



Review

Detection of microorganisms using biosensors—A smarter way towards detection techniques

Madhura Nayak¹, Akhil Kotian¹, Sandhya Marathe¹, Dipshikha Chakravorty*

Centre for Infectious Disease Research and Biosafety Laboratories, Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore, 560012, India

ARTICLE INFO

Article history:

Received 8 July 2009

Received in revised form 22 August 2009

Accepted 25 August 2009

Available online 31 August 2009

Keywords:

Biosensors

Microorganisms

Biodefence

Agriculture

Healthcare

ABSTRACT

Along with useful microorganisms, there are some that cause potential damage to the animals and plants. 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. Here, we aim to review the use of various biosensors, developed by integrating the biological and physicochemical/mechanical properties (of transducers), which can have enormous implication in healthcare, food, agriculture and biodefence. We have also highlighted the ways to improve the functioning of the biosensor.

© 2009 Elsevier B.V. All rights reserved.

Contents

1. Introduction.....	661
2. Biosensors and their application.....	662
2.1. Biosensors in healthcare	662
2.2. Biosensors for food and water borne microorganisms	663
2.3. Biosensors in agriculture	664
2.4. Biosensors in defence	664
3. Ways to improve the functioning of biosensors.....	666
4. Future prospects	666
Acknowledgement.....	666
References	667

1. Introduction

Microorganisms (bacteria) since time immemorial have been an integral part of our life on earth. The property of microorganisms to multiply rapidly has been exploited to produce diverse range of products since ancient time. However, some microorganisms cause diseases that have disastrous effects on humans and can cause widespread damage. The ability of some microorganisms to evolve rapidly allows them to adapt and grow under stressful con-

ditions. With this property of microorganisms and the advances in genetic engineering technology, the use of these harmful microbes to cause intentional damage to the life and property cannot be ruled out. Under such a scenario the control measures that we currently possess, seem time-consuming and inappropriate. The best emerging technology to counteract this problem is the use of biosensors that provide us with a tool to rapidly detect the presence and amount of microorganisms in any given environment. International Union of Pure and Applied Chemistry (IUPAC) defines biosensor as a “device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals”.

* Corresponding author. Tel.: +91 80 23133142/86; fax: +91 80 23602697.

E-mail address: dipa@mcbl.iisc.ernet.in (D. Chakravorty).

¹ These authors have contributed equally.

A biosensor detects chemical and biological compounds in a living environment with the help of a specific biological recognition element whose property changes upon binding of the compound. This change is converted into a signal that can be conditioned and quantified. Dr. Leland C Clark, the father of biosensors, established the concept of utilizing a biological sensing element for the detection of different analytes. He developed an 'enzyme electrode' in 1960 for the measurement of glucose levels using immobilized glucose oxidase enzyme. This paved ways for the development of a wide array of sensors to detect biological compounds using a variety of enzymes, examples being urea detection using urease, NADH using NAD⁺, glutamate dehydrogenase and lactate dehydrogenase (Turner, 2007). While these sensors are based on relatively simple principle, the next generation of sensors incorporated more sensitive recognition elements and complex methods of detection. These include antigens, antibodies, nucleic acids, whole cells, and proteins. The changes in these elements upon sensing a signal are detected via optical, electrochemical, calorimetric, acoustic, piezoelectric, magnetic and micromechanical transducers.

2. Biosensors and their application

The basic biosensor framework includes a substrate such as silicon, glass or polymers such as polymethyl methacrylate, polydimethyl siloxane, etc. coated with a conductive layer like polysilicon, silicon dioxide, silicon nitride, metal like gold, and metal oxides; specific capture molecules like antibodies, enzymes, DNA/RNA probes, phage-derived biomolecular recognition probes and a suitable detection system. Highly sensitive sensors (e.g. thickness shear mode and immunosensor) can be fabricated using piezoelectric materials such as quartz crystal, potassium sodium tartrate, lithium niobate, etc. as a substrate, coupled with electromechanical detectors (Fengjiao He et al., 2002). High sensitivity, ease of operation, high accuracy and wide detection capacity can be achieved with optical biosensors (Surface plasmon Resonance based, resonant mirror and fiber-optic biosensors) that utilize fiber-optics, optoelectronic components, complementary metal oxide semiconductors and fluorescence/phosphorescence, reflectance, chemiluminescence, light scattering or refractive index for the detection purpose (Velasco-Garcia, 2009).

The expeditious growth in the development of biosensors and the involvement of multidisciplinary research activities in this field have led to the immense application of this technology. Conservative detection techniques like fluorimetry and colorimetry have given way to plasmon resonance, microcantilevers, electrochemical methods, etc. Hence, the range of analytes which could previously be detected has expanded, with improved sensitivity and reduced time of detection. Here, we restrict ourselves to the application of biosensors mainly in the detection of bacteria.

2.1. Biosensors in healthcare

Work leading to the sensitive, quick and accurate measurements using biosensors began in the 1980s. Researchers speculated that this technology would dominate the hospitals and home diagnostics by 1990s. While this did not happen, the last two decades have seen significant advances and developments in the biosensors for detection of the common pathogens like *Mycobacterium tuberculosis*, *Vibrio cholerae*, *Treponema pallidum*, etc.

