Chapter 14
Biological Biosensors for Monitoring and Diagnosis

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Abstract Quantification and detection of various contaminants in the ecosystem have become critically important in the past few decades due to their exhaustive use in soil and aquatic ecosystems. The contamination by both organic and inorganic contaminants in the ecosystem has drawn attention due to their persistence, biological accumulation, and toxicity. Organic contaminants reach the air, water, food, soil, and other systems through drift mechanism and have detrimental effect on various life systems after entering the food chain, thus interfering the normal biological process of the ecosystem. Inorganic contaminants have less solubility, primarily get adsorbed, and accumulate on lower sediments. The sources of both organic and inorganic contaminants include anthropogenic activities which dispose industrial and sewage effluent directly into water bodies. Most of the contaminants are very much toxic and have tumorigenic, carcinogenic, and mutagenic effect on various life-forms. Biosensors have various prospective and existing applications in the detection of these compounds in the environment by transducing a signal. It also has immense applications in the detection of different contaminants in the food industry, environmental monitoring, disease diagnosis, etc. where reliable and precise

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analyses are required. This chapter points out a comprehensive glimpse on different biosensors and their characteristics, operating principles, and their designs, based on transduction types and biological components. Efforts have been made to summarize various applications of biosensors in food industry, environmental monitoring, drug delivery systems, and clinical diagnostics etc.

Keywords Biosensors, environment monitoring · Disease diagnosis · Drug delivery

14.1 Introduction

Biosensors are devices comprising of a biological and physicochemical component to detect an analyte by producing a signal which can be measured (Mishra et al. 2019; Rovira and Domingo 2019; Mehrotra 2016). The first biosensor was invented by American biochemist "L.L Clark" in the year 1950 and the term "biosensor" was first introduced by "Cammann" in 1977 (Bhalla et al. 2016; Krishnaperumal and Lakshmanan 2013; Tchounwou et al. 2012). According to IUPAC nomenclature, biosensors are integrated receptor–transducer devices, which are able to provide selective quantitative or semiquantitative analytical information using a biological recognition element (Tangahu et al. 2011; Thévenot et al. 2001). A typical biosensor usually comprises of a biosensing element and a transducer. It has various biological applications and is used for the detection of several components such as pollutants, microbial load, metabolites, control parameters, and various other substances (Neethirajan et al. 2018). It also has immense applications in food industry, clinical diagnostics, and various other areas where reliable and precise analyses are required (Rasheed et al. 2019; Mishra et al. 2018). During the last few decades, numerous biosensing elements and devices have been developed (Grieshaber et al. 2008). Biosensors have numerous applications in various fields such as bio-monitoring of pollutants, disease diagnostics etc. The most common used biosensor is blood glucose biosensor which is used to check blood glucose levels (Nilsen et al. 2019; Yoo and Lee 2010).

Detection of various contaminants such as chemical and hazardous pollutants, drug detection, and detection of toxins in food, water, and soil ecosystems are some applications where biosensors are regularly used (Kimmel et al. 2007). Recent advancement in recombinant DNA technology led to the development of DNA-based or aptamer-based biosensors which act as a diagnostic tool in clinical assessment (Zhu et al. 2015). Incorporation of nanoparticles in biosensors helps in improving its parameters such as reliability, validity, lower detection limit, residence time, stability, sensitivity, etc. (Malekzad et al. 2017) (Fig. 14.1).

