as the biorecognition elements are well-developed and widely studied area. These enzymes are chosen based on their specific binding capability and their catalytic activity. The chosen enzyme with a suitable substrate should provide sufficient electron transfer to the working electrode [35].

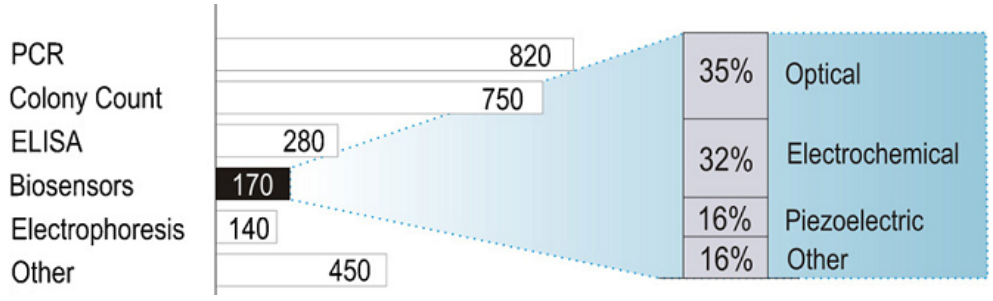

Figure 2: Biosensor technology ranks fourth in the area of pathogen detection.

In the field of pathogen detection, using enzymes as bio-receptors provides biosensors with a high degree of specificity. Additionally, their catalytic activity can amplify the sensitivity of the assay. However, there are some disadvantages found when using enzymes as labels, which include multiple assay steps and the possibility of interference from endogenous enzymes [36].

Biosensor utilizing enzymes as bio-recognition receptor have been developed successfully for the detection of pathogenic bacteria's such as L. monocytogenes, E. coli and C. jejuni in food samples [33].

Nucleic acids

In this type biosensor, the bio-recognition mechanism involves hybridization of Deoxyribonucleic Acid (DNA) or ribonucleic acid with the target analyte forms the basis for the detection. These types of biosensor are also referred as genosensors [37].

Biosensors based on DNA, RNA and peptide nucleic acid gain their high sensitivity and selectivity from the very strong base pair affinity between complementary sections of lined up nucleotide strands [1].

Nowadays, mainly synthetic Oligode-Oxyribonucleotides (ODNs) are being used as probes in the DNA hybridization sensors [38]. The electrochemical DNA biosensors convert the base pairing mechanism into a measurable electrical signal. This makes this type of biosensor a suitable candidate for the rapid and inexpensive diagnosis of genetic disease and for the compatibility with micro-fabrication technology [39]. DNA based biosensors are being used for the determination of the level of drug in blood serum matrix [40], detection of the DNA damage and detection of cancerous cells [38,41-43].

Cellular structures/cells

Cellular structures and cells comprise a broad category of bio-receptors that are being used in the development of biosensors and biochips. These bio-receptors are either based on bio-recognition by an entire cell/microorganism or a specific cellular component that is capable of specific binding to certain species [44].

There are presently three major subclasses of this category: 1) cellular systems, 2) enzymes and 3) non-enzymatic proteins. Due to the importance and large number of biosensors based on enzymes, they have been given their own classification and were previously discussed. One of the major benefits associated with using this class of bio-receptors is that, the detection limits can be very low because of signal amplification. Many biosensors developed with these types of bio-receptors rely on their catalytic or pseudo-catalytic properties [45].

Microorganisms such as bacteria and fungi are being used as indicators of toxicity or for the measurement of specific substances. For example, cell metabolism (e.g. growth inhibition, cell viability, substrate uptake), cell respiration and bacterial bioluminescence were used as to evaluate the effects of toxic heavy metals [46].

Many cell organelles can be isolated and used as bio-receptors. Since cell organelles are closed systems, they can be used over a long period. Whole mammalian tissue slices or in vitro cultured mammalian cells are also used as biosensing elements in bio-receptors [47].

Bilitewski and coworkers have developed a microbial biosensor for the monitoring of short chain fatty acids in milk. Arthrobacter nicotianae microorganisms were immobilized in a calcium alginate gel on an electrode surface. By monitoring the oxygen consumption of the Arthrobacternicotianae electrochemically, its respiratory activity was monitored, thereby providing an indirect means of monitoring fatty acid consumption (Figure 3).

