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Basics of Biosensors and Nanobiosensors

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1.1 Introduction

The conventional analytical methods, both qualitative and quantitative, based on the measurements of species in complex matrices dominated the era of chemical sensing. These methods were based on the complete separation of sample components followed by the identification and quantitation of the target analytes. However, (i) expensive nature of the measurement techniques both financially and temporally, (ii) difficulty in the analysis of complex samples within a limited sample concentration, and (iii) the employment of separation methods limiting real-time analysis during *in vivo* applications subtly challenged its future development [1]. At present, an inexpensive and facile way of biosensor fabrication for the real-time detection and/or quantification of biologically relevant analytes provides an analytically powerful tool over conventional techniques [2]. These biosensors can surpass the major limitations of traditional sensors such as sensitivity, speed, and sensibility. Such biosensors typically function by combining a biomolecular recognition unit that is capable to sense the biochemical reaction and a transducer that can convert the concentration of the target analytes into a measurable signal. In 1977, Karl Camman first coined the term *biosensor*, but the IUPAC (International Union of Pure and Applied Chemistry) disagreement led to the conception of a new standard definition in 1997 [3]. A standard definition of biosensor now is as follows: "*A biosensor is a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor), which is retained in direct spatial contact with a transduction element. Because of their ability to be repeatedly calibrated, we recommend that a biosensor should be clearly distinguished from a bioanalytical system, which requires additional processing steps, such as reagent addition. A device that is both disposable after one measurement, i.e., single use, and unable to monitor the analyte concentration continuously or after rapid and reproducible regeneration should be designated as a single-use biosensor*." Since the earliest enzymatic electrode-based biosensors developed by Clark, there has been a rapid development/improvement in the design and application of these biosensors (Figure 1.1). Recently, biosensors

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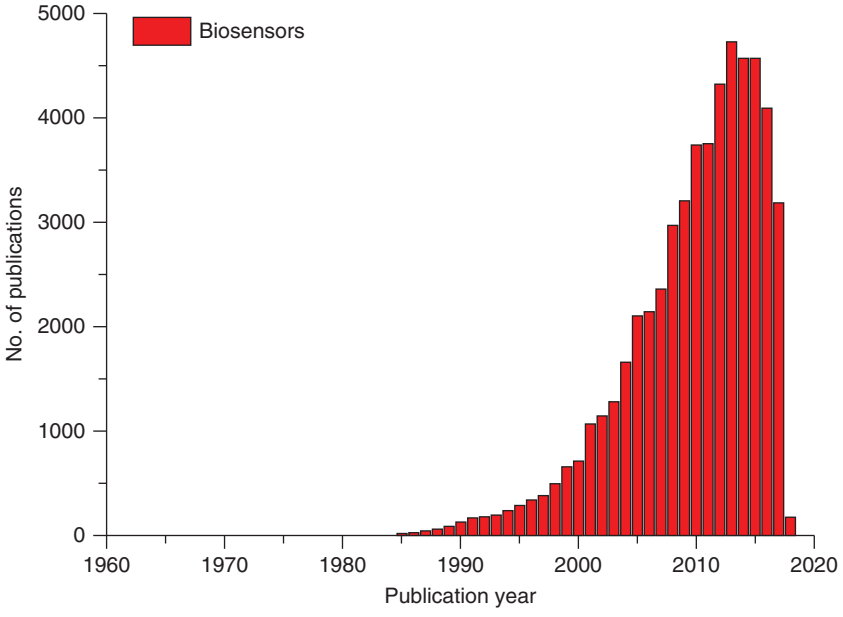

Figure 1.1 Recent publication trend in biosensors.

(electrochemical, optical, electronic, and piezoelectric) comprising various biorecognition molecules such as enzymes [4], aptamers [5], whole cells [6], antibodies [7], and deoxyribonucleic acid (DNA) [8] are widely applied in health care, food quality management, forensics, pharmaceutical industries, and several other areas (Figure 1.2). Improvised methods in the fabrication of biosensors have greatly augmented the characteristics of a biosensor measured in terms of selectivity, reproducibility, stability, sensitivity, and linearity. Moreover, rapid advancement in the fabrication technology together with electronic components has ushered miniaturization of such devices resulting a huge surge in the biosensor market. Notably, the use of nano-sized materials (having at least one dimension <100 nm) in the fabrication of biosensors leading to nanobiosensors have gained high momentum lately. The unique properties (mechanical, chemical, structural, and electrical) of these nanomaterials used in nanobiosensors have not only helped to overcome challenges based on the sensitivity and detection limit of the devices but has also improved the interfacial reaction owing to the better immobilization of biorecognition molecules [9, 10]. In addition, hybridization of nanomaterial-based strategies with a microscale system has allowed a new type of biomolecular analysis together with a high level of sensitivity that can leverage nanoscale binding events to detect circulating tumor cells (CTCs) or sense rare analytes [11]. In brief, this chapter comprehends all the basic information about biosensors and also provides in-depth knowledge of the design, components, characteristics, and applications of biosensors.

