Chapter 17
Introduction to Biosensing

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17.1 Introduction

Various analytical methods are used as detection methods for routine analysis in medicine, agriculture, pharmaceuticals, food production, etc. However, most of these methods are time consuming, expensive, require well-trained experts, and often suffer from false-positive or false-negative results [146, 149]. Many experts believe that the advent of biosensors can solve these problems. Biosensors can become alternative analytical tools with high efficiency, high sensitivity, and selectivity. In this chapter, we consider various approaches and materials that are used to develop biosensors, as well as evaluate the prospects for their application in various fields.

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17.2 Biosensors. What Is It?

The first biosensor was invented in the 1950s by the American biochemist L.L. Clark [38]. This biosensor is used to measure the oxygen content in the blood and the electrode used in this sensor is called Clark electrode or oxygen electrode. Then, in 1962, Clark and Lyons [37] applied a gel with an enzyme that oxidizes glucose to an oxygen electrode, which led to the development of the first glucose meter to measure blood sugar levels. It is from this time that the era of biosensors begins [53].

Currently, a biosensor is understood as an analytical device that combines a physicochemical transducer with a biomolecule or biological active element such as proteins, enzymes, nucleic acids, and microorganisms to generate a signal proportional to the analyte concentration [4, 105, 154, 155, 160]. Thus, a biosensor can be represented as a device shown in Fig. 17.1.

In the first segment, the sensor is a bio-receptor with recognition elements for selective binding of target analytes contained in biological fluids such as whole blood, serum, plasma, urine, saliva, sweat, tissue, and cell culture extracts [149]. The second segment is the detector part, a transducer that converts interaction with a biological object into an electrical or optical signal. The biological component identifies and also interacts with the analyte to generate a signal that can be measured. These receptor biomolecules at concentrations of 1–5 μg/mm^2 are properly immobilized on the transducer surface using different physical and chemical methods, which depend on the choice of the measurement method used and the type of biosensor. Schematically, the main methods of immobilization are presented in Fig. 17.2. The last section is an electronic device that measures this signal, amplifies it, converts it, and displays it in an accessible form. It includes an amplifier, which is known as a signal conditioning circuit, a display unit, and a processor. The main characteristics of biosensors are stability, cost, sensitivity, and reproducibility of parameters. Linearity, selectivity, and response time are also important biosensor parameters.

As our knowledge about the operation of biosensors improves, the requirements for sensor systems based on them become more complex. Currently, scientists identify three requirements for the sensory system of the next generation [126]: (1) a change in the system caused by interaction with the analyte must lead to a visible result; (2) the change in the system should not depend on the target molecule, so that the system as a whole can be used for the detection of various substances; and

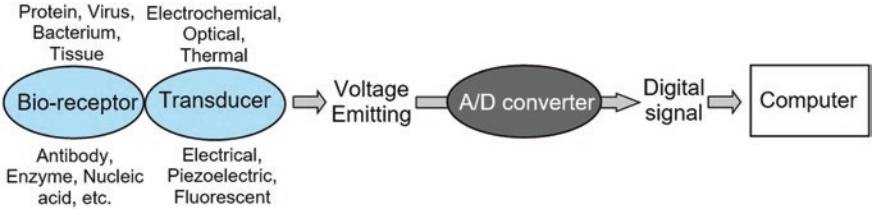

Fig. 17.1 Typical configuration of biosensor. (Idea from Yoon [177])

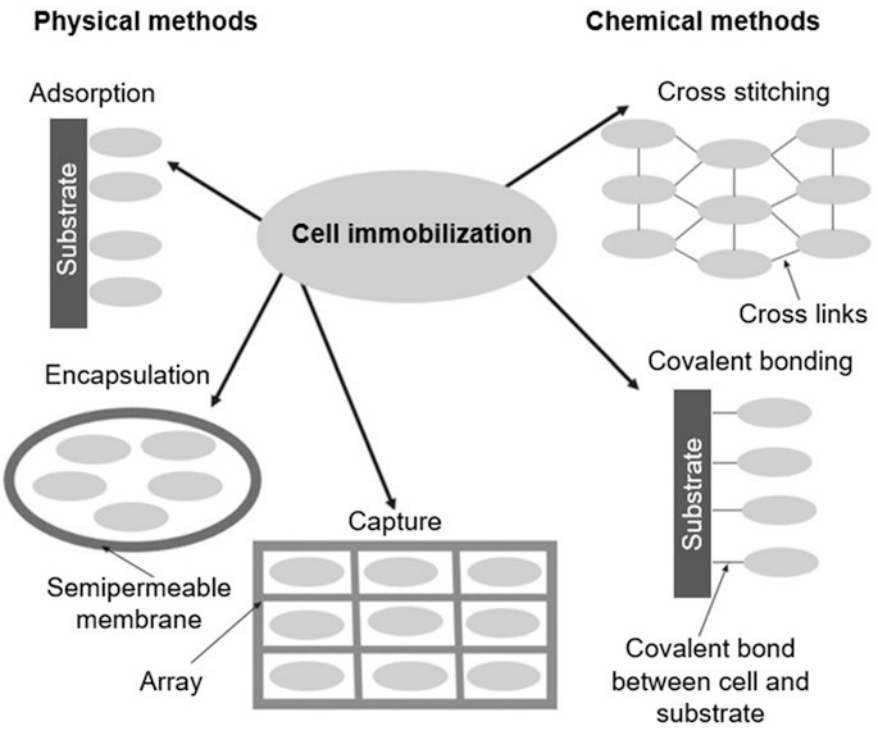

Fig. 17.2 Schematic representation of the main methods of cell immobilization. (Idea from Plekhanova and Reshetilov [122])

finally (3) the system must be easily configurable so that substances with different energy binding characteristics to the sensor can be detected, and so that the minimum detectable concentration can be adjusted.

17.3 Types of Biosensors

As mentioned earlier, all types of biological elements such as enzymes, antibodies, receptors, and living cells, can be used as a bio-selective element. Various transducers can also be used: electrochemical, optical, gravitational, calorimetric, and resonant systems. Theoretically, any bio-selective element can be combined with any of the possible transducers. As a result, there is a wide variety of different types of possible biosensors, which are classified depending on the transducer type, the materials used, as well as the biological element immobilized in the biosensor.

Biosensors that can quickly and reproducibly recover are reusable. Using reusable biosensors, direct monitoring of an increase or decrease in the concentration of the biological agent being determined is carried out. Biosensors that cannot be

reproducibly and quickly restored are called disposable, and these include bioassays and bio-indicators. The potential use of disposable biosensors, especially in environmental monitoring, is more focused on warning and signalling systems that do not require accurate determination of the concentration of the analyte.

17.3.1 Electrochemical Biosensors

This type of biosensor produces an electrical signal proportional to the analyte concentration when it reacts with it [35, 66]. Such biosensors typically have three separate electrodes: a working electrode (WE), a counter electrode (CE), and a reference electrode (RE). One of these electrodes (WE), on the surface of which a layer of biomaterial is deposited via the immobilization method, generates a potential, while others generate an electric current resulting from the reaction of the biomaterial with the analyte. As a rule, the operation of an electrochemical biosensor is based on an enzymatic catalysis reaction that occurs on the surface of the active electrode and consumes or generates electrons, forming a signal that corresponds to the concentration of the investigating analyte [28, 127]. The enzymes involved in this reaction are called redox enzymes.

Electrochemical biosensors are classified into four types:

- Amperometric biosensors
- Potentiometric biosensors
- Impedimetric biosensors
- Voltammetric biosensors

  – An *amperometric biosensor* is a device that measures the magnitude of the current produced due to the oxidation-reduction reaction occurring on the surface of the working electrode [18, 23, 81, 121]. Typically, these biosensors have a response time, energy range, and sensitivity comparable to potentiometric biosensors. The Clark oxygen electrode is a simple amperometric biosensor. More complex biosensors also exist. For example, Singh et al. [141] have developed a DNA-based amperometric sensor for one of the most common human pathogens—*Streptococcus pyogenes.*
  – The main types of *potentiometric biosensors* are membrane-based ion-selective electrodes (ISE) and ion-selective field-effect transistors (ISFET). These biosensors are often equipped with electrodes coated with a polymer to which an enzyme is bound. Enzyme-analyte interactions result in a change in potential. Therefore, the transducer used here measures the variations that exist between the potential of reference electrode and working electrode [102]. This type of biosensor provides a logarithmic response.
  – *Conductometric* or *impedimetric biosensors*: In a sensor of this type, due to biocatalytic reactions, the ionic composition of the solution varies, as a result of which a change in the conductivity of the solution in the sensor is observed [54]. This effect is used in various biosensors for the detection of urea [158], *Escherichia coli* [69], microbes [81], and many other biological analytes.