Bio-mimetic Receptors

Receptors that are being fabricated and designed to mimic a bio-receptor (antibody, enzyme, cell or nucleic acids) are often termed a biomimetic receptor. Though there are several methods, such as genetically engineered molecules and artificial membrane fabrication, the molecular imprinting technique has emerged as an attractive and highly accepted tool for the development of artificial recognition agents. Molecular imprinted polymers (MIPs) can, in principle, be synthesized for any analyte molecule and are capable of binding target molecules with affinities and specificities on par with biological recognition elements [48].

One of the major advantages of the molecular imprinting technique is the rugged nature of a polymer relative to a biological sample. The molecularly imprinted polymer can withstand harsh environments such as those experienced in an autoclave or chemicals that would denature a protein. On the other hand, due to their rigid structures, molecular imprint probes do not have the same flexibility and selectivity as compared to actual bio-receptors [49,50].

However, MIPs possess many disadvantages as well. For example, it is hard to remove the template from MIPs, the imprinted polymer is insoluble, and although the polymer contains many imprinted cavities, only some are good and match the template molecule [51].

Bacteriophages

From the past decades to date, enzymes, antibodies, nucleic acids and bio-mimetic materials are being used as bio-molecular recognition elements. They have both merits and demerits when compared to one another. Recently, bacteriophages are being employed as biorecognition elements for the identification of various pathogenic microorganisms. These powerful bacteriophages (phages) are viruses that bind to specific receptors on the bacterial surface in order to inject their genetic material inside the bacteria [52].

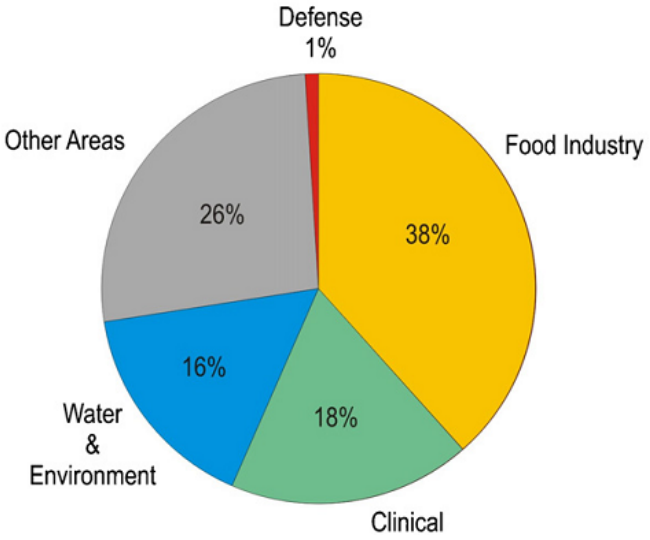

Figure 3: The areas of interest for pathogen detection.

Phages recognize the bacterial receptors through its tail spike proteins. Since the recognition is highly specific, it is used for the typing of bacteria and opened the path for the development of specific pathogen detection technologies. Researchers have reported the application of phage as a biorecognition element for the detection of various pathogens such as E. coli [53], S. aureus [54] and B. anthracisspores [55,56] by using different sensing platforms.

The ability to distinguish between live and dead bacteria is the biggest advantage of reporter phages. Since the phages will be unable to infect and express the reporter gene in dead bacteria. Reporter phages, however, suffer from limitations such as phage multiplication inhibition (due to prophage presence), DNA restriction-modification system, presence of specific phage inhibition genes and antiviral bacterial immunity system [57].

Biotransducers

A biotransducer converts the biochemical signal to an electronic signal. It gives the biosensor selectivity and specificity. Typically, biotransducers consist of two main parts: a biorecognition layer and a physico-transducer [58].

The biorecognition layer typically contains an enzyme or another binding protein such as antibody. A physico-transducer has contact with the recognition layer. A physico-chemical change that produced by the recognition layer due to the analyte, is measured by the transducer [59].

Biosensors can be classified according to the transduction methods they utilize [60]. The transduction methods such as optical, electrochemical and mass based are given importance here since they are the most popular and common methods [33].

Optical-based biosensors

Optical biosensors have received considerable interest for the bacterial pathogen detection due to their sensitivity and selectivity. The most commonly employed techniques of optical detection are surface plasmon resonance due to their sensitivity.

The output transduced signal that is measured is light. The biosensor can be based on fluorescence or optical diffraction. Fluorescence is often used for biosensing due to its selectivity and sensitivity. A fluorescence-based device detects the change in frequency of electromagnetic radiation emission. Single molecules may be repeatedly excited to produce a bright signal, which can be measured even at the single cell level [60].

