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Biosensors and Nanobiosensors: Design and Applications

Ahmed Touhami

Physics & Astronomy Department, University of Texas at Brownsville
One west university boulevard, Brownsville, Texas, 78520, USA

Outline:

Introduction: What is a Biosensor?

The most widely accepted definition of a biosensor is: "**an analytical device which incorporates a biologically active element with an appropriate physical transducer to generate a measurable signal proportional to the concentration of chemical species in any type of sample**" [1-5].

Type of Biosensors: Biosensors can be categorized according to the basic principles of signal transduction and biorecognition elements. In the general scheme of a biosensor (figure 15.1), the biorecognition element responds to the target compound and the transducer converts the biological response to a detectable signal, which can be measured electrochemically, optically, acoustically, mechanically, calorimetrically, or electronically, and then correlated with the analyte concentration. Biological elements include enzymes, antibodies, micro-organisms, biological tissue, and organelles. When the binding of the sensing element and the analyte is the detected event, the instrument is described as an **affinity sensor**. When the interaction between the biological element and the analyte is accompanied or followed by a chemical change in which the concentration of one of the substrates or products is measured the instrument is described as a **metabolism sensor**. Finally, when the signal is produced after binding the analyte without chemically changing it but by converting an auxiliary substrate, the biosensor is called a **catalytic sensor** [5, 6]. The method of transduction depends on the type of physicochemical change resulting from the sensing event [5]. Often, an important ancillary part of a biosensor is a membrane that covers the biological sensing element and has the main functions of selective permeation and diffusion control of analyte, protection against mechanical stresses, and support for the biological element.

The research field of biosensors started with the introduction of the first generation glucose oxidase (GOx) biosensor in 1962 by Clark and Lyons [7]. Since then, biosensors have been intensively studied and extensively utilized in various applications, ranging from public health and environmental monitoring to homeland security and food safety [5, 8-11]. According to the Web of Knowledge, over 4000 research papers about biosensors have been published each year for the past three years (2011-2013). Though a lot of research activity has been involved in developing biosensors for various purposes the time has come to bring this technology to the forefront and make it commercially available [12-14]. Efforts and funds need to be mobilized to manufacture biosensors on a large scale so as to benefit and be of use to the general public. With exposure to the commercial market the applications of this technology would be greatly enhanced. A few such applications could be detection of virulence of a vaccine just before it is injected so as to prevent accidental acquisition of a disease, bandages detecting a septic wound, deadly viruses in the environment or from the patient sample (rapid and early detection) and so forth in the medical field. Real time monitoring of dairy products and breweries might help foster a cleaner and hygienic environment and experiment with different tastes imparted by specific microorganisms in specific concentrations giving rise to new products. A farfetched and plausible use of this technology could be in space exploration where if present the concentration of the living organisms would be very low and might lead to answering many of the long standing questions regarding the presence of life in space.

The goal of this chapter is to cover the full scope of biosensors. It offers a survey of the principles, design, operation, and biomedical applications of the most popular types of biosensing devices in use today. By discussing recent research and future trends based on many excellent books and reviews, it is hoped to give the readers a comprehensive view on this fast growing field.

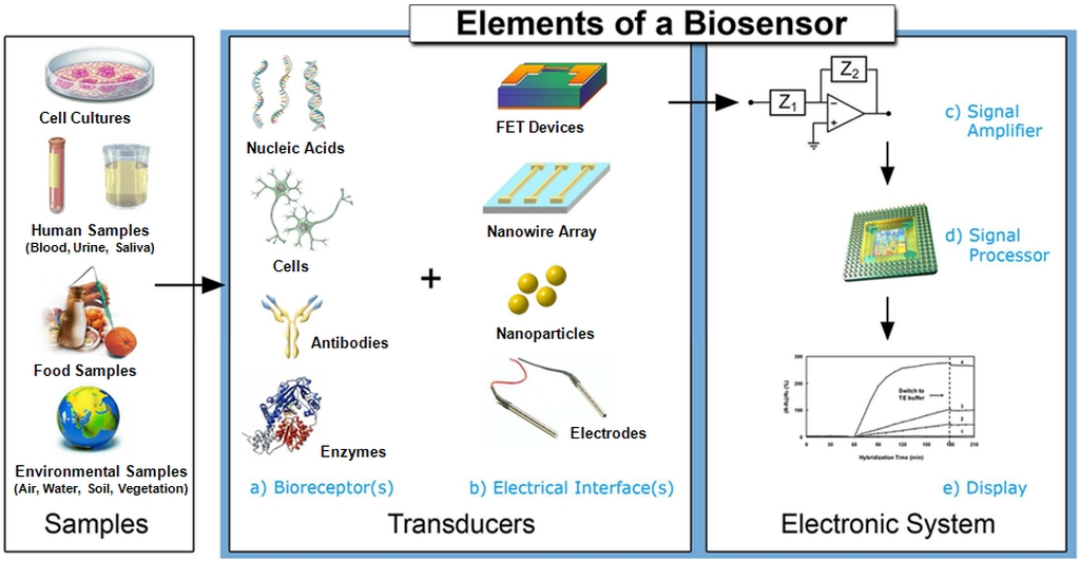

FIGURE 15.1
Schematic of a typical biosensor: a) biorecognition element that specifically bind to the analyte; b) an interface architecture where a specific biological event takes place and gives rise to a signal picked up by c) the transducer element; the transducer signal (which could be anything from the in-coupling angle of a laser beam to the current produced at an electrode) is converted to an electronic signal and amplified by a detector circuit using the appropriate reference and sent for processing by d) computer software to be converted to a meaningful physical parameter describing the process being investigated; finally, the resulting quantity has to be presented through e) an interface to the human operator. (Modified and adopted from reference 11)