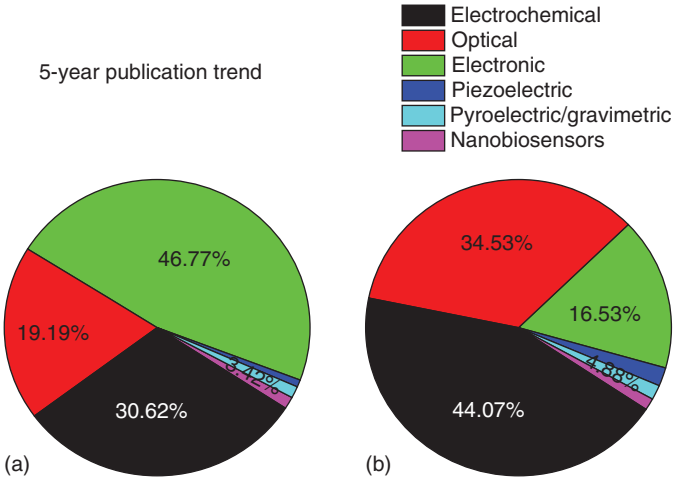

Figure 1.2 Five-year publication trends of various types of biosensors. (a) 2017–2013 and (b) 2012–2008.

1.2 Biosensor and Its Working Principle

A simple design of any biosensors basically comprises four major components: (i) a bioreceptor, (ii) a transducer, (iii) electronic components, and (iv) a read-out/display unit (Figure 1.3). Briefly, a bioreceptor is an external component of a biosensor that comes in direct contact with the target analyte during operation. The major function of a bioreceptor is to capture the target analytes with high specificity and selectivity [12]. Some examples of bioreceptors commonly used in the construction of biosensors are enzymes [4], aptamers [5], whole cells [6], antibodies [7], and DNA [8]. Construction mechanism typically follows the adsorption/immobilization of a biorecognition element on the surface of a biosensor. Therefore, techniques deployed for the adherence of such biorecognition elements remain central to the sensitivity and selectivity of a biosensor.

A most common approach for the immobilization of biorecognition elements includes adsorption, microencapsulation, entrapment, covalent bonding, and cross-linking [13–15]. Immobilization serves one or more of the following

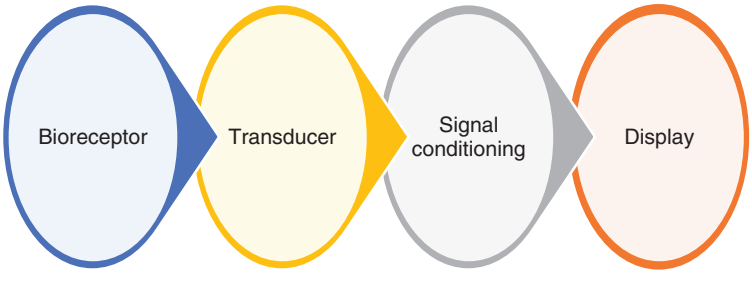

Figure 1.3 Schematic of biosensor components.

purposes: (i) continuous monitoring of analytes in flowing samples such as environmental samples, biological fluids having less amount of target molecules or bioreactor fluids, (ii) the biosensor can be used repeatedly, (iii) enhances the performance of biosensors in terms of reproducibility and sensitivity owing to the advancement of the biorecognition unit, and (iv) simplicity and flexibility of the immobilization technique. Toward a closer look in the fabrication strategies, (nano)biosensors confer multivariate interfacial region ranging between 1 and 10 nm, especially for the recognition of target analytes [11]. The detection of various biological molecules including protein–protein interactions can occur in this region. However, complexity during immobilization of such nanoscale components may be a challenging task. The chemical reaction at the site of bioreceptor, also termed as biorecognition, results in the generation of various signals such as light, changes in pH, heat generation, or changes in mass, which can be perceived by the physical component, transducer. The transducer can be defined as a device that can convert one form of energy to another. Therefore, depending on the type of biochemical reactions, several types of transducers can be used during construction of a biosensor; for instance, if the biorecognition process yields output in the form of light, then an optical transducer (e.g. photodetector) can perceive the incoming light and convert into a measurable electrical form [16]. Notably, all of the conversion processes are directly proportional to the amount of analyte–bioreceptor interactions at the biorecognition unit. The signals generated by the transducer (usually electrical) are in analogous form and cannot be read directly. Therefore, a signal conditioning unit assimilating various high pass/low pass/notch filters, amplifiers, and analogs to digital converters usually quantifies the signal that can be displayed directly in a readable format [17]. Biosensors may consist of different types of display units such as liquid crystal display (LCD), computer, or directly to the printer that comprises a pictorial representation of the measured signal. Depending on the user's requirement, the format of output signals may vary, e.g. the final data can be either numeric, tabular, graphics, or an image.

1.3 Characteristics of a Biosensor

The design of a biosensor defines the intended purpose of the application; however, other key factors are still central to manipulate the performance of these biosensors (Figure 1.4) [18].

1.3.1 Selectivity

Selectivity is the ability of a biosensor to detect a specific target analyte from a pooled sample containing mixtures of unwanted contaminants. The best classical example to explain selectivity is the interaction between an immobilized antibody and an antigen that is highly specific in nature.