Surface Plasmon Resonance (SPR)

In a typical SPR biosensing experiment, a ligand or biomolecule will be immobilized on an SPR-active gold-coated glass slide, which forms a thin flow-cell. The other interactant in an aqueous buffer solution is induced to flow across this surface, by injecting it through this flow-cell. When light (visible or near infrared) is shined through the glass slide and onto the gold surface at angles and wavelengths near the so-called "surface plasmon resonance" condition, the optical reflectivity of the gold changes very sensitively with the presence of biomolecules on the gold surface or in a thin coating on the gold [61].

The extent of binding between the solution-phase interactant and the immobilized interactant can be easily observed and quantified by monitoring this reflectivity change. An advantage of SPR is its high sensitivity without any fluorescent or other labeling of the interactants [62].

Bioluminсent Sensor

Recent advances in bioanalytical sensors have led to the utilization of the ability of certain enzymes to emit photons as a byproduct of their reactions. This phenomenon is known as bioluminescence. The potential applications of bioluminescence for bacterial detection were initiated by the development of luciferase reporter phages [63,64].

The genes encoding luciferase will be introduced into the genome of a bacterial virus (bacteriophage). If this virus infects a host bacterium, a bioluminescent phenotype can be conferred to previously nonbioluminescent bacteria.

Bioluminescence systems have been used for detection of a wide range of microorganisms [65,66]. The TM4 bacteriophage to detect Mycobacterium avuim and Mycobacterium paratuberculosis, however, a concentration of 104 cells was required to produce a detectable luciferase signal and the response declined after 2h [67].

Electrochemical biosensors

Electrochemical-based detection methods are another possible means of transduction that has been used for identification and quantification of food borne pathogens. Electrochemical biosensors can be classified into amperometric, potentiometric, impedimetric and conductometric based on the observed parameters such as current, potential, impedance and conductance respectively [68].

Although the electrochemical detection has several advantages like low cost, ability to work with turbid samples and easy miniaturization, their sensitivity and selectivity are slightly limited when compared to optical detection. However, electrochemical biosensors have been found coupled with other biosensing techniques for enhanced detection [33].

This technique is very complementary to optical detection methods such as fluorescence. Since many analytes of interest are not strongly fluorescent and tagging a molecule with a fluorescent label as often labor intensive, electrochemical transduction can be very useful [50].

Amperometric sensor

Amperometry is the most widely used technique in electrochemical microbial biosensors. Amperometric microbial biosensors have been extensively exploited for environmental applications [69,70].

Potentiometric sensor

The application of these devices in the area of biosensors is reasonably new [71]. Their use is not spreading as quickly as other electrochemical techniques due to problems related to production, which include incompatibility of most biomolecule immobilization, poor detection limits, linear range and reproducibility and inadequate device stability [72,73].

Potentiometric microbial biosensors detect the number of analytes by measuring the potential difference between the working electrode and the reference electrode separated by a selective membrane. Recently, a potentiometric biosensor based on the pH electrode modified by permeabilized P. aeruginosa was developed

for selective and rapid detection of the cephalosporin group of antibiotics [74].

Piezoelectric sensor

The Piezoelectric effect is an effect in which energy is converted between mechanical and electrical forms. Specifically, when a pressure ('piezo' means pressure in Greek) is applied to a polarized crystal, the resulting mechanical deformation results in an electrical charge [75].

Piezoelectric microphones serve as a good example of this phenomenon. Microphones turn an acoustic pressure into a voltage. Alternatively, when an electrical charge is applied to a polarized crystal, the crystal undergoes a mechanical deformation, which can in turn create an acoustic pressure [76] (Figure 4).

Quartz Crystal Microbalance (QCM)

In these devices, living cells are attached to the gold surface of the quartz crystal and serve as the sensing element, where cellular mass and viscoelastic properties affect the oscillation frequency of the crystal. Cells as the sensing element have the advantage that they possess a wide range of intelligent system properties resulting from the interplay of their integral membrane receptor-cytoskeletal-nuclear membrane systems to alter their mass distribution or viscoelastic properties due to external (i.e. cytokines, chemotactic agents, toxins, pathogens, chemical signals, pH or ionic alterations) or internal (i.e. DNA damage, mitochondria activity, cell polarity, new gene expression) signals [77].

The bottom diagram illustrates how the frequency of the oscillating sensor crystal (gold) changes when the mass is increased by the addition of a molecular layer. Here antibodies (line) are added to a layer of protein (dots) (Figure 5).