Micro-Biosensors

The major progress in microsystem technologies for creating small, integrated and reliable microtransducers devices in combination with biological sensing elements has revolutionized the field of biosensors during the last decade. Such micro-biosensor systems raised the expectation to get a comprehensive insight into dynamic cellular metabolic events and subsequently a complete understanding of the metabolism of human biology. Currently, cancer can be detected by monitoring the concentration of certain antigens present in the bloodstream or other bodily fluids, or through tissue examinations [13]. Correspondingly, diabetes is monitored by determining the glucose concentrations in the blood over time [14]. However, despite their widespread clinical use, these techniques have a number of potential limitations. For example, a number of diagnostic devices have slow response times and are burdensome to patients. Furthermore, these assays are expensive and cost the health care industry billions of dollars every year. Therefore, there is a need to develop more efficient and reliable sensing and detection technologies.
Optical transduction will be the focus of this article

Biosensing Techniques

Biosensors can be classified either by the type of biological signaling mechanism they utilize or by the type of signal transduction they employ. Transduction can be accomplished via a great variety

of methods. Most forms of transduction can be categorized in one of three main classes. These classes are: 1) electrochemical detection methods, 2) optical detection methods and 3) mass detection methods. However, new types of transducers are constantly being developed for use in biosensors. Each of these three main classes contains many different subclasses, creating a nearly infinite number of possible transduction methods or combination of methods. Here, we will discuss each of these three detection mechanisms.

Electrochemical Biosensors

The first scientifically proposed as well as successfully commercialized biosensors were those based on electrochemical sensors for multiple analytes [7, 14-17]. At present, there are many proposed and already commercialized devices based on the electrochemical principle including those for pathogens and toxins [18]. This stems from a number of attributes of electrochemistry including the high sensitivity of electrochemical transducers, their compatibility with modern miniaturization/microfabrication technologies, minimal power requirements, economical cost, and independence of sample turbidity and color [16]. The basic principle for this class of biosensors is that chemical reactions between immobilized biomolecule and target analyte produce or consume ions or electrons, which affects measurable electrical properties of the solution, such an electric current or potential [15, 16]. The electrochemical signal produced is then used to relate quantitatively to the amount of analyte present in a sample solution. Potentiometry, amperometry, voltammetry, and, more recently, electrochemical impedance spectroscopic measurements are among the electrochemical detection techniques often used in conjunction with immunoassay systems and immunosensors, leading to their respective categories according to the type of signal measured. The fundamental principles of each of these techniques are presented below, followed by discussions based on some recent work that have specifically addressed problems encountered in these areas.

Potentiometric Biosensors

These biosensors are based on ion-selective electrodes (ISE) and ion-sensitive field effect transistors (ISFET) [14-18]. The primary outputting signal is possibly due to ions accumulated at the ion-selective membrane interface. Current flowing through the electrode is equal to or near zero. The electrode follows the presence of the monitored ion resulting from the enzyme reaction. For example, glucose oxidase can be immobilized on a surface of the pH electrode. Glucose has only minimal influence on pH in the working medium; however, the enzymatically formed gluconate causes acidification. A biorecognition element is immobilized on the outer surface or captured inside the membrane. In the past the pH glass electrode was used as a physicochemical transducer [19]. Nowadays, semiconductor based physico-chemical transducers are more common. ISFETs and LAPS (light addressable potentiometric sensor) based systems especially are convenient for biosensor construction. The ISFET principle is based on a local potential generated by surface ions from a solution [16, 20]. This potential modulates the current flow across a silicon semiconductor. The transistor gate surface in ISFET is covered by a selective membrane; for pH detection this could be made from compounds such as silicon nitride (Si_3N_4), alumina (Al_2O_3), zirconium oxide (ZrO_2) and tantalum oxide (Ta_2O_5). The LAPS principle is based on semiconductor activation by a light-emitting diode (LED). The sensor is made from an n-type silicon typically coated with 30 nm of silicon oxide, 100 nm of silicon nitride, and indium-tin oxide. The LAPS measures a voltage change as a function of medium pH in the LED activated zone 9figure 15.2). This opens the way for multiposition sensing

and construction of an array of biorecognition zones. A good example of a potentiometric immunosensor involves the detection of enzyme-labeled immunocomplexes formed at the surface of a polypyrrole coated screen-printed electrode [20].
In potentiometric measurements, the relationship between the concentration and the potential is governed by the *Nernst equation*, where E_{cell} represents the observed cell potential at zero current. This is sometimes also referred to as the electromotive force or *EMF*. E^0_{cell} is a constant potential contribution to the cell, *R* the universal gas constant, *T* the absolute temperature in degrees Kelvin, *n* is the charge number of the electrode reaction, *F* is the Faraday constant and *Q* is the ratio of ion concentration at the anode to ion concentration at the cathode [21].

$$EMF\ or\ E_{cell} = E^0_{cell} - \frac{RT}{nF} lnQ$$

The direct determination of the analyte ion concentration with the *Nernst equation* is referred to as direct potentiometry. The lowest detection limits for potentiometric devices are currently often achieved with ion-selective electrodes (ISE). Therefore, by definition the detection limit is analyte specific and current devices have limits of detection in ranges between 10^{-8} to 10^{-11} M. Potentiometric sensors prove suitable for measuring low concentrations in tiny sample volumes, since they ideally offer the benefit of not chemically influencing a sample. The variety of ions, for which low detection limits are possible, is currently quite limited and missing such important analytes as: nickel, manganese, mercury and arsenate ions. Detailed information about potentiometry and their limit of detection (LOD) is provided in the review by Bakker et al. [22].

