Chapter 3
Overview of Biosensors and Its Application in Health Care

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Abstract In recent years, biosensor is referred to as most impressive and ingenious analytical tool consisting biosensing element with wide range of medical utilizations like detection of diseases, diagnosis, treatment, patient well-being monitoring and individual fitness management. In addition, these sensing devices have been emerged as the prospective apparatus of cardiac segment. In modern medicinal avenue, biomedical analysis of diagnostic report is of thriving interest because biosensors have excellent potential, easy to use, ascendable and impressive in construction procedures of medical devices. Additionally, improved qualities of biosensing technology grant the competence to recognize disease and track the body's reaction to utmost concern. Scientists and doctors always prefer secure and lucrative modes of operating their exploration, assuring people health & security and freight patient specific fitness choice. As a result, present and future trends of medical science have shifted into implementation of low-cost biosensors to examine foodstuff & water pollutants, monitoring human physiological systems, appraise explicit health analysis and so on.

3.1 Introduction

By definition, biosensor is a measuring device or system which incorporates a probe united with either biologically sensitive element as perception material or bioreceptor as physicochemical identifying element and a transducer for the recognition of a chemical reagent that blends a biological component with a physicochemical detector [9, 14]. So, this is an interpretive tool combining an entrapped biological component like antibody, enzyme, DNA/RNA, hormone, whole cell, etc. that precisely bind with an analyte to develop physical, chemical or electrical signal as an output which is comparable to amount of analyte in the specimen solution (e.g. glucose, urea, drug, pesticide) in the reaction process whose concentration has to be measured [2, 36].

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Various sensitive biological elements like nucleic acids, enzymes, antibodies, tissue, microorganisms, organelles, cell receptors, etc., which is either a biologically synthesized material or biomimetic substances that can easily reacts or binds with, or recognizes the measurand of the testing sample [48]. The transducer is considered as detector element to converts one form of signal into another using physicochemical approach, i.e. optical, piezoelectric, electrochemical, etc., after combining of marked analyte with the bioelement, for easy measurement and also compute the measurand [35].

Biosensor produces some detectable physical changes after combines with measured analytes and transformed into a measurable electrical output with the help of a transducer. Output signal is then intensified, analysed and displayed as analyte concentration in the sample [48, 51].

The mechanism of action between the analyte and biorecognition material of biosensors is of two categories:

(a) Catalytic biosensors in which the measurand may be transformed into a synthetic product by enzymes and
(b) In affinity biosensors, the analyte may directly react with the biological element embedded on the biosensor (e.g. to antibodies, nucleic acid).

3.2 Components of a Biosensor

A biosensor typically composed with a bioreceptor in the form of aptamer, nucleic acid, enzyme, antibody, cell, etc., a transducer made of either semi-conducting material or nanomaterial, and also an electronic system includes signal amplifier, processor & display unit [22].

So, a biosensor is developed with two distinct components mentioned below:

1. Biological element such as enzyme, antibody, etc.
2. Physical instruments like transducer, amplifier, processor, display, etc.

The following components are essential for a typical biosensing device which is displayed in Fig. 3.1.

- Analyte—It is a chemical component or substance or that is of interest in a biosensing procedure which requires identification. For sugar level measurement in bloodstream, biosensor is designed to identify the analyte glucose.
- Bioreceptor—A biological molecule (e.g. enzyme, antibody) is considered as bioreceptor that is sensitive to recognize the specific chemical substance. Enzymes, proteins, nucleic acid and antibodies are common examples of bioreceptor. Biorecognition is the step of signal generation as heat, light, pH, charge or mass change, etc. when interaction takes place between bioreceptor and the analyte.
- Transducer—Devices which mould one form of energy into other have played vital role in biosensing system that transforms biological interaction into a measurable

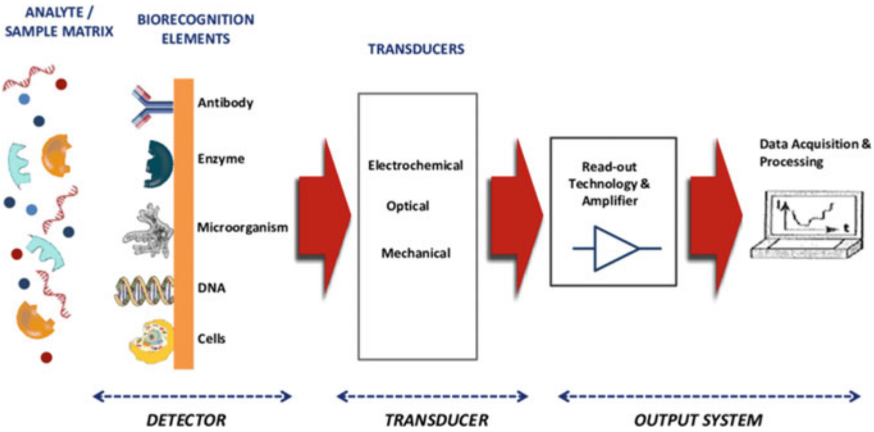

Fig. 3.1 Basic components of a biosensor

signal. Most biosensors produce either optical or electrical signal as an output after interaction with substrates, and the magnitude of such outcome is corresponding to the amount of analyte–bioreceptor reactions.

- Electronics: Signal produced after transduction is generally refined and keep ready for exhibit by electronic components of the biosensor. It composed of complex electronic circuit that operates as signal processing system to amplify the signal as well as change it from analogue to digital phase. After that the display unit of biosensor will quantify the processed signals.
- Display: This section composed of a translation system such as liquid crystal display of a computer or direct printer as a numbers or graphical representation or image according to the demands of the final user which is easily recognizable by the customer. The outcome component usually composed of amalgamation of hardware with software that customizes output of the biosensor in a user-friendly manner.

Three basic segments, namely detector, transducer and output system, are shown in the block schematic diagram of the biosensor. In the initial part, the sensing element acts as a responsive biological factor to identify analyte, whereas the later section is considered as the identification portion that modifies output data from the interaction with analyte and provide result as a display form by an accessible way. The concluding part encompasses with a signal conditioning unit, a demonstrating segment & a processor to amplify and display the signal in a suitable manner [35]. The biorecognition element first admits and binds with target analyte to generate signal resulting from substantial change that can be identified by transducer present in biosensor. In general, bioreceptor is suitably embedded on transducer, and thus, so-called biosensors are regularly used number of times for a long span [29].

3.3 General Features and Characteristics of Biosensors

(a) Features of Biosensor

Biosensors are utilized in number of medical applications like detection of disease, drug revelation, identification of contaminants, illness causing pathogens, markers, etc. are signals of disorders in blood, urine, saliva, sweat, etc.

Successfully designed biosensors must have certain features as follows:

- It should be specific and selective towards analyte.
- Biosensor must have sufficient sensitivity and resolution.
- It should be highly accurate and repeatable.
- It must have satisfactory speed of response and acceptable dynamic range.
- The mechanism of interaction should be free from external factors such as pH of sample solution, temperature, exhilarating speed, etc.
- Chemical reaction should be linear for a specific length of analyte concentrations.
- Output signal must be relevant to measurement environment.
- Device should be highly biocompatible and miniaturized so that it can be easily implemented within the body.
- This system must be cost effective, portable, user friendly and suitable for repeated use.

(b) Characteristics of Biosensor

Every biosensing element possesses certain static and dynamic attributes that are essentials requirements for any biosensor. The performance of each biosensor depends on the optimization of these properties. Biosensors are characterized by eight essential static and dynamic parameters, which includes-

Selectivity—Perhaps the most decisive quality of any biosensor is selectivity which indicates the strength of a bioreceptor to identify and respond only to a destiny analyte within a sample combined with other combinations. The simplest way of selectivity is explained by the coupled of an antigen with marked antibody. Generally, antibodies act as bioreceptors and are embedded on face of the transducer to detect specific analyte, i.e., antigen. A solution containing desired pathogen is then exposed to the transducer where antibodies combine only with such pollens. So, during design and development of a biosensor, selectivity is one the primary attributes of attention while selecting bioreceptors.

Sensitivity—It defines as the ratio of biosensor response and change in analyte concentration in the sample. In number of monitoring applications in healthcare sector, a biosensor is mandatory to expose analyte concentration in the range of ng/ml to confirm the presence of minute quantity of antigen in solution. For example, a prostate-specific antigen (PSA) concentration of 4 ng/ml in blood indicates prostate cancer for which doctors prescribe biopsy tests. Therefore, this character treated as one the essential features for biosensor fabrication.