Despite some advantages, such as low-cost thin-film applications [135], real-time direct monitoring [19], no need for a reference electrode, and the possibility of miniaturization, this method gives less sensitive responses than other electrochemical methods [79].

– *Voltammetry* is a widely adaptable method of electrochemical analysis, and there are a large number of reports on this type of biosensor [44, 124]. During the measurement process, current and voltage are simultaneously monitored. Voltammetric methods can be divided into: cyclic voltammetry (CV), differential pulse voltammetry (DPV), stripping voltammetry, AC voltammetry, polarography, linear sweep voltammetry (LSV), etc. However, CV, DPV, and LSV are most commonly used. The difference lies in the way the potential is changed at the electrodes [79]. The simplest is LSV, where at WE the applied potential increases linearly with time. The position of the current peak is related to the nature of the detected analyte, and the magnitude of the peak is related to its concentration. One of the main advantages of this type of sensor is that this system has a low noise level, which makes it possible to create a biosensor with high sensitivity. Moreover, many compounds with different characteristic potentials can be found in one measurement [81].

The use of electrochemical sensing offers significant advantages that include high sensitivity, high accuracy when the surface of electrodes is well modified with for examples biomarker this could also be used for rapid detection as well as suitability for field applications. The advantages of these devices also include simple measuring equipment and the possibility of developing portable devices. In addition to their cost-effectiveness, high sensitivity, and large range of detection linearity, electrochemical sensors are capable of handling small sample volumes. In addition, the measurement result is not affected by the turbidity of the samples, unlike optical methods based on spectroscopic transduction [17, 142].

17.3.2 Physical Biosensors

Any biosensor that uses a response to a change in the physical properties of the medium has been called a physical biosensor. Physical biosensors are the most fundamental, widely used sensors. Physical biosensors are divided into three types: piezoelectric biosensors, thermometric, and magnetic biosensors.

- *Thermometric biosensors* are type of biosensor in which the heat is released as a result of various biological reactions [2]. A heat-sensitive enzyme sensor known as “thermistor” was developed by Mosbach and Danielsson in [111]. This type of biosensor measures changes in the temperature of a solution containing an analyte resulting from enzymatic reactions. The thermometric biosensor is also used to measure or evaluate serum cholesterol levels. When cholesterol is oxidized by the enzyme cholesterol oxidase, heat is released that can be calculated. Similarly, glucose, urea, uric acid, and penicillin G levels can be assessed using these biosensors [2].

- *Piezoelectric biosensors* work on a principle affinity by interaction of recording [123, 156]. Piezoelectric biosensors typically have a surface modified with an antigen or antibody. When an analyte molecule is attached to a membrane, cantilever, or surface of a piezoelectric crystal, their mass changes, resulting in a change in the resonant frequency of the oscillation. This change in frequency, proportional to the change in mass of the adsorbed analyte, can be measured. Piezoelectric biosensors are also known as acoustic biosensors. These sensors offer many advantages [103]. Based on acoustic biosensors, immunosensors have been developed for the detection of *Salmonella typhimurium*, the herpes virus [45]. Using specific immunoglobulins and *Francisella tularensis* antigens, piezoelectric biosensors have been developed to detect the causative agent of tularemia in water and milk [125].
- The *magnetic biosensor* is another type of biosensor. This type of sensor is used to detect changes in magnetic effects or magnetic properties caused by the interaction of a bio-receptor with an analyte [114]. Recently, with the development of magnetic nanoparticles and their use in biosensors, new diagnostic methods at the nanolevel have appeared that allow diagnosing diseases at an early stage [173, 176]. For example, Yang et al. [176] developed a core-shell aptarsensor containing a magnetic core, an aptamer, and gold nanoparticles. After the addition of the target molecule, an interaction occurs between the target molecule and the aptamer thrombin, which leads to the release of gold nanoparticles from the magnetic ball. The amount of thrombin can be calculated based on the amount of released gold nanoparticles. The resulting limit of detection was 2.54 fM.

17.3.3 Optical Biosensors

An optical biosensor is another type of biosensors most commonly used for bio-recognition [11, 24, 33, 63, 72]. Optical detection is usually based on the measurement of luminescent, fluorescent, colorimetric, or other optical signals arising from the interaction of microorganisms with analytes and correlating with their concentration. Optical biosensors typically use a light source, a light detector, and a sensitive material that allow interaction with the analyte in the gas and liquid phases [1]. Antibodies and enzymes are mainly used as bio-receptors in these sensors.

- *Fluorescent biosensors*: The basic principle of operation of this biosensor is based on the change in the absorption/emission of light when a detectable analyte is added. Fluorescence can be defined as an optical phenomenon in which shorter wavelength photons are absorbed, resulting in longer wavelength emission. Due to its high performance in terms of selectivity, sensitivity, and low detection limit, the luminescent sensors are most applicable for both chemical and biological analyses [91, 92]. Fluorescent microbial biosensors can be divided into two categories: in vivo and in vitro [63]. The in vivo fluorescent microbial biosensor uses genetically modified microorganisms with a transcriptional fusion between an inducible promoter and a reporter gene encoding a fluorescent pro-

tein. The green fluorescent protein (GFP), encoded by the gfp gene, is one of the most popular tools due to its attractive stability and sensitivity, and the fluorescence emitted by GFP can be conveniently detected by modern optical equipment with little or no damage to the host system.

- *Biosensors based on bioluminescence*: Bioluminescence is associated with the emission of light by living microorganisms and plays a very important role in real-time monitoring of the process. The bacterial luminescence *lux* gene is widely used as a reporter, either in an inducible or constitutive manner. In the inducible manner, the reporter *lux* gene is fused to a promoter controlled by the concentration of the analyte of interest. As a result, the concentration of the analyte can be quantitatively analyzed by determining the intensity of the bioluminescence [58].
- The operation of a *colorimetric biosensor* is based on the formation of a colored compound during the interaction of a bio-receptor with an analyte, the color of which can be assessed and correlated with the concentration of analyte [39]. For example, a whole cell colorimetric biosensor has recently been designed to detect arsenite with high sensitivity. His work is based on the following; the photosynthetic bacterium, *Rhodovulum sulfidophilum,* synthesizes carotenoids via the spheroiden (SE) pathway, where yellowish SE is catalyzed by SE monooxygenase (CrtA) to form reddish spheroidenone, which is the predominant carotenoid under semi-aerobic conditions. In this biosensor, a genetically engineered photosynthetic bacterium, crtA-deleted R. sulfidophilum, was used as the host strain, which accumulated SE and therefore displayed yellow color [63].
- *Surface Plasmon biosensors*: Among the family of optical label-free biosensors, surface plasmon resonance (SPR) is one of the most accessible, developed, and most successfully used technologies for medical diagnostic applications in recent years. This is due to the high sensitivity and versatility of this type of sensors. In addition, they allow real-time detection and direct measurement of the kinetics of molecular ligand-receptor interaction [113, 118]. The principle of operation of such biosensors is based on the effect of surface Plasmon resonance. If gold (or silver) is deposited onto a hydrogel plate, then a phenomenon arises associated with the presence of free electrons in this metal, which, when illuminated, begin to be affected by an alternating electric field. These electrons are able to collectively oscillate and resonate, adjusting to the frequency of the incident light. Such electron oscillations in noble metal nanoparticles are called plasmonic oscillations [65, 115]. In addition, the unique properties of gold and silver nanoparticles that can be used instead of a continuous film increase the sensitivity and selectivity of biosensors [118, 179] For example, in recent years, a SPR-based biosensor has been proposed for the detection and monitoring in urine of biomolecules of *M. tuberculosis* and nontuberculous mycobacteria, such as CFP10 and MPT64 [36, 51, 65]. Based on the principle of surface Plasmon resonance, biosensors have also been developed to detect anthrax spores [168] and serogroup O1 *Vibrio cholerae* [78].
- *Chemiluminescent-based biosensors:* Chemiluminescent is the process in which emission of light takes place due to chemical reaction. Either the emission can be

produced/generated by direct oxidation of the chemiluminescent reagent, resulting in the formation of emitting substances, or indirectly through the enhancing/inhibiting effects of certain phosphor compounds. Recently, chemiluminescence research has been extended to nanomaterials to improve intrinsic sensitivity and extended to new detection applications [49]. For example, Luo et al. [93] developed a chemiluminescence-based biosensor to detect DNA using graphene oxide, which exhibited high sensitivity and selectivity with a limit of detection of 34 pM.