According to the WHO statistics one third of the world population is infected with Tuberculosis bacilli, with 55% of the incidence reported in Asia (WHO, 2009). *M. tuberculosis* has been reportedly detected by a wide variety of biosensors like nucleic acid sensors, surface plasmon resonance (SPR), etc. (Sawata et al., 1999). Recent report uses a nucleic acid sensor (genosensor) for detection of *M.*

tuberculosis by SPR. The genosensors contain two probes, a cysteine modified NH₂-end peptide nucleic acid (PNA) and 5'-thiol end labelled DNA probes, immobilized on BK-7 gold-coated glass plates, that have complementary target sequence in genomic DNA of *M. tuberculosis* (Prabhakar et al., 2008). The shift in the resonance angle when the target (DNA of *M. tuberculosis*) binds the biological recognition element (probes) immobilized on the surface of the sensor is used as a signal for the presence of the pathogen. The PNA/Au bioelectrode is more stable against nuclease attack and it showed detection at 1 ng/ml level of *M. tuberculosis* DNA. He et al. has described a thickness shear mode (TSM) immunosensor using styrene-butadiene-styrene (SBS) copolymer as the matrix to immobilize antibodies specific to *M. tuberculosis* on the quartz crystal. SBS was more stable and of better quality than poly (vinyl chloride) and polystyrene tested. Binding of the *M. tuberculosis* antigens to the antibody immobilized on the surface changes the resonance frequency of the quartz crystal surface due to increase in mass. This technique proved to be simple and accurate (Fengjiao He et al., 2002). A piezoelectric immunosensor has been described for the rapid diagnosis of *M. tuberculosis*. It is based on the frequency shift caused by antigen binding to rabbit IgG against *M. tuberculosis* (He and Zhang, 2002) immobilized in an ordered orientation onto the crystal surface. Detection range of 10⁵–10⁸ cells/ml was obtained after eliminating non-specific absorption by using anti-honey bee venom antibody. A difference of greater than 15% between the experimental and control frequencies was considered as a positive test for TB. Experiments carried out on saliva and sputum samples of TB and non TB patients gave accurate and reliable results (He and Zhang, 2002). The response of the piezoelectric sensor is affected by viscosity, density, conductivity and permittivity of the liquid. Shen et al. constructed the 'series piezoelectric crystal quartz sensor' that sensitively responded to the changes in liquid conductivity with excellent frequency stability against operating voltage and the density and viscosity of the solution. It was able to detect a small conductivity change even in the presence of foreign electrolytes (Shen et al., 1993a,b). Other methods like the bulk acoustic wave impedance biosensor (He et al., 2003) and multi-channel series piezoelectric quartz crystal sensor system (Ren et al., 2008) has been developed for rapid detection of *M. tuberculosis*. The changes in the growth media caused by the metabolism of *M. tuberculosis* (release of NH₃ and CO₂) have been exploited for the detection purpose. The metabolites released by *M. tuberculosis* changes the conductivity of the medium (selective for *M. tuberculosis*). The change in conductivity used to measure the presence of *M. tuberculosis*. In both the systems NaOH pretreatment, that kept *M. tuberculosis* alive, was used to eliminate the contaminants that may be present in the sputum of patient.

Cholera is another disease which usually occurs in the areas with minimal laboratory facilities hence portable, rapid and minimal equipment is needed for the detection (Rahman et al., 1987). A technique using quartz crystal microbalance was used to detect *V. cholerae* in the range of 10⁵ cells per ml of *V. cholerae* O139 strain versus a background of O1 (Ogawa) serotype (Carter et al., 1995). This technique can be used for the detection of contaminated samples directly without any pretreatment and is not hampered by a dirty matrix. The sensor measures the change in mass caused by the binding of antigen to antibody as a corresponding change in the resonant frequency of the crystal. Immunosensor based detection utilizing SPR technique was also used for *V. cholerae* O1 (Jyoung et al., 2006) detection. An amperometric immunosensor using a screen printed electrodes (SPEs) has been developed for the detection of *V. cholerae* O1 in various environmental water samples. SPEs were fabricated by sequentially screen printing a layer of silver ink and then carbon ink (prepared by mixing polystyrene dissolved in mesitylene and graphite powder) on the alumina substrate. A layer of insulation ink was then screen printed over the electrode to

expose an area of 7 mm^2 . The variation in the amperometric current after the application of the samples was used as the detection signal. The spiked samples of ground water, sea water, sewer and tap water were filtered through polycarbonate membrane filters ($0.22 \mu\text{m}$) which were enriched in alkaline peptone water for 6 h. The detection limits reported were 8 CFU/ml in ground and sea water and 80 CFU/ml in sewer and tap water (Sharma et al., 2006). An optical biosensor that monitors the interactions between the analytic (bacteria) and the evanescent field of an optical fiber passing through the culture media has been developed for monitoring *Streptococcus pneumoniae* and *Staphylococcus aureus* (Ferreira et al., 1999). An example of a light-addressable potentiometric sensor (LAPS) used in immunofiltration procedure for detection of *Yersinia pestis* and *Neisseria meningitidis* has been given by Libby and Wada (Libby and Wada, 1989). The authors showed that fewer than 1000 cells could be detected within a 20 min assay time.

One of the most important applications of biosensors is the rapid detection of pathogens in the blood stored in the blood banks. To this extent, an immunosensor based on SPR technique has been described for the screening of syphilis (Sevars et al., 1993). This sensor however does not directly detect the microorganisms in blood but detects the presence of antibodies thereby concluding the presence of the causative organism *T. pallidum*. The selectivity was achieved by the use of recombinant *T. pallidum* membrane protein A (Tpm A). 'Sandwich SPR' method was employed to provide real time detection and reproducible results (Sevars et al., 1993). One step SPR can be used for the real time detection of *T. pallidum* in the serum samples (Sevars et al., 1993), e.g. SPR competition assay based on latex beads of submicrometer size, coated with antibodies against *T. palladium*. While many biosensors have been developed in the research phase, only few of them have been commercialized. Ivnitski et al. (1999) in their review provide a well founded overview of all the detection methods employed and commercialized biosensors till 1980s.