1.3.2 Reproducibility

Reproducibility, on the other hand, is the ability of a biosensor to yield identical end results regardless of the number of times experiment is repeated. This is

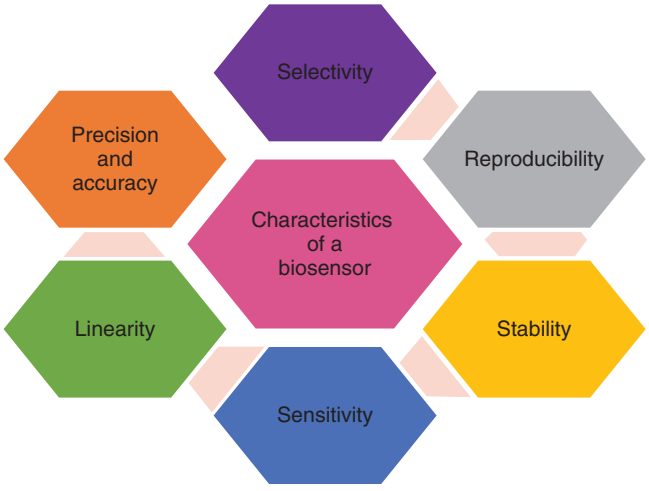

Figure 1.4 Biosensor characteristics.

mainly determined by the precision and accuracy of the transducer or electronic components in a biosensor. The reliability of biosensor output is highly dependent on the reproducibility of the biosensor devices.

1.3.3 Stability

Although precision and accuracy regulate the ability of biosensors to yield highly reproducible results, nevertheless, stability is another key aspect that may also undermine the performance of biosensors. In brief, stability refers to the ability of biosensors to circumvent ambient disturbances that are likely to alter the desired output response during measurement. This is more critical in the fabrication of biosensors that may require longer time or continuous monitoring to give a final result. Several factors such as temperature, the affinity of the bioreceptor, and fouling of membranes can influence the stability of a biosensor.

1.3.4 Sensitivity and Linearity

Sensitivity and linearity are two major properties of biosensors that determine the application and robustness of the device. Moreover, in a clinical setting, these basic characteristics of biosensors cannot be overlooked and should be dealt with utmost care. Sensitivity refers to the lowest detection limit of an analyte by a biosensor. This may range from nanogram per milliliter to even femtogram per milliliter. Basically, in case of biosensors designed for medical or environmental monitoring applications, sensitivity can be attained in the lowest possible value such as nanogram per milliliter or even femtogram per milliliter. Alternatively, linearity represents the accuracy of the obtained output within a working range where the concentration of the analyte in the sample is directly proportional to the measured signal. The straight line in the form of $y = mx + c$ represents the linearity of a biosensor. Here, y = output signal, m = sensitivity of the biosensor, and c = concentration of the analyte. Generally, detection of high-substrate concentration is usually better if the dynamic range or the linearity of the sensor is higher.

1.4 Biosensor Evolution: A Brief Outlook

The biosensor has a long history of development and also experienced huge transformation encompassing design strategies, working mechanisms, and most importantly reduction in the size of the biorecognition unit to a nanoscale. Until now, glucose biosensors are a role model to exemplify changes that took place since the advent of the term "*biosensor*." Herein, we present a brief snapshot on the evolution of biosensors while considering glucose biosensors as a backbone of our discussion. The success story of glucose biosensors and their subsequent evolution with the passage of time have become a role model in the history of biosensors [19]. Since past 50 years, a variety of transformations have been attributed to the design and construction of these glucose biosensors [20]. The first-generation glucose biosensors were based on the use of natural oxygen substrate and relied mainly on the detection of the hydrogen peroxide [21]. However, several limitations such as restricted solubility of oxygen in biological fluids resulting in "oxygen deficit" and limited selectivity of hydrogen peroxide severely compromised the accuracy of measurement. The limitations of first-generation glucose biosensors were overcome by replacing oxygen with nonphysiological electron acceptors. These redox mediators were able to carry electrons from the enzyme to the surface of the working electrode [22]. A variety of electron mediators such as ferrocene, ferricyanide, quinines, tetrathiafulvalene (TTF), tetracyanoquinodimethane (TCNQ), thionine, methylene blue, and methyl viologen were used to improve the sensor performance [19, 23]. In addition, the second generation of glucose biosensors experienced a huge paradigm shift in the sensor performance and design strategies such as (i) introduction of commercial screen-printed strips for self-monitoring of blood glucose, (ii) use of modified electrodes and tailored membrane, and (iii) the first electrochemical pen-sized blood glucose monitor for self-monitoring of diabetic patient [22, 24, 25]. Various strategies to enable electron transfer were primarily adopted for enhancing the sensor performance: (i) enzyme wiring of GOx developed by Heller's group, (ii) chemical modification of GOx with electron-relay groups, and (iii) application of nanomaterial as electrical connectors [19, 26, 27]. The third generation or the concurrent glucose biosensors are reagentless and based on direct transfer (without mediators) between the enzyme and the electrode [28]. This has led to the development of an implantable, needle-type device for continuous *in vivo* monitoring of blood glucose [29, 30].

1.5 Types of Biosensors

Biosensors can be classified either based on the mechanism of transduction or on the biological signaling mechanism (Figure 1.5).