Optical biosensors can be label-free or label-based depending on the choice of detection method as well as the analyte of the biosensor. In label-free detection method, there is no need for any probe mediator and the interaction of analyte with bio-receptor in the sensor is converted either directly into electric signal that is measurable or combined intrinsically with another transduction for the production of signal for detection of the analyte [139].

Optical-based biosensors offer advantages of compactness, flexibility, resistance to electrical noise, and a small probe size [169]. Optical biosensors allow for safe nonelectrical noncontact sensing. An additional advantage is that they often do not need reference sensors, since a reference signal can be obtained using a light source. However, one of the big problems with an optical sensor is the sensitivity to humidity, which can vary widely. Another disadvantage of label-free optical biosensors using the effects of prismatic light refraction and SPR is their relatively large size, which is incompatible with mobile use in point-of-care mode [113, 164]. Therefore, a modern promising alternative to this type of optical biosensors are fiber-optic biosensors, which are excellent for creating miniature portable devices, which have a low cost and have successfully proven themselves for clinical diagnostics [113]. For example, fiber-optic-based immunosensors have been developed to detect the capsular antigen "fraction 1" of the plague microbe [10] and antibodies to it [9].

Various approaches can be used for immobilization in fiber optic biosensors. But in recent years, the use of hydrogels for these purposes has become most widespread. Hydrogels (polyacrylamide) are hydrophilic cross-linked polymers that can be molded into a variety of shapes for immobilization, ranging from thin films to nanoparticles. Hydrogels are considered to be a simple substrate for DNA immobilization, with a number of advantages, such as the ability to retain molecules, their controlled release, enrichment of analytes, and protection of DNA. These characteristics are unique for hydrogels compared to other materials suitable for biomolecular immobilization. Compared to other materials, immobilization in a hydrogel occurs in three dimensions, which ensures the loading of a large number of sensitive molecules. Moreover, the good optical transparency of hydrogels allows for a convenient visual detection strategy. Methods for immobilizing DNA biosensors in monolithic polyacrylamide gels and gel microparticles are often regarded as a technical achievement in the field of biosensor technologies.

At the end of the consideration of biosensors using various transducers, we will give a comparative description of several biosensors and test systems for enzyme immunoassay (Table 17.1) and biosensors for the detection of influenza virus (Table 17.2).

Table 17.1 Comparative characteristics of biosensors and test systems for enzyme immunoassay

Sensor group	Analysis time, min	Advantages	Limitations
Potentiometric biosensors	30–45	Simplicity, reliability	Slow response, sensitive to electrical noise
Amperometric biosensors	55	Low cost, small size, stable response	Low sensitivity, low selectivity
Piezoelectric biosensors	5–10	Speed, stability of response	Low sensitivity, nonspecific binding error
Biosensors based on SPR	40	High sensitivity, noncontact measurement	Optical interference, the need for photodetectors
Fiber-optic biosensors	1–3	Fast response, little influence of electrical interference	The need to use photodetectors
Immunoenzymatic test systems	180–240	Specificity	Duration of analysis, the need to use labels, chromogen

Source: Data extracted from Utkin et al. [162]

Table 17.2 Comparison of the sensitivity of the developed biosensors for the detection of influenza virus

Sensor	Probe	Virus	Sensitivity or affinity	Remark
SPR	Glycan-ligands	H1N1	K_d 1.5 μM (10 μg/mL, ~17 μM)	Low affinity and nonspecific interaction
	RNA aptamer	H1N1	K_d 67 fM	High affinity.
	Glycan	H5N1	K_d 1.6 nM	High affinity; specific substrate (Biacore chip) required
	DNA aptamer	H5N1	K_d 4.65 nM	Good affinity.
	RNA aptamer	H3N2	K_d 120 pM	High affinity; unstable RNA aptamer probe
QCM	Glycan	H5N3	K_d 14.4 nM	Good affinity
WG	Antibody	H1N1, H5N1	1 nM	Dissociation of dye from antibody may decrease sensitivity.
IF	RNA aptamer	H3N2	10 nM	Relative low sensitivity
EC	Immunoliposome-Ru	H1N1	3×10^{-14} g/mL (12 fM)	Very high sensitivity; complex probe preparation step
FET	Glycan	H5N1, H1N1	50 aM	Very high sensitivity

Source: Reprinted from Chen and Neethirajan [32]. Published by MDPI as open access. Table 1
EC Electrochemical, *FET* Field Effect Transistor, *IF* Interferometry, *QCM* quartz crystal microbalance, *WG* Waveguide

17.3.4 Sensors Based on Specific Biological Material

17.3.4.1 Enzyme Biosensor

An enzyme biosensor is a sensor on the surface of which an enzyme is immobilized [95]. Enzymes are biological catalysts that have a pronounced ability to selectively catalyze many chemical transformations both in a living cell and outside the body. When enzymes are adsorbed on solid surfaces (metals, ceramics, polymers, semiconductors), they usually retain their structure and catalytic activity. The conjugation of enzymatic catalytic and electrochemical reactions occurring on electrically conductive materials immersed in an electrolyte solution has made it possible to develop many modern biosensors. The simplest case in the design of an enzyme biosensor is realized under the condition that either the substrate or the product of the enzymatic reaction is electrochemically active, that is, they are able to quickly and preferably reversibly oxidize or reduce on the electrode when an appropriate voltage is applied to it. It is most convenient to carry out measurements on enzyme electrodes in the amperometric mode. The best known enzyme biosensor is the glucose biosensor discussed earlier. They use the enzymes glucose oxidase or glucose dehydrogenase as a bio-recognizing component, which are immobilized on the electrode surface and break down glucose. The products of enzymatic reactions are transformed into a physicochemical signal [55]. It is important to note that, when designing an enzyme biosensor, the main task is to increase the duration of the enzyme action. The fact is that the native enzyme retains its properties only for a relatively short time. Therefore, the operation of the so-called immobilization of enzymes was developed (see Fig. 17.2). In the process of immobilization with the help of special reagents, the enzyme is "fixed" either on the surface of adsorbents, such as silica gel, coal, or cellulose, or is introduced into a porous polymer film, or is "attached" covalently through chemical bonds to some substrate. Upon fixation, the enzyme ceases to be mobile, is not washed out of the bio-layer, and its catalytic activity lasts much longer.

17.3.4.2 DNA Biosensors or Aptasensors

An aptasensor is a special class of biosensors in which the element of biological recognition is a DNA or RNA aptamer. In the aptasensor, the aptamer recognizes the molecular target against which it was previously selected in vitro. The development of DNA biosensors has been based on nucleic acid identification techniques for simple, rapid, and cost-effective testing of genetic and infectious diseases. In addition, the accurate detection of a DNA series is important in several fields such as food quality analysis, clinical analysis, environmental monitoring, etc. It is believed that aptasensors are very promising analytical devices due to the high specificity and stability of nucleic receptors, their low cost, and the potential for creating various sensor platforms [161, 180]. For example, SAM [16] and SELEX [40] technologies, which were developed to more efficiently recognize DNA biosensors, can

be used to improve detection methods. Self-assembled monolayer (SAM) is the method for DNA surface immobilization and a paradigm for the attachment of unmodified DNA of any length or sequence. Systematic evolution of ligands by exponential enrichment (SELEX) is a well-established and efficient technology for the generation of oligonucleotides with a high target affinity. These SELEX-derived single-stranded DNA and RNA molecules, called aptamers, were selected against various targets, such as proteins, cells, microorganisms, and chemical compounds.