2.2. Biosensors for food and water borne microorganisms

Quality control expenses amounts to about 1.5–2% of the total sales of the food industry (Luong et al., 1997). Electrochemical, optical (SPR) and piezoelectric based immunosensors are the common approaches used to detect microorganisms in food and water (Ricci et al., 2007). *Escherichia coli* has a notorious reputation of causing food poisoning. It mainly contaminates poultry, vegetable and dairy products, which constitutes a large fragment of staple diet. Gfeller et al. (2005) made use of an oscillating cantilever for the detection of active bacteria (*E. coli*) in less than 1 h. The detection is through the measurement of the change of resonance frequency of the cantilever array which is a result of increase in mass caused due to adsorption of the pathogen on the cantilever. The reference cantilever was used to exclude any undesired environmental changes. By altering the nutritive layer and gas phase in which the detection takes place, the use of the sensor can be extended for the detection of different microbes. Binding of bacteria to a surface patterned with specific antibodies and detection of the pattern using laser has been described by St. John et al. (1998). Such rapid detection methods would save the food and health industry billions of dollars annually spent on the wastage and treatment cases of food poisoning. Another deadly strain of *E. coli* responsible for causing global disease outbreaks is *E. coli* O157:H7. Only a few 100 cells are sufficient to cause the infection and hence very sensitive methods are required for the detection. Poitras and Tufenkji (2009) have developed a biosensor based on quartz crystal microbalance with dissipation monitoring. The biological recognition element used was polyclonal antibodies immobilized on the gold-coated quartz crystal using a self assembled monolayer of cysteamine. The

biosensor was found to have a wide detection range from 3×10^5 to 1×10^9 cells/ml. A highly sensitive method of an electrochemical immunoassay has been described by Zhang et al. for the rapid detection of *E. coli* in surface water. Anodic stripping voltammetry based on Cu@Au nanoparticles as antibody labels was used for the detection of *E. coli*. The Cu@Au bimetallic nanoparticles have high stability, good biocompatibility and fine voltammetric activity for anti-*E. coli* antibody. It has a sensitivity to detect 30 CFU/ml which can further be increased to 3 CFU/ml by doing a pre-enrichment step where the sample is passed through $0.45 \mu\text{m}$ pore size filter (Zhang et al., 2009). This biosensor can be used for the detection of *E. coli* for environmental monitoring and biomedical requirement. A biosensor utilizing an acousto-gravimetric flexural plate wave transducer has been developed for the detection of *E. coli* (Pyun et al., 1998). The transducer is an elastic membrane which gives out flexural plate waves. The sensing is done by an immunoaffinity layer made of bound antibodies, specific for the bacteria, present on the transducer membrane. The binding of the bacteria to the transducer causes shift in the flexural plate wave frequency which is used to ascertain the presence of the bacteria. It has a detection range of 3×10^5 to 6.2×10^7 cells/ml (Pyun et al., 1998). A micro-electromechanical systems (MEMS) based biosensor for *E. coli* has been described by Gau et al. It is highly specific as it utilizes ssDNA to capture *E. coli* RNA. This method utilizes screen printing of multiple electrodes, self assembled monolayers of streptavidin attached to the capture ssDNA and enzyme amplification using amperometric methods. It has a sensitivity of detecting 1000 cells and an assay time of 40 min (Gau et al., 2001). The self assembled monolayers of streptavidin are stable in the presence of 1 M KCl, 0.1 M HCl, and 40% formamide whereas 8 M urea, 0.5% SDS, and 0.1 M NaOH cause 2–35% of desorption of streptavidin from the surface. Au/biotin-SH/streptavidin proved to be the optimal streptavidin surface with low non-specific binding (Gau et al., 2001).

Salmonella serovars are associated with 26% of all food borne diarrhea that lead to hospitalization (Joshi et al., 2009). These organisms that are shed in animal faeces and under unhygienic conditions contaminate dairy products, poultry and water resources. A novel Au/Si hetero nanorod based structure has been devised by Fu et al. for *Salmonella* detection. The Si-nanorod is coated with dye molecules through silanization. The Si-nanorods are then sputter coated with Au which is conjugated with anti-*Salmonella* antibodies (Fu et al., 2008). The bacterium is captured by the antibody and detected via enhanced fluorescence of the dye molecule. The same principle could be applied for the detection of other food borne pathogens like *E. coli*, *Staphylococcus* and *Campylobacter*. Another detection method for *Campylobacter jejuni* has been described by Che et al. using an enzyme linked immunoassay coupled with an enzyme electrode. In this method they use immunomagnetic separation in order to isolate the bacteria with the help of streptavidin labelled magnetic beads (2.8 mm) coated with anti-*C. jejuni* antibody. The enzymatic reaction at the electrode helps in the amplification of the signal. However, refreshing of the electrode leads to fluctuations in the base line signal. This was minimized by considering the peak current ratio of the test sample and the control (sterile phosphate buffer solution). This method was used to detect *C. jejuni* in chicken carcass wash water and ground turkey meat samples. However, pretreatment of samples was required to remove non-specific inhibitors like fat, plasma, etc. which led to 1% loss of *C. jejuni* cells. This detection method for *C. jejuni* was completed within 2.5 h and had a detection limit of 2.1×10^4 CFU/ml (Che et al., 2001). The detection limit could be further reduced by increasing the capture ability of the immunomagnetic beads.

Drinking water is usually contaminated with more than one pathogen and their toxins; hence biosensors aimed at simultaneously detecting these would be highly expedient. Wolter et al. described the first flow through chemiluminescence microarray for

simultaneously detecting *E. coli* O157:H7, *Salmonella typhimurium*, and *Legionella pneumophila* in water samples. The technique being simple in principle consists of antibody microarrays produced on poly(ethylene glycol)-modified glass, the presence of biotinylated antibodies that provides the necessary specific trap for the bacteria. The detection is accomplished by a streptavidin-horseradish peroxidase catalyzed reaction of luminol and hydrogen peroxide. The authors reported an overall assay time of 13 min and detection limits of 3×10^6 , 1×10^5 and 3×10^3 cells/ml for *S. typhimurium*, *L. pneumophila* and *E. coli* O157:H7, respectively (Wolter et al., 2008). This technique allowed parallel and quantitative measurement of the three pathogens tested. This system can be integrated in an inline setup with pre-enrichment modules (filtration and immunomagnetic separation) so as to detect single cell per 100 ml of drinking water.