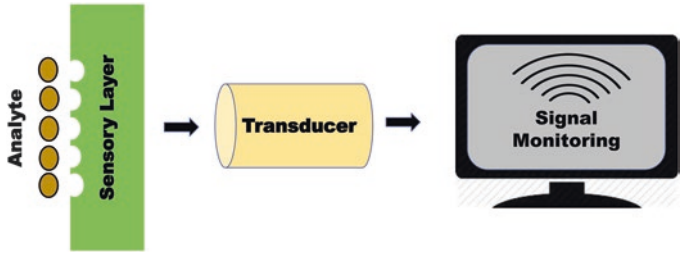

Fig. 14.1 General components of a biosensor

14.2 Types of Biosensors

The biological sensing material can be DNA probes, antibodies and enzymes, and cell receptors which interact with the analyte. The transducer may be optical, physicochemical, or piezoelectric material that translates the biological signals to optical and electrical signals (Nguyen et al. 2019). Biosensors are divided into different groups depending on signal transductions (Rocchitta et al. 2016). The electrochemical sensors were first introduced in 1962 by Leland C. Clark during the NASS symposium (Bhalla et al. 2016). In this sensor, molecules interact with the reactants and transduce an electrical signal which is directly proportional to the concentration of the analyte (Grieshaber et al. 2008). Based on this principle, it employs impedimetric, amperometric, and potentiometric sensors converting sensing information into a measurable signal (Malhotra et al. 2017).

14.2.1 Voltammetric Biosensors

Voltammetric biosensors measure the current produced during reduction or oxidation of electro-active reactant or product (Hung Tzang et al. 2001). In voltammetric biosensors, the electroactive species reduction or oxidation current is measured by producing the potential drop (Goyal et al. 2010). Here, the reference electrode and constant potential is applied to the working electrode which measures the current which is either directly proportional to the formation of a biocatalytic layer or to the volume concentration of electroactive species (Sangamithirai et al. 2018).

14.2.2 Potentiometric Biosensors

They usually measure the potential of the biosensor electrode with respect to a reference electrode (Bundschuh et al. 2018; Pisoschi 2016). The potential drop carries the analytical signal between the two reference electrodes or between the reference

electrode and the analytical electrode separated by the membrane. The transducer used in potentiometric transducers is the ion-selective electrode (ISE) (Cuartero et al. 2019).

14.2.3 Conductometric Biosensors

These biosensors involve the change in conductance arising due to the biochemical reaction (Jaffrezic-Renault and Dzyadevych 2008). These biosensors measure the electrical conductivity of the solution during a biochemical reaction. The sensing element is an enzyme and less discounted for the detection of affine interactions (Velychko et al. 2016).

14.2.4 Optical Biosensors

It measures light absorbed or emitted during a biochemical reaction (Damborský et al. 2016). In these biosensors, the optical fibers detect the analytes on the basis of light scattering fluorescence or absorption (Long et al. 2013). These biosensors measure both the affinity and catalytic reactions. The sensing element causes a change in absorbance or fluorescence which changes the refractive index between two media having different densities (Pospíšilová et al. 2015). They are superior to nonelectrical biosensors as they allow multiple analyte detection using various monitoring wavelengths (Dey and Goswami 2011). The adaptability of using optic probes is because of their ability to transmit signals that account on changes in polarity, time, wavelength, wave propagation, distribution of the spectrum, or intensity of the light (Peltomaa et al. 2018). They are solely based on the principle of light scattering absorption, internal reflection, fluorescence, surface plasmon resonance, or luminescence spectroscopy (Fan et al. 2008).

14.2.5 Calorimetric Biosensors

Also known as thermal biosensors, they work on the change in enthalpy during a reaction (Y. Zhang and Tadigadapa 2004). These are developed by integrating biosensor components into a physical transducer. It is used for the detection of a pathogen in water and food by measuring the change in optical density or color of the test sample upon a chemical reaction (Park et al. 2007).

14.2.6 Enzymatic Sensors

These sensors include biological material having certain antibiological activities (Hwang et al. 2018). The simplest form of enzymatic biosensors is capable of reversible reduction or oxidation on the electrode upon application of electrochemically active potential (Bernards et al. 2008). Enzymatic sensors were further categorized into inhibitor and substrate sensors. Inhibitor sensors often tend to determine the reducing activity of the enzyme or substances, while substrate biosensors tend to determine selected substrates and its enzymatic reactions (Campanella et al. 2000).

14.2.7 Impedimetric Biosensors

It measures the variation impedance of an electrochemical cell with AC frequency (Guan et al. 2004). These involve ion-sensitive silicon field-based sensors which enhance the resolving power of the transducer by raising its sensitivity. Mostly, biologically modified impedimetric biosensors are used to determine small proteins and peptides on the basis of a net charge on it (Kim et al. 2019).