17.3.4.3 Immunosensors or Antibody-Based Biosensors

Immunosensors were created based on the fact that antibodies have a high affinity for their specific antigens. For example, antibodies specifically bind to toxins or pathogens or interact with toxins or pathogens through components of the host's immune system. Antibodies, antibody fragments, or antigens are used for the monitoring of bioelectrochemical reactions in immunosensors [66]. Immunosensors are widely used, in particular, to detect *Soybean* rust [106], hepatitis B antigen [60], and virus [92].

17.3.4.4 Protein Biosensors

Protein biosensors are created on the basis of naturally occurring proteins. To do this, a protein domain is selected that can bind to the target molecule and connect to a reporter domain that produces a readable signal. In particular, biologists have created a class of proteins that glow in the presence of a given pathogen or disease marker [84]. The main drawback of this approach is laboriousness, because a lot of bioengineering transformations are required to obtain an effective biosensor from these two domains. Therefore, researchers are looking for a universal platform on the basis of which it will be possible to easily create biosensors for various pathogens or disease markers using protein molecules, simply by replacing the necessary "details" in it. Studies performed by Baek and Baker [15] have shown that in order to create biosensors for new target molecules, it is enough to carry out computer analysis and replace one of the protein domains. Quijano-Rubio et al. [126] tested this approach when developing sensors for botulinum toxin, coronavirus, a marker of myocardial infarction, and other clinically significant targets. In fundamental research, protein sensors are used to study the processes occurring in cells, and in medicine, for example, they are used to predict the effectiveness of therapy for patients with cancer.

17.3.4.5 Cell-Based Biosensors

One of the achievements of biotechnology and bioengineering is associated with the development of methods for incorporating living cells into polymers and solid carriers of various nature and the use of such materials for the development of

biosensors for various purposes [41, 129, 163, 174]. Several surprising properties of immobilized cells can be noted. First, cells are available biological material. Cells of plants, animals, and humans are used, but the cells of microorganisms that are cultivated, easily reproduced, and maintained in pure culture have found the greatest use. Second, unlike enzymes, the use of cells does not require expensive purification steps. Third, the available methods of immobilization make it possible to obtain cells that retain about 100% of enzyme activity and are able to function for sufficiently long periods of time. Features of cell metabolism make it possible to create biosensors both for individual molecules and for very wide classes of compounds. To create cell-based biosensors, as well as for enzyme sensors and aptasensors, a wide variety of physical transducers can be used: from electrochemical to optical and acoustic.

Currently, cell-based biosensors have been created for the determination of phenols, glutamine, tyrazine, and lactic acids. There are also cell-based biosensors for water and wastewater quality analysis. An amperometric sensor for ammonia (in wastewater) based on immobilized nitrifying bacteria and a Clark oxygen electrode can be mentioned as an example of such devices. The advantages and disadvantages of microbial biosensors compared to enzymatic biosensors are presented in Table 17.3. The main advantage of cell-based biosensors is that the cell is the most comfortable environment for enzymes, since they are in their natural state and therefore have the highest stability when functioning in a biosensor. Significant

Table 17.3 Advantages and disadvantages of cell-based biosensors compared to enzymatic ones

Advantages	Limitations
Broad substrate specificity of microorganisms	Low speed of analytical signal generation
Lack of labor-intensive techniques such as isolation, purification, and immobilization of pure enzymes	Low selectivity
At several stages of substrate oxidation, the electrochemical signal when using whole cells is higher than in the case of an isolated enzyme	High adaptability and variability of properties
Some enzymes lose their activity upon isolation or immobilization, if these processes lead to damage to the active center or to the disintegration of the enzyme complex. By using whole cells, this danger is minimized	The problem of maintaining the activity of microorganisms unchanged for a long time
Enzymes in cells are in their natural environment, so many biosensors based on microorganism cells are characterized by high stability	
The cells contain coenzymes and activators of biochemical processes, so there is no need to add them to the measurement medium	
Many microorganisms have been genetically characterized in detail	
The use of mutations makes it possible to further increase the activity, selectivity, and specificity of the cell-based biosensor	
For a biosensor based on microbial cells, it is possible to create conditions under which the receptor element is regenerated due to their growth; this technique can be used to restore the sensitivity of the biosensor	

Source: Data extracted from Plekhanova and Reshetilov [122]

disadvantages of such biosensors include the low selectivity of determination due to the fact that the cells of living organisms are in fact a source of a wide variety of enzymes. In addition, the response time of biosensors based on tissues and microorganisms can be quite long, which also reduces their practical value.

17.4 Features of Biosensor Design

17.4.1 Bio-Interfaces

As follows from the previous sections, a biosensor is a kind of composite formed by transducer materials and a biomolecule or cell. At the same time, the materials that form the transducer can have a very different shape from 0D to 3D, which introduces its own specifics into the formation of the interface between the transducer and biomolecule or cell used for the biofunctionalization of the transducer surface. Figure 17.3 shows how different this interface can be. First, the processes occurring at bio-interfaces depend on the function of semiconductors during their interaction with the cell (Fig. 17.3e). Second, the semiconductor-cell interface can be both extracellular and intracellular bio-interface. In particular, nanosized semiconductors can enter the cell and thus form intracellular bio-interfaces for either perception or stimulation [75, 153]. Intracellular semiconductors are in direct contact with the organelles and/or cytosol, allowing for highly accurate studies of subcellular activity [75]. Unfortunately, a clear understanding of semiconductor internalization mechanisms is still difficult to achieve.

When designing highly sensitive biosensors, the correct choice of not only the matrix, but also the conditions for bio-receptor immobilization is of key importance [27]. When using noncovalent binding, the receptor is held on the sensor substrate of the transducer by electrostatic, van der Waals, or ionic interactions. The main advantage of this type of immobilization is that the matrix does not affect the biological properties of the receptor [55, 133]. However, noncovalent binding does not always provide strong retention of the biomolecule. For example, biosensors with adsorbed enzymes have low stability during operation and storage. Therefore, adsorption in combination with crosslinking is very often used for enzyme immobilization [87].

Modification of electrode surfaces by covalent bonding is advantageous because it produces a more stable biosensitive material. Covalent bonding results in irreversible bonding and high surface coverage [80]. In this case, receptor biomolecules in a sensor are firmly held, which prevents them from being washed out of the sensor matrix, and this is of key importance in the development of a reusable biosensor [129, 161, 174]. But covalent bonding requires a large amount of bioreagent and is generally poorly reproducible [87], as such bonding can potentially adversely affect the active site. Covalent bonding is carried out due to the functional groups of the exposed amino acids of the enzyme, such as amino, carboxyl, imidazole, thiol, and