Listeria monocytogenes is the causative agent of listeriosis and one of the most virulent foodborne pathogens. Twenty percent of clinical infections results in the death. In United States it is responsible for approximately 2500 illnesses of which 500 die annually (CDC, 2008). Different systems have been developed for the detection of *Listeria*. The ELISA system, *Listeria*-tek was formulated in the United States and tested using milk and meat. A direct immunofluorescence kit (DIR) from UK is used for detecting *Listeria* in cheese samples. Detection from 1×10^4 to 1×10^6 cells/ml after enrichment of the sample was achieved by using commercially available kit from Transia-Diffchamb S.A. (Lyon, France). The total assay time was 48–72 h (Minunni et al., 1996). A biosensor for the detection of *Listeria* in milk has been described using quartz crystal microbalance displacement assay (Minunni et al., 1996). The antibody specific for binding *Listeria* was immobilized on the gold coating of the quartz crystal plate using different methods and the antibody antigen binding was monitored real time using a liquid flow cell. The detection range was from 2.5×10^5 to 2.5×10^7 cells/crystal with a detection time of 15 min (Minunni et al., 1996). Banada et al. used light scattering sensors for the detection of target bacteria viz. *L. monocytogenes*, *E. coli* O157:H7 and *Salmonella* in vegetable and meat samples spiked with these bacteria. The forward scattering was able to detect the presence of contaminants accurately based on the distinct colony/scatter signature. The detection limit of this system was single cell per 25 g portion of test specimen. The method was able to recognize colonies of target bacteria in the presence of natural background microflora in clinical specimen (Banada et al., 2009).

Impedance based biosensors has been developed for the detection of foodborne pathogens. The applications and development of these biosensors is extensively reviewed by Yang and Bashir (2008) with a focus on microfabrication with the aim to increase the speed and accuracy of pathogen detection under different conditions.

2.3. Biosensors in agriculture

Agriculture includes the production of crops and the rearing of livestock producing various products which are used in daily life. These elements have always been susceptible to damage in the form of pests and diseases causing a loss in the profits (Fletcher et al., 2006). Hence, a way of increasing profits would be to reduce the loss of crops and livestock by such natural threats. With the advancement in bioterrorism, the need for biosecurity becomes very necessary. There have been cases where bacteria such as anthrax and virus such as the small pox have been deliberately propagated through livestock in order to infect the nation's population and inflict damage (O'Toole and Inglesby, 2003). Also, the need for biosecurity is essential when agricultural produce or any living object is to be transported across the international borders. Hence, the need for biosecurity is very high in order to preserve the safety and confidence of the public. Biosensors may play a

major role in this field as they provide rapid and specific detection compared to the older techniques. A biosensor has been developed for the detection of the fungus *Phakopsora pachyrhizi* that causes Asian rust or Soybean rust, using the SPR technique. In this case, antibody against *Phakopsora pachyrhizi* was used as the biological recognition element. The biosensor had a response range of 3.5–28 mg/ml of antigen solution (leaf extract) and a detection limit of 800 ng/ml (Mendes et al., 2009). The non-specific binding to the antibody was prevented by blocking the reactive sites with BSA. Such rapid and simple methods can be developed for world's most acute crop diseases thus preventing damage and spread. It is also important to develop biosensors for monitoring agricultural by-products. A biosensor for the detection of aflatoxin in olive oil has been developed (Amine et al., 2006). Aflatoxins produced by molds *Aspergillus flavus* and *Aspergillus parasiticus* are carcinogenic to humans. Aflatoxin has inhibitory effect on acetylcholinesterase (AChE) and its detection is coupled with the decrease in the activity of AChE which is measured using a choline oxidase amperometric biosensor. The choline oxidase enzyme is immobilized on screen printed electrodes. The residual activity of the enzyme is calculated after the application of sample and is used for the indirect detection of aflatoxin that may be present in the sample (Ben Rejeb et al., 2009). The AChE activity is highly pH dependent with best results obtained at pH 7.4. Amperometric method allows the detection of low aflatoxin concentration that cannot be detected by the classical spectrophotometry because of the omission of the dilution step used in the classical method (Ben Rejeb et al., 2009).

2.4. Biosensors in defence

The threat to the loss of life and agriculture due to biological warfare agents came to the forefront in 2001 with the anthrax attack on the US Postal Service creating panic among the general population (Xie et al., 2009). This incident led to the rapid development of biosensor technology for the detection of such agents at an early stage, in low concentrations to thwart serious damage to life and property. Although the detection techniques mentioned earlier can be used for this application, specific sensors have been developed to suit this purpose. The main problem in detecting a biological warfare agents lie in distinguishing them from normal or natural infective agents. Chemical markers of known biological agents are employed to reveal the identity of these agents in order to provide a solution to the problem.

The Bioterrorism Guide issued by the Interpol declares *Bacillus anthracis* and *Y. pestis* as the main bacterial agents in biological warfare (ICPO-Interpol, 2007). The commonly used biosensors for the detection are the ones based on nucleic acid and a comprehensive review for it has been provided by Gooding (2006). A 36° YX cut LiTaO₃ based Love-wave device has been described for detection of pathogenic spores in aqueous conditions. 100 Å titanium (Ti) layer (for binding) was evaporated onto single-side polished 36° YX LiTaO₃ wafers. Over this 800 Å gold layer was applied. The wafers were diced and patterned. Polyimide/polystyrene waveguide with protein G was applied followed by the activation of sensor side with the antibodies (adsorbed on the surface). Polyimide was used as the waveguide material so as to have a lower mass sensitivity (calculated using ellipsometry technique after injecting BSA in the test cell) with detection limit of 1.0–2.0 ng/cm² over polystyrene with detection limit of 2.0 ng/cm². The cross-linked nature of polyimide produces a highly stable film suitable for aqueous conditions. For experimental purpose *Bacillus thuringiensis* spores were used which selectively bound to the monoclonal antibody BD8 (anti-B8 spore) coupled to protein G (Branch and Brozik, 2003). Magnetoelastic biosensors are based on the principle of change in the frequency of magnetoelastic materials. The shape change caused gives rise