14.2.8 Piezoelectric Biosensors

It involves measurement of mass change during biomolecular interaction. They are also considered as mass-based biosensors and are based on the principle of sound vibrations and also called as acoustic biosensors (Tombelli 2012). They produce an electrical signal when mechanical force is applied (Pohanka 2017). Sensing molecules are directly attached to a piezoelectric surface which is piezoelectric in nature. Hence, mechanical vibrations arise from the interaction between the sensing molecules and analyte and translate them to electrical signals (Pohanka 2018).

14.2.9 Immunosensors

They are widely used to detect the immunochemical reaction which occurs between antigens and antibodies (Piro and Reisberg 2017). Hence, they are employed to detect the presence of antibodies and used as a diagnostic indication for toxic substances. They determine the antigens in both media (biological liquids and natural environment) (Balahura et al. 2019). They detect any compound having high selectivity and specificity against specific antibodies (Wen et al. 2017).

14.2.10 DNA Sensors

The major component of DNA sensors is nucleic acids, mostly DNA. These sensing materials are the fragments commonly called DNA primers or DNA probes which reflect specificity of the whole DNA structure. These probes or primers are synthesized by amplification of DNA by PCR (polymerase chain reaction) (Campàs i Homs 2002). They are modified to increase the stability or to facilitate introduction of probes into biosensors. This type of biosensors helps in revealing non-macromolecular and protein compounds which interact with specific DNA fragments (Diculescu et al. 2005). On the basis of the type of biorecognition unit used, they are classified as nucleic acid-based, enzymatic, whole cell-based, antibody-based, or aptamer-based biosensors (Rasheed and Sandhyarani 2017) (Table 14.1).

14.2.11 Biosensors Based on Supramolecular Structures of a Cell

These biosensors inhabit intermediate between DNA enzyme and sensors, since they have a hierarchical structure. Sometimes these entities are composed of lipid membranes, poly-enzyme complexes, and cell organelles (chloroplasts and mitochondria) (Duan et al. 2013). These biosensors are less stable as they are derived from their natural component and cannot maintain operating parameters during procedures that are time consuming (Wajs et al. 2016).

14.3 Biosensor Design and Operation

It is an analytical device that employs certain biological compounds to recognize some molecules by providing their presence and concentration as a signal for processing and recording (Vigneshvar et al. 2016). Usually, biosensors contain three components: the first one is the recognition element which is a membrane having various biological structures. The second one is the transducer and the last one is the electronic system which amplifies the signal and records the signal for data presentation (Kozitsina et al. 2018).

The basic part of any sensor is the recognition element which responds to one or many analytes among various substances (Luka et al. 2015). The commonly used recognition elements include biological structures such as living cells, nucleic acids, receptors, antibodies, and enzymes (Rahaie and Kazemi 2010). Transducers convert the variations into optical or electric signal when a reaction occurs between the analytes and the selective biological layer. This signal is further measured using an electronic or light-sensitive device (Rahaie and Kazemi 2010). The biological component is immobilized by membrane or physical entrapment, covalent binding, or

Table 14.1 Different types of biosensors, their transducers, specifications, and applications

Biosensor	Transducer type	Specifications	Applications	References
Amperometric	Electrochemical	Usually redox reaction which brings change in current between reference and working electrode	Quantification and detection of alcohols, cholesterol, urea, amino acids, and glucose	Goriushkina et al. (2009)
Baroxymeter	Others	Measures respiration of bacterial cells by pressure measurements	Detection of toxic components in wastewater	Tzoris et al. (2002)
Bioluminescent	Optical	Light emission principle in which viable bacteria responds to any physical chemical or biological change	Quantification and detection of heavy metal, food toxicants, and environmental monitoring	Alloush et al. (2006)
Colorimetric	Optical	Monitors the change in optical density of a sample during a reaction	Water- and foodborne pathogens	Radhakrishnan et al. (2014)
Conductometric	Electrochemical	Measures change in the electrical conductivity of the medium upon entry of any analyte that changes the concentration of ionic species by measuring electrical conductivity when analyte reacts with medium	Detection of protein markers, chemicals, heavy metals	Jaffrezic-Renault and Dzyadevych (2008)
DNA sensors	DNA primers or DNA probes	Synthesized by amplification of DNA by PCR (polymerase chain reaction)	Measures non-macromolecular and protein compounds	Rasheed and Sandhyarani (2017)