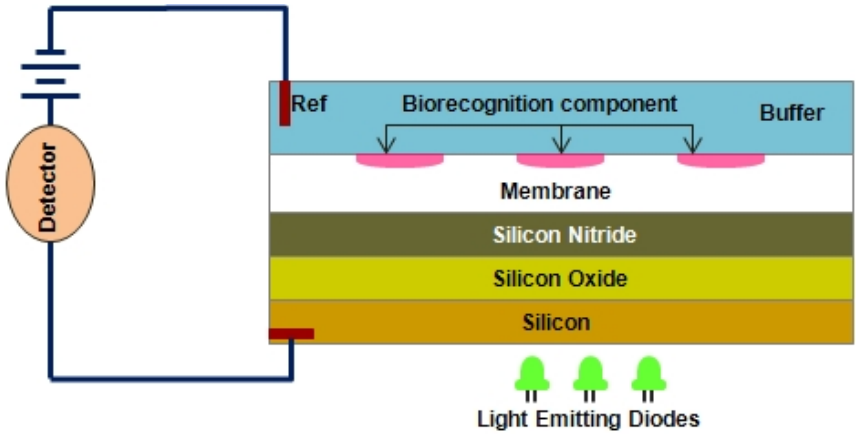

FIGURE15.2
Block diagram of the light addressable potentiometric sensor with biorecognition component bound into membrane and with buffered reaction cell

Amperometric Biosensors

In amperometry, the current produced by the oxidation or reduction of an electroactive analyte species at an electrode surface is monitored under controlled potential conditions. The magnitude of the current is then related to the quantity of analyte present [16]. Clark oxygen electrodes perhaps represent the basis for the simplest forms of amperometric biosensors, where a current is produced in proportion to the oxygen concentration. This is measured by the reduction of oxygen

at a platinum working electrode in reference to a Ag/AgCl reference electrode at a given potential [23]. Typically, the current is measured at a constant potential and this is referred to as amperometry. If a current is measured during controlled variations of the potential, this is referred to as voltammetry. Furthermore, the peak value of the current measured over a linear potential range is directly proportional to the bulk concentration of the analyte, i.e. the electroactive species [15]. However, not all protein analytes are intrinsically capable to serve as redox partners in electrochemical reactions, a suitable label must be introduced to promote the electrochemical reaction of the analyte at the working electrode [23] (figure 15.3). Despite the disadvantage of this often indirect sensing system, it is claimed that amperometric devices maintain a sensitivity superior to potentiometric devices [24]. An example of an amperometric device is the aforementioned glucose biosensor, which is based on the amperometric detection of hydrogen peroxide. A very tangible application of amperometry is used in combination with immunosensing techniques to measure levels of the human chorionic gonadotropin β-subunit (β-HCG) in advanced pregnancy testing.

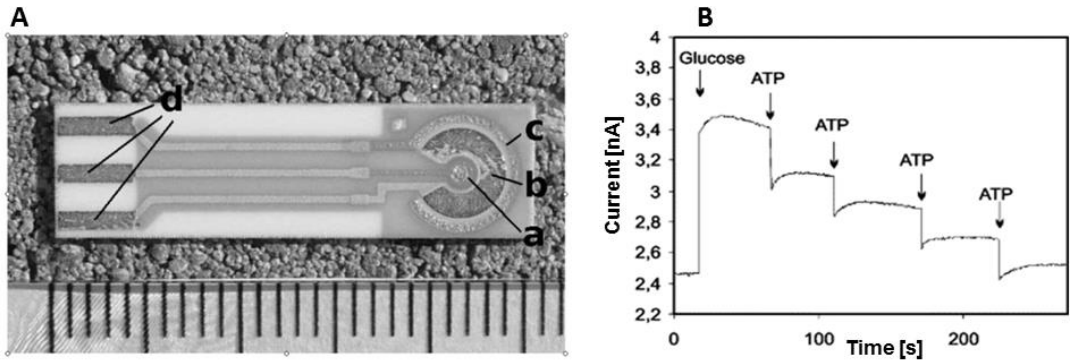

FIGURE15.3
(A) Example of the three-electrode screen-printed sensor produced by BVT (Brno, Czech Rep.). The sensor body is made from ceramics. A gold working electrode (a) is surrounded by an Ag/AgCl reference electrode (b) and gold auxiliary electrode (c). Letter d means silver output contacts. *The ruler in the bottom is in millimeter scale* (from refer [25]). (B) A sample amperometric measurement: This is a typical hydrodynamic response of their biosensor to glucose followed by several injections of ATP measured in phosphate buffer at 650 mV in reference to Ag/AgCl. The change in current response is proportional to the ATP concentration as glucose is consumed at the glucose oxidase (GOD) and hexokinase (HEX) modified electrode surface (from refer [26])

Voltammetric Biosensors

Voltammetry belongs to a category of electro-analytical methods, through which information about an analyte is obtained by varying a potential and then measuring the resulting current. It is, therefore, an amperometric technique. Since there are many ways to vary a potential, there are also many forms of voltammetry, such as: polarography (DC Voltage) [25], linear sweep, differential staircase, normal pulse, reverse pulse, differential pulse and more. Cyclic voltammetry is one of the most widely used forms and it is useful to obtain information about the redox potential and electrochemical reaction rates (e.g. the chemical rate constant) of analyte solutions. More recently, interdigitated array (IDA) microelectrodes have gained popularity as an alternative transducer in electrochemical immunoassays. In general, a simple design of an IDA consists of a pair of interdigitated microelectrode “fingers”. When an IDA is used as a sensing electrode in a

voltammetric experiment, the two interdigitated electrodes are usually held at different potentials to achieve “redox” cycling of the electroactive species to be detected. A major advantage of this redox cycling is that it improves the signal-to-noise ratio by enhancing the Faradaic current relative to the background current, resulting in lower detection limits and improved sensitivity. These features opened up many opportunities in which IDA electrodes were applied as electrochemical detectors in analytical chemistry and biosensor systems [26].