1.5.1 Electrochemical Biosensors (ECBs)

These biosensors are basically a subclass of chemical sensors that hybridize the sensitivity of electrochemical transducers and high specificity of biological

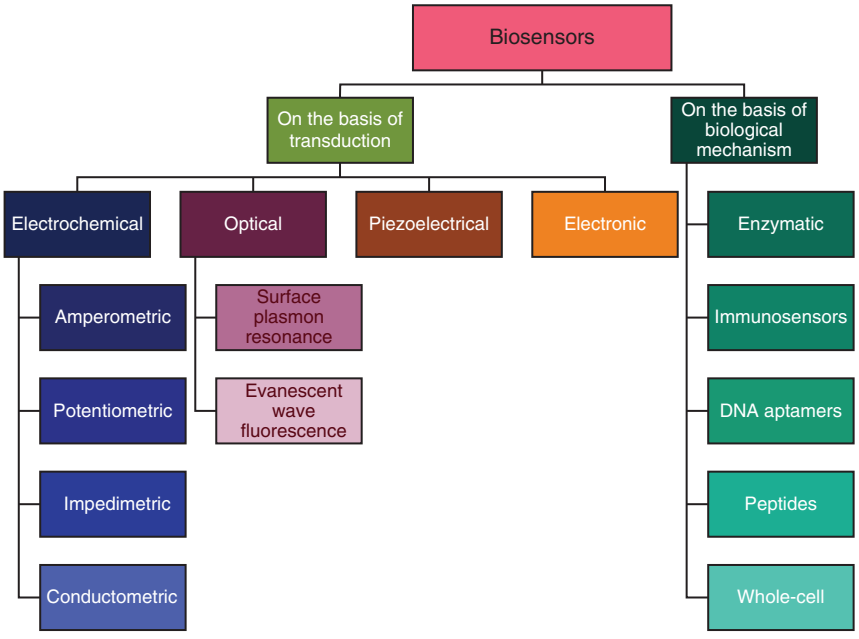

Figure 1.5 Biosensors classification.

recognition processes [31, 32]. Both of these features make electrochemical sensors as the best choice in a variety of clinical applications. The inherent selectivity of the biological components in electrochemical biosensors (ECBs) is mainly achieved by the use of enzymes (most commonly used), antibodies, proteins, cells, nucleic acids, receptors, or tissues. In principle, ECBs can selectively react with the target analyte to produce an electrical signal proportional to the concentration of the target analyte. Over the past several decades, ECBs have received overwhelming attention and are increasingly adopted in routine diagnosis of diseases or important areas, e.g. industrial, agricultural, and environmental analysis [33]. Advantages of ECBs over conventional detection techniques are (i) low detection limits, (ii) a wide linear response range, (iii) good stability and reproducibility, (iv) experimental simplicity, (v) low cost, (vi) portability, and (vii) small sample volumes [34, 35]. So far, various experts in this field have greatly reviewed subsequent progress, improvised concepts, and future applications of ECBs and opined about multitude ways of ECB classification [31]. For example, ECBs can be classified based on biological signaling mechanism (e.g. enzyme-based biosensor, immunosensors, DNA/nucleic acid sensor, cell-based biosensor, and biomimetic biosensor) or signal transduction mechanism (e.g. amperometric, potentiometric, conductometric, electrical impedance spectroscopy [EIS], and calorimetric). Because the detailed concept of individual biosensors and their types will be greatly discussed elsewhere in this chapter, we aim to briefly summarize the basic principles that can help readers to understand the most commonly used ECBs.

1.5.1.1 Potentiometric Biosensors

Potentiometric biosensors characterized by simplicity, low cost, and familiarity have been widely used since the early 1930s [36]. In general, potentiometric biosensors can exist in three different types: ion-selective electrodes (ISEs), coated wire electrodes (CWES), and field-effect transistors (FETs) [37]. However, the most common examples of these classes are the glass pH electrode and ISEs for detection of potassium, calcium, sodium, or chloride ions. In contrast to ISE, the CWES received attention lately around the 1970s after it was introduced by Freiser [38, 39]. The CWES responses are very much similar to those of classical ISE, but elimination for the need of an internal reference electrode allowed flexibility in miniaturization of the device. A more advanced form of potentiometric sensors comprises an ion-selective field-effect transistor (ISFET), which is more like an upgraded version of CWES [40]. The advantage of this type of biosensor is to easily fabricate smaller sized devices that are greatly useful for the *in vivo* testing of several ions. The fabrication of these biosensors is similar to the one used in manufacturing microelectronic chips. In brief, potentiometric biosensors have been greatly successful in clinics, industries, and other major sectors. Since the advent of a glass electrode, an increasing number of potentiometric biosensors (ISEs) have come into existence, resulting in successful analysis of various inorganic ions that were initially thought as difficult to analyze. Interestingly, commercialization trend has revealed the maximum use of potentiometric sensors in the clinics and industries where accuracy, speed, and simplicity are a primary focus.

1.5.1.2 Voltammetric/Amperometric

This type of ECB is mainly responsible for the continuous measurement of current resulting from the oxidation/reduction process during a biochemical reaction [41]. The current produced at the working electrode as a result of electrochemical reduction or oxidation proportional to the oxygen concentration that is measured at a constant potential is referred as an amperometry [42]. In contrast, voltammetry is the technique that measures current during controlled variations of the potential or over a set potential range. Despite limited mass transport of the molecules to the electrodes, it is claimed that amperometric devices facilitate a wide dynamic range suitable for low-level quantitation and superior sensitivity compared to potentiometric devices. Examples of such sensors include glucose biosensors, human chorionic gonadotropin β-subunit (β-HCG)-based pregnancy testing, adenosine triphosphate (ATP) sensors, and so on [43, 44].