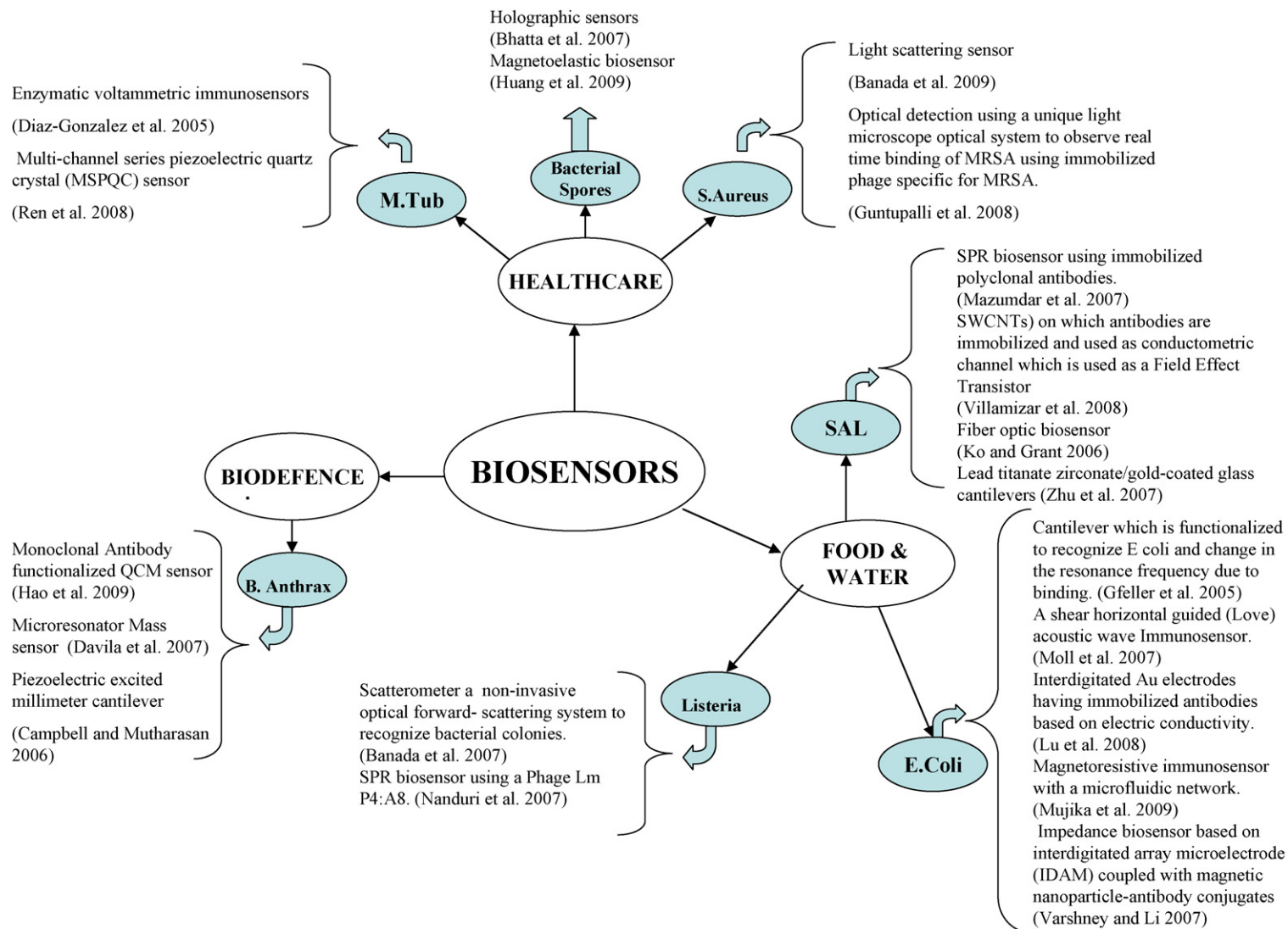


Fig. 1. Newer ways of detection of microorganisms using biosensors and its application in agriculture, health, biodefence and food quality control sectors.

to a characteristic mechanical vibration designed for detection of bacillus spores in the range of 10^2 CFU/ml. Phage-based magnetoelectric wireless biosensors for the detection of *B. anthracis* spores has been proposed by Wan et al. (2007). This biosensor utilizes a filamentous phage-derived biomolecular recognition probes to capture target agents multivalently. The use of phage as a biomolecular recognition element improves the sensitivity and detection limit as it is robust and has strong resistance against heat (up to 80°C), organic solvents like acetonitrile, urea (up to 6 M), acid, alkali and other chemicals. The detection limit of the biosensor was 10^3 spores/ml. The sensors can be stored for period of time with about 51%, 60% and 75% loss in binding affinity for temperatures of 25, 45 and 65°C , respectively, after 3 months of storage (Wan et al., 2007). An adaptation of microchip technology using a membrane based optical analysis system has been reported for the detection of anthrax spores (Floriano et al., 2005). Experiments were carried out using *Bacillus globigii* a common substitute for anthrax and the system could detect less than 500 spores within an assay time of 5 min. The postal samples are commonly contaminated with dust and many other types of organisms. The possibility of getting false positive results due to these contaminants was ruled out by testing the system against yeast, talc and powdered detergents. The system could also withstand filter clogging by dust particles up to 60 mg of dust corresponding to a pressure greater than 60 psi (Floriano et al., 2005). Another novel strategy developed by Wang et al. uses multiplex PCR to amplify virulence genes pag in PXO1 strain and cap gene in PXO2 strain. The detection of the PCR products is through the colourimetric reaction with the use of alkaline phosphatase (Wang et al., 2004). Pohanka et al. report in their review, the commercialized biotectors namely 'Bio-Detector based on 8 channel LAPS' developed by 'Smith Detection' for *B. anthracis*. It has a limit of detection 10^3 CFU/ml in 15 min. 'Midas Pro' with amperometric sensor produced by 'Biosensori SPA', Italy can detect cells in the range of 10^6 in an assay time of 20 min and SPR biosensor 'Spreeta 2000' from 'Texas Instruments' can detect bacillus spores in the range of 3.2×10^2 spores/ml in less than an hour (Pohanka et al., 2007).