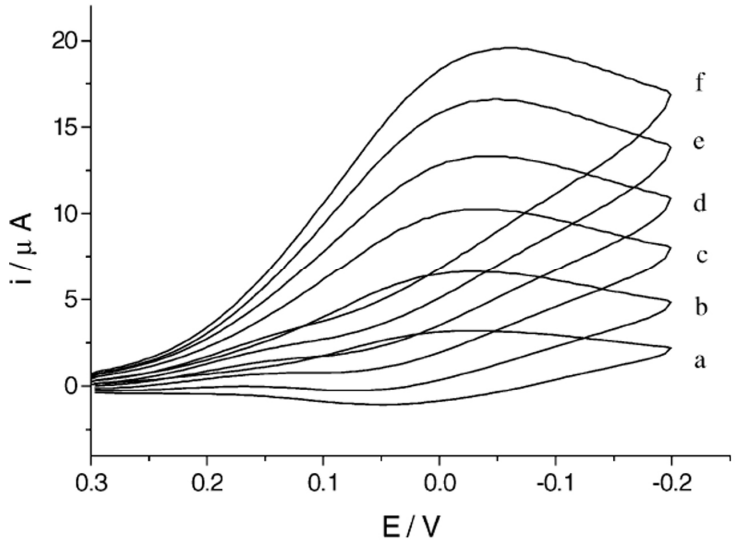

FIGURE15.4
An example of cyclic voltammogram. The cholesterol/PB sol-gel modified glassy carbon electrode in a phosphate buffer (pH 6.8) at varying concentrations of the analytic solution: a) blank solution; b-f) blank solution + cholesterol 10^{-5}, 2.10^{-5}, 3.10^{-5}, 4.10^{-5}, 5.10^{-5} mol/L. (from reference [27])

Optical Biosensors

Optical detection biosensors are the most diverse class of biosensors because they can be used for many different types of spectroscopy, such as absorption, fluorescence, phosphorescence, Raman, SERS, refraction, and dispersion spectrometry. In addition, these spectroscopic methods can all measure different properties, such as energy, polarization, amplitude, decay time, and/or phase. Amplitude is the most commonly measured as it can easily be correlated to the concentration of the analyte of interest [28]. In optical biosensors, the optical fibers allow detection of analytes on the basis of absorption, fluorescence or light scattering. Since they are non-electrical, optical biosensors have the advantages of lending themselves to in vivo applications and allowing multiple analytes to be detected by using different monitoring wavelengths. The versatility of fiber optics

probes is due to their capacity to transmit signals that reports on changes in wavelength, wave propagation, time, intensity, distribution of the spectrum, or polarity of the light. In general, acquisition of the signal from these devices is accomplished through flexible cables, which can transmit light to the biological component. Optical methods are readily multiplexed; samples can be interrogated with many wavelengths simultaneously without interfering with one another. A large variety of optical methods have been used in biosensors, however, those devices based on fluorescence spectroscopy, surface plasmon resonance, interferometry and spectroscopy of guided modes in optical waveguide structures (grating coupler and resonant mirror) are the most common. However, other emerging optical sensing technologies have been under investigation, such as optical ring resonators and photonic crystals.

Fluorescence-based Biosensors

Fluorescence is a widely used optical method for biosensing due to its selectivity and sensitivity. A fluorescence-based device monitors the frequency change of electromagnetic radiation emission stimulated by previous absorption of radiation and subsequent generation of an excited state that only exits for a very short time. Single molecules could be repeatedly excited and detected to produce a bright signal easily measured even at single-cell level. There are three types of fluorescence biosensing. The first is direct sensing when a specific molecule is detected before and after a change or reaction takes place. The second form is indirect biosensing when a dye is added that will optically transduce the presence of a specific target molecule. The use of green fluorescent protein (GFP) is a powerful fluorescent tag that has enabled investigators to study the location, structure and dynamics of molecular events within living cells. However, binding interactions between an activated signalling molecule and its target could be difficult to detect due to the difficulty of seeing this localized interaction over background fluorescence. A third type of fluorescence biosensing, called fluorescence energy transfer (FRET), can be used and it generates a unique fluorescence signal. In a typical fluorescence measurement, the fluorophore is excited by a specific wavelength of light and emits light at a different wavelength. However, when two fluorophores are paired in such a way that the emission wavelength of one overlaps with the excitation wavelength of the other, the excitation of one of them will stimulate fluorescence of the complementary pairing one (if they reside within about few Angstroms from eachother). FRET has tremendous utility because the unique fluorescence signal generated under these circumstances can be used to visualize and quantify the position and concentration of interacting fluorophores. Two major strategies have been used to develop FRET biosensors: (i) two chain probes in which the fluorophores are on two different molecules resulting in intermolecular FRET when the two molecules come into proximity, or (ii) single chain probes in which different regions of a single molecule are tagged and undergo FRET due to intramolecular, conformational changes [29].

Surface Plasmon Resonance Biosensors

Surface plasmon resonance (SPR) biosensor was first demonstrated for biosensing in 1983 by Liedberg et al. [30]. Since then it has been extensively explored and has gradually become a very powerful label-free tool to study the interactions between the target and biorecognition molecules. The principle, development, and applications of SPR biosensors have been well described in several excellent review papers [31, 32]. A quick survey of the literature points to the success of surface plasmon resonance (SPR) biosensors in a wide range of fields from fundamental biological studies to clinical diagnosis applications. In SPR biosensing, the adsorption of a targeted analyte by a

surface bioreceptor is measured by tracking the change in the conditions of the resonance coupling of incident light to the propagating surface plasmon wave (SPW). The SPW is a charge density oscillation that occurs at the interface of two media with dielectric constants of opposite signs, such as a metal and a dielectric. The existence of this surface plasmon wave is dictated by the electromagnetic (EM) properties of the metal, typically gold or silver, and the dielectric interface (sample medium). The resonance coupling appears as a dip in the reflectivity of the light spectrum, which is traditionally tracked by measuring the wavelength, the incident angle or the intensity of the reflected light (figure 15.5). The coupling of the light to the SPW requires, for electromagnetic reasons, a high-index prism or a periodic grating surface. The sensitivity of the SPR lies in the strong electromagnetic enhancement of the SPW. Commercial SPR biosensors are generally capable of detecting 1 pg/mm^2 of absorbed analytes. This sensitivity is strongly dependent on many parameters, but is particularly dependent on surface functionalization. Sensor detection limit (DL) is another important parameter to characterize the sensor performance. The DL can be deduced by taking into account the noise in the transduction signal,σ, i.e., the minimum resolvable signal: DL = σ/S, where S is the sensitivity. Improvement in the DL can be accomplished by increasing the sensitivity or reducing the noise level. Sensitivity can be enhanced by increasing the light-matter interaction. Today, the key challenge in the SPR biosensor development lies not primarily in the integration of the various components of the biosensor (sampling handling, control electronics, etc.) but on providing robust integrated SPR biosensors that are as or more sensitive (<pg/mm^2) than their current counterparts, such as interferometer, optical ring resonator, and optical fiber based biosensors.