1.5.1.3 Impedance (Electrical Impedance Spectroscopy, EIS)

EIS was first described by Lorenz and Schulze in 1975 where a sinusoidal potential, U (2–10 mV), was applied to measure the resulting current response, I, representing both resistive and capacitive properties of materials [45]. To obtain the impedance spectrum, the excitation frequency of the applied potential is varied over a range of frequencies resulting in the sum of a real and an imaginary impedance component (complex impedance). This technique is more powerful in terms of sampling electron transfer at a high frequency and mass transfer at a low frequency. An example of the impedimetric detection to monitor immunological

binding events of antibody (Ab)–antigen (Ag) on an electrode surface includes measurement of the small changes in impedance that are proportional to the concentration of Ag in the specimen [46]. However, limitation of such immunosensors may constitute damage and release of the bound immunoreagent from the surface of transducer, resulting in a compromised efficiency of the sensor. Moreover, nonspecific binding of Ab–Ag should also be carefully minimized.

1.5.1.4 Conductometric

These are basically a subset of impedimetric devices that monitor changes in the electrical conductivity of the sample solution with respect to the change in the composition of solution/medium during the process of chemical reaction. Conductometric sensors have been used in chemical analysis, environmental monitoring, or detection of foodborne pathogens such as *Escherichia coli* or *Salmonella* spp. [47]. Some major limitations of enzyme-based conductometric biosensing devices that limit their wide applications are the variable ionic background of clinical samples and the obligation to measure small differences in the conductivity of high ionic strength media. However, the rapid advent of electronic-based technologies such as semiconductors and integration of sensors to microelectronic devices (e.g. FETs) have greatly surpassed these limitations [48]. The most successful examples in this category highlights detection of drugs in human urine and pollutant detection in the testing of environmental specimen [49].

1.5.2 Optical Biosensors

Optical biosensors are the most preferred type because of the ease of real-time, direct, and label-free detection of various chemical and biological substances [50]. In comparison to conventional measurement techniques, optical measurement strategies are mainly preferred because of higher sensitivity, specificity, low cost, and portability [51]. In the recent trend, optical measurement technologies have received profound attention for the development of new optical biosensors that integrates microelectronics or micro-electro-mechanical system (MEMS)-based technologies together with molecular biology, chemistry, and biotechnology [16, 52, 53]. An exponential growth in the design and fabrication of optical sensors over the past decade has paved its way for worldwide application in the field of health care systems, biotechnology industry, and other environment-related applications. Optical biosensors following an optical detection technique mostly exploit the interaction of the optical field with a biorecognition element, which can be either labeled or label-free. In brief, label-free optical detection technique follows interaction of analyzed sample or analytes with the transducer; however, labeled detection technique involves the interaction of the label and analytes to generate signals such as colorimetric, fluorescence, or luminescence, followed by the detection with the transducer of a specific type. The design of the latter one comprises a sensing element (biorecognition unit) that is integrated with an optical transducer system capable to generate signal proportionate to the concentration of the measured analyte. The biorecognition unit of optical biosensors may also include biological

materials similar to the other types of sensors. The interaction of biorecognition unit and the target analytes results in the generation of an optical signal that can be further deployed for measurements via surface plasmon resonance (SPR) [54], optical waveguide interferometry [55], and evanescent wave fluorescence imaging [56]. Optical biosensors offer huge variations based on the construction types, of which (i) SPR biosensors including localized SPR and (ii) evanescent wave fluorescence biosensors are of particular interest and require further discussion.

1.5.2.1 Surface Plasmon Resonance

Surface plasmon resonance biosensors that could detect biomolecular interactions came into existence after 80 years of discovery of SPR phenomenon (first observed in 1902) [54]. The basic physics involved in the fabrication of SPR biosensors is the generation of surface plasmons after illuminating metallic surfaces (or similar conducting materials separated at the interface by a glass or a liquid) by a polarized light directed at a specific angle [57]. The subsequent generation of surface plasmons and reflected light of reduced intensity at a specific angle (known as resonance angle) therefore provides information about the proportionate mass attached to the surface of the transducer. Unlike conventional techniques, SPR is widely acknowledged as a primary tool to provide direct information on biomolecular interaction without the use of any labeling strategies. However, limitations resulting from nonspecific binding, limited mass transfer, and avidity can often complicate SPR analysis. Despite this, SPR has received a profound application in drug development, clinical diagnosis, food industry, biological sciences, and many more [58–60]. An advanced version incorporating the sensitivity of SPR and imaging technique to yield spatially resolved images of biointeractions has opened a new avenue in medicine, especially for the screening or identification of biomarkers and therapeutic targets [61]. On the other hand, localized surface plasmon resonance (LSPR) is a strikingly similar technique to SPR but of higher importance for nanostructures, mainly metallic nanoparticles such as Au and Ag exhibiting unique optical properties that are normally absent in larger metallic structures [62]. The major difference between these two techniques is the oscillation of plasmons, which is confined locally on the nanostructured surface rather than along the metal/dielectric interface as in SPR. Biosensors based on LSPR technology are mainly popular because of the ease of miniaturization resulting in increased throughput and lower operational costs. Moreover, LSPR biosensors known as the state-of-the-art analytical devices have demonstrated excellent performance compared to the SPR systems even at significantly lower surface densities of interacting molecules [63]. However, fabrication strategies may require additional care to control factors such as shape, material types, dimension, and also the interparticle distance that may otherwise compromise sensitivity of LSPR sensors.