Reproducibility—It means capability of a biosensor to produce similar reply for an identical condition and procedure. It is expressed by two parameters of the transducer named precision and accuracy with which the sensor's output depends because precision of sensor means exhibiting analogous results always when same sample measured repeatedly and accuracy expresses sensing ability of transducer to obtain a mean value close to the actual value when a specimen is measured more than once. The reliability of the reproducible signals is very high, and it provides compactness to the assumption made on the basis of biosensor.

Stability—This factor characterizes the shifting of its baseline or sensitivity over a specific time duration. By definition, it is the degree of sensitivity against surrounding interruption on biosensing environment that create flutter in output signals of a biosensor. As a result, it can create a mistake in measurand concentration and affect the precision and accuracy characters of biosensor. So, stability is the most influential factor in monitoring applications where a biosensor requires long incubation steps. The response of a biosensor depends on two factors such as (a) influence of temperature and (b) affinity of the bioreceptors. Deterioration of bioreceptor over a period of time is added factor that alters the stability of any instrument.

Linearity—This property exhibits the accuracy of the measured response of any biosensor in a straight line, and it is closely related with the verdict of biosensor and territory of analyte concentrations under inspection. Linearity of any biosensor should be steep for the detection of high analyte concentration. Based on operation, good resolution is appropriate because most biosensing applications need not only analyte detection but also measure the concentrations of chemical constituent over a large range of operation.

Operating Range—Operation range in biosensor is termed as concentration range over which response of biosensing elements changes linearly with the concentration, i.e. the sensitivity of such sensor is good. Occasionally, this character is also known as dynamic range.

Response Time—In other word, it is called as reaction time and can be expressed as time required to respond from no load to a step change in load to illustrate more than 60% of its final response for step input change in analyte concentration.

Life Time—Life time of a biosensor is specific time frame on which sensor can perform without severe degradation in functional characteristics.

3.4 Basic Principle and Working Mechanism of Biosensor

Usually, a specific enzyme is considered as desired biorecognition element which is deactivated by some natural process known as electroenzymatic approach and immobilized bioelement is in touch with the transducer. The analyte coupled with biological substance to shape a clear analyte which converts enzymes into corresponding electronic reaction with the help of a transducer that can be calculated. Oxidation of enzyme is most common biological response in biosensor where oxidation catalyses the reaction and alters the pH of the biological material. Alterations in pH will automatically influence the current carrying ability of the enzyme that has direct relation with the enzyme being measured. In some instances, analyte is altered when it connected to a device that is associated with release of gas, heat, electron ions or hydrogen ions. Outcome of the transducer in form of current is a direct rendering of the enzyme that being measured. The current is usually transformed into voltage for proper analysis and depiction.

Biosensors are operated on signal transduction principle for recognition of biological element. The transducer measures analyte–bioreceptor interaction and provides an output signal. Power of output signal is related to the amount of the analyte. After that output is amplified and processed by the associated electronic circuit. The flow diagram of biosensing principle is shown in Fig. 3.2.

A number of bioreceptors is used as sensing element in this biosensing device, but the most suitable biomolecule is usually a specially deactivated enzyme which immobilized at the tip of the transducer. The target analyte connects to the specific biological material embedded on transducer (bioreceptor) by conventional methods and inducing an alteration in biochemical nature of enzyme which results an electronic response through electroenzymatic approach. Now, the electrical signal comes from transducer is a direct depiction of biological substance that can be calculated. The block diagram of biosensors principle is shown in Fig. 3.3.

In some instance, antigen is transformed into a product that may connected with the liberation of heat, oxygen, electrons or hydrogen ions. The consolidation of sensitive organic molecule and transducer is liable to disciple the biological material to a corresponding electrical signal which can be amplified and measured. Based on nature of enzyme, the output of the transducer is different, i.e. either current or voltage. If output is current instead of potential, a converter needed to change into an equivalent voltage before proceeding further. Working mechanism of biosensor is depicted in Fig. 3.4.

The output voltage signal obtained from chemical reaction is sufficiently low in intensity and overlaid on a high-repetition commotion signal. Electrical impulse is then intensified and moved through a low-pass RC Filter. A signal conditioning or processing entity of the electronic circuit is responsible for amplification and filtration

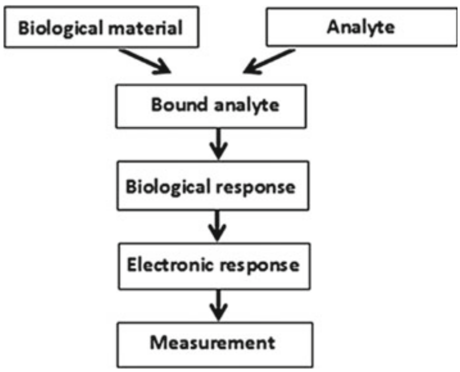

Fig. 3.2 Flow diagram of biosensing principle

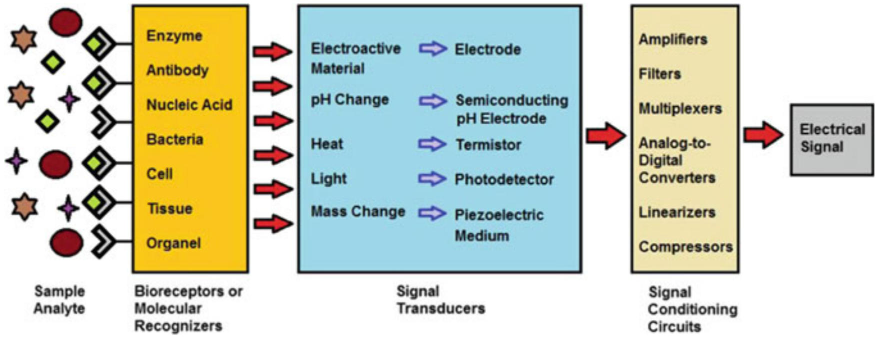

Fig. 3.3 Operating principle of biosensor

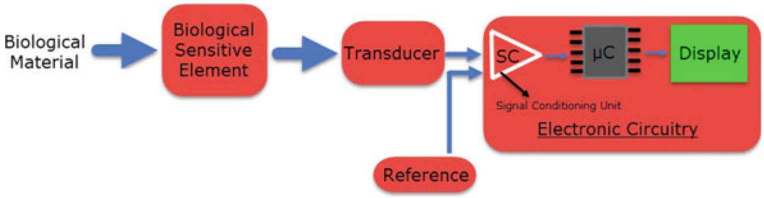

Fig. 3.4 Working mechanism of biosensor

of the signal. An output signal obtained from conditioning unit is proportional to the organic material which is being measured. After that the filtrate signal is fed straight to the LCD for presentation, but normally, this analog signal is transmitted to a microcontroller unit, where this output is transformed into digitized mode that is easier to analyze, process or storage.

Therefore, three elements are mainly involved in the basic working mechanism of any biosensor:

(i) At first, highly specific biorecognition element interacts with the analytes present in the sample.
(ii) Then, transducer detects and transforms signal into electrical one from biological target receptor molecule after electrochemical reaction.
(iii) Finally, transduction output is converted from biological to electrical signal where its elaboration takes place and processed values are displayed in monitor.

3.5 Evolution of Biosensor

Biosensor's development becomes the broadly studied research discipline due to simple, faster, cost-effective, highly sensitive and selective biosensor contribution leading to advances in imminent medicines such as ultrasensitive pin point detection of markers for diseases and health inspection. Aptamer biosensors can identify a vast spectrum of target analytes of small molecules such as ions, vitamins, large molecules like proteins, whole cells, etc.

In the year 1950, an American Biochemist named LL Clark invented the first biosensor for use. This device is applied to measure the O_2 content in blood, and later this electrode is known as Clark electrode or O_2 measuring electrode. After that, the enzyme glucose oxidase incorporated gel was coated on the O_2 electrode to determine the glucose level. Similarly, enzyme urease was also applied with an electrode for NH4++ ion measurement useful for calculation urea concentration in blood and urine.

Three geneses of biosensors accessible in the retail according to the degree of incorporation of biological components like the technique of adherence of the biorecognition molecule towards element of the base transducer.

For first generation, biorecognition element is physically enticed within the environs of the sensor backside of a semipermeable sheath such as dialysis membrane. In the successive evolutions, the entrapment of bioreceptor can be accomplished by covalent bonds on an appropriately customized transducer junction otherwise inclusion within a polymer matrix of transaction surface. In this stage of biosensors, the return of the product dissipates to sensor and causes the electrical reaction, and subsequently, electrons are transferred to O_2 molecule and the analysis is made when reduction in O_2 concentration and/or increase in H_2O_2 production.