There are four basic methods to excite the SPR, as shown in figure 15.5: prism coupling, waveguide coupling, fiber optic coupling, and grating coupling. In the prism coupling configuration (Fig. 5A), the incident light is totally reflected at the prism–metal interface and generates an evanescent field penetrating into the metal layer. At the resonant angle or resonant wavelength, the propagation constant of the evanescent field matches that of the SPW as described in Eq. (2), and as a result, the photon will be coupled into the SPW.

$$\frac{2\pi}{\lambda} n_p \, sin\theta = \beta_{sp}$$

Where λ is the incident wavelength, n_p the prism refractive index, the incident angel and β_{sp} is the propagation constant of the SPW. Prism coupling is the most convenient SPR configuration and generally has the best sensing DL; however, the prism is bulky and it is difficult to integrate. Waveguide coupling offers a good alternative to the prism; it is robust and easy to integrate with other optical and electrical components. The light propagates in a waveguide through total internal reflection and generates an evanescent field at the waveguide–metal interface, which excites the SPW in the same way as in the prism configuration (Fig. 5B).Although these basic SPR structures have good DL that can satisfy most research requirements, there still exist two potential problems that will limit their applications in some fields. First, the evanescent field in those basic SPR structures only penetrates into the surrounding medium for about 100 nm, and thus it is very difficult to detect the large target molecules like cells and bacteria. Second, there is only one SPW to detect the RI change. It is impossible to differentiate the surface RI change and the bulk solution RI change. As a result, the sensing performance is deteriorated when detecting the target molecules in a complex solution, such as blood samples. To overcome those two problems, a new optical structure has been developed, as illustrated in figure 15.5F, where the metal layer is sandwiched by two dielectric layers with a similar RI [33–34]. Two new surface plasmon modes

called long range surface plasmon (LRSP) and short range surface Plasmon (SRSP) form, which are bound to both metal–dielectric interfaces. Using this dual mode of the LRSP and SRSP, the sensor is able to differentiate the background RI change and surface bound RI change [35]. Furthermore, the LRSP has a longer penetration length in the surrounding medium, making it suitable for cell and bacterium detection. The LRSP also exhibits an excellent DL as low as 2.10^{-7} to 2.10^{-8} RIU [33, 36].

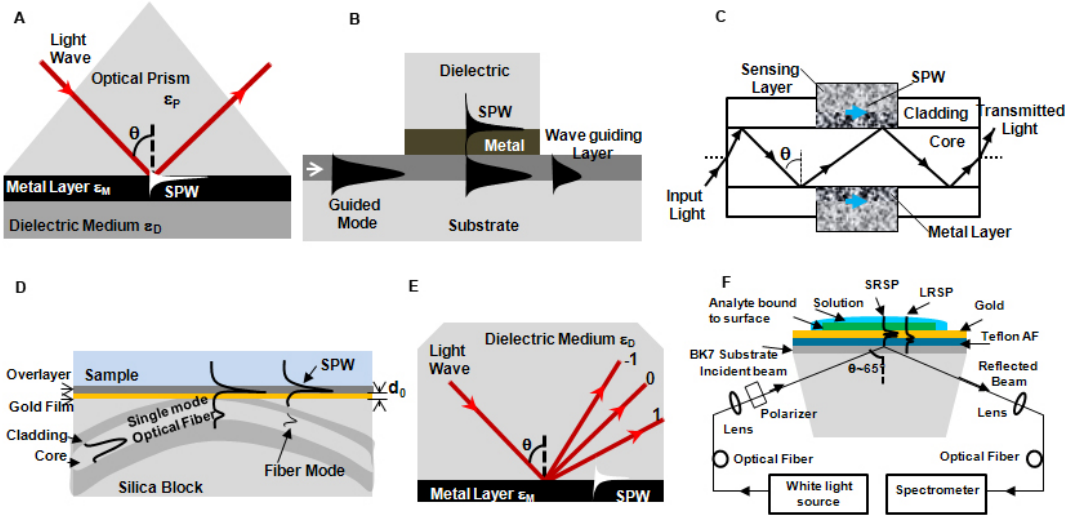

FIGURE 15.5
Various SPR sensor configurations. (A) Prism coupling, (B) waveguide coupling, (C) optical fiber coupling, (D) side polished fiber coupling, (E) grating coupling and (F) long-range and short-range surface plasmon (LRSP and SRSP). (from reference [39])

Optical fiber based biosensors

Among optical-based biosensors, optical fibers are new, tiny, flexible platforms that are being used with increasing frequency as biosensor transducers. Optical fibers are able to make quick and sensitive responses, and can be employed as an intrinsic or extrinsic biosensor [37]. Optical fibers are a convenient material for optical sensor design because they can be inexpensive and provide easy and efficient signal delivery. Fiber Bragg gratings (FBGs), while developed as a tool for the telecommunications industry, have flourished as a versatile sensor with a wide breadth of applications. They are currently among the most popular of all fiber-based optical sensors for analyzing load, strain, temperature, vibration, and RI [38].

Optical fibers transmit light on the basis of the principle of total internal reflection (TIR). Fiber optic biosensors are based on the transmission of light along silica glass fiber, or plastic optical fiber to the site of analysis. Optical fiber biosensors can be used in combination with different types of spectroscopic technique, e.g. absorption, fluorescence, phosphorescence, surface plasmon resonance (SPR), etc. Optical biosensors based on the use of fiber optics can be classified into two different categories: intrinsic sensors, where interaction with the analyte occurs within an element of the optical fiber; and extrinsic sensors, in which the optical fiber is used to couple light, usually to and from the region where the light beam is influenced by the measurand. In practice, fiber optics can be coupled with all optical techniques, thus increasing their versatility.

