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Biosensors: A Novel Approach for Pathogen Detection

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The detection of pathogenic bacteria is the key in prevention and identification of problems related to health and safety. Biosensors are analytical devices that allow the detection of biomolecules in a label-free, real-time, specific, highly sensitive format and can essentially serve as low-cost and highly efficient devices for diagnosis of pathogenic diseases. Different types of biosensor are being employed for detection of pathogenic microbes. Biosensors attach biological materials to a physiochemical transducer. The transducer is a device that produces the optical, calorimetric, acoustic, electrochemical, or magnetic signal of interaction between specific interaction partners such as target analyte and the immobilized biological materials. Immobilized layer produces a physicochemical change that is detected by the transducer. The transducer then yields a digital electronic signal proportional to the concentration of a specific analyte. Different types of biosensor are being employed for detection of pathogenic microbes. Diagnosis of pathogens by biosensors may become more popular than the standard methods, although achieving high-throughput and sensitivity are still important challenges.

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INTRODUCTION

Bacteria, viruses and other microorganisms are found widely in nature and environment. Worldwide, infectious diseases account for nearly 40 % of total 50 million estimated deaths annually. Microbial diseases constitute the major cause of deaths in developing countries. The diseases caused by bacterial agents are severe and highly fatal in nature. The development of rapid, accurate, and sensitive diagnostic methods for detecting these pathogens is the basis for treating, controlling, and eradicating infectious diseases of veterinary importance. Scientific and technological advancements have revolutionized the field of veterinary diagnostics. Genome sequencing has allowed efficient, sensitive, and specific diagnostic assays to be developed based on the detection of nucleic acids. The integration of advances in biochemistry, proteomics, engineering, and medicine offers enormous potential for the rapid and accurate diagnosis of viral, microbial, genetic, and metabolic disease. Detection and identification of these harmful organisms in a cost and time effective way is a challenge for the researchers. The future of detection methods for microorganisms shall be guided by biosensor, which has already contributed enormously in sensing and detection technology. Biosensors as an approach for detection of pathogen have got greater attention from researchers and agencies controlling the spread of disease at hospital, field and point of attack.

Biosensors are analytical devices that allow the detection of biomolecules in a label-free, real-time, specific, highly sensitive format. Biosensors attach biological materials such as cell receptors, antibodies, micro-organisms, organelles and tissues, to a physiochemical transducer. The transducer is a device that produces the optical, calorimetric, acoustic, electrochemical, or magnetic signal of interaction between specific interaction partners such as target analyte and the immobilized biological materials. Immobilized layer produces a physicochemical change that is detected by the transducer. The transducer then yields a digital electronic signal proportional to the concentration of a specific analyte or group of analytes. Label-free detection methods, which rely on optical, acoustic, and other types of biosensors, may improve on the standard methods, although achieving high-throughput and sensitivity are still important challenges.

Detection is not in natural proteins but with altered one. Disadvantages include lack of sensitivity, interference and cross-reactivity. Efficiency of labeling varies from protein to protein, making comparison a challenge. In addition, attaching fluorophores may influence the way in which proteins bind to other molecules and cause background signals.

TYPES OF BIOSENSORS

Optical biosensors

Optical transducers are particularly attractive for application to direct (label-free) detection of bacteria. These sensors are able to detect minute changes in the refractive index or thickness which occur when cells bind to receptors immobilized on the transducer surface. They correlate changes in concentration, mass or number of molecules to direct changes in characteristics of light. Several optical techniques have been reported for detection of bacterial pathogens including: monomode dielectric waveguides, surface plasmon resonance, ellipsometry, the resonant mirror and the interferometer etc. It is used for direct detection of Staphylococcus aureus by resonant mirror (Watts et al, 1994) and *Salmonella typhmurium* by evanescent wave interferometer (Schneider et al, 1997). Indirect detection of *Escherichia coli* O157:H7 by flourescent labeled antibody method (Pyle et al, 1995) and *Salmonella typhmurium* by immuno-magnetic assay system(Bruno, 1996)

Surface plasmon resonance (SPR) biosensor

This is an evanescent field based optical sensors using thin gold film for sensing applications. The interaction between analyte flowing over immobilized interactant on gold surface is probed through the detection of reflection minima on photo-detector array sensors. The SPR biosensors are versatile tool for monitoring biomolecular interactions and for detection of small, medium and large analyte.

Piezoelectric biosensors

Piezoelectric (PZ) biosensor offers a real-time output, simplicity of use and cost effectiveness. The general idea is based on coating the surface of the PZ sensor with a selectively binding substance, for example, antibodies to bacteria, and then placing it in a solution containing bacteria. The bacteria will bind to the antibodies and the mass of the crystal will increase while the resonance frequency of oscillation will decrease proportionally. PZ immunosensors were developed for Listeria monocytogenes and members of the Enterobacteriaceae family (Plomer et al, 1992) etc. In the immunogravimetric microbial assay (Muramatsu et al, 1986), a PZ crystal coated with anti-*C. albicans* antibody was used for the detection of *C. albicans* concentrations in the range of 106–108 cells/ml. The sensor showed no response to other yeast species, and frequency shifts due to nonspecific adsorption were not significant.