In second generation, the individual components remain noticeable or stay separate like control electronics, electrode and biomolecule. Second-phase biosensors use synthetic, partially toxic mediators or nanomaterials to transit the electrons towards electrode in a better way.

In third generation, biorecognition elements are indispensable part of base sensing material, whereas these explanations were possibly expected for enzyme electrode systems, akin gradations are pertinent to biosensors usually can be made. It is basically in between second and third generation families that the major developmental attraction which currently observed. In this generation of biosensors, no mediator is directly associated to initiate the reaction. As a result, the electrons are directly moved to electrode from enzyme without any intermediate stages or use of nanoparticles.

3.6 Types of Biosensors

Different classes of biosensors are available depending on the sensing devices and type of biochemical substances used [32]. The different types of biosensors are described below.

1. Electrochemical Biosensor

These are the simplest biosensing devices according to measurement of electric current, ionic or conductance changes imposed by electrochemical transducer. They can easily identify the biological materials such as enzymes, whole cells, specific

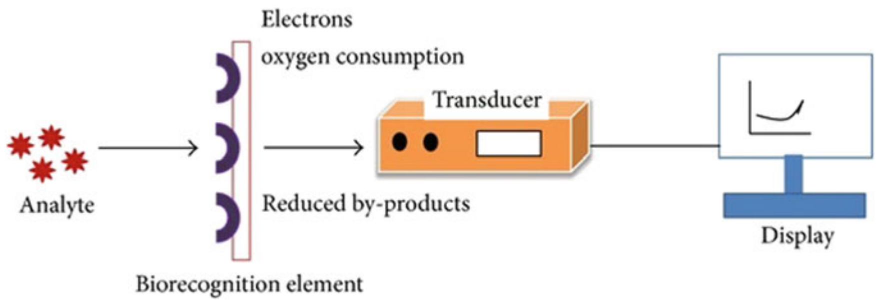

Fig. 3.5 Schematic diagram of electrochemical biosensor

ligands and tissues, along with non-biological matrixes [43]. Generally, the principle of electrochemical biosensor depends on enzymatic catalysis that evolves or consumes electrons [15]. This biosensor usually composed of three electrodes such as measuring, reference and counterelectrode. Block schematic diagram is represented here as Fig. 3.5.

In this sensor, target substance is committed towards response that takes place on the base of measuring electrode which is the origin for electron transmission through the dual-layer potential. Electrode potential or current can be determined at definite voltage [17].

A number of electrochemical biosensors available in the market depend on their working mechanism to secure organic selectivity or their signal transduction procedure or both [54]. The bioreceptors are specific for particular biocatalytic event such as a reaction that is catalysed by enzymes, or for a selective bioaffinity, such as the interaction between an analyte and a biorecognition element irrespective of their biological environment. Electrochemical biosensors are generally categorized into four types [50] such as follows.

(a) Amperometric Biosensor

These kinds of biosensors are self-supporting unified devices based on the developing current from redox reaction of an electroactive biological element offering exact quantitative analytical information. The current developed in amperometric biosensors is corresponding to the concentration of the target substances [5]. In comparison with potentiometric biosensors, it shows less response times, maximum energy spectrum and higher sensitivities. Clark oxygen electrode is the simplest amperometric biosensor which is frequently used.

The functioning principle of such biosensors depends on the movement of electrons as a result of enzyme-catalysed interaction and oxidation/reduction reactions [8]. Normally, a pre-set bias voltage applied through the electrodes being measured. In an enzyme-based reaction, the substrate or product can easily transport an electron-to-electrode surface which are either oxidized or reduced (Fig. 3.6).

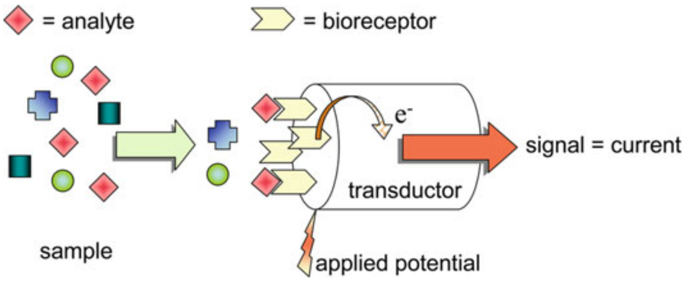

Fig. 3.6 Working principle of amperometric biosensor

When a voltage difference is created between two electrodes, a current is produced which is equivalent to substrate concentration. These are the first-generation biosensors. A typical example of such sensor is glucose sensor where we can measure the blood glucose level by redox reactions using glucose oxidase enzyme [20]. The considerable drawback is their affinity to dissolved O_2 content in analyte solution. This problem can be solved using mediators that relocate the electrons generated by the interaction directly to the electrode other than slashing dissolved O_2 in analyte solution. That's why these are referred as second-generation biosensors. Modern electrodes directly expel the electrons from the reduced enzymes without any help of mediators and are coated with power-conducting organic salts [19].

(b) Potentiometric Biosensor

It is a class of chemical sensor where biosensing element is attached with an electrochemical potential transducer applied to find the analytical concentration of some components of the gaseous substrate or solution [52]. These sensors usually measure the electrode potential without applying voltage as it relies on a biochemical reaction leading to a simpler chemical species and its subsequent electrochemical detection of NH_4OH, CO_2, pH, H_2O_2, etc. Potentiometric biosensors utilize ion selective electrodes to transduce the biological reaction into an analytical electrical output. For example, in pH measuring device a specific enzyme immobilized on membrane which surrounds the probe.

The electrical current is calculated as voltage difference between the active & reference electrode and is critically rely on the analyte concentration in gas or solution phase.

Potentiometric biosensors have been categorized under three distinct groups.

(i) Type I sensors contains an ionic solution having free ions inside chemical component of gas phase which is to determine. The example of commercially available type I sensor is YSZ oxygen sensor.
(ii) Although type II sensors having no free ions in the chemical compound that to be assessed, an ion pertinent to target gas can diffuse in the solid conductor to maintain equilibration with the surroundings. Thus, both type I and II sensors have the similar design with gas electrodes united with metallic strip

and an electrolyte where oxidized or reduced ions are in equilibrium through the electrochemical cell.

(iii) Type III sensors consist an auxiliary phase to the electrodes for promoting the selectivity and stability of the potentiometric biosensors. In the designing of a solid-state sensor, the ancillary phase acts as of part of the electrode but due to less electrical conducting nature of the auxiliary phase materials, it is not considered as an electrode. Irrespective of this distraction, these sensor designs attempt more usefulness in designing of various sensors with different accessory materials and electrolytes.

The main classes of potentiometric biosensors include membrane-based ion-selective electrodes, ion-selective field effect transistors (ISFETs), solid-state devices, screen-printed electrodes & electrodes modified through chemical reaction like metal oxides otherwise electrodeposited conducting polymers like sensitive layers. The cost-effective ISFET devices can be easily practised for miniaturization of potentiometric biosensors [26].

In potentiometric biosensors, alterations in ionic concentrations are usually calculated through ion-selective electrodes (Fig. 3.7). pH glass electrode is the most frequently used ion-selective electrode as lots of enzymatic reactions involve in the formation of hydrogen ions. Other important example includes ammonia-selective electrode and CO_2 selective electrodes.

The power variation obtained among the measuring and the reference electrode can be determined which is proportional to the concentration of the substrate. The main drawback of potentiometric biosensor is the sensitivity of enzymes towards H + and NH4 + concentrations.