The simplest optical biosensors use absorbance measurements to determine any changes in the concentration of analytes that absorb a given wavelength of light. The system works by transmitting light through an optical fiber to the sample; the amount of light absorbed by the analyte is detected through the same fiber or a second fiber. The biological material is immobilized at the distal end of the optical fibers and either produces or extracts the analyte that absorbs the light. Illuminating the fiber with two focused intersecting laser beams, RI perturbations can be written into the fiber core that have periodicities Λ on the order of the wavelength. The resulting structure functions as a band rejection filter, reflecting a narrow band of light at the Bragg wavelength (λ_B) according to the following relationship:

$$\lambda_B = 2n_{eff}\Lambda$$

Where n_{eff} is the effective RI encountered by the fiber core mode. By monitoring λ_B, the system functions as a RI sensor which serves as the foundation for biochemical sensing functionality.

As described in figure 15.6, in order to expose the evanescent field from the FBG, several strategies have been pursued. One approach is to create a surface grating on the side of the fiber. This usually requires bending and polishing until the fiber's core is exposed, and then a grating is physically patterned on that surface. Another way of exposing the evanescent field involves chemically etching the optical fiber down to its core (see Fig. 6B)

Long-period gratings (LPGs), have attracted a great deal of attention in recent years for biochemical sensing applications although the advantages of LPGs have been known since the early 1990's. These devices are manufactured with periodicity on the order of 100 µm to 1mm, three or four orders of magnitude larger than traditional FBGs. They therefore have the advantage of being able to sense the RI changes around the cladding without etching or complex structural design. Due to the large grating pitch, they are also far easier to manufacture. LPGs can also be customized by chemically etching the cladding material down in order to enhance the sensitivity.

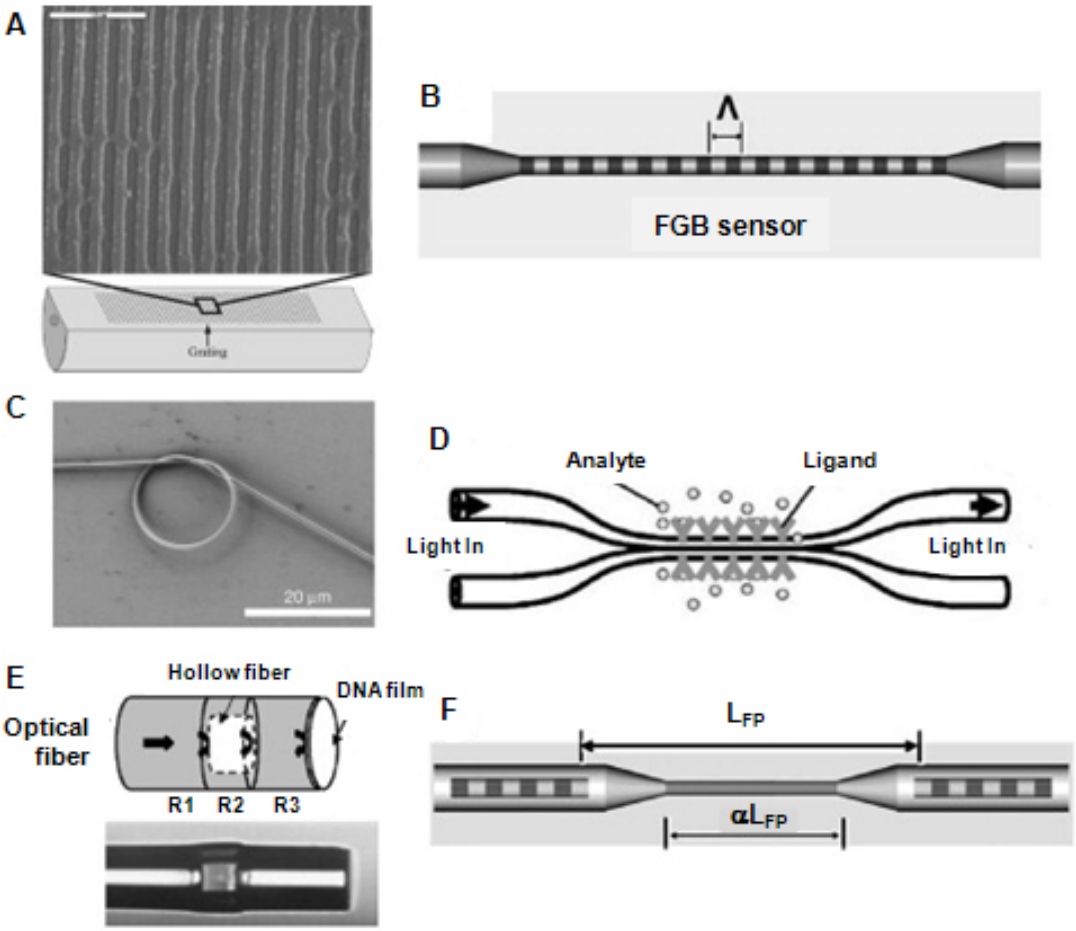

FIGURE 15.6
Overall, FBG and LPG sensors are very attractive due to their simple design, low manufacturing costs, and high compatibility with standard optical fibers. Most applications, however, focus on physical and basic RI sensing, because they lend themselves so readily to these purposes. For biochemical detection, these sensors cannot compete with other types of optical sensors in terms of their DLs even though their sensitivities can exceed 100 $nmRIU^{-1}$. (from reference [39])