Quartz Crystal Microbalance as Biosensor

It is a type of PZ biosensor. The status of the quartz crystal microbalance (QCM) technique for applications in biochemistry, food, environmental and clinical analysis is of a biosensor technique having capability similar to SPR in terms of sensitivity and selectivity, but reproducibility and stability is yet to be at SPR level. It has been developed for detection of *C. albicans* by

Muramatsu et al, *E. coli* by Plomer et al, and Salmonella by Koenig and Gratzel.

Electrical impedance biosensors

Microbial metabolism usually results in an increase in both conductance and capacitance, causing a decrease in impedance. Impedance usually is measured by a bridge circuit. Often a reference module is included to measure and exclude nonspecific changes in the test module. This method is well suited for detection of bacteria in clinical specimens, to monitor quality and detect specific food pathogens, also for industrial microbial process control, and for sanitation microbiology (Silley and Forsythe, 1996). This technique has been used for estimating microbial biomass, for detecting microbial metabolism and for detecting the concentration and physiological state of bacteria. Viable cells are commonly measured microscopically after suspending the cells in a dye such as Trypan Blue. Bulk acoustic wave impedance sensor has been developed for *Proteus vulgaris* by Deng et al.

Amperometric Biosensors

Amperometric biosensors produce a current proportional to the concentration of the substance to be detected. The most common amperometric biosensors use the Clark Oxygen electrode. Amperometric biosensors have been developed for indirect detection of *E. coli* by Nakamura et al. Brooks et al, developed amperometric biosensor for Salmonella detection.

Thermal Biosensors

This type of biosensor is exploiting one of the fundamental properties of biological reactions, namely absorption or production of heat, which in turn changes the temperature of the medium in which the reaction takes place. They are constructed by combining immobilized enzyme molecules with temperature sensors. When the analyte comes in contact with the enzyme, the heat reaction of the enzyme is measured and is calibrated against the analyte concentration. Common applications of this type of biosensor include the detection of pesticides and pathogenic bacteria.

Potentiometric Biosensors

In this type of sensor the measured parameter is oxidation or reduction potential of an electrochemical reaction. The working principle relies on the fact that when a voltage is applied to an electrode in solution, a current flow occurs because of electrochemical reactions. The voltage at which these reactions occur indicates a particular reaction and particular species. Light-addressable potentiometric sensor array have been developed for *Neisseria meningitidis*, *Brucella melitensis* (Lee et al, 1993) and *Francisella tularensis* (Thompson and Lee, 1992)

Nucleic Acid-based Biosensors or Genosensors

A nucleic acid biosensor is an analytical device that integrates an oligonucleotide with a signal transducer. The nucleic acid probe is immobilized on the transducer and acts as the bio-recognition molecule to detect DNA/RNA fragments. Several DNA based biosensors have recently been developed for the detection of virus-related sequences and other infectious agents. DNA or RNA ligands can bind to their targets with affinity ranging from the micromolar to the nanomolar level and can discriminate between closely related targets. Nucleic acid hybridization based biosensor schemes are being developed for pathogens such as *E. coli* and *Mycobacterium tuberculosis* (Wang et al, 1997).

Flow cell based displacement fluorescent biosensor

With the advent of pre-coated crystals, used in a flow cell with the microprocessor controlled PZ 106 Immunobiosensor System, assays can be performed with the QCM in a manner equivalent to the SPR (surface plasmon resonance) technique. Flow cell based displacement immunobiosensor are developed for pesticides and environmental pollutants using fluorescent labels. Advantages with such system are that there is no incubation and detection time is reduced considerably.

Bioluminescence sensors

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. Bioluminescence systems have been used for detection of a wide range of microorganisms. TM4 bacteriophage was used to detect *Mycobacterium avium* and *Mycobacterium paratuberculosis*. The use of the A511 bacteriophage led to the construction of a polyvalent system for the detection of a wide range of Listeria strains. Using this bacteriophage, it was possible to detect one viable cell/gram of *Listeria monocytogenes* within 24 h. The bioluminescence approach is a new attractive approach due to its extremely high specificity and the inherent ability to distinguish viable from non-viable cells. However, the main disadvantages is the relatively long assay time as well as its lack of sensitivity that becomes apparent when low numbers of bacteria are to be detected.

CONCLUSION

Global bio-security threats such as the spread of emerging infectious diseases (i.e., avian influenza, SARS, etc.) and bioterrorism have generated significant interest in recent years. There is considerable effort directed towards understanding and negating the proliferation of infectious diseases. Biosensors are an attractive tool which has the potential to detect the outbreak of a virus and/or disease at the point of attack and consequently alert us for counter prophylactic measures to contain the disease. Development of biosensors for diagnosis of animal diseases is still at preliminary stage in India. Although there is a host of technologies available, either from commercial line or in the scientific arena, the development of biosensors for the detection of emerging infectious diseases (EIDs) is still in its infancy. There is no doubt that the glucose biosensor, the gene chip, the protein chip, etc. have played and are still playing a significant role in monitoring various biomolecules.

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