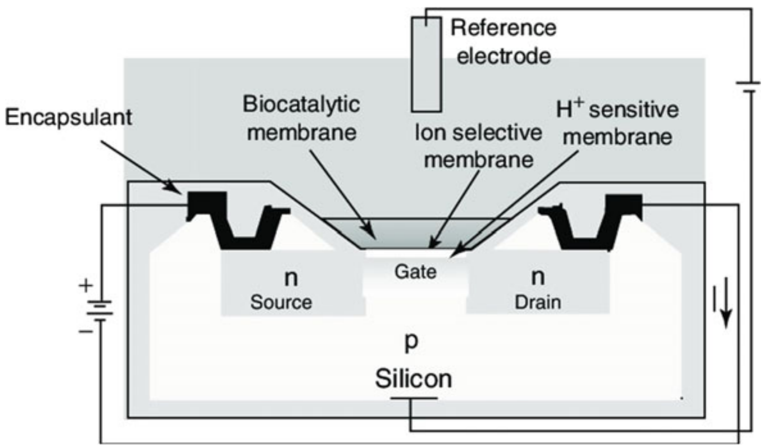

Fig. 3.7 Components and principle of potentiometric biosensor

(c) Impedimetric Biosensor

Impedimetric biosensors generally measure the conductance and capacitance changes at sensor surface due to selective binding of the target analytes (Fig. 3.8) In impedance spectroscopy, the hindrance of electrochemical cell surface is inspected which is dependent and proportional with the concentration of analyte [21]. Electrochemical impedance spectroscopy (EIS) is applied for a wide range of physical and chemical properties measurement. At present, there is ascending tendency towards the inflation of impedimetric biosensors in medical field. Impedimetric technique has been implemented to distinguish the invention of the biosensors and examine the catalysed reactions of various biological element such as lectin enzyme, nucleic acids, receptors, whole cells and antibodies [47].

(d) Voltammetric Biosensor

This biosensor was developed with a carbon glue electrode customized with Hb (haemoglobin), which includes four prostatic groups of the hem (Fe). This type of electrode shows a reversible oxidation or reduction procedure of Hb (Fe) [28]. This device detect analyte by calculating the change in current as a function of applied voltage. The peak current value is used for identification, while the peak current density is corresponding to the concentration of the analogous species. The advantages of this type of electrochemical biosensor are highly sensitive measurements, and multiple analytes can detect simultaneously.

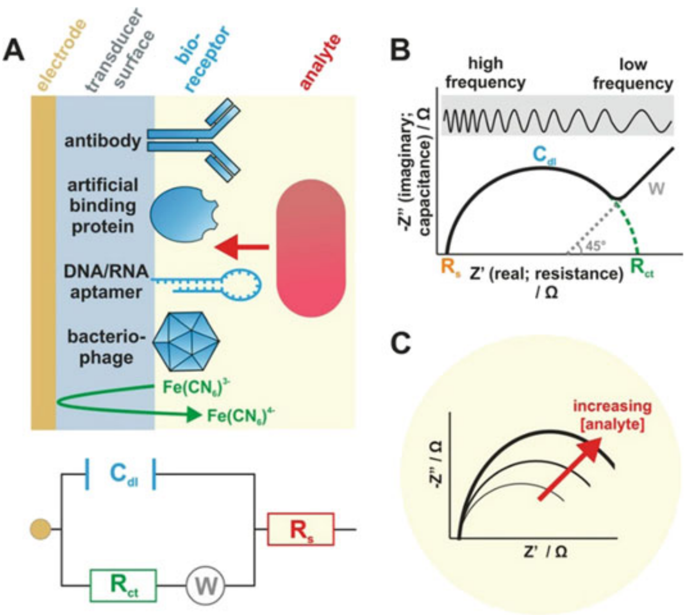

Fig. 3.8 Structure and function of impedimetric biosensor

2. Piezoelectric Biosensors

Piezoelectric biosensor, class of analytical tools, works on the principle of "affinity interaction recording", i.e. by measuring the shift in frequency when the antigen interacts with the antibody receptor [39]. The platform of a piezoelectric biosensor made of sensing element that works on the vibrations transform law due to collection jump on the surface of a piezoelectric crystal. In this measurement, biosensors having their modified surface with specific antigen or antibody, a molecularly stamped polymer, and genetic information [40]. The piezoelectric platform appears to be ideal for the construction of biosensors. It can simply record affinity interactions without the necessity to apply any specific reagents.

Piezoelectric biosensors are also known as acoustic biosensors as they work on the principle of acoustics (sound vibrations) (Fig. 3.9). In this sensor, the crystals having positive and negative charges oscillate with attribute frequencies [55]. Consumption of some molecules on crystal surface amends the resonance frequencies which can be recorded by electronic apparatus. Enzymes with gaseous substances or hindrances can also attached to these crystals.

These biosensors utilize ion-selective electrodes to disciple the biological return into electronic signal. The electrodes employed are most commonly glass pH electrodes coated with a specific gas selective membrane (for CO_2, NH or H_2S) or solid-state electrodes [13].

Many reactions produce H+ ion which is identified and measured by gas sensing electrode where very weak buffered solutions are used. An example of such ion selective electrode is based on urease which catalyses the following reactions:

$$CO(NH_2)_2 + 2H_2O + H^+ \rightarrow 2NH_4^+ + HCO^{-3}$$

The above-mentioned reaction can be measured by either a pH or ammonium ion or NH_3 or CO_2-sensitive electrode. Biosensors can now be prepared by placing enzyme-coated membranes on the ion-selective gates of ISFET which are extremely small.

Piezoelectric biosensors are readily available and reliable devices suitable for the determination of analytes by interactions without application of any reagents [41]. When it is compared with surface plasmon resonance methods having the same character, piezoelectric biosensors can be performed same function with low costs and simple analysis devices. In modern bioanalysis, diagnosis based on the determination of macromolecules can attain popularity for piezoelectric biosensors.

3. Thermometric Biosensors

These are also termed as thermal or calorimetric biosensors. Numerous organic reactions are responsible for the liberation of heat, and this form of calorie generation is the basis of thermometric biosensors [42]. A simple diagram of a thermal biosensor is shown in the following Fig. 3.10. It composed of a heat insulated box along with aluminium cylinder as heat exchanger. In thermometric biosensors, the reaction occurs in a small enzyme-packed bed reactor. When substrate penetrates the

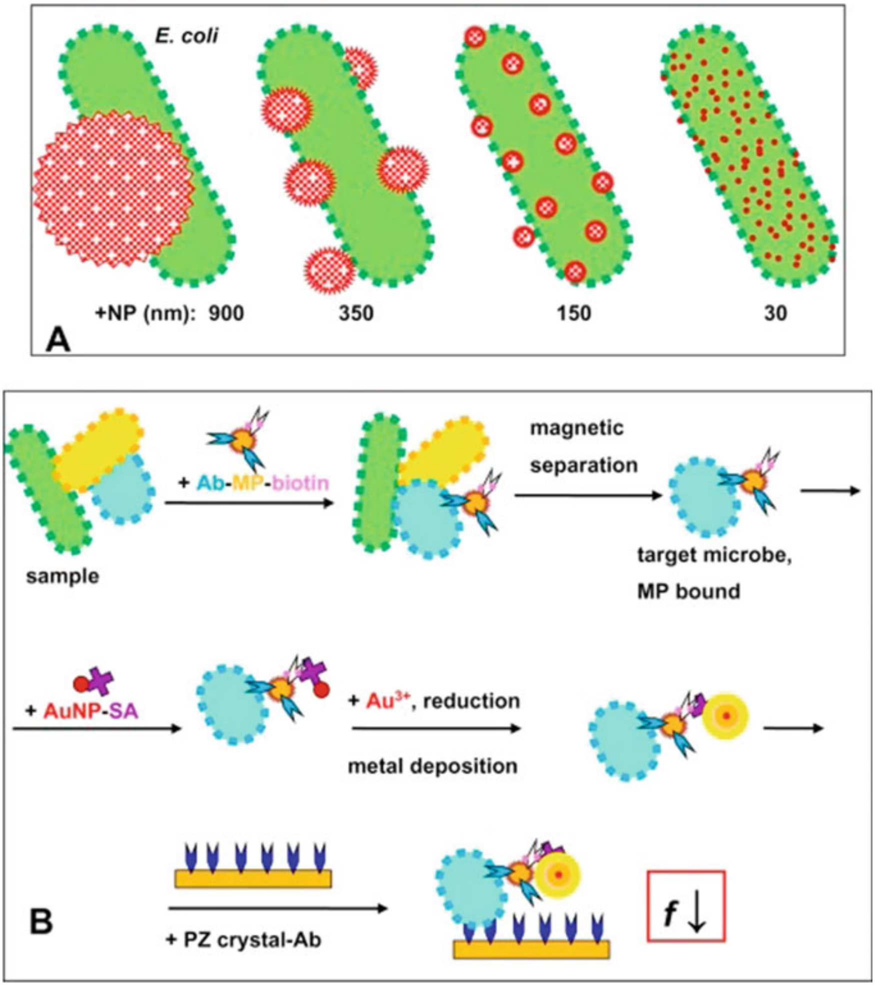

Fig. 3.9 Working principle of piezoelectric biosensor

bed, it transformed to a product and generated heat. The temperature difference between substrate and product is measured by thermistors because a small variation in temperature can easily be detected by thermal biosensors.

