

Review Article

Role of Biosensors in the Field of Veterinary Practice

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Abstract

The early and on-site detection of pathogen is of utmost important as far as rapid diagnosis of disease is concerned. We also need to be prepared for the re-emerging diseases of livestock. The conventional methods of pathogen detection like immunoassays, PCR needs to be replaced with a very accurate, highly specific, on the door, rapid methods as the biosensors does. A lot of research work is being carried out all over the world to develop various biosensor techniques for early detection of veterinary pathogens. The success of this technique lies in their validation and commercialization. It can be a great aid to the veterinarian and scientist to diagnose and control the outbreaks. However, many hurdles still need to be cleared before transferring this technology from the laboratory to the field.

Keywords: Biosensor; Bioreceptors; Transducers; Pathogens.

Introduction

A biosensor is an analytical device with a specific bio-recognition element (enzymes, hormones, nucleic acid, and cells) immobilized on the surface of a sensor which is connected to a transducer that transmits and interprets the signal. Biosensors comprises mainly of two components: a biorecognition element or a bioreceptor which recognize the desired analyte and a transducer yielding a digital electronic signal which is proportional to the concentration of a specific analyte.

Conventional methodologies for pathogen detection require specialized technical staff, equipment, and is arduous, time taking and costly. There is lack of uniformity and lack the convenience of on-site testing. By the time a diagnosis is made, the disease condition may aggravate affecting more animals and even death may occur. Although molecular methods of pathogen detection have better

sensitivities and rapid than antibody based assays but these methods require skilled personals and equipment, and are costly (Arora et al., 2006).

Therefore we need a cost effective, less time consuming, highly specific analytical tools for the rapid disease diagnosis and the Biosensors fits into that. Biosensors are becoming important in a broad range of analysis. Miniaturisation, reduced cost and the enhanced processing power increased the analytical capabilities of such devices. Biosensors are highly specific, easy to use, require only small sample volume, rapid, accurate, stable and capable of yielding onsite results.

Basic characteristics (Stoytcheva, M et al 2009) of a biosensor includes linearity (linearity of the sensor should be high for the detection of high substrate concentration), sensitivity (value of the electrode response per substrate concentration), selectivity (chemicals interference must be minimized for obtaining the correct result) and the response time (time necessary for having 95% of the response). The

first description of a biosensor was made in 1962 by Clarke which was an amperometric enzyme electrode for glucose.

Classification of Biosensors

Biosensors are mainly classified based on the basis of bioreceptors and the transducers.

Bioreceptors

A bioreceptor is a molecular species that exploits a biochemical mechanism for recognition (Sharma H et al 2013). Biorecognition elements have recognition properties, which can be applied to produce either an affinity or a catalytic sensor. A diverse range of molecules such as nucleic acids, enzymes, antibodies, cell receptors etc. can be used as the sensing element in biosensors (Ibtisam E. Tothill, 2001). Bioreceptors are accountable for binding the concerned analyte to the sensor for measurement. In catalytic sensors, the change in the concentration of a component resulting from the catalysed reaction is detected to yield a signal. The binding event between the receptor and the desired analyte is monitored, in an affinity sensor. Bioreceptors can broadly be classified into following distinct classes:

Antibody-Antigen Bioreceptor

Affinity sensors use mainly antibody-antigen binding reactions. Antibodies probes can be polyclonal, monoclonal or recombinant. Antibodies are the most popular class of biorecognition probes because of their high binding affinity and target specificity.

Enzymatic Bioreceptor

Enzymes are used extensively in biosensors as the catalytic component; the most important group has been the oxido-reductases.

Nucleic Acids (DNA) Bioreceptor or Genosensors

It integrates an oligonucleotide with a signal transducer. The DNA probe is immobilized on the transducer and acts as a biorecognition molecule to detect DNA/RNA fragments. DNA biosensors are currently used in the detection of infectious diseases and the genetic abnormalities.

Cellular Structures or Cellular Bioreceptor

Whole cells such as bacteria, yeast, fungi, plant

and animal cells have also been used as the bioreceptors by integrating their general metabolic status. This usually involves detecting oxygen or substrate consumption, the production of carbon dioxide or metabolites, detection of bacterial luminescence (Tothill and Turner, 1996).

Biomimetic Bioreceptor

Biomolecules are poorly stable. Various methods are being tried to improve the stability of these molecules such as by using diethyl amino ethyl (DEAE) dextran, lactic acid, and sugar derivatives or artificial receptors or biomimics like molecularly imprinted polymers (MIP).

Transducers

A transducer converts the biorecognition event into a measurable signal. They are mainly classified as electrochemical, optical, mass based and calorimetric with the first three being the most commonly employed and universal for pathogen detection.

Electrochemical Biosensors

It measures the change in electrical properties following biorecognition, as a result of change in ion concentration during a reaction. Electrochemical biosensors are further classified into amperometric, potentiometric and impedimetric or conductometric.

Amperometric biosensors measure the generated current at a constant voltage and are the most commonly used class of electrochemical biosensors. Potentiometric biosensors measure difference in voltage at zero current. Impedimetric or conductometric biosensors function by measuring the change in electrical resistance/conductance of the solution.