(continued)

Table 14.1 (continued)

Biosensor	Transducer type	Specifications	Applications	References
Fluorescence	Optical	Emit fluorescence during immobilization of fluorescent-tagged biomolecules during reaction with analyte	Water or iron availability in plants or cell populations, BOD measurement, water in microbial habitat	Lei et al. (2006)
FET-based biosensor	Others	Measures change in conductance of field effect biosensor	IV blood pH recording, clinical investigation	Xu et al. (2005)
Impedance	Electrochemical	Measures the changes in conductivity or resistivity due to biorecognition event in medium	Polychlorinated biphenyls, milk toxins, PCBs, endocrine-disrupting hormones	Jaffrezic-Renault and Dzyadevych (2008)
Immunosensors	Based on ELISA technique	Amplifies and detect an antigen–antibody reaction	Clinical chemistry, antigen–antibody reaction	Balahura et al. (2019)
Piezoelectric	Others	Monitors changes in resonating frequency due to absorption or desorption of analyte results in current generation in piezoelectric material	Cellular studies, nucleic acid sensing	Skládal (2016)
Potentiometric	Electrochemical	Measures charge or potential accumulation between a reference and an ion-selective electrode	Determination of carbon dioxide, urea, pesticides, neurotransmitters, sugars, etc.	Pisoschi (2016)

(continued)

Table 14.1 (continued)

Biosensor	Transducer type	Specifications	Applications	References
Pyroelectric		Measurement of change in current induced by an analyte upon a temperature difference	Diagnostics	Spain and Venkatanarayanan (2014)
Supramolecular		Entities composed of lipid membranes, poly-enzyme complexes, cell organelles, immobilization of organic thin film	Inhabit intermediate between DNA enzyme and sensor	Wajs et al. (2016)

noncovalent interactions. Analytes bind with biological material resulting in the generation of an electronic response. Sometimes these reactions may be exogenous or release oxygen, hydrogen, or electrons ions (Nguyen et al. 2019). The transducer amplifies the changes in the product linked into the signal. Signal processing involves minimizing the reference signal arising from a similar transducer without any biological component and also smoothens the unwanted signal noise (Semenova et al. 2019).

14.4 Biosensors for Monitoring and Diagnostic Purposes

Biosensors exhibit numerous promising applications in various fields such as environmental monitoring, molecular diagnostics, pathogen detection, food industries, etc. (Mehrotra 2016). Biosensors monitor the presence of various contaminants in order to ensure the quality of drinking water, food, and soil. Biosensors detect contaminants at a low concentration which is a matter of priority for environmental protection and disease prevention as well (Rodriguez-Mozaz et al. 2006) (Fig. 14.2).

14.4.1 Biosensors for Monitoring Water Quality

Presently, water quality monitoring has become a primary environmental concern due to anthropogenic activities that are deteriorating the quality of water (Bi et al. 2018). To improve it, different preventive measures need to be taken like enhancing