Thermometric biosensors are used to measure or estimate the serum cholesterol. When cholesterol gets oxidized by the enzyme cholesterol oxidase, the heat will produce which can be calculated. Similarly, assessments of glucose (enzyme-glucose oxidase), urea (enzyme-urease), uric acid (enzyme-uricase) and penicillin G (enzyme-P lactamase) can be done by these biosensors. In general, their utility is, however, limited. Thermometric biosensors can be used as a part of enzyme-linked immunoassay (ELISA), and the new technique is referred to as thermometric ELISA or TELISA.

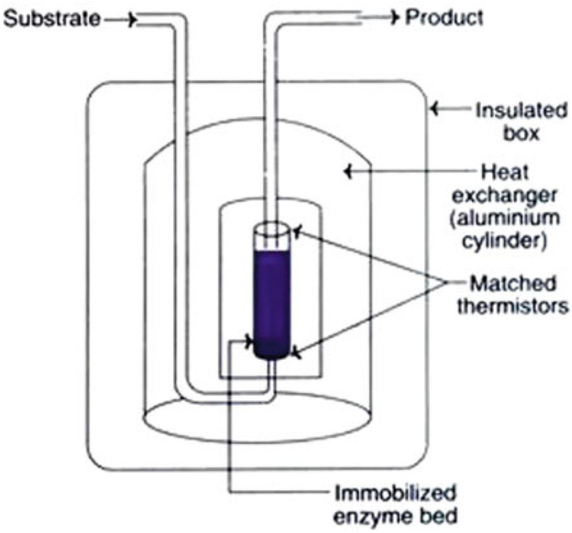

Fig. 3.10 Components and structure of thermometric biosensor

4. Optical Biosensors

Another type of analytical tools implemented using fibre optics and optoelectronic transducers and utilizes the principle optical measurement, i.e. absorbance, fluorescence, chemiluminescence, etc. [11] (Fig. 3.11). Antibodies and enzymes are considered as two main transduction molecules in optical biosensors [3]. The main advantages of optical biosensors include it allows a safe non-electrical remote sensing materials and usually do not require any reference sensors as the correlative signal can be generated using a similar light source like sampling sensor.

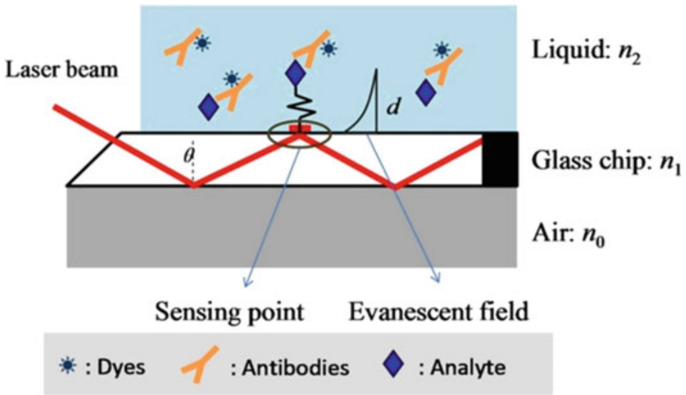

Fig. 3.11 Working mechanism of optical biosensor

Both catalytic and affinity reactions are measured with these biosensors. Optical biosensors measure the fluorescence deviation or absorbance caused by the developed products after every catalytic reaction. Alternately, they determine the alterations induced in the inherent optical properties of biosensor exterior due to loading of dielectric molecules like protein in affinity-type reactions. A most encouraging luminescence biosensor uses firefly enzyme luciferase for identification of bacteria in food or biological samples [10]. The bacteria are specially degraded to release ATP, which is used by luciferase in presence of O_2 to generate light that is measured by such biosensor.

Optical biosensors allow secure non-electrical inaccessible sensing of equipment. The optical biosensors are categorized as non-labelled and labelled optical detection biosensors.

5. Immunosensors

It is a class of biosensor that incorporates a biological recognition mechanism with a transducer which produces a measurable output in reply to alterations in the concentration of given biomolecule [27]. Immunosensors are compact device that reveal specific immunoreaction between antibody and antigen, i.e. creation of a stable immunocomplex is detected by using a transducer and an electrical signal is measured [30]. This distinct immunoreactions between these specific molecules makes immunosensor very interesting and attractive in recent years as a tool for several applications in different fields such as kits for clinical diagnosis & health monitoring, food management, industrial analyses and environment monitoring. These biosensors use antibodies as bioreceptor and a transducer which changes the antibody–antigen interaction event to a measurable outcome. Some examples of immunosensors are optical, evanescent wave, surface-plasmon resonance, fluorescence and chemiluminescence that used for clinical and environmental monitoring. Continuous and selective detection of analyte, yielding a response in real time, are the major advantages of these sensors.

Based on working principle, immunosensors can be classified into two types named nonlabelled and labelled immunosensors. First group of immunosensors are designed in such a way that the antigen–antibody immunocomplex is directly determined by measuring the physical changes that induced by the formation of this compound. On the other hand, a sensitive label is incorporated into second category for detection is referred as labelled immunosensor. As a result, the immunocomplex is sensitively determined through the label identification.

Label-free immunosensors are suitable for point-of-care analysis as devices work very fast and use simple technique [33].

Labelled immunosensors are designed to identify the immunochemical complexation that takes place on the exterior of the sensor matrix. There are number of variations in the procedure to form an immunocompound on this matrix.

3.7 General Working Principle of Immunosensors

Immunosensors are solid-state biosensing devices, where immunochemical reaction is coupled with a suitable & pertinent transducer and considered as most interesting categories of affection-based biosensors depends on the specific recognition of antigens by antibodies to form a stable immunocomplex. Based on the type of transducer used, there are three types of immunosensor available as electrochemical, optical and piezoelectric (Fig. 3.12). All these types can operate as either set of non-labelled or labelled immunosensors.

In recent years, most commonly used bioelements for the development of electrochemical immunosensors are antibodies (Ab), followed by aptamers (Apt) and microRNA (miRNA). Here, the highly specific reaction occurs between variable regions of an antibody and the epitopes of an antigen involving different types of bonding/interactions such as hydrophobic & electrostatic interactions, van der Waals force and hydrogen bonding. The immuno-reaction is reversible due to the relative weakness of the forces that holds the antibody and antigen together; the formed complex would dissociate easily based on reaction environment, i.e. pH and ion strength. The binding strength of immunocompound could be characterized by its affinity constant (K), ranges between 5×10^4 and 1×10^{12} L mol^{-1}. The high affinity and specificity of this antigen–antibody binding reaction confirms the unique immunosensor characteristics.

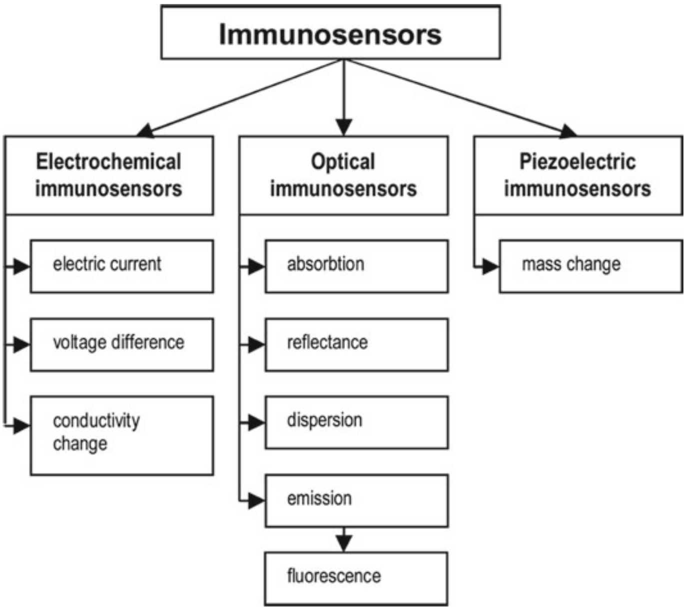

Fig. 3.12 Types of immunosensor

The recent developments made in the immunosensor field include incorporation of nanomaterials for increased sensitivity, multiplexing or microfluidic-based devices, may have potential for promising use in industry and clinical analysis. Some examples of assays for several commercially available biomarkers will be presented. The main application fields, beside biomedical analysis, are drug abuse control, food analysis and environmental analysis.