1.5.2.2 Evanescent Wave Fluorescence Biosensors

Biosensors constructed on the basis of evanescent wave principle have become particularly useful in the development of immunosensors (different from the

enzymatic biosensors) [64]. So far, total internal reflection fluorescence (TIRF) is often applied to evanescent wave spectroscopy (EWS). Guided light in an optical waveguide or fibers surrounded by a low refractive index medium is totally internally reflected after striking the interface. This results in the generation of a wave that normally extends out from the interface into the lower index medium called as an evanescent wave. Such evanescent waves have a very short lifespan and are subjected to decay exponentially over a distance of 100 nm to an approximate wavelength. The important attraction for the use of such technique lies in the minimization of background signals to a large extent because the detection limit of the excited fluorophore by irradiating light is very narrow and is largely captured only at the surface excluding unwanted background signals from the bulk. The construction of devices based on EWS geometry offers a wide range of applications from clinical diagnostics to the food safety and biodefense [56]. The very near future may experience a larger number of ESW biosensors in health care areas. Such biosensors, while minimizing the background interference, would also offer advantages such as specificity attributed only to the labeled species or performance improvement besides the use of turbid or absorbing media similar to the biological solutions.

Based on the transduction mechanism, optical biosensors can be further classified into several types such as absorption, fluorescence, or luminescence that have received immense market priorities. All these three methods represent a unique property of detecting the output light intensity in reference to the incoming light beam, also called self-referenced (exception may include fluorescence measurement at a single wavelength). Design of these biosensors also follows a similar strategy comprising a biomolecule-immobilized surface/transducer for receiving and processing information based on the optical properties such as absorption, emission, reflectance, or change in an interferometric pattern. The only difference in the construction of such biosensors is the use of photodetectors that can transform incoming light into electrical signal. Absorption, fluorescence-based biosensors are a common example in the family of spectroscopy and are extremely convenient to use compared to other important types of spectroscopic techniques such as optical waveguide light mode spectroscopy, reflectometric interference spectroscopy, light scattering, or supercritical angle fluorescence [51]. Altogether, the advantage of the optical biosensor is the flexibility to combine with (micro)fluidic devices regardless of the applied voltages up to several kilovolts. This is a major limitation for most of the ECB, which makes electrochemical detection difficult.

1.5.3 Piezoelectric Biosensors

Piezoelectric biosensors are basically composed of a mechanical component or a piezoelectric material that can transform the mass or thickness of an analyte into an electrical signal [65, 66]. This is mainly possible because of the use of noncentric piezoelectric materials that can resonate at a natural resonance frequency under the application of an external alternating electrical field. In most of the cases, quartz crystals can serve this purpose and are used most widely. Typically, construction of these sensors is relatively easy and integrates the use

of a biosensing material coated with the piezoelectric material and an external electronic device that produces an electric signal that resonates at the natural frequency of quartz crystal. However, during the time of detection, when the biosensing component encounters the target analyte, there is a shift in frequency, resulting in the changes of output current with respect to the mass of the target analyte. In general, piezoelectric biosensors can be classified as two main types: (i) bulk wave (BW) and (ii) surface acoustic wave (SAW). BWs are studied most widely and are represented by various names such as quartz crystal microbalance (QCM) or thickness shear mode (TSM) referred to the mass sensitivity and motion of crystal vibration, respectively [67]. In a particular example of QCM, the antigen–antibody interaction occurring at the surface of the crystal leads to the changes in the loading of mass resulting in the corresponding decrease in the resonant frequency. Such changes can be measured down to the nanogram (ng) level depending on the sensitivity of the QCM. In contrast, SAW devices are a different class of biosensors in which the physical deformation of the wave is limited only to the crystal surface. Although SAW biosensors are known to be sensitive than piezoelectric quartz crystal (PQC), attenuation of acoustic waves in a biological environment might be problematic and requires more attention.

1.5.4 Electronic Biosensors: Based on Field-Effect Transistor

Electronic biosensors have gained immense popularity in the detection of biological and chemical compounds. The rapid development of electronic devices has helped to widen its application from electronic paper, low-cost photovoltaics, and organic light-emitting diodes (OLEDs) to the design of the state-of-the-art biosensors [68]. The recent trend in the fabrication of these biosensors typically focuses on minimization of cost, size, and higher throughput. This is possible because of the improvised synthetic methodology in the field of organic electronics by virtue of which has led to the yield of novel materials and abridged the knowledge gap in semiconductor–analyte interactions. Other advantages such as the elimination of bulky components used in the construction of optical or electrochemical biosensors such as photodetectors and excitation sources by the use of simple electrical sensing unit have revitalized its success. At present, electronic biosensors are mainly constructed by the use of FETs and require further explanation. FETs are commonly used semiconductor devices comprising three major components, the source (s), the drain (D), and the gate (G), which therefore functions as an on/off switch based on the applied electrical field [69]. Unlike most of the conventional biosensors, FET-based biosensors follow a different construction mechanism. In brief, the source and the drain terminals of semiconductor consist of nanowire channels to establish a connection; however, during construction of biosensors, these nanowire surfaces can be further modified by a biorecognition element. This can eventually lead to the generation of an electric field after binding with target analytes, similar to the control electric field applied to a conventional FET. An electronic circuitry connected to the FET sensor helps to monitor the specific conductance of the surface based on the type of interaction mechanism. In a particular example of a traditional metal oxide semiconductor field-effect transistor (MOSFET)-based

biosensors, the gate is biologically modified by an enzyme, receptor, antibody, DNA, or other similar recognition units that can capture the target analyte. Upon interaction with the target analyte, there is an accumulation of carriers, which is analogous to applying a voltage to a gate. Therefore, FET whose conductance is controlled by the gate voltage can be used to fabricate a similar type of electrical biosensors [68]. In brief, electronic biosensors are the most widely explored biosensors; however, the future realization should concentrate on the fabrication strategy, choice of electrodes, device stability, reproducibility, and sensitivity.