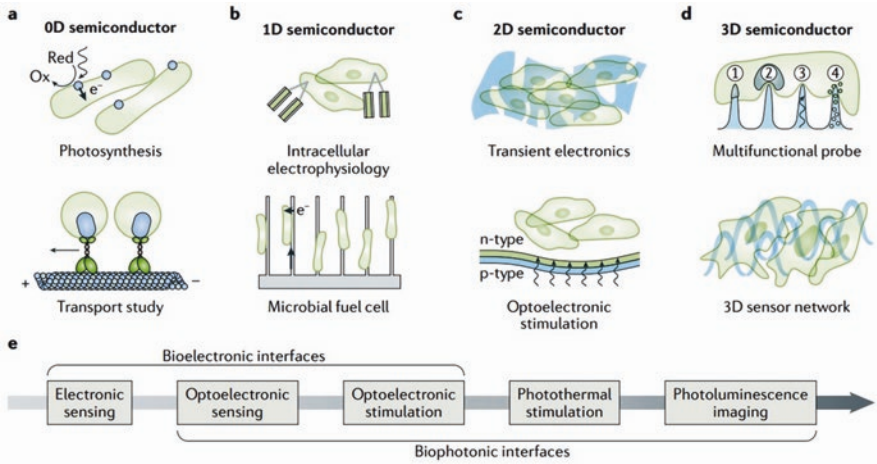

Fig. 17.3 Semiconductor geometries and possible modes for bio-interfaces. (**a**) 0D semiconductors can be used to mimic photosynthesis, for example, by using CdS nanoparticles that are precipitated on the cell wall of a bacterium to sensitize non-photosynthetic bacteria through photoinduced electron transfer pathways. Photoluminescent quantum dots can be coupled to motor proteins to enable the tracking of intracellular transport mechanisms. (**b**) 1D semiconductors, for example, nanoscale kinked Si nanowire field-effect transistors, allow intracellular recordings of single cell action potentials. Nanowire–bacteria hybrids can photoelectrochemically fix carbon dioxide and produce value-added chemicals. (**c**) 2D semiconductors, for example, biodegradable Si, provide a physically transient form of electronic devices. The photovoltaic effect of thin-film Si diode junctions can be used for the optical control of biological activities. (**d**) 3D semiconductors, for example, semiconductor micropillar or nanopillar arrays, can detect cellular electrophysiological signals, potentially probe nucleus mechanics, deliver optical stimuli for photo-stimulation, and release drugs. Strain-engineered 3D mesostructures of Si can serve as electronic scaffolds for neural networks. (**e**) Typical signal transduction mechanisms of semiconductor devices involve electrical or optical inputs and outputs. Ox - oxidation; Red - reduction. (Reprinted with permission from Jiang and Tian [74]. Copyright 2018: Springer Nature)

hydroxyl groups, which do not significantly affect its catalytic activity. These groups create a covalent bond with the functional groups present on the surface of the solid substrate, or which can be obtained by chemical or electrochemical processing. Many procedures for covalently binding an enzyme to a solid substrate have been reported [80, 132].

17.4.2 Nanomaterial-Based Biosensors

Nanomaterials [104, 131], whose unique catalytic activity, high surface-to-volume ratio, and excellent adsorption properties provide optimal physicochemical characteristics of the sensor substrate surface, are increasingly being used in the development of new generation biosensors [55, 83, 107, 119]. Nanomaterials are considered important components of bioanalytical devices due to their ability to increase

sensitivity and detection thresholds, which is important for single molecule detection. Nanomaterials ultimately provide efficient ligand-receptor interaction, which is transmitted as a specific equivalent enhanced signal [55, 83, 131]. It is important to note that in this case, neither bio-receptors nor analytes undergo conformational changes and loss of biological activity.

17.4.2.1 Biosensors Using Semiconductor QDs

Quantum dots (QDs) are semiconductors, which are crystalline nanostructures ranging in size from 2 to 10 mm. They play a significant role in modern biosensor technologies, especially in medical and biological applications [97]. QDs play an important role in the creation of biosensors due to the amazing properties they possess [5]. They have fluorescent superior behavior. Semiconductor QDs have the higher fluorescence efficiency compared to other traditional fluorophores. Due to size-dependent properties of QDs [59, 70, 97, 134], emission can be controlled over a very wide spectral range from IR to blue fluorescence by changing the size of the QDs. QDs also have a strong biological probe and are more photostable compared to other common traditional organic dyes [5, 172]. All these properties of QDs make it possible to develop on their basis various approaches to biosensing, immunoassays, mapping, and visualization, including those in multicolor mode. QDs can be synthesized based on various semiconductors. However, II-VI compounds are of the greatest interest. These compounds have a particularly high quantum yield, which contributes to an increase in the efficiency of biosensors being developed [140].

QD-based biosensors can be designed in several ways, depending on the requirements for their sensitivity and the type of analyte being detected [90]. Figure 17.4 shows examples of preparation protocols used in the fabrication of QD-based optical sensors. In all cases, the protocol begins with the appropriate modification of the QD surface to achieve the desired selectivity for a particular analyte. An important aspect is also the preparation of substrates that can be used during measurements. Strategies (a) and (b) differ in steps III and IV, which occur in reverse order. Whereas in strategy (a), step III is the deposit of QDs, in strategy (b) it is step IV. This step can be performed by methods such as layer-by-layer [42], sol-gel [165], or the electrochemical method [73]. Step IV in strategy (a) and step III in strategy (b) represent the conjugation of the analyte, which may be possible due to the pre-prepared and targeted substrate. Jie et al. [76] proposed the binding of an analyte to a pre-prepared substrate based on CdSe nanocomposite QDs using antibodies selective for an antigen called human IgG. The last step in all strategies is QD stimulation, which is used to detect the analyte. As a result, both qualitative and quantitative assessment of the presence of the analyte is possible. As seen in Fig. 17.4, the strategies presented differ in the number of steps and require specific preparation of both the QD and the substrates used, which significantly complicates the measurements and imposes increased requirements on the measurement specialists.

As for the mechanisms of analyte detection by QD-based sensors [90], the most popular mechanism is QD emission quenching. In this mechanism, due to the

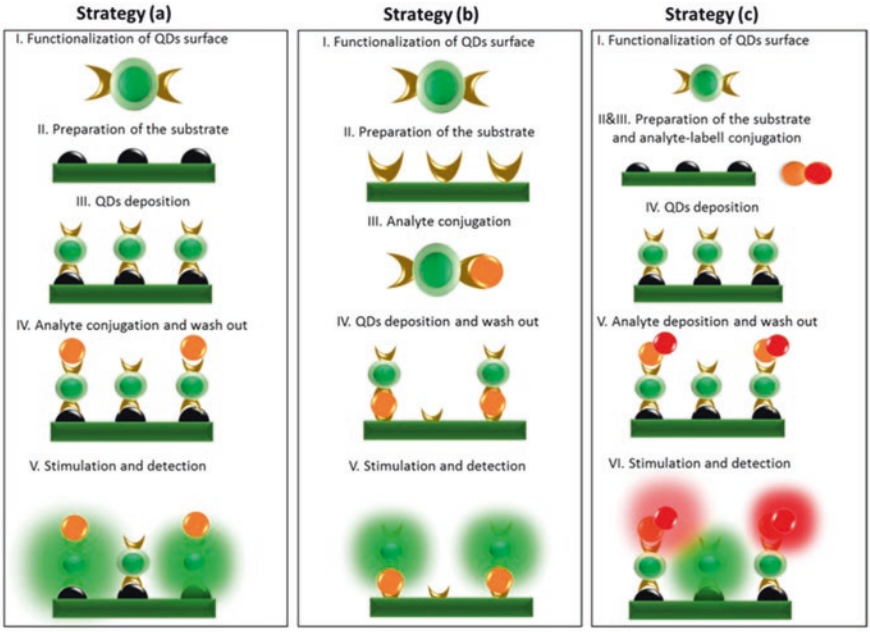

Fig. 17.4 Three examples of the strategy of QDs-based optical sensors (strategy a—modification of substrate with QDs directed to detection of analyte, strategy b—modification of substrate for detection of analyte-QDs complex, strategy c—using the analyte labeled with appropriate fluorophore). (Reprinted from Lesiak et al. [90]. Published by MDPI as open access)

interaction of the QD surface with the analyte, the QD emission intensity decreases (Fig. 17.5a) [144]. For example, Weng and Neethirajan [172] developed a QD-based biosensor for sensitive and rapid detection of food allergens. In this probing method, QDs-Aptamer-GO acts as a probe in which interaction with food allergens results in fluorescence quenching. Another mechanism is based on an increase in the emission of QDs due to passivation of the QD surface with an analyte (Fig. 17.5b). For example, addition of bovine serum albumin or nucleic acids resulted in increased emission of mercaptoacetic acid-coated CdS QDs [170]. The third mechanism that can be used to detect an analyte is stimulated aggregation of QDs (Fig. 17.5c). In this mechanism, due to the interaction of the analyte with the QD surface, surface ligands are cleaved and QDs are aggregated. Aggregation can also be caused by analyte-stimulated bonding between functionalized QDs [82]. There is also a very rarely used analyte detection mechanism based on the modification of the nanostructure growth process by introducing an analyte during the nanostructure growth process. Because of this perturbation, nanostructures can have different emission or other detectable properties (Fig. 17.5d). There is also a fifth detection mechanism based on the transfer of excitation energy from the QD to another optical center (QD or dye). As a result, the color of the emission or the decay time of the donor radiation changes (Fig. 17.5e) [12, 157].