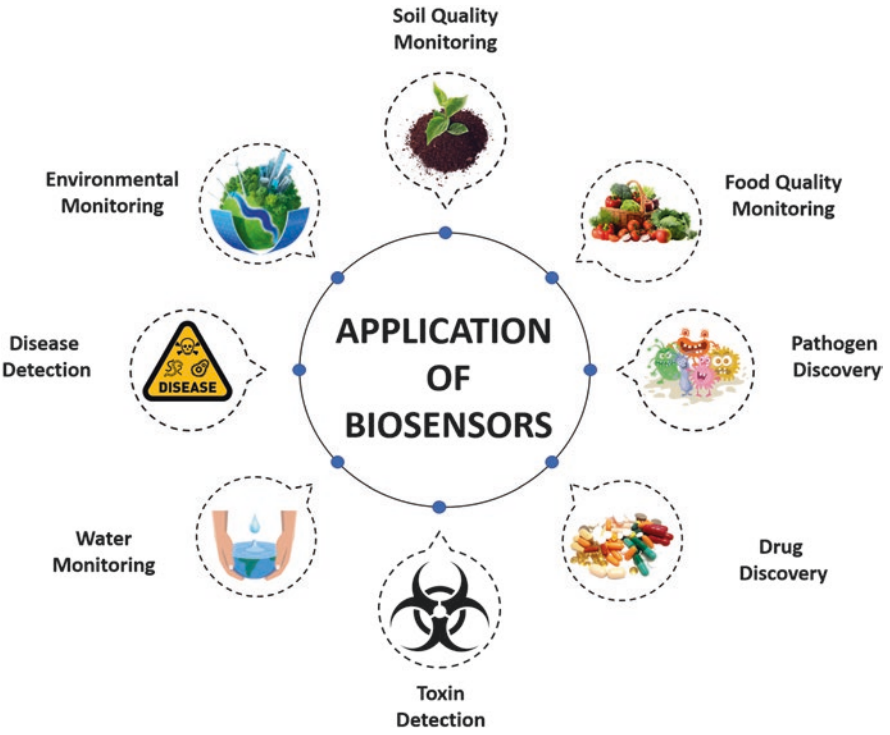

Fig. 14.2 Different applications of biosensors

the methods for sustaining natural resources, controlling the release of toxic compounds into the environment, and treating the localized sources of contamination (Ezeonu et al. 2012), as different contaminants like insecticides, pesticides, and surface-active molecules (SAM) enter the water sources and affect the life of marine organisms. These interventions disturb the water equilibrium and reduce the natural ability of self-purification. As a result, it induces harmful effects on humans upon consuming the contaminated water (Agrawal et al. 2010). Hence, there is need for the monitoring of toxic compounds in water. Regrettably, conventional methods like chromatography, spectrometry, etc. have their limitations as they work on chemical and physical principles. Moreover, these approaches are high-priced and labor-intensive, have narrow range of sensitivity, and do not robustly provide an effective result of the ecological situation (Starodub et al. 2005). Therefore, sensors involving a biological component/organism have gained attention and can serve as reliable and effective analytical techniques for assessing environmental contamination (Kovalchuk and Kovalchuk 2008).

Animals, culture of different tissues, microbes, protozoa, and water plants can function as biological indicators for checking toxicity (Parmar et al. 2016). At the same instance, it may also aid in determining group-specific substances that are

highly sensitive to specific groups present on the contaminant (Parmar et al. 2016). From different living organisms, *Daphnia magna St.* is an effective candidate for determining the total toxicity level of ecological contaminants as it has chemiluminescence (ChL) property. In an experiment, 5 organisms of *Daphnia* in 10 mL were found to be optimum for producing ChL response in the presence of H_2O_2. Using this idea, a *Daphnia*-based ChL biosensor has been created, which is aided with a computer controlling device for automatic detection of ChL signal. This biosensor especially detects the presence of SAM and is able to give measurement in the range of 20 mgL^{-1} or less (Starodub 2009). Another biosensor has been developed using bioluminescent bacteria. For this, bacteria were isolated from the Azov Sea and Black Sea according to their tolerance to SAM and other toxic entities to assess the toxicity of water samples. To develop this computer-controlled biosensor, bioluminescent bacteria like *Photobacterium phosphoreum* K3 and *Vibrio fischeri* F1/Sh1 are used. This biosensor works on bioluminescence inhibition as majority of SAM and toxic compounds inhibit bioluminescence activity. This inhibition response determines the presence of toxic compounds (Kuznetsov et al. 2002). Various other biosensors that possess the ability to determine specific toxic substances like chlororganics, cyanides, and phosphororganics have also been developed working on the principle of the electrolyte–insulator–semiconductor (EIS) system (Starodub et al. 2012). One such system is surface plasmon resonance (SPR)-based biosensor, which involves ethoxylated nonylphenol (NphEO) and immunoglobulin gamma (IgG) antibodies for detection. This approach measures the response of an SPR sensor against the NphEO concentration in the test sample (Bakhmachuk et al. 2017). Another powerful system using same component is ISFET biosensor, which has five times higher sensitivity than SPR-based biosensor and is the preferred biosensor for water assessment (Grieshaber et al. 2008).