Immunosensors are often used to identify or quantify the disease-related substances in clinical diagnosis, providing their increased attraction towards antibody–antigen complex with greater selectivity. For example, a novel strategy for AFB1 detection based on DNA tetrahedron-structured probe (DTP) and horseradish peroxidase (HRP) triggered polyaniline (PANI) deposition was developed. In this process, the carboxylic group was associated with the AFB1 monoclonal antibody (mAb) to fabricate DTP.

3.8 Wearable Biosensor

Wearable biosensor is a digital electronic device that can mainly attached on the human limb to monitor and transmit physiological information continuously and non-invasively [24]. Various wearable devices in the form of smart clothing, smart watch, ring, smart shoes, belt, arm strip or band, etc. allow the person to monitor vital signs and detect crucial biological anomalies such as glucose level in blood, blood pressure, heart rhythm, body temperature and other physiological variables that is necessary to calculate regularly and invariably (Fig. 3.13) [31]. Advanced biosensors are able to track targeted biomarkers and help to understand the disease in better way.

Presently, we observe that wearable sensors are providing an indication of development for better universe. Suitable application of such user-friendly sensors permits an authentic degree of knowledge into a patient's real-time well-being stature [6]. This real-time data convenience will furnish preferable clinical choices and will affect embellished fitness results and added proficient use of health systems [7].

For human subjects, wearable sensors may support in incomplete realization of health actions as well as avoid the chance of hospitalization [46]. The major advantages of such sensors are curtailed hospital stays and readmissions that will obviously create positive awareness in upcoming days. Furthermore, investigated report reveals that these sensors will undeniably provide low-cost wearable health apparatus to the world [1].

Fig. 3.13 Wearable biosensors

3.9 Types of Wearable Biosensors and Their Applications

3.9.1 Smart Socks

A pressure sensor is infused within comfortable, textile shocks that can restrict GAIT pattern by which feet-ground interactions and foot-landing technique in various activities and positions like walking, running, standing or sitting position (Fig. 3.14). It is really a wonderful supportive tool to guidance the elders having difficulty in walking and training kit for children who trying to learn walk, inhibit potential injuries during walking, etc. Athletes can also apply such socks to modify training arrangement. User's mobile can store the data recorded by pressure sensors and transmitted wirelessly to the cell phone for satisfactory analysis through a proprietary program, and necessary alarming indicator is set for the subject if necessary. They inform us in real time when we are striking with the heel on the ground.

Fig. 3.14 Smart socks

3.9.2 Ring Sensors

Ring sensor is basically a pulse sensor that allows us to monitor heart rate and oxygen saturation in real time. Light-emitting diode (red & infrared) and photodiode are entrapped into the ring-shaped device and can be worn for long period of time for constant monitoring (Fig. 3.15). When heart muscles contract in each beat, blood ejects from the ventricles with pressure pulse that transferred through the circulatory system. During transmission, pressure pulse travels through the blood vessels as a result vessel wall displacement takes place which can be measured at various points to detect pulse or blood volume changes by the optical method. Generally, photo conductors are applied, but sometimes photo resistors are used to amplify the signals. The entire system is designed and guarded by a special processor. The transmitted waves are conveyed through a digital wireless transmission network to home computer and analysed after receiving.

3.9.3 Smart Shirt

This unique shirt is designed & developed at Georgia tech incorporating special sensors and optical bears to detect wounds and interconnected with the monitor to measure the vital signals of our body (Fig. 3.16). Smart shirt caters a structure for monitoring, instruction processing systems and sensing of the shocks. The sensors can be fixed on the right positions of the shirt for all the users, and it can be washed without damage.

Fig. 3.15 Smart ring sensor

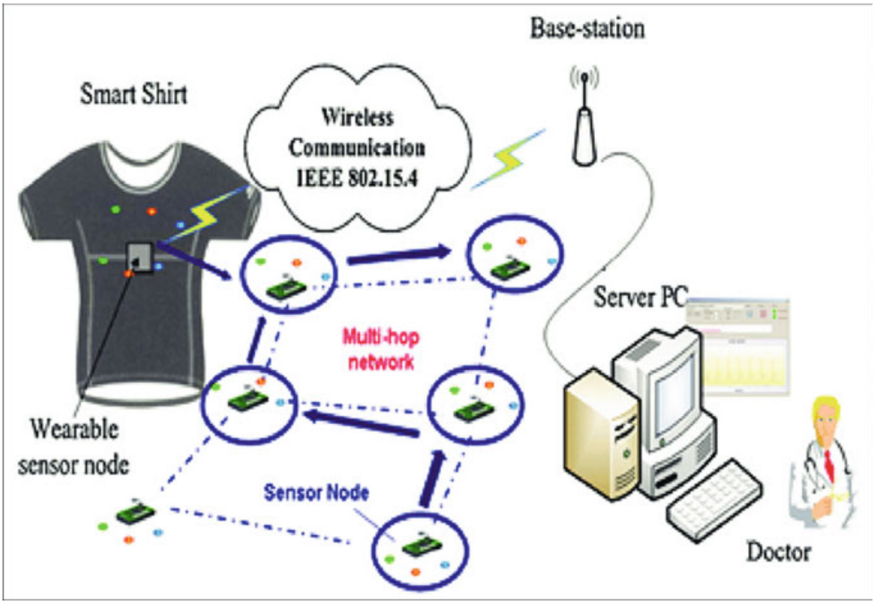

Fig. 3.16 Smart shirt as wearable biosensor

Primary advantages of wearable smart shirt are to help to monitor the physiological variables like heart rhythm, respiration rate, physique temperature, etc. Integrated sensors and conductive fibre grid are accustomed in smart shirt having band connectors that recognize important parameters and transmit signals to wireless server system that carries out future analysis for medical care.

3.9.4 Smart Clothing for Premature Babies

Over 15 million premature babies are born annually in this world, out of which more than one million of this populations dies or suffers physical and psychological complications due to the loss of body water. Researchers from Poland have successfully designed an intelligent clothing for premature babies to prevent the unwanted fluid loss. This clothing is composed of two different layers in which first layer is made of ordinary fabric and second layer is basically a membrane which counters excessive sweating in the baby.

3.9.5 Digital Clothing for Examining Mental Status

Infused miniature sensors in digital clothing can monitor the physiological indicator for determining the psychological state of the people, i.e. heart rhythm, body temperature, as well as skin conductance very efficiently [44]. After that, data are fed to a database through a cell phone, where an appropriate reply is sent considering the present situation and general interest of people. The display curtain in the clothing is equipped with LED lamps for displaying cheerful symptoms when people are in regret or scare.

3.9.6 Benefits of Wearable Biosensors

The biosensors with wearable technology offered most crucial role in managing fitness by sending useful information about performed activities and physical status of the body. Medical sensors in wearable form are the revolution in many application avenues ranging from cardiovascular monitoring to battlefield personnel monitoring and sports mechanics including medicines. Recent advancements of such tiny devices have favoured a sensational gain of attraction in wearable technology.

The usage of wearable biosensors facilitates consistent investigation of physiological signals crucial for upgrading both diagnosis and treatment of diseases. Further, this portable device will help to restrict the use of expensive technologies in patient monitoring for days, weeks or months.

3.9.7 Enzyme-Based Biosensors

An enzymatic biosensor is one kind of analytical device used where enzyme is merged with transducer for recognition and then reacts with the destiny analyte to form chemical signal. The produced output is comparable with the amount % of target analyte which can be amplified, stored, processed for future analysis.

Over the past several decades, development of enzymatic biosensing devices has been extensive for overall investigation of variety target substrates on a vast range of applications [34]. The noticeable benefits that enzyme-depended biosensors provide are high sensitivity & specificity, portability, low cost and the possibilities of miniaturized form and point-of-care diagnostic testing which makes them one of the most extensive and attractive areas for clinical assays, maintained food safety or disease monitoring.

The principle behind the sensing mechanism of the enzymatic biosensor is to identify the existence of some substrates by measuring alterations like H+ ions number, release or consumptions of gases such as CO_2, NH_3, O_2, etc., emission or absorption of light, heat generation and so on, which substrate is consumed or formed a bi-product due to an enzymatic reaction [23]. After that, the transducer transfers those transformation into measurable signals as electrical, thermal or optical that are utilized to find the analytes of concern. Enzyme-dependent biosensors are classified into various groups based on transduction categories such as electrochemical, optical, thermal and piezoelectric biosensors. The working principle of enzyme-based biosensors is shown as block schematic in Fig. 3.17.