1.6 On the Basis of the Use of Biorecognition Elements: Catalytic Versus Affinity Biosensors

Catalytic biosensors mainly resemble the use of (bio)chemical species to obtain a product mainly via a chemical reaction. A most common example of this category is an enzymatic biosensor that is fabricated by using either specific or a combination of enzymes. In a stark contrast, affinity biosensors are specific in nature and confer binding of an analyte to a specific biorecognition element [70]. Examples of such biosensors mainly include immunosensors that facilitate binding of specific antibody–antigen or nucleic acid-based biosensors that assist binding of complementary oligonucleotide sequences or ligand–receptor interaction-based biosensors [71].

1.6.1 Enzymatic Biosensors

Enzymatic biosensors received profound interest after Leland C. Clark, Jr. first invented the oxygen electrode that was later used for the fabrication of glucose biosensors [72]. The general overview of enzymatic biosensors mainly constitutes of an enzyme as the essential components that determine the specificity when used as an electrochemical detection tool. The time lapse of an enzymatic biosensor mainly spotlights on three major types: oxygen-based (first generation), mediator-based (second generation), and direct electrochemistry-based (third generation) electrodes. Enzymes that are commonly used in the construction of biosensors include globular proteins, nucleases (both RNase and DNase), and nucleic acid molecules such as ribozymes/DNAzymes. In the history of biosensors, enzyme-based detection methodology is the most commonly used as a biorecognition element [4]. Nevertheless, enzyme-based biosensors are still susceptible to several limitations owing to the poor stability, stringent operational requirements, and variations in pH/temperature that limits the detection ability of the enzymatic biosensors. Recent progress to overcome existing challenges has been mostly addressed by the use of recombinant enzymes in the aid of genetic engineering that can help to modify the catalytic enzymatic sites of the target enzyme [73].

1.6.2 Immunosensors

Biosensors that consider the use of antibody or antibody fragment as a biorecognition element is basically referred as immunosensors. Normally,

immunosensors are considered as a highly specific sensor type because the recognition process involved at the interface takes account of an antigen–antibody interaction [74]. Immunosensors have experienced a remarkable popularity in a very short span of time. This is mainly because of the ability of immunosensors to detect analytes at a lowest possible concentration with high specificity and selectivity. Moreover, rapid technological advancement in the field of biology and electronics observed by the miniaturization of the transducer and purity of antibodies has helped to foster the application and performance of immunosensors remarkably. However, there is still room for improvements in the design of such biosensors. The few limitations may include poor solubility, aggregation induced by changes in temperature, retention of binding affinities at a higher temperature, and limited thermal stability. Recent development in the isolation of recombinant antibody fragments using phage display libraries have greatly improved the performance of immunosensors.

1.6.3 DNA Aptamer Biosensors

Aptamer-based biorecognition technique is also highly specific because it uses complementary DNA strands or oligonucleotides as a recognition element [5, 75]. Moreover, in comparison to antibodies as a recognition unit, aptamers are relatively convenient to use because of easy fabrication technique (using selective evolution of ligands by exponential enrichment [SELEX]) and no need to depend on cells or animals. The affinity of aptamers to the target has a dissociation constant almost close to the nano–picomolar range and can be used for detection of a wide range of target analytes [76]. The application of aptamer-based biosensors may include the detection of mycotoxins, cyanotoxins, and bacterial toxins. Recent progress in the field of aptasensors includes detection of multiple toxins simultaneously.

1.6.4 Peptide-Based Biosensors

Peptides have gained immense popularity because of its inherent nature to self-assemble in 1D, 2D, and 3D structures via noncovalent interactions (H-bonding, electrostatic, aromatic, π-stacking, hydrophobic, and Van der Waals) [77]. Such unique properties have helped to fabricate various flexible and supramolecular frameworks for a variety of applications including the biosensor. Other advantages might also include their ability to transfer electrons and conductive nature for electrical applications (helical conformation), easy synthesis of peptides sequences, excellent biocompatibility, and so on. The application of peptide-based biosensors includes detection of several analytes such as proteins, cells, small molecules, and ions [78]. The general immobilization techniques such as adsorption, covalent attachment, or self-assembled monolayers allow preparing the biorecognition unit of biosensors.

1.6.5 Whole-Cell Biosensors

In recent years, enzyme-based biosensors are gradually being replaced by the use of whole cell as a biorecognition element in a biosensor. The relatively low-cost

design and higher stability have offered a great advantage and are widely used in the detection of multiple targets [6, 79–81]. Moreover, whole-cell-based approach provides additional flexibility such as the massive production of microbes via simple cell culturing strategies, ability to withstand harsh environments, and elimination of the need for purification as in enzyme-based biosensors. The application area for whole-cell-based biosensors can vary from clinical diagnosis and detection of heavy metals and chemicals as a part of environmental monitoring to the drug discovery. General strategies to incorporate microbes on the surface of transducers may follow both physical and chemical methods, e.g. entrapment, adsorption, or cross-linking. Because the adsorption of microbes is the most critical step to determine the sensitivity and stability of the biosensors, various surface adhesion techniques are currently under investigation, which may assist in the improvement of biosensors in the near future. In contrast, improvement in the type of microbes can also correspondingly enhance the performance of these sensors. Recent improvements based on the genetic modification or protein engineering together with the use of synthetic biology have greatly influenced the sensitivity and selectivity of biosensors. This may be achieved intracellularly by coupling an inducible promoter and a transcriptional regulator or externally by displaying the molecules of interest in the surface of microbes or use of G-protein-coupled receptors against target analytes.