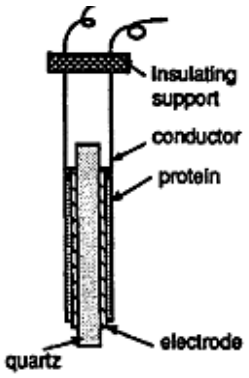

Figure 4: Piezoelectric Biosensor.

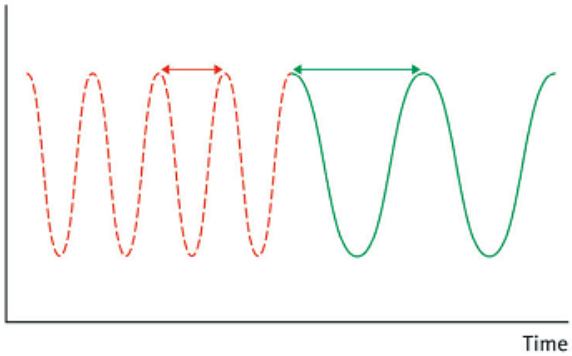

Figure 5: The diagram illustrates how the frequency of the oscillating sensor crystal (gold) changes.

Other biosensors

Electronic nose

The greatest advantage of using E - nose is that it can be calibrated and can give objective data for important functions like quality and safety control. These instruments can also test samples that are unfit for human consumption [78].

The disadvantage of the E -nose is that, the environment including temperature and humidity affects them. This can cause sensor drift and/or chemical interactions to occur when the volatile compounds flow over the sensor [78-81].

The ideal sensors to be integrated in an electronic nose should fulfill the following criteria (79,80,81): (i) high sensitivity towards chemical compounds; (ii) low sensitivity towards humidity and temperature;(iii) selectivity to respond to different compounds present in the headspace of the sample; (iv) high stability; (v) high reproducibility and reliability; (vi) short reaction and recovery time; (vii) durability; (viii) easy calibration and (ix) easy to process data output [82].

Unlike other analytical instruments, these devices allow the identification of mixtures of organic samples as a whole (identifiable to a source that released the mixture) without having to identify individual chemical species within the sample mixture. Hundreds of different prototypes of artificial nose devices have been developed to discriminate complex vapor mixtures containing many different types of Volatile Organic Compounds (VOCs). Identification and classification of an analyte mixture is accomplished through recognition of this unique aroma signature (electronic fingerprint) of collective sensor responses. The identity of a simple or complex mixture represented by a unique aroma signature pattern may be determined without having to separate the mixture into its individual components prior to or during analysis. A reference library of digital aroma signature patterns for known samples is constructed prior to analysis of unknowns. Identification of unknowns is based on the distribution of aroma attributes or elements that the analyte pattern has in common with patterns present in databases of the reference library [83].

The development of an 'electronic nose' for pathogen detection has received considerable attention in recent years. Balasubramanian et al. [84] used a commercially available Cyranose-320™ electronic nose system to identify S. Typhimurium in inoculated beef samples.

Surface Enhanced Raman Spectroscopy (SERS)

Raman signals are inherently weak, especially when using visible light excitation and so a low number of scattered photons are available for detection. One method to amplify weak Raman signals is to employ Surface-Enhanced Raman Scattering (SERS). SERS uses nano scale roughened metal surfaces typically made of gold (Au) or silver (Ag). Laser excitation of these roughened metal nanostructures resonantly drives the surface charges creating a highly localized (plasmonic) light field. When a molecule is absorbed or lies close to the enhanced field at the surface, a large enhancement in the Raman signal can be observed [85].

Hardly the biological sample has been installed in the device that the result appears on the screen in the form of characteristic lines. By comparing a baseline data already in progress, the technician can not only detect the presence of a virus, but also identify whether a seed is already known, like influenza HIV (AIDS virus),

RSV (Respiratory Syncytial virus), H5N1 (avian influenza) or swine influenza (H1N1) [86].

IMMOBILIZATION OF BIOLOGICAL RECEPTORS

The three most frequent antibody immobilization routes, (i) Adsorption on gold, (ii) The Avidin–biotin system, (iii) Self-Assembled Mono Layers (SAMs).The biomolecule immobilization step is critical in the development of any sort of biosensor. It provides the core of the biosensor and gives it its identity. Moreover, the immobilized biomolecule needs to keep its original functionality as far as possible in order for the biosensor to work. This means that care must be taken so that the recognition sites are not sterically hindered. Another common reason for biosensor failure or under performance is the chemical inactivation of the active/recognition sites during the immobilization stages. There is no universal immobilization method suitable for every application imaginable. When it comes to choosing the immobilization method, there are other important factors that need careful consideration, e.g., the type of transduction used, the nature and composition of the sample and the possibility of multiple use of the biosensor [87].