In these biosensors, enzyme particles are entrapped over solid support or matrix transforming substrate into the desired product. Thus, confinement of enzyme is considered as protection of catalyst on base. To make it more workable, the enzyme has to heed within the matrix of ascertaining action. Biosensors are anticipated for high stimulant storing in such a way that a sufficient amount of biocatalyst gets accustomed to the surface to assure the biocatalyst receives the appropriate environment to continue their enzymatic activities.

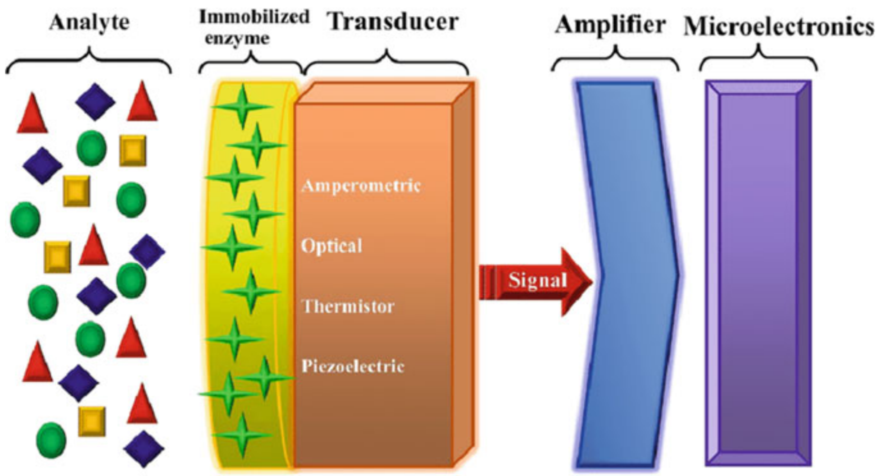

Fig. 3.17 Schematic diagram of working of an enzyme-based biosensor

3.9.8 DNA Biosensor

Based on nucleic acid realization technique, DNA biosensors are being fabricated for the rapid analysis, easiest and cost-effective examination of genetic and contagious diseases. Nucleic acid recognition layers can be easily produced & refurbished for numerous applications that is not possible for enzymes or antibodies [53].

DNA-based sensing devices are considered as application biosensors and play decisive role in disparate domain such as environmental surveillance, foodstuff control, drug exploration, forensic and biomedical research.

Nowadays, application of nucleic acids has become the utmost modern tools for identification and monitoring lots of relevant complexes. As a result, tremendous requirement of detection systems is not only for specific DNA fragments determination but also calculate the total amount of nucleic acid present in sample without error [37]. Over a decade, DNA biosensor technologies have gained immense interest with immense pledge for rapid and inexpensive detection of definite DNA arrangements in human, bacterial and viral nucleic acids [12].

The analysis of nucleic acids has accomplished vast acknowledgement in various domains including diagnostic test, pharmacological findings and various other areas such as animal farming & determination of transgenes. The increasing number of DNA-based tests has accelerated requirement for mechanized, and low-cost testing kit provides miniaturized inspection platform and exquisitely the associated instrumentation.

The functioning of electrochemical DNA biosensor is based on principle of biological synergy between biomolecules & marked analytes that can either generate or consume electrons, which alters the electric current, potential or any other electrical properties of the solution being tested.

The working mechanism of these widely used biosensors depends on hybridization technique via unplanned H bonding between the specific DNA and its reciprocal strand (Fig. 3.18). This principle is usually consummate by entrapping the single-stranded DNA onto a suited platform. Amalgamation episode usually invented by two distinct process: (i) identification of certain electroactive indicator (labeling) and (ii) detection of signal produced by the maximum electroactive base of DNA as adorned in Fig. 3.19.

3.9.9 Biosensors Applications in Medical Field

Healthcare or clinical service sectors are the considerable dominant area where biosensors found their best possible applications. Figure 3.20 illustrates a number of noticeable applications of biosensors that fit under one umbrella of healthcare services and allied areas. Major application area of biosensors includes detection of disease, prosthetic eye, divergent imaging during MRIs, cardiovascular diagnosis, medical mycology, health monitoring, etc. These potential abilities further exhilarate

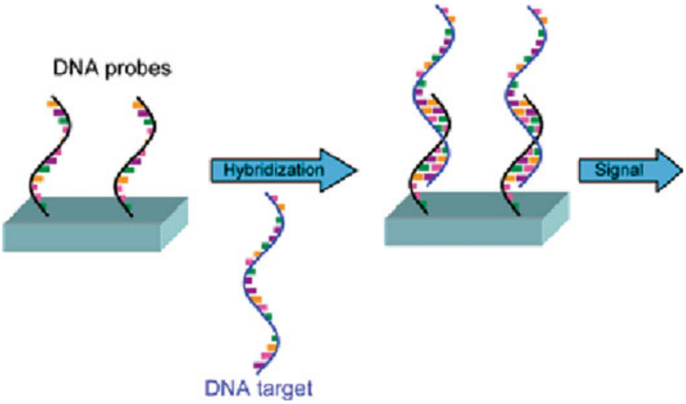

Fig. 3.18 DNA biosensor

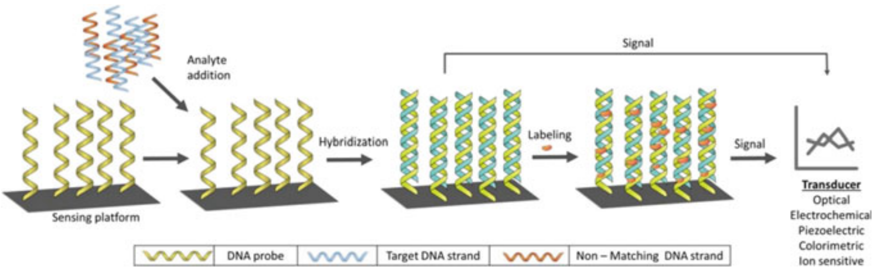

Fig. 3.19 Design and working principle of DNA biosensor

health care to a new pinnacle with admirable social services [25, 49]. The virulent diseases such as avian influenza, SARS, Hendra, Nipah, etc. have gained significant interest in recent years along with latest COVID-19 disease which is highly infectious and is caused by a newly discovered coronavirus that has impacted the world. Thus, biosensors have mammoth power & promise to identify the outbreak caused by virus and/or any disease.

Presently, a number of biosensing devices are used in the medical field not only for regular healthcare monitoring, clinical analysis, diagnosis of disease, etc. but also useful for treatment purposes [18]. These apparatuses are useful to expose a molecule in sensitive bioelements utilizing microorganisms, cell receptors, enzymes, antibodies and DNA/RNA [16]. Biosensors' design and fabrication have been especially symbolic and contributed towards exceptional developments in healthcare sector, leading to exploring new horizon of decisive and potential analytical sensors [38]. In present condition, nanomaterial-immobilized biosensors are used for the COVID-19 detection. Some of the significant applications of biosensors in health care are as:

(i) Tracking of Biological Flaws—Biosensors can solve various medical problems as it measures the indispensable symptoms of a subject and identifies the

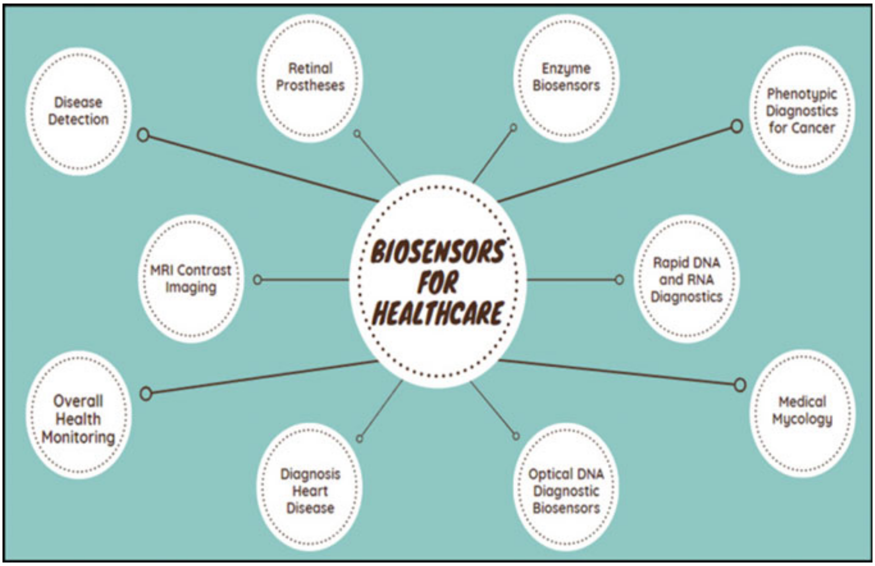

Fig. 3.20 Biosensors in healthcare services

biological abnormalities & irregularities in an efficient manner. Early intervention drive for detection and supervision of danger factors are very essential to reduce the medical costs and prevention of flaws as well. Effective time execution on diagnosis is promising as patient will recover their disorders by faster and effective treatment. With help of medical biosensor, physiological changes can be identified quickly which leads to proactive, earlier treatment. Recent technological advancements in monitoring systems using biosensors can be expanded at lesser cost than hospital remedy.