1.7 Application of Biosensors

Biosensors have been widely applied in a variety of applications such as in the diagnosis of diseases including cancer biomarkers, monitoring quality of food, environmental monitoring applications in both human and animal species, detection of infectious disease and outbreaks, and several more (Figure 1.6). However, this chapter will mainly focus only in some major applications that are used routinely and are of high practical importance.

1.7.1 Biosensors in Microbiology

Microbe detection is important to areas such as clinics, industries, or environmental sectors [82]. With the rise in disease outbreaks or food

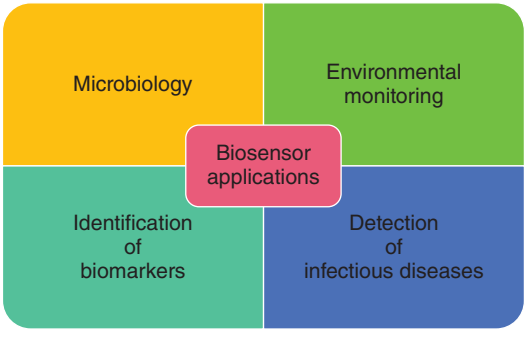

Figure 1.6 Application of biosensors.

contamination-related hazards, the development of such biosensors has become essential. Emerging classes of biosensors such as a laboratory-on-a-chip (LOC) or micro total analysis systems have been a promising strategy in the family of automated biosensors with a high throughput for microbiology applications. However, the major challenge to be addressed during the fabrication of biosensors for microbial detection should include real-time detection with high specificity and sensitivity.

1.7.2 Biosensors for Environmental Monitoring Applications

Environmental pollution has raised a global concern and can have a major impact on human health and animal species as well. Various types of biosensors have been evaluated as a monitoring tool to prevent environment-induced hazards in the day-to-day life of an individual. Based on the previous surveys, water pollutants contain a handful amount of harmful substances that have very low molecular weight (<1000 Da), resulting in difficulty to design an appropriate biosensor. Therefore, a sensitive and highly specific biosensor that can be fabricated in a most facile and inexpensive way for the monitoring of environmental hazards including wastewater effluents and other natural sources has remained as an ideal choice [83]. At present, biosensors based on spectroscopic, electrochemical, or chromatographic techniques have been widely acknowledged for this application. However, recent trends highlight fabrication of aptamer-based biosensors or aptamers with high affinity and specificity for environmental monitoring applications.

1.7.3 Biosensors for Cancer Biomarker Identification

Biosensors for health care applications alone have retained almost more than 80% of the total market. The conventional glucose biosensors have almost reached to every individual doorstep. However, a greater milestone has been achieved apart from those conventional glucose monitoring devices. The development of newly designed biosensors in health care systems mainly concentrates to minimize the death of million people because of cancer [84]. Early detection of cancer cells has been a hot topic for past several decades. Most importantly, identification of tumor biomarkers in a sensitive, accurate, and precise manner has mainly driven the biosensor market at present. Various types of ECBs have been routinely investigated for the determination of tumor markers such as gastric cancer, liver cancer, prostate-specific antigens, and many more [85].

1.7.4 Biosensor in the Detection of Infectious Diseases

Prevention of infectious diseases responsible for the morbidity of thousands of people has led to the rapid development of biosensors. Conventional modes of pathogen detection such as culture, microscopy, reverse transcription-polymerase chain reaction (RT-PCR), or immunoassays are usually time-consuming and are less sensitive. Recent progress in the design of biosensors based on electrochemical detection technique has gained major

attraction because of the low cost, noninvasive, relatively faster, and portable detection method. For example, immunobiosensors based on nucleic acid for targeting bacterial 16S rRNA and nanomaterial-based electrodes based on an immunosensing strategy for the detection of *Streptococcus pneumoniae* have received a profound application for the detection of such infectious pathogens [86]. Similar detection strategies have also been used successfully for the detection of viral infections such as human enterovirus 71, p24 from HIV, NS1 from dengue, viral antigens such as pseudorabies, avian influenza virus H5N1, and several others [87].

1.8 Conclusion

This chapter briefly summarizes the basic working mechanism, design principles, types, and applications of biosensors. In brief, biosensors are a powerful analytical tool for the inexpensive and rapid detection/analysis of several analytes in a variety of applications. In the past 50 years, these biosensors have witnessed a lot of transformations, resulting in the sophistication of sensing technology driven by the state-of-the-art detection strategies and miniaturization of devices together with the nanoscale-based recognition units. However, compared to the exponential outgrowth of biosensors publication in academics and research centers, the emerging market trend is yet far behind from the equivalent technological advancements. Therefore, the future work in biosensors should concentrate on the minimization of cost and the difficulties associated with the technological shift from academics to industries. Other works such as the design of simplified, sensitive, and robust biosensing devices for real-world applications, implementation of advanced nanomaterial-based bioanalysis tools for the alleviation of "noisy" bioenvironments, and significant improvements in throughput rate, specificity, long-term stability, and integrity might be an important step for the next generation of biosensors.

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