Optical Biosensors

It measure changes in intensity of light. Detection elements in such biosensors are frequently based on luminescence, fluorescence, phosphorescence, colorimetry, reflectance, interference, spectroscopy, and surface plasmon resonance (SPR). Fluorescence and SPR-based biosensors are most commonly used. SPR biosensors are used for monitoring biological interactions and for detection of small, medium and large analyte.

Mass-Based Biosensors

It detects a change in mass that occurs following

the interaction between the biorecognition element and the target analyte. The change in frequency is proportional to the mass of absorbed material. It generally uses piezoelectric materials that change their resonant frequency, following the change in mass, generating acoustic waves. The most commonly used piezoelectric biosensors make use of Quartz Crystal Microbalance (QCM). QCM was used for detection of *Candida albicans*. (Muramatsu et al 1986).

Role of Biosensors in Diagnosis of Diseases of Livestock and Poultry

A number of antibody based biosensors have been developed for detection of viral pathogens of veterinary significance like Avian influenza virus (AIV) subtype H5N1, Bovine viral diarrhoea virus (BVDV), Rabies virus, Swine origin influenza virus (S-OIV) subtype H1N1 by using electrochemical and optical transducers and Duck hepatitis virus serotype1 (DHV1), Foot and mouth disease virus (FMDV), Infectious bursal disease virus (IBDV), Porcine Rotavirus using optical transducers, Cocksackie virus B4 using mass based transducers (Ayyar et al 2013). Biosensors are used for the detection of *E. Coli* O157:H7 in food samples (Li D et al 2011). A cell based biosensor technique was used to detect various numbers of pathogens and toxins (Banerjee, P et al 2011). Biosensor technique for *Salmonella* detection was developed by Seo et al. (1999). Ye et al. (1997) described a piezoelectric biosensor for detection of *Salmonella typhimurium*. Piezoelectric immunosensors were developed for detection of *Vibrio cholera*, *Candida albicans*, *Salmonella typhimurium*, *L. monocytogenes*.

Other Applications

An immuno-affinity fluorimetric biosensor was developed for detecting and quantifying Aflatoxins. Biosensor has also been used for detection of aflatoxin in milk samples (Parker, C.O et al 2009). Biosensors can be used for the quality control of milk and meat. A multi-enzymatic amperometric biosensor for estimation of lactose in fresh raw milk was developed by Eshkenazi et al. (2000). This method may be used as a cheaper on-line lactose measurement technique in the milking parlour. An amperometric glucose sensor named meatcheck has been successful commercialized. The meatcheck is a four-electrode array attached to a knife, which is inserted into meat to measure the glucose gradient immediately below the surface. The extent of the gradient is related to microbial activity on the surface

of the meat and is regarded as a sound indicator of meat quality. This device provides results in seconds whereas laboratory based microbiological tests takes days. Concentration of lactic acid is an important parameter for the meat industry as it indicates the state of fresh meat. Bergann et al. (1999) reported an enzymatic biosensor based on immobilised lactatoxidase as bioreceptor and an amperometric transducer. The biosensor estimates lactic acid without special sample preparation, very quickly and at low cost. A disposable screen printed amperometric progesterone biosensor was developed by Pemberton et al. (1998) for the detection of estrous cycle. The basic principle is the reduction in the binding of alkaline phosphatase labelled progesterone to the sensor surface in the presence of endogenous milk progesterone. Similar effort was made (Claycomb et al. 1998) for estrous detection by using optical transducer. Setford et al. (1999) developed a field based screening method using amperometric biosensor for detection of beta lactams in milk.

A surface plasmon resonance biosensor (SPR) was used for detection of sulfamethazine enrofloxacin and its metabolite, ciprofloxacin residues in milk in milk (Mellgren et al., 1996). SPR developed by the Pharmacia BIA core indicated the occurrence of less than 0.9 mg of sulfamethazine per kg of milk with the advantages of freedom from sample preparation, high sensitivity, rapid and full analysis in real time for the control of residues and contaminants in food (Maria et al 2003). The drug residue of salbutamol was analysed in the urine samples of calves using SPR (Elliot et al 1998).

Conclusion

Biosensors can be proved as an efficient analytical tool to the animal disease diagnosis, quality control of meat, milk. This review summarizes the present developments in the field of veterinary science. Since the inception of biosensors some fifty years back, its commercialization in the field of pathogen detection is in its nascent stage. The main reason being the lack of sensitivity, stability and applicability to unprocessed samples. Stability has been a concern while working with the antibody-based biosensors which mainly uses polyclonal or monoclonal antibodies. Lots of lab work has been done on development of biosensors for detection of veterinary viral pathogens but hardly any find its market value and it can be concluded that biosensing for veterinary pathogens is still a distant dream. As biosensors is a fast, simple, have an on-site

application and cost efficient technique, it has an evident advantages compared to traditional analytical techniques and therefore future hold great promises provided all the concerns are fully addressed.

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