3. Ways to improve the functioning of biosensors

PCR has been used to accurately detect low numbers of bacteria (Jensen et al., 1993), viruses (Schwab et al., 1996) and simultaneous detection of different pathogens with multiple sets of primers. The disadvantage with the technique being the inhibition of the polymerase enzyme by the contaminants from the sample, difficulties in quantification, false positives resulting from the detection of naked nucleic acids, non-viable microorganisms or contamination of samples in the laboratory (Toze, 1999). These problems can be overcome by incorporating an enrichment step so as to decrease the contaminants, by coupling the PCR with other methods, e.g. PCR has been coupled with piezoelectric biosensor for the specific detection of *Aeromonas hydrophila* (Tombelli et al., 2000). Alternative methods for PCR can be immunological methods like ELISA, immunosensors, etc. or the biosensors with combination of biological and physical/physicochemical transducers (SPR, piezoelectric, acoustic, amperometric biosensors). However, the problems remain associated with these biosensors too, like the chemical/physical stability of the transducers in the biological samples tested, the difficulty in production of highly specific antibodies, poor signal, etc. These problems can be overcome by coating the surface with Au or SiO_2 so as to make the transducer compatible with the biological samples, use of highly specific monoclonal antibodies, incorporation of amplification step to generate stronger signal. The detailed account for the same has been highlighted in the review by Leonarda et al. (2003).

The production of the biosensor able to function in the harsh working conditions is another challenge. This can be achieved by making modifications in the working parameters. Dong and Wang (2002) has discussed this with illustrative examples in their review. For example, to use biosensors, involving enzymes, under different conditions can be achieved by adopting enzymes either from extremophiles or other strains with enzymes resistance to the conditions in which the biosensor is to be used. The soyabean peroxidase from Chinese soyabean was used in the amperometric biosensor utilized for the determination of H_2O_2 in the samples from fermentation and food industries and environmental analysis which are slightly acidic. This enzyme maintained its biocatalytic activity in the pH range 3–10. The acid stability was further improved by encapsulating the enzyme in a graft copolymer of poly (vinyl alcohol) and 4-vinylpyridine. This biosensor can be applied in the system with high H_2O_2 concentration. Detection of H_2O_2 in the culture media has been used as an indirect method for the detection of *E. coli* and *S. pneumoniae* (Serra et al., 2008). So, amperometric biosensors with acid stable peroxidase can prove to be useful for quality control in food industry.

It is also important to distinguish microbial cells from the bacteriomorphic particles that may be present in the sample. This can be achieved by checking for the presence and absence of sulphur (S) and/or phosphorus (P) in the particle (S and P are absent in abiotic material) using X-ray microanalysis (Muliukin et al., 2002). X-ray microanalysis can also be used to distinguish between viable and non-viable microbes based on presence of high or low concentration of potassium (K), iron (Fe), silicon (Si) in the microbial cells (normal levels of K–0.065–0.920, Si–0–0.12, and Fe–0.079–1.1332). X-ray microanalysis can also be used for the ecological monitoring of the environment in order to access the physiological state of the microbes.

The applications of biosensor techniques are plenty and the detection techniques used are ever advancing to suit the purpose of these applications. To discuss all the techniques available is beyond the scope of this review. Fig. 1 illustrates few newer detection techniques employed for the applications discussed above.

4. Future prospects

Though a lot of research activity has been involved in developing biosensors for various purposes the time has come to bring this technology to the forefront and make it commercially available. Efforts and funds need to be mobilized to manufacture biosensors on a large scale so as to benefit and be of use to the general public. With exposure to the commercial market the applications of this technology would be greatly enhanced. A few such applications could be detection of virulence of a vaccine just before it is injected so as to prevent accidental acquisition of a disease, bandages detecting a septic wound, deadly viruses like H1N1 in the environment or from the patient sample (rapid and early detection) and so forth in the medical field. Real time monitoring of dairy products and breweries might help foster a cleaner and hygienic environment and experiment with different tastes imparted by specific microorganisms in specific concentrations giving rise to new products. A farfetched and plausible use of this technology could be in space exploration where if present the concentration of the living organisms would be very low and might lead to answering many of the long standing questions regarding the presence of life in space and the most elusive question to date vis-à-vis life on earth.

Acknowledgement

We acknowledge "Society For Integrated Circuit Technology And Applied Research" (SITAR), Bangalore, India for the constant sup-

port. This work was supported by the grant, Provision (2A) Tenth Plan (191/MCB) from the Director of Indian Institute of Science, Bangalore, India, and Department of Biotechnology (DBT 197 and DBT 172) and the grant from SITAR.