Acoustic Biosensors

Electroacoustic devices used in biosensors are based on the detection of a change of mass density, elastic, viscoelastic, electric, or dielectric properties of a membrane made of chemically interactive materials in contact with a piezoelectric material. Bulk acoustic wave (BAW) and surface acoustic wave (SAW) propagation transducers are commonly used. In the first, a crystal resonator, usually quartz, is connected to an amplifier to form an oscillator whose resonant frequency is a function of the properties of two membranes attached to it. The latter is based on the propagation of SAWs along a layer of a substrate covered by the membrane whose properties affect the propagation loss and phase velocity of the wave. SAWs are produced and measured by metal interdigital transducers

deposited on the piezoelectric substrate as shown in figure 15.7 [40]. Even though SAW-based biosensor systems have been the focus of academic and industrial research for a number of years, most of these approaches only feature laboratory setups that are suitable for proof-of-principle evaluation and first experimental tests. For real commercial success, two crucial issues need to be solved: an appropriate production process is required, as is an applicable handling process for future SAW based biosensors. Most contributions to the scientific community relating to SAW-based sensor technology do not suggest overall system designs but rather basic approaches limited to the sensor element itself. Apart from the sensor, there are a number of additional issues which must be addressed when considering a market-compatible overall system [40]. However, surface acoustic wave biosensors are inexpensive devices to manufacture, and require inexpensive electronics to run and to disseminate data. They offer a flexible approach to point-of-care, real-time diagnostics and their small size allows flexibility in the samples to be analyzed; the devices can be incorporated into airway tubing to capture proteins and other molecules in breath condensate. Therefore SAW-based biosensor technology is a promising approach which may eventually be able to compete against established but much more complex optical- based biosensing techniques, like surface plasmon resonance (SPR).

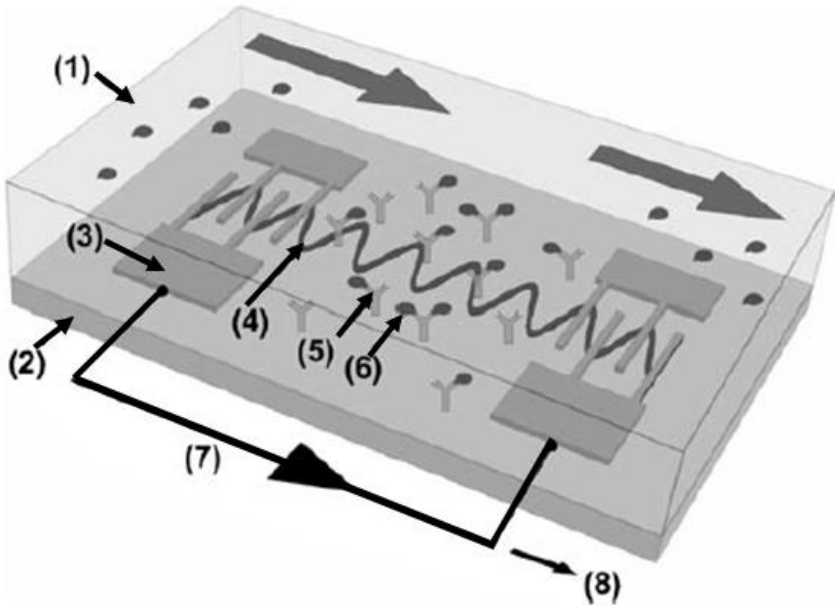

FIGURE 15.7
Basic SAW biosensor setup exemplified by a SAW immunosensor. The arrows at the top indicate the flow of the liquid sample (1) in which the sensor is immersed. The elements of the SAW biosensor are a piezoelectric crystal (2), IDTs (3), the surface acoustic wave (4), and immobilized antibodies (5) corresponding to the analyte molecules (6) in the sample. The driving electronics (7) operate the SAW biosensor and generate changes in the output signal (8) as the analyte binds to the sensor surface. (From reference [40])

Quartz Crystal Microbalance

The Quartz Crystal Microbalance (QCM) has been the most used acoustic device for sensor applications since 1959, when Sauerbrey established the relation between the change in the resonance frequency and the surface mass density deposited on the sensor face [41]. The classical QCM sensor typically consists of an oscillator circuit containing a thin AT-cut quartz disc with circular electrodes on both sides of the quartz (figure 15.8). Due to the piezoelectric properties of the quartz material, an alternating voltage between these electrodes leads to a mechanical oscillations of the crystal. These oscillations are generally very stable due to the high quality of the quartz. If a mass is adsorbed or placed onto the quartz crystal surface, the frequency of oscillation changes in proportion to the amount of mass. Therefore, these devices can be used as high sensitivity microbalances intended to measure mass changes in the nanogram range by coating the crystal with a material which is selective towards the species of interest. The quartz crystal acts as a signal transducer, converting mass changes due to the hybridization process into frequency changes. One of the main advantages of this device is the ability to control a QCM's selectivity by applying different coatings, which makes this sensor type extremely versatile. Despite of the extensive use of QCM technology, some challenges such as the improvement of the sensitivity and the limit of detection in high fundamental frequency QCM, remain unsolved; recently, an electrodeless QCM biosensor for 170MHz fundamental frequency, with a sensitivity of 67 Hz cm^{-2} ng^{-1}, has been reported [42]; this shows that the classical QCM technique still remains as a promising technique.

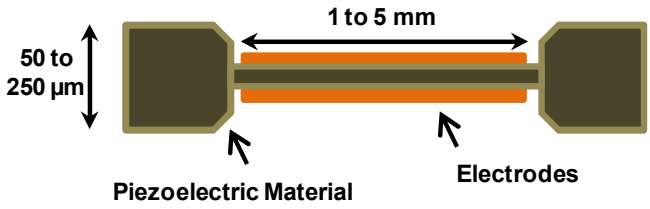

FIGURE 15.8
Schematic of a classical Quartz Crystal Microbalance

Application and Examples of Micro-Biosensors

In Vivo Biosensor

The field of biosensors may be viewed as comprising essentially two broad categories of instrumentation: i) sophisticated laboratory machines capable of rapid, accurate and convenient measurement of complex biological interactions; ii) easy-to-use, portable devices for use by non-specialists for in situ or home analysis. Although biosensor development made a huge progress in recent years, their application in clinical diagnosis is not very common, except for glucose biosensors which represent about 90 % of the global biosensor market. Interferences with undesired molecules during measurements with real samples and also high selectivity and accuracy are still serious issue. This is very important, since treatment is often dependent on individual levels of clinical markers. The emergence of semi-synthetic and synthetic receptors is yielding more

robust, versatile and widely applicable sensors, while nanomaterials are facilitating highly sensitive and convenient transduction of the resulting binding and catalytic events. Escalating healthcare costs together with consumer demand is likely to generate a new generation of inexpensive wearable, integrated and less-invasive sensors amenable to mass production to support the maintenance of wellbeing, care of the elderly, pharmaceutical development and testing, and distributed diagnostics.