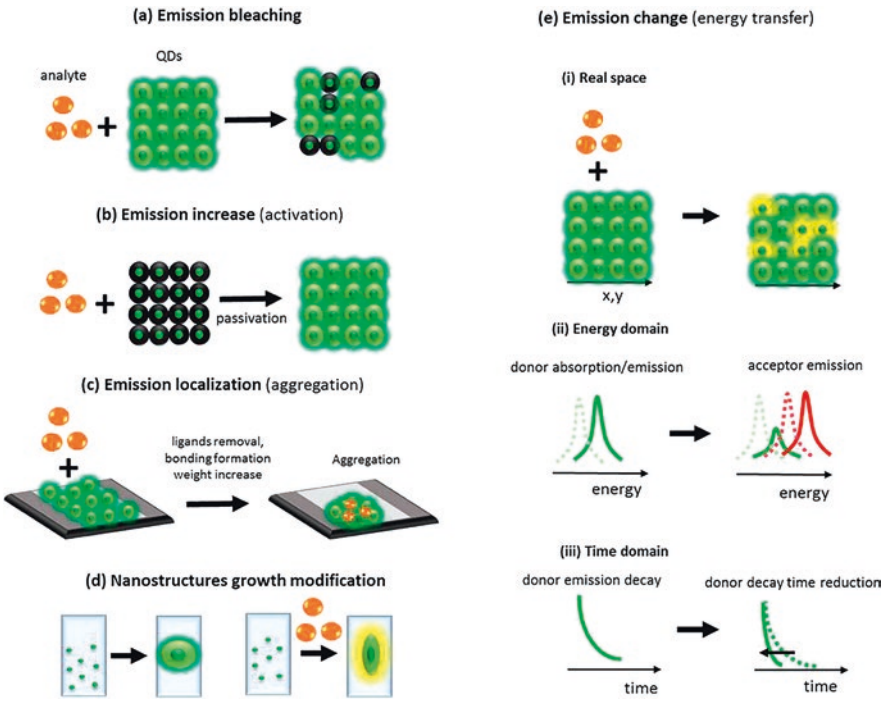

Fig. 17.5 Examples of physicochemical mechanisms used for analyte optical detection—emission bleaching (**a**), increase of emission (**b**), emission localization (**c**), nanostructures growth's modification (**d**), emission change (**e**). (Reprinted from Lesiak et al. [90]. Published by MDPI as open access)

17.4.3 Biosensor Using Metallic Nanoparticles

Metallic nanoparticles (NPs) are another example of nanoparticles widely used in biosensor development [167]. Lower toxicity and the possibility of fairly simple surface modification make noble metals such as gold and silver nanoparticles with excellent biocompatibility ideal for use in biological analysis [62]. At the same time, the experiment showed that their size and shape are of great importance for achieving high bio-recognition efficiency. For example, a biosensor based on a tilted fiber Bragg grating (TFBG) coated with noble metal nanoparticles showed improved analyte (protein) selectivity and a low detection limit compared to other biosensors that do not contain gold NPs [89]. It has also been reported that many metal nanoparticles are effectively used for immobilization in order to increase the sensitivity of electrochemical biosensors. This is due to the fact that they are able to stimulate direct electron transfer after capture by biomolecules, thereby maintaining the biological activity of the biosensor for a long period [31, 181, 120].

It is important to note that localized surface Plasmon resonance (SPR) is of great importance for the use of metal nanoparticles in biosensors. Particularly effective is the use of metal nanoparticles in SPR-based biosensors developed on the basis of

metal-enhanced fluorescence (MEF) and surface-enhanced Raman scattering (SERS). MEF and SERS remain the most sensitive detection methods for many analytes such as biomolecules, ions, macromolecules, and microorganisms, respectively. In particular, anisotropic metal nanoparticles, which have a good improved electric field with a large number of excitation wavelengths, are very suitable for developing biosensors with ultrasensitive Plasmon amplification capability [120].

17.4.4 Biosensor Using Polymers and Polymeric Nanoparticles

To develop biosensors, various categories of polymeric materials, both bulk and in the form of nanoparticles, are used. It is noted that the modification of electrodes with conductive polymeric materials in electrochemical sensors provides good stability, high sensitivity, low applied potential, low detection limit, as well as fast electron transfer. The use of polymers also promotes the immobilization of enzymes on the electrode surface [112]. Conductive polymers have been found to be an excellent matrix for the functionalization of many biological molecules. Various methods have been used for these purposes. This can be either direct adsorption, covalent bonding, cross-linking with glutaraldehyde, or simple mixing of enzymes with polymers.

The field of application of polymer nanoparticles is growing rapidly and plays a significant role in areas such as biosensors and nanomedicine. Recently, biomedical applications such as bioimaging, diagnostics, and drug delivery using polymer nanoparticles have been developed. The unique chemical and physical properties of polymer-based nanoparticles can become the basis for the development of new nanomaterials for biosensors [98]. In particular, the integration of biocompatible polymers into a biosensor using nanoparticle-based nanotechnologies as well as nanoengineered smart polymers can lead to composites with new and desirable characteristics that provide high sensitivity and stable immobilization of biomolecules. The integration of conductive polymers with metals, metal oxides, or semiconductors such as II-VI compounds also increases the sensitivity and stability of the biosensor [56, 94]. Together, this can form the basis for the development of advanced analytical devices for various applications [152].

17.4.5 Biosensor Using Core-Shell Materials

Core-shell nanoparticles can be considered as a kind of QD containing two or more materials, one of which forms the core, and the second - the shell. Core-shell nanostructures are very important [86, 117], since the creation of core-shell structures can significantly increase the reactivity, chemical and thermal stability, solubility, and reduce the toxicity of II-VI compounds. In addition, the formation of core-shell structures gives such structures many new properties suitable for use in biosensors.

The nanostructure of core-shell nanoparticles can have a different shape and size of the core with different surface morphology and shell thickness. Their shapes can be centric, eccentric, spherical, star-shaped, and tubular [30, 64, 86]. Metals, semiconductors, and polymers can be used to form core-shell structures. In biosensors, polymers are especially often used to form shell in core-shell structures. To obtain a homogeneous core-shell structure, as well as to improve the properties of the material, the synthesis technology of core-shell nanoparticles is of great importance. The synthesis of these nanostructures can be organized in various ways. Possible technologies for the formation of core-shell structures based on II-VI compounds are described in the Chap. 12 (Volume 1).

17.4.6 Biochips

Biosensors can be designed using a variety of approaches. For example, electrochemical biosensors can be manufactured using the so-called bulk technology, in which the individual components listed in the diagram (Fig. 17.1) are assembled into a single physical ensemble as they are manufactured. Such biosensors are currently dominant and are used in practice. But they have disadvantages associated with difficulties in organizing their mass production and microminiaturization. Advances in the development of microelectronic technologies have pushed the developers of electrochemical biosensors to new solutions.

In particular, it turned out that planar and printing technologies offer great opportunities for microminiaturization of electrochemical biosensors. Screen-printed three-minielectrodes are deposited or printed onto polymer substrate forming ultrasmall measuring system. Taking advantage of these opportunities, laboratory-on-a-chip (LOC) devices were developed in which the three-electrode system was miniaturized to a platform of several square centimeters with many laboratory functions. Such devices can handle very small volumes of liquid (picoliter level) [19]. Currently, LOC devices are mass-produced with high reproducibility and low cost. This kit makes it easy to modify the WE surface [79, 175]. With the help of this technology, it has also become possible to manufacture a so-called biochip that combines a sensor system, a converter, an analog-to-digital converter, and a microprocessor for measuring an analytical signal and calculating analysis results. This approach has allowed the development of a large number of portable and wearable biosensors that analyze the biochemical composition of body fluids, such as sweat from the skin surface or tears. Such devices have been created by directly transferring sensors to the skin (using E-skin or temporary printed tattoos), embedding sensors in bracelets, patches, or textiles to ensure close contact with the skin. Such portable biosensor platforms provide insight into the dynamic processes in biological fluids, providing continuous real-time monitoring, which, in turn, is of great importance for maintaining the health of the user. Evaluation of indicators in real time allows you to monitor the state of health and the course of treatment and warns the patient and the attending physician about sudden violations. Although such

biochips can be replicated, the main problem in this case will be the reproducibility of the microstructure of the surface with the deposited layer of biologically active enzyme.