(ii) Heart Rate Monitoring—A sensor-based watch or band can repeatedly track the heart rhythm and other physiological information. Modern biosensors can identify the targeted biomarkers quite effectively and help the medical personnel to understand the disease clinically. A bioreceptor senses a biomarker's activity and generates associated electrochemical and optical signals. A transducer transforms this crude data to an electric signal which can relay biological data. These technologies are being utilized as an evolutionary algorithm to perform various essential assignments regarding healthcare applications.

(iii) Biochemistry Tracking—Implantable biosensor confined beneath the skin would allow tracking a patient body's chemistry uninterruptedly. The applications of such investigation technology can easily carry out for blood sugar tracking and exercise habits. With this revolution, medical professional is able to monitor the health of the patient from home.

(iv) Diet Inspection—Currently, public can examine the foodstuff with diet sensor, which can also monitor the supplied calorie [45]. Biosensors are being developed for expeditious detection of food pathogens, carcinogens, toxins and pesticides like contaminants [4]. Apart from their quick response, selectivity, easy function, and low cost make biosensors a potential tool for diet monitoring. Health care uses modern nanosensor to test an acetone molecule inpatient breath.

(v) Air Quality Inquiry—Implementing most recent sensor technologies, people can easily monitor air quality. Biosensing technology aids to track everyday atmosphere and measures the human body's core temperature to notify users and healthcare professionals about disease diagnosis. Wireless biosensors meticulously monitor internal body temperature, sleep pattern, sports physiology, clinical testing and hospital uses applications.

(vi) Sugar Level Measurement—For diabetic patient, glucose monitoring is performed by applying electrochemical test strips. Prior to design and development of biosensor in miniature form, blood collected from fingertip was used to determine glucose levels, but nowadays researchers are looking for biosensor-based wearables devices that will monitor blood glucose level continuously. Therefore, biosensors are suitable for some common monitoring applications such as glucose monitoring and diagnostics purpose like maternity and fertility testing. They are also convenient for lifestyle devices like cholesterol monitoring. In recent years, biosensors are also incorporated as medical instruments for cancer and genetic tests.

(vii) Authentic Results and Decision Authoring—These devices provide original information as well as instant and accurate data. Multimodal testing using biosensing instruments can be valuable assets in several medical applications as it has been investigated for decades in decision-making. These devices are used to measure, early diagnosis and treatment of diagnosed disease and future study in allied areas of health care. It facilitates the possible advantages of a multimodal approach to medical applications.

(viii) Heart Rate Tracking for Cardiac Patient—The potential applications of biosensor have increased extensively after screen-printed electrodes make the wearable biosensing devices portable and furnish maximum customized data. By this portable biosensor, heart rate of cardiac patients can be tracked rapidly. It also records the degree of tension levels for service people, legislation enforcement bureau, engineman and many more. Therefore, to continue persistent prosperity and advanced outcomes, these mechanisms can explore to check athletes' strength before and after physical exercise.

(ix) Patient Condition in Medical Unit—Biosensors embedded smart watch or arm band can support children, athletes, older people, and many others. In large context, the healthcare sector is responsible for the exponential development of biosensor technology that biosensors have been used for patient status surveillance in hospitals. These tools easily track the biological processes and provide insightful analytical data for both doctors and patients. Biosensors also have the advantage of making biological activity such as the levels of blood oxygen minimally invasive. The requirement for biosensing process increases many folds due to various conditions including sleep apnoea, peripheral artery disorders and pulmonary obstruction.

(x) Disease Management—Healthcare unit relies on the identification and management of effective diseases through the applications of biosensors. Innumerable clinical disorders such as infection and cancer can be detected via biosensor technology. Biosensors are gain immense opportunity for medical applications with higher sensitivity and accuracy at a lower cost. Therefore, there is increasing requisition for better sensors. As a result, biosensors gain leading role in medical equipment, as well as very common in smart cities, IoT-based devices and wearable equipment. Wearable biosensor can analyse individuals' health features including heart rate, sleep habits, recognition of personality and many more.

(xi) Monitoring of Physiological Parameters—Medical patch on patient's body can investigate physiological variables like skin temperature and respiration rate. In recent years, home monitoring kits for biosensor producers have flourished all over world. These kits allow patients to track their health status every day without facing difficulties. Many critical illnesses such as cancer may be monitored at home. Patients would potentially make the device accessible in a high percentage of medical facilities when biosensors are easily available in hospitals. It can greatly enhance the patient experience because they continue to play a pivotal role in personalizing health care.

(xii) Tracking Cell Protein—The biosensor is highly effective to measure live cell proteins without labels in real time. A microfluidic system consists of a cell module and a biosensor module structures. It is positioned middle on a single zigzag channel where the architectural module of microfluidic cell provides the secreted proteins with a straight-forward tube network to nearby biosensor module. The dynamics of protein secretion can be pursued by invariably transcript the spectral shifting. The proposed microfluidic stage has explicit capacities in small footprint for multiplex and label-free disclosure that pledge to be miniature and integrated into lab-on-chip instruments.

(xiii) Symptom Detection in Patient—For quick identification of symptoms in patient, medical practitioners use biosensors rather than standard procedure. Biosensor manufacturers have given a special attention on enhancing it application in health sector to improve the quality of life. Hospitals and healthcare centres routinely used this to control blood pressure & sugar and cardiac rhythm in many distinct ways. These devices facilitate the physicians to execute patient diagnostic analysis in real time. A number of biosensors in miniature form can be tattered to collect patient data meticulously which makes it simple for medical set-up to grasp smart wearable technology without delay.

(xiv) Biomolecule Detection and Evaluation—Biosensors are fundamental tools of analytical process for biomolecular recognition and analysis. It allows the researcher to observe the levels of O_2 continuously in chips in actual time. This grants our body systems to reproduce the function of essential systems more precisely. The main objective of sensor research and design of new drugs with small-scale biomolecules mimics the specific organ functions, including O_2 exchange into bloodstream from the air.

(xv) Postsurgical Treatment Monitoring—Biosensing devices also contribute the opportunity to strengthen follow-up treatment for postoperative surgery by using dissolved pressure sensors for brain and sensors which control implants infection & inflammation. Working principle and process for getting more systematic and logical processing, storing and exchanging this information with appropriate stakeholders are decisive to make this data a significant influence on patients' treatment. Successful application of biosensors in medical care has very imperative for growing public confidence during handling of patient information.

3.10 Conclusion

Detection and diagnosis of several human diseases at early phases of their growth grant successful treatment of the patients at the initial stage and are most essential. Therefore, it is indispensable to design and establish a cost-effective, elementary, highly responsive, user-friendly and competent diagnostic tools like biosensors that efficiently detect the diseases. Biosensors are analytical devices that are engaged in various medical operations such as disease monitoring, drug exploration, pollutant disclosure, identification of disease-creating microbes and disease-indicating markers in body fluids mainly in blood, urine, saliva, sweat, etc. Biosensing device is considered as highly decisive tool which grants people to regulate the target glucose levels in bloodstream and resolve green feedback to the disease. Due to its rapid diagnosis of diseases as well as accurate inspection of patient health, biosensors can help medical practitioners and patients in many ways like control sickness, clinical care, precautionary treatment, patient health information and disease scrutiny more accurately. Recently, wearable biosensors have gained considerable enthusiasm as they are highly capable of providing uninterrupted, real-time physiological information via dynamic, non-invasive measurements of biochemical markers in in sweat, tears, saliva and interstitial fluid. Present and future evolution have been focused on amalgamation of electrochemical and optical biosensors along with the advancement in the monitoring of biomarkers including metabolites, microbes, enzymes, DNA, hormones, etc. Thus, fusion of multiplexed biosensing, microfluidic sampling and transport systems must be interspersed, shortened and combined with flexible materials for improved wearability, better performance and simple operation process.

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