14.4.2 Biosensor for Infectious Disease Detection

The spread of various infectious diseases like avian influenza, Hendra, Nipah, and SARS has become a global threat, which demands considerable effort to regulate their proliferation (Karesh et al. 2012). As there are various challenges associated with these infectious diseases, there is need for the development of diagnostic tools for eradicating/minimizing the chances of virus outbreak beforehand (Reyes et al. 2013). Biosensors have emerged as an attractive tool for providing robust information on these diseases. Usually, biosensors are characterized on the basis of their biological component and nature of the process like biocatalytic agent (such as enzyme), immunological agent (such as antibody), and nucleic acid material (such as DNA) (Mehrotra 2016). Majority of the biosensors developed for detecting pathogens involved in causing infectious disease are based on the principle of electrochemical reaction. This type of biosensor holds the majority as they are cost effective, independent of solution turbidity, low power requirement, high sensitivity, and simple instrumentation (Srinivasan and Tung 2015).

Different electrochemical methods like amperometric, impedance, and potentiometric are used to examine the changes which take place during disease detection (Hammond et al. 2016). The amperometric biosensor generally involves biosensor marker, antibody–antigen, and DNA hybridization reactions with an electrochemical transducer which amplifies the signal to a significant level for detection (Belluzo et al. 2008). One of the most common amperometric sensors is a glucometer (Yoo and Lee 2010). Previously, Gong and his colleagues have developed an amperometric-based immunosensor for detecting Newcastle disease (Gong et al. 2003). Another amperometric-based immunosensor has also been developed to diagnose forest-spring encephalitis with great precision (Brainina et al. 2003). Biosensors based on label-free amperometric immunosensor have been developed for Japanese B encephalitis vaccine (Yuan et al. 2005). Another group of researchers developed an optical biosensor to check the presence of Newcastle disease virus with sensitivity to 10 ng/ml (Lee and Thompson 1996). SPR-associated immunosensors have also been developed to detect the coronavirus responsible for severe acute respiratory syndrome (SARS) (Huang et al. 2009). Another type of biosensor, i.e., piezoelectric biosensors, has also been developed by the research group to assess the food and mouth disease virus (Gajendragad et al. 2001). Moreover, piezoelectric-associated DNA biosensor has also been developed by a group of researchers to detect hepatitis B virus infection with limited concentration range of about 0.02–0.14 μg/ml (Yao and Fu 2014).

14.4.3 Biosensor for Pathogen Detection

Foodborne illnesses have become a major health issue worldwide and raised the health safety concern in both food industries and regulatory bodies (Fung et al. 2018). Microbes have also been found to perform a function that is beneficial to food and its production, whereas some of them have been found to be associated with food spoilage (Rawat 2015). Few of food pathogen microbes are *Aeromonas* spp., *Bacillus anthracis, Bacillus subtilis, Brucella* spp., *Campylobacter* spp., *Clostridium* spp., *Escherichia coli, Listeria monocytogenes, Salmonella* spp., *Shigella* spp., *Staphylococcus aureus,* and *Yersinia enterocolitica* (Mody and Griffin 2015). These microbes have been found to produce cell metabolites and toxins which can cause severe diseases. These issues have prompted the researchers to develop a cheap, portable, and robust detector for pathogens (Abbasian et al. 2018). They developed different biosensors to detect these pathogenic microbes in food sources. Therefore, robust detection approaches for assessing foodborne pathogens are classified into antigen–antibody-based, bacteriophage-based, and nucleic acid-based biosensors (Mehrotra 2016). The rapid detection of foodborne pathogens has increased the popularity of these biosensors in the food sector, as these biosensors are effective in examining the food quality and microbial contamination in food products (Law et al. 2014).