References

- Amine, A., Mohammadi, H., Bourais, I., Palleschi, G., 2006. *Biosens. Bioelectron.* 21 (8), 1405–1423.
- Banada, P.P., Guo, S., Bayraktar, B., Bae, E., Rajwa, B., Robinson, J.P., Hirleman, E.D., Bhunia, A.K., 2007. *Biosens. Bioelectron.* 22 (8), 1664–1671.
- Banada, P.P., Huff, K., Bae, E., Rajwa, B., Aroonnu, A., Bayraktar, B., Adil, A., Robinson, J.P., Hirleman, E.D., Bhunia, A.K., 2009. *Biosens. Bioelectron.* 24 (6), 1685–1692.
- Ben Rejeb, I., Arduini, F., Arvinte, A., Amine, A., Gargouri, M., Micheli, L., Bala, C., Moscone, D., Palleschi, G., 2009. *Biosens. Bioelectron.* 24 (7), 1962–1968.
- Bhatta, D., Christie, G., Madrigal-Gonzalez, B., Blyth, J., Lowe, C.R., 2007. *Biosens. Bioelectron.* 23 (4), 520–527.
- Branch, D.W., Brozik, S.M., 2003. Low-Level Detection of a *Bacillus anthracis* Simulant using Love-Wave Biosensors on 36 Degree YX LiTaO₃. Other Information: PBD: 1 Mar 2003, p. Medium: ED; Size: 33 pages.
- Campbell, G.A., Mutharasan, R., 2006. *Biosens. Bioelectron.* 21 (9), 1684–1692.
- Carter, R.M., Mekalanos, J.J., Jacobs, M.B., Lubrano, G.J., Guilbault, G.G., 1995. *J. Immunol. Methods* 187 (1), 121–125.
- CDC, 2008. Listeriosis, http://www.cdc.gov/nczved/dbmd/disease_listing/listeriosis_gi.html.
- Che, Y., Li, Y., Slavik, M., 2001. *Biosens. Bioelectron.* 16 (9–12), 791–797.
- Davila, A.P., Jang, J., Gupta, A.K., Walter, T., Aronson, A., Bashir, R., 2007. *Biosens. Bioelectron.* 22 (12), 3028–3035.
- Diaz-Gonzalez, M., Gonzalez-Garcia, M.B., Costa-Garcia, A., 2005. *Biosens. Bioelectron.* 20 (10), 2035–2043.
- Dong, S., Wang, B., 2002. *Electroanalysis* 14 (1), 7–16.
- Fengjiao He, L.Z., Zhao, J., Hu, B., Lei, J., 2002. *Sensors and Actuators B* 85 (3), 284–290.
- Ferreira, A.P.W., Marcelo, M., Ribeiro, R.M., Lins, U.G., 1999. In: Glass, A.J., Goodman, J.W., Chang, M., Guenther, A.H., Asakura, T. (Eds.), *Proceedings of the 18th Congress of the International Commission for Optics*, pp. 395–396.
- Fletcher, J., Bender, C., Budowle, B., Cobb, W.T., Gold, S.E., Ishimaru, C.A., Luster, D., Melcher, U., Murch, R., Scherm, H., Seem, R.C., Sherwood, J.L., Sobral, B.W., Tolin, S.A., 2006. *Microbiol. Mol. Biol. Rev.* 70 (2), 450–471.
- Floriano, P.N., Christodoulides, N., Romanovic, D., Bernard, B., Simmons, G.W., Cavell, M., McDevitt, J.T., 2005. *Biosens. Bioelectron.* 20 (10), 2079–2088.
- Fu, J., Park, B., Siragusa, G., Jones, L., Tripp, R., Zhao, Y., Cho, Y.J., 2008. *Nanotechnology*, 19.
- Gau, J.J., Lan, E.H., Dunn, B., Ho, C.M., Woo, J.C., 2001. *Biosens. Bioelectron.* 16 (9–12), 745–755.
- Gfeller, K.Y., Nugaeva, N., Hegner, M., 2005. *Biosens. Bioelectron.* 21 (3), 528–533.
- Gooding, J.J., 2006. *Anal. Chim. Acta* 559, 137–151.
- Guntupalli, R., Sorokulova, I., Krumnow, A., Pustovyy, O., Olsen, E., Vodyanov, V., 2008. *Biosens. Bioelectron.* 24 (1), 151–154.
- Hao, R., Wang, D., Zhang, X., Zuo, G., Wei, H., Yang, R., Zhang, Z., Cheng, Z., Guo, Y., Cui, Z., Zhou, Y., 2009. *Biosens. Bioelectron.* 24 (5), 1330–1335.
- He, F., Zhang, L., 2002. *Anal. Sci.* 18 (4), 397–401.
- He, F., Zhao, J., Zhang, L., Su, X., 2003. *Talanta* 59 (5), 935–941.
- Huang, S., Yang, H., Lakshmanan, R.S., Johnson, M.L., Wan, J., Chen, I.H., Winkle 3rd, H.C., Petrenko, V.A., Barbaree, J.M., Chin, B.A., 2009. *Biosens. Bioelectron.* 24 (6), 1730–1736.
- ICPO-Interpol, 2007. *Bioterrorism Incident Pre-Planning and Response Guide*. ICPO-Interpol.
- Ivnitski, D., Abdel-Hamid, I., Atanasov, P., Wilkins, E., 1999. *Biosens. Bioelectron.* 14 (7), 599–624.
- Jensen, M.A., Webster, J.A., Straus, N., 1993. *Appl. Environ. Microbiol.* 59 (4), 945–952.
- Joshi, R., Janagama, H., Dwivedi, H.P., Senthil Kumar, T.M., Jaykus, L.A., Schefers, J., Sreevatsan, S., 2009. *Mol. Cell Probes* 23 (1), 20–28.
- Jyoung, J.Y., Hong, S., Lee, W., Choi, J.W., 2006. *Biosens. Bioelectron.* 21 (12), 2315–2319.
- Ko, S., Grant, S.A., 2006. *Biosens. Bioelectron.* 21 (7), 1283–1290.
- Libby, J.M., Wada, H.G., 1989. *J. Clin. Microbiol.* 27 (7), 1456–1459.
- Leonarda, P., Heartya, S., Brennana, J., Dunnea, L., Quinna, J., Chakraborty, T., Kennedy, R.O., 2003. *Enzyme Microb. Technol.* 32 (1), 3–13.
- Lu, Y.C., Chuang, Y.S., Chen, Y.Y., Shu, A.C., Hsu, H.Y., Chang, H.Y., Yew, T.R., 2008. *Biosens. Bioelectron.* 23 (12), 1856–1861.