There is another approach to the development of biochips, which has recently become very widespread. In this case, a biochip is understood as a set of diminished microarrays placed on a solid substrate, which allows many experiments to be carried out simultaneously to obtain a high performance in less time. This device contains a set of sensor elements or biosensors. Unlike microchips, these are not electronic devices. Each biochip can be considered as a microreactor that can detect a specific analyte like an enzyme, protein, DNA, biological molecule, or antibody. The main function of this chip is to perform hundreds of biological reactions in a few seconds like decoding genes (a sequence of DNA).

Three types of biochips are currently available, namely DNA biochip, protein microarray, and microfluidic chip. The first DNA biochip or DNA microarray was developed by the American company Affymetrix, and the product of this company is the GeneChip. A DNA biochip is a set of tiny DNA spots fixed on a solid surface with an array of nano-wells (see Fig. 17.6b). For example, the SMARTer ICELL8 multisample nanodispensor from Takara (https://www.takarabio.com) uses a biochip with isolated 5184 nano-wells for specific DNA labels. Each DNA tag contains picomoles of specific genes called probes. This may be a short segment of genetic material under conditions of high rigidity. Typically, probe-target hybridization is noticed and counted by recognition of fluorophore-labeled or chemiluminescent targets to determine the relative number of nucleic acid series in the target [68, 108].

Protein microarray or protein chips are used to track the actions and relationships of proteins and to elucidate their functions in large scale studies [77, 171]. The main advantage of protein microarrays is that we can track a large number of proteins in parallel. Protein chips are automated, fast, economical, very sensitive, and require fewer samples. The first protein array methodology was presented in scientific publications on antibody microarrays in 1983. Subsequently, protein chip technology has been fairly easily adapted to DNA microarrays, which have now become the most widely used biomicroarrays. Despite the large number of successful examples of the use of protein biochips in biomedical and biotechnological research, many problems remain to be solved. Most protein biochips are currently made using traditional strategies that result in the random orientation of proteins on the surface of the chip. This random arrangement can adversely affect protein activity or ligand binding due to steric hindrance and thus can lead to reduced assay performance or even falsified assay results [147]. The problem can be solved by introducing more advanced methods for preparing protein biochips. Many such methods have been developed over the past few years [77].

Microfluidic biochips or “laboratory on a chip” are an alternative to traditional biochemical laboratories. Microfluidic biochips combine various biochemical analysis functions (e.g., dispensers, filters, mixers, separators, detectors) on a chip, miniaturizing the macroscopic chemical and biological processes that are often handled by laboratory robots to the submillimeter scale. There are several types of microfluidic biochips, each with its own advantages and limitations [88]. In flow biochips

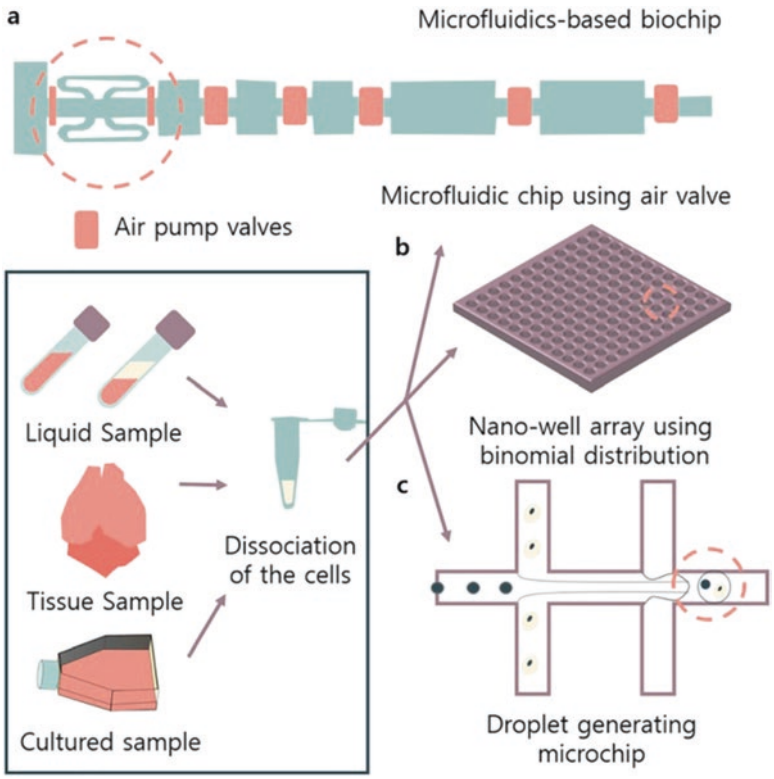

Fig. 17.6 Representative microfluidics-based biochips and representative references. Microfluidics-based biochips can be categorized into three major platforms: (**a**) microfluidic channel-based, (**b**) well-array-based, and (**c**) droplet-based. After biological samples are dissociated into solution, the samples are processed through these biochips and can be applied to various fields in biology and medicine. (Adapted from Lee et al. [88]. Published by AIP as open access)

(Fig. 17.6a), the on-chip microfluidic channel circuit is equipped with microvalves built into the chip, which are used to control the flow of fluid on the chip. By combining multiple microvalves, it is possible to create more complex devices such as mixers, micropumps, multiplexers, etc., with thousands of devices on a single chip. In droplet-based biochips, liquid is treated as individual drops on an array of electrodes (Fig. 17.6c).

Microfluidic biochips have a number of advantages over conventional chemical analyzers. These include reduced sample and reagent volumes, faster biochemical reactions, ultra-sensitive detection, and higher system throughput allowing multiple analyzes to be performed simultaneously on a single chip. Microfluidic biochips have great potential in many applications such as clinical diagnostics, advanced sequencing, drug discovery, and environmental monitoring. The use of microfluidic biochips also facilitates tasks such as DNA analysis, protein studies, and disease diagnosis (clinical pathology) [34, 57, 61, 100].

Of course, biochips are expensive. But the positive effect of their application is obvious. Biochips have helped to significantly accelerate the identification of approximately 80,000 genes in human DNA as part of the international Human Genome Project. In addition to genetic applications, the biochip is suitable for toxicological, protein, and biochemical research. Biochips can also be used to quickly detect chemical agents used in biological warfare so that protective measures can be taken.

17.5 Applications of Biosensors

Currently, biosensors have a very wide field of applications (see Fig. 17.7). Environmental monitoring and pollution control using biosensors is also of great importance [7, 130], since human activities and industry create many environmental hazards that worsen the quality of human life. Agriculture and veterinary medicine, military affairs, drug development and quality improvement, disease diagnosis, treatment, and clinical research are also areas where biosensors should be widely used [11]. Several examples of the use of the biosensors are shown in Table 17.4. According to the experts' forecasts, in 10–15 years the market for these analytical devices will exceed $ 70 billion [55]. Companies such as Roche Diagnostics,

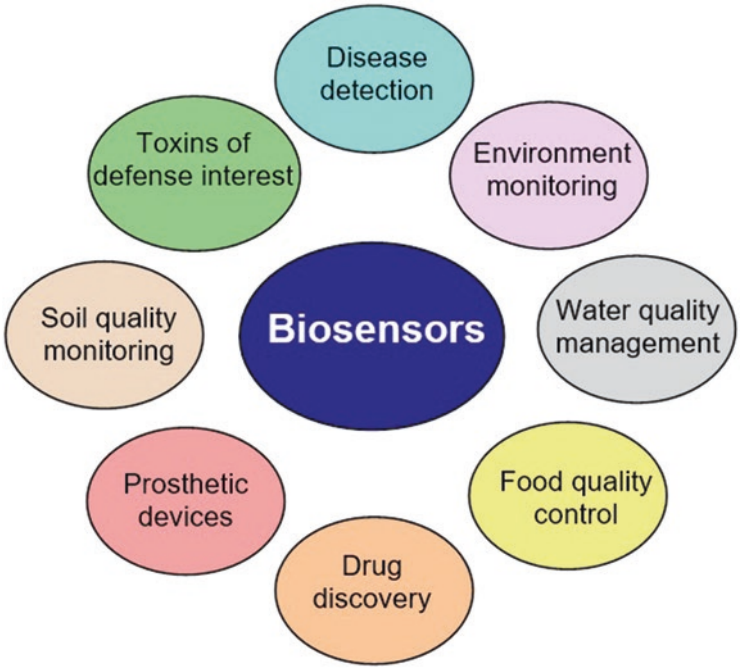

Fig. 17.7 Major areas of applications for biosensors. (Data extracted from Bhalla et al. [22])