The most commonly used immobilization techniques for construction of biosensors are physical adsorption, covalent binding, matrix entrapment, inter molecular cross-linking and membrane entrapment [88-90].

1. Adsorption: The physical adsorption utilizes a combination of Van der Waals and hydrophobic forces, hydrogen bonds, and ionic forces to attach the biomaterial to the surface of the sensor. Many substrates such as cellulose, silica gel, glass, hydroxyl apatite and collagen are well known to adsorb bio-components. This method is very simple, however, employed forces are not very strong and biomolecules attached by this method may be released or not persist [91].

2. Covalent binding: The sensor surface can be modified to acquire a reactive group to which the biological materials can be attached. This method improves uniformity, density and distribution of the bioelements, as well as reproducibility and homogeneity of the surfaces [92].

3. Matrix entrapment: In this case biomolecules are trapped within the polymeric gel matrix. For this method the polyacrylamide, starch, alginate, pectate, polyvinyl alcohol, polyvinyl chloride, polycarbonate, polyacrylamide, cellulose acetate and silica gel are often be used [93].

4. Cross-linking: For intermolecular cross linking of biomolecules bifunctional or multi-functional reagents such as glutaraldehyde, hexamethylene di-isocyanate, 1,5-difluoro 2,4-dinitrobenzene and bisdiazobenzidine-2,2'-disulphonic acid, etc., are used. The most common cross-linking agent in biosensor applications is glutaraldehyde, which couples with the lysine amino groups of enzymes [94].

5. Encapsulation: In this method, a porous encapsulation matrix (e.g. lipid bilayers) is formed around the biological material and helps in binding it to the sensor. Other approach for encapsulation uses sol-gel method for the immobilization of biological molecules in ceramics, glasses, and other inorganic materials [95-97] (Figure 6).

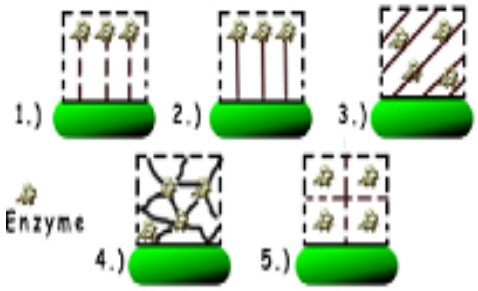

Figure 6: Methods used for immobilization of enzymes and other bioreceptors in biosensors. 1.) Adsorption, 2.) Covalent binding, 3.) Matrix entrapment, 4.) Cross-linking, 5.) Encapsulation.

CONCLUSION AND FUTURE PERSPECTIVE

Though conventional pathogen detection methods are sensitive, they lag behind the analytical methods by detection time. The Biosensor will replace all the other conventional and molecular method of diagnosis in the near future. However, certain biosensors like optical and electrochemical detection have some disadvantages as well, considering the sensitivity and cost.

Optical techniques possibly provide better sensitivity relative to electrochemical detection, but they are expensive and complicated. In contrast, electrochemical techniques involve much simple procedures, but for the detection of pathogens, it requires enhanced performance.

Nevertheless, commercial microbial biosensors are just the tips of the iceberg compared to the great amount of academic research on them. The intrinsic disadvantages (slow response, low sensitivity, and poor selectivity) using microorganisms as the biosensing element limit the widespread interest of microbial biosensors on the market. Fortunately, with the development of biotechnology, micro/nanotechnology, and novel immobilization strategy in the past years, microbial biosensors are becoming more powerful in the field of analytical chemistry.

Microbial biosensors typically suffer from the poor selectivity because of the nonspecific cellular response to substrates. With the development of biotechnology and the availability of genome sequence for more microorganisms, selectivity to specific targets can be increased. Another way to improve the selectivity of microbial biosensors is to develop microbial sensor arrays.

In spite of some disadvantages and difficulty in developing, biosensor has several advantages compared to other conventional and molecular techniques. It also opens new areas for research and exploitation in near future. Lot of researches were done in biosensor, in case of medical field especially in food microbiology. However, it is not well studied or exploited in the case of veterinary field.

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