- Luong, J.H., Bouvrette, P., Male, K.B., 1997. *Trends Biotechnol.* 15 (9), 369–377.
- Mazumdar, S.D., Hartmann, M., Kampfer, P., Keusgen, M., 2007. *Biosens. Bioelectron.* 22 (9–10), 2040–2046.
- Mendes, R.K., Carvalho, R.F., Stach-Machado, D.R., Kubota, L.T., 2009. *Biosens. Bioelectron.* 24 (8), 2483–2487.
- Minunni, M., Mascini, M., Carter, R.M., Jacobs, M.B., Lubrano, G.J., Guilbault, G.G., 1996. *Anal. Chim. Acta* 325 (3), 169–174.
- Moll, N., Pascal, E., Dinh, D.H., Pillot, J.P., Bennetau, B., Rebiere, D., Moynet, D., Mas, Y., Mossalayi, D., Pistre, J., Dejous, C., 2007. *Biosens. Bioelectron.* 22 (9–10), 2145–2150.
- Mujika, M., Arana, S., Castano, E., Tijero, M., Vilares, R., Ruano-Lopez, J.M., Cruz, A., Sainz, L., Berganza, J., 2009. *Biosens. Bioelectron.* 24 (5), 1253–1258.
- Muliukin, A.L., Sorokin, V.V., Vorob'eva, E.A., Suzina, N.E., Duda, V.I., Gal'chenko, V.F., El'-Registan, G.I., 2002. *Mikrobiologiya* 71 (6), 836–848.
- Nanduri, V., Bhunia, A.K., Tu, S.I., Paoli, G.C., Brewster, J.D., 2007. *Biosens. Bioelectron.* 23 (2), 248–252.
- O'Toole, T., Inglesby, T.V., 2003. *Biosecure. Bioterror.* 1 (1), 1–3.
- Pohanka, M., Skládal, P., Kroča, M., 2007. *Defence Sci. J.* 57 (3), 8.
- Poitras, C., Tufenkji, N., 2009. *Biosens. Bioelectron.* 24 (7), 2137–2142.
- Prabhakar, N., Arora, K., Arya, S.K., Solanki, P.R., Iwamoto, M., Singh, H., Malhotra, B.D., 2008. *Analyst* 133 (11), 1587–1592.
- Pyun, J.C., Beutel, H., Meyer, J.U., Ruf, H.H., 1998. *Biosens. Bioelectron.* 13 (7–8), 839–845.
- Rahman, M., Sack, D.A., Mahmood, S., Hossain, A., 1987. *J. Clin. Microbiol.* 25 (11), 2204–2206.
- Ren, J., He, F., Yi, S., Cui, X., 2008. *Biosens. Bioelectron.* 24 (3), 403–409.
- Ricci, F., Volpe, G., Micheli, L., Palleschi, G., 2007. *Anal. Chim. Acta* 605 (2), 111–129.
- Sawata, S., Kai, E., Ikebukuro, K., Iida, T., Honda, T., Karube, I., 1999. *Biosens. Bioelectron.* 14 (4), 397–404.
- Schwab, K.J., De Leon, R., Sobsey, M.D., 1996. *Appl. Environ. Microbiol.* 62 (6), 2086–2094.
- Serra, B., Zhang, J., Morales, M.D., Guzman-Vazquez de Prada, A., Reviejo, A.J., Pingarron, J.M., 2008. *Talanta* 75 (4), 1134–1139.
- Sevares, A.H., Schasfoort, R.B., Salden, M.H., 1993. *Biosens. Bioelectron.* 8 (3–4), 185–189.
- Sharma, M.K., Goel, A.K., Singh, L., Rao, V.K., 2006. *J. Microb. Biotechnol.* 22, 1155–1159.
- Shen, D.Z., Li, Z.Y., Nie, L.H., Yao, S.Z., 1993a. *Anal. Chim. Acta* 280, 209–216.
- Shen, D.Z., Zhu, W.H., Nie, L.H., Yao, S.Z., 1993b. *Anal. Chim. Acta* 276, 87–97.
- St. John, P.M., Davis, R., Cady, N., Czajka, J., Batt, C.A., Craighead, H.G., 1998. *Anal. Chem.* 70 (6), 1108–1111.
- Tombelli, S., Mascini, M., Sacco, C., Turner, A.P.F., 2000. *Anal. Chim. Acta* 418 (1), 1–9.
- Toze, S., 1999. *Water Res.* 33 (17), 3545–3556.
- Turner, A.P.F., 2007. In: Robert, S., Marks, D.C.C., Karube, I., Lowe, C.R., Weetall, H.H. (Eds.), *Historical Perspective of Biosensor and Biochip Development*. John Wiley & Sons.
- Varshney, M., Li, Y., 2007. *Biosens. Bioelectron.* 22 (11), 2408–2414.
- Velasco-Garcia, M.N., 2009. *Semin. Cell Dev. Biol.* 20 (1), 27–33.
- Villamizar, R.A., Maroto, A., Rius, F.X., Inza, I., Figueras, M.J., 2008. *Biosens. Bioelectron.* 24 (2), 279–283.
- Wan, J., Shu, H., Huang, S., Fiebor, B., Chen, I.H., Petrenko, V.A., Chin, B.A., 2007. *IEEE Sens. J.* 7 (3), 470–477.
- Wang, S.H., Wen, J.K., Zhou, Y.F., Zhang, Z.P., Yang, R.F., Zhang, J.B., Chen, J., Zhang, X.E., 2004. *Biosens. Bioelectron.* 20 (4), 807–813.
2009. Global tuberculosis control: epidemiology, strategy financing: WHO report 2009, http://www.who.int/tb/publications/global_report/2009/pdf/full_report.pdf.
- Wolter, A., Niessner, R., Seidel, M., 2008. *Anal. Chem.* 80 (15), 5854–5863.
- Xie, F., Yang, H., Li, S., Shen, W., Wan, J., Johnson, M.L., Winkle, H.C., Kim, D.J., Chin, B.A., 2009. *Intermetallics* 17 (4), 270–273.
- Yang, L., Bashir, R., 2008. *Biotechnol. Adv.* 26 (2), 135–150.
- Zhang, X., Geng, P., Liu, H., Teng, Y., Liu, Y., Wang, Q., Zhang, W., Jin, L., Jiang, L., 2009. *Biosens. Bioelectron.* 24 (7), 2155–2159.
- Zhu, Q., Shih, W.Y., Shih, W.H., 2007. *Biosens. Bioelectron.* 22 (12), 3132–3138.