The different types of immunosensors have been developed which measure the signal induced on the interaction of specific antibodies with a targeted antigen. An immunosensor has been found effective in detecting two species of *Salmonella* (i.e., *S. gallinarum* and *S. pullorum*) in chicken meat and eggs (Cinti et al. 2017). Another immunosensor based on a screen-printed interdigitated microelectrode has been developed to detect *E. coli* O157:H7 as well as *S. typhimurium* in chicken and milk sample within the range of 10^3–10^6 CFU/mL (Xu et al. 2016). An enzyme-based biosensor is another category, in which an enzyme works as a bioreceptor for detection. One of the most common enzyme-based biosensors is a glucometer which measures the level of glucose with the help of the immobilized glucose oxidase enzyme (Yoo and Lee 2010). Hesari and his colleagues developed an enzyme-based biosensor to robustly detect the *E. coli* contamination in drinking water (Hesari et al. 2016). Another category of biosensor, i.e., optical biosensor, has also been developed in which the toxins or pathogens are labeled with fluorescent compound, which on interaction with surface biosensor gets triggered by laser wave and induces a signal for detection (Bosch et al. 2007). Based on this principle, Adak and his group have developed an optical biosensor for *S. aureus* detection with detection limit in the range of 10^2–10^3 CFU/mL (Adak et al. 2013). Fluorescence resonance energy transfer (FRET)-based biosensors are also a type of optical biosensor. This type of biosensor has been found to be effective in detecting *S. aureus* in spilled milk and buffer (He et al. 2014). Moreover, Zhang with his colleagues developed a surface plasmon resonance (SPR) biosensor for the rapid detection of *E. coli O157:H7, L. monocytogenes,* and *S. enteritidis* in food products (Zhang et al. 2014). A colorimetric biosensor, another type of optical biosensor method, has allowed us to robustly detect pathogens like *Listeria* spp. and *S. aureus* in food products as well as the environment (Oluwaseun et al. 2018).

On the other hand, different electrochemical biosensors have also been developed to detect the microbes in contaminated food. Recently, amperometric biosensors with high sensitivity have been developed for identifying different foodborne pathogens like *C. jejuni, E. coli* O157:H7, *L. monocytogenes,* and *Salmonella* species (Arora et al. 2018), although a field effect transistor-based potentiometric biosensor has been developed to detect the presence of *E. coli* with detection limit as low as 10 cells/mL (Moran et al. 2016). Another electrochemical impedimetric biosensor based on aptasensors has been developed by Sheikhzadeh and his colleagues to detect the presence of *Salmonella typhi* in apple juice having a detectable range of 10^2–10^8 CFU/mL (Sheikhzadeh et al. 2016), whereas Zhang and his team developed aptasensors based on surface-enhanced Raman spectroscopy to detect *Staphylococcus aureus* and *Salmonella typhimurium* in pork with a working range of 10^2–10^7 CFU/mL (Zhang et al. 2015). Moreover, magnetoelastic sensors have also been developed to detect the foodborne pathogens like *Staphylococcus aureus* and *Salmonella typhimurium* in food items like spinach and tomato (Byeon et al. 2015; Li et al. 2010).

14.5 Conclusion

Biosensors have various applications in different fields such as disease diagnosis, environment monitoring, food control, drug discovery, biomedical research, forensics, etc. These devices need the interaction of various disciplines and are dependent on very special features like interaction of biomolecular analytes with recognition elements, device fabrication and design, on-chip electronics, sampling techniques, microfluidics, etc. Incorporation of nanoparticles in biosensors provides an opportunity to build a new generation of sensing technologies. Nanoparticles improve the magnetic, optical, electrochemical, and mechanical properties of the biosensors. No studies have been reported on understanding the mechanism of interaction between biomolecules and nanomaterials on nanofilms or surface of electrodes for fabrication of new-generation biosensors. However, nanoparticle-based biosensors show attractive prospects which will be applied in process control, food analysis, environmental monitoring, and clinical diagnosis in the near future.

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