Table 17.4 Various approaches to the detection of biological objects

Approach	Target bio-analyte	Detection limit	References
Electrochemical	Protein	10 pM	[96]
	Carcinoembryonic antigen (CEA)	3.3 fg/mL	[166]
	Xanthine,	0.25 mM	[46]
	Antipsychotic clozapine	24 nM	[138]
	Salmonella	10^1 CFU mL^{-1}	[48]
Amperometric	Aromatic hydrocarbons	0.5 µm	[28]
	Baeyer–Villiger (BV)	–	[137]
Electrical	Hepatitis B-antigen	20 aM	[29]
Voltammteric	Fluvoxamine	3.5×10^{-9} Mol L^{-1}	[21]
Potentiometric	Lead	8.5 pM	[151]
	Zinc	510^{-4} M	[41]
Conductometric	Diazinon	60 µg/L	[182]
Impedemetric	Bacterial endotoxin	2 µg mL^{-1}	[26]
	Escherichia coli 0157:H7	10^2 CFU mL^{-1}	[25]
Piezoelectric	Pesticide residue	–	[143]
Optical	Bacterial endotoxin	0.4 ng/mL	[101]
Electrical/Fluorescent	Bacteria	1 CFU/mL	[71]

Johnson and Johnson Innovative Biosensors Inc., Cranfield Biotechnology Centre, EG and IC Sensors, Inc., Biosensor BV, QTL Biosystem, Molecular Devices Corporation, AZUR Environmental, Pharmacia Biosensor AB, Strategic Diagnostics, Affinity Sensor, Pinnacle Technologies Inc., DuPont Biosensor Materials, and others work in the field of biosensors and biochips development.

17.5.1 Biosensor Detection of Diseases

Biosensors are of great importance for monitoring and detection/diagnosis of diseases and subsequent treatment [11, 109, 136, 159]. This is because the biosensor approach is easy to use, has high sensitivity, fast detection time, real-time analysis and miniaturization, as well as low cost. The use of biosensors, which make it possible to detect diseases at an early stage, dramatically increases the effectiveness of therapy. In addition, rapid and accurate laboratory diagnosis of infectious diseases can reduce the incidence and prevent the formation and spread of dangerous epidemics. Therefore, biosensors designed for these purposes can serve as an alternative tool for the development of diagnostics of epidemic diseases [3, 50, 146]. For example, a disposable electrochemical immunosensor using a screen electrode with functionalized gold nanoparticles was developed by Rama and colleagues [128] to detect amyloid beta 1–42 in situ. This identification is significant because Alzheimer's disease is one of the most common forms of dementia. Testing showed

that these immunosensors had detection limits of 0.1 ng/mL. Biosensors can also be used for early detection of heart disease, cancer, diabetes, and many other infectious diseases. QD-based biosensors make it possible to detect viral, bacterial, and fungal diseases in human. Some other examples of such applications of biosensors are listed in Table 17.5. All these indicate that biosensor technologies that have appeared in recent years and are actively developing are innovative platforms for the analysis of infectious process biomarkers. They have a high potential to become affordable, fast and reliable, highly specific and sensitive tools for timely and reliable diagnosis and monitoring of bacterial and viral diseases [85, 178].

17.5.2 Biosensor for Detection of Toxins and Pathogens

In the last decade, more and more attention has been paid to the use of biosensors for monitoring toxins and pathogens. Microbial pathogens such as bacteria, fungi, viruses and protozoa can have a major impact on public safety and human health [110]. Therefore, the detection of microorganisms as well as microbial toxins is of great importance for security and public health care systems [150]. In these applications, sensitivity, accuracy, and speed of detection are important. The developed biosensors solve this problem. For example, Sheng et al. [145] developed a label-free biosensor with an RNA aptamer that allows for rapid quantitative recognition of foodborne pathogens. In the proposed aptosensor, the RNA aptamer acts as "anti-nucleic acid antibodies" of target microorganisms. The oligonucleotide nature of aptamers makes it possible to amplify or chemically synthesize the desired pool rather quickly and in any quantities, which makes it possible to create highly specific homogeneous sensors that perform accurate quantitative detection of pathogen nucleic chains [47, 113].

Table 17.5 Use of biosensors in disease diagnosis

Biosensor	Disease diagnosis or medical applications
Glucose oxidase electrode-based biosensor and HbA1c biosensor	Diabetes
Uric acid biosensor	Cardiovascular and general disease diagnosis
Microfabricated biosensor	Optical corrections
Hydrogel (polyacrylamide)-based biosensor	Regenerative medicine
Silicon biosensor	Cancer biomarker development and applications
Nanomaterials-based biosensors	For therapeutic applications

Source: Reprinted from Vigneshvar et al. [160]. Published by Frontiers as open access

17.5.3 Biosensor for Environmental Monitoring

Biosensors play an important role in environmental monitoring, in particular in the control of water, air, and soil pollution [7, 28, 52, 112]. For example, Bidmanova et al. [20] have developed a portable biosensor to detect halogenated pollutants (halogenated aliphatic hydrocarbons). The developed optical biosensor with a fluorescent indicator makes it possible to detect pollutants with halogen-containing compounds in water samples in the pH range of 4–10 and the temperature range of 5–60 °C. In addition, the biosensor with detection limits of 2.4, 1.4, and 12.1 mg/l had a short measurement response time (1 min) and small dimensions ($60 \times 30 \times 190$ mm^3), which is an additional advantage of the developed sensor. The functionalized nanopolymer immunosensor developed by Deep et al. [43] had high sensitivity specificity for atrazine and gave a detection limit of 0.01 ng/mL. A biosensor based on the Au@Ag core-shell for SERS detection of arsenic (III) was successfully developed [148]. This biosensor had high efficiency in the range of arsenic III concentrations from 0.5 to 10 ppb and a detection limit of 0.1 ppb. This detection limit is below the maximum value established by the US Environmental Protection Agency (EPA) and the World Health Organization (WHO), respectively. In addition, the results showed that the detection of As(III) did not interfere with other ions present in the water sample.

17.5.4 Biosensor for Food Quality Control and Agriculture

Biosensors are of great importance in food quality control and agriculture [6, 8, 13, 14, 67, 110, 116], etc. The agricultural sector plays an important role in the sustainable economic development of the country and the world community, in connection with which it is of great importance to develop methods that allow early detection of plant diseases and prevent their spread over large areas. To achieve regional and global food security goals, there is also an urgent need for food control at all stages of their production and storage. The experiment showed that biosensors have such an opportunity. For example, it has been reported that biosensors can be used to detect plant pathogens [70, 99]. Wang et al. [163] showed that a biosensor can be used to monitor plant health. Fang et al. [58] developed electrochemical biosensors to detect *p-ethylguaiacol*, a fungus that infects fruit plants with *Phytophthora cactorum* pathogens and causes root rot during growth. To do this, they investigated the effect of various treatments on specific plant stress. In the field of quality control in the food industry, the importance of express methods for assessing shelf life, spoilage, and contamination of products is studied. Advances in biotechnology are stimulating the development of methods for monitoring fermentation processes, which also expands the possibilities of continuous monitoring of these processes.

17.6 Conclusion

Many approaches can be used to detect analytes. However, traditional methods are labor-intensive, expensive, and require well-trained staff and personnel. This chapter shows that biosensors are ideal for this task. Biosensors can provide real-time detection of various analytes with high efficiency, sensitivity, low detection limits, and good linearity. Therefore, biosensors can become a promising analytical tool for routine analysis and monitoring in various fields such as medicine, environmental monitoring, food quality assurance, the agricultural sector, etc. Unfortunately, most biosensors are disposable or have a short lifespan. Therefore, the goal of the next level of development should be to create more stable regenerative biosensors for long-term use.

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