Electrochemical biosensors currently dominate the field, but are focused mainly on metabolite monitoring, while bioaffinity monitoring is carried out principally using optical techniques. However, both transducers find utility across the whole field, along with piezoelectric, thermometric, and micromechanical transducers.

The Glucose Biosensor

Blood glucose measurement for the management of diabetes comprises approximately 90% of the world market for biosensors. Millions of diabetics test their blood glucose levels daily, making glucose the most commonly tested analyte. Such huge market size makes diabetes a model disease for developing new biosensing concepts. Glucose concentration is also one of the most monitored indicators in many endocrine metabolic disorders. The glucose biosensor is the most widely used example of an electrochemical biosensor which is based on a screen-printed amperometric disposable electrode.

The first developed glucose enzyme electrode relied on a thin layer of glucose oxidase (GOx) entrapped over an oxygen electrode via a semi-permeable dialysis membrane. Measurements were made based on the monitoring of the oxygen consumed by the enzyme-catalyzed reaction [14]:

$$Glucose + O_2 \rightarrow Gluconic\ acid + H_2O_2 \qquad (1)$$

The entire field of biosensors can trace its origin to this original glucose enzyme electrode. A wide range of amperometric enzyme electrodes, differing in electrode design or material, immobilization approach, or membrane composition, has since been described. In 1973, Guilbault and Lubrano described an enzyme electrode for the measurement of blood glucose based on amperometric (anodic) monitoring of the hydrogen peroxide product [43]:

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \qquad (2)$$

The resulting biosensor offered good accuracy and precision in connection with 100 µl blood samples. The most suitable concept for glucose determination rely on the use of the natural oxygen cosubstrate and generation and detection of hydrogen peroxide (equations 1 and 2). The biocatalytic reaction involves reduction of the flavin group (FAD) in the enzyme by reaction with glucose to give the reduced form of the enzyme (FADH2) followed by re-oxidation of the flavin by molecular oxygen to regenerate the oxidized form of the enzyme GOx(FAD). The resulting electric current from the re-oxidation on the working electrode at applied constant potential is proportional to the glucose concentration:

$$GOx(FAD) + Glucose \rightarrow GOx(FADH_2) + Gluconolactone$$
$$GOx(FADH2) + O_2 \rightarrow GOx(FAD) + H_2O_2$$

This model is now utilized in the most commercially successful glucose biosensors utilizing glucose oxidase or glucose dehydrogenase. Varieties of enzymes were used for biosensor construction, for

example oxidoreductase enzymes were used for lactate, malate, and ascorbate. Extensive review of commercially available biosensors for glucose, cholesterol, lactate, triglycerides and creatinine determination can be found in the review by Monošík et al. [44]. This type of biosensor has been used widely throughout the world for glucose testing in the home bringing diagnosis to on site analysis.

Array-type Biosensors

The rapid detection and monitoring of toxins in clinical fluids require new approaches in order to expedite appropriate countermeasures. For example, many toxins are secreted by bacteria during the course of infection and should be detected in low quantities ($ngmL^{-1}$) in urine or blood products following intoxication. In recent years, the fabrication of biosensors able to distinguish multiple analytes in a single sample has become an increasingly well recognized research goal. In such bioassays, it is imperative to implement the sensor in an array format. Instruments for reading microarray systems and microplate readers are a few examples (figure 9). The detectors for such systems need to measure the analyte quantity at different locations, which is typically carried out sequentially by a single detector across the array (scanning) or to dedicating an individual detector to each site. Most array-biosensors require a single measurement (usually when the assay reaches its biochemical equilibrium) per detection site (pixel) [45]. Others require the capturing of the reaction kinetics, necessitating multiple measurements per pixel. For instance, fluorescence detection in microarray systems is extensively used for a variety of applications. These include but are not limited to nucleic acid/protein detection and quantification, DNA sequencing, blotting, and real-time PCR analysis. The single frequency excitation induces photon emission (at a different frequency) from the fluorescent label in the sample material. Filtration of the emission spectrum and detection follow to form the fluorescence image of the sample. There are two types of fluorescence detection systems for array-based platforms. In one system, the excitation source is scanned across the array and a single-pixel very high-performance detector such as a photomultiplier tube is used for detection. The other approach is to use a homogeneous excitation light source for the entire assay at once and measure fluorescence emission at different pixels through a 2D array of detectors e.g. a CCD camera. In these implementations, the resolution of the CCD camera and the number of photosensitive pixels determine the possible array size [46].

Array-type biosensors offer many advantages over the conventional analytical methods, the most significant of which are: i) a variety of analytes can be investigated simultaneously in the same sample, ii) the required sample quantities are minimal, iii) low consumption of scarce reagents, iv) high miniaturization and v) high sample throughput. These advantages become very evident if we consider the workflow during a typical drug screening process. At the beginning there is the need for a selection of few eligible compounds out of a large variety of molecules for a given purpose. Since the most limiting factor is the ratio between the number of data points per day and the cost per data point, this first mass-selection is best done at a molecular level where DNA-chips and protein microarrays find their most common application.

Microarrays are not limited to DNA analysis; protein microarrays, antibody microarray, chemical compound microarray can also be produced using biochips. Randox Laboratories Ltd., the first protein Biochip Array Technology analyzer in 2003. In protein Biochip Array Technology, the biochip replaces the ELISA plate or cuvette as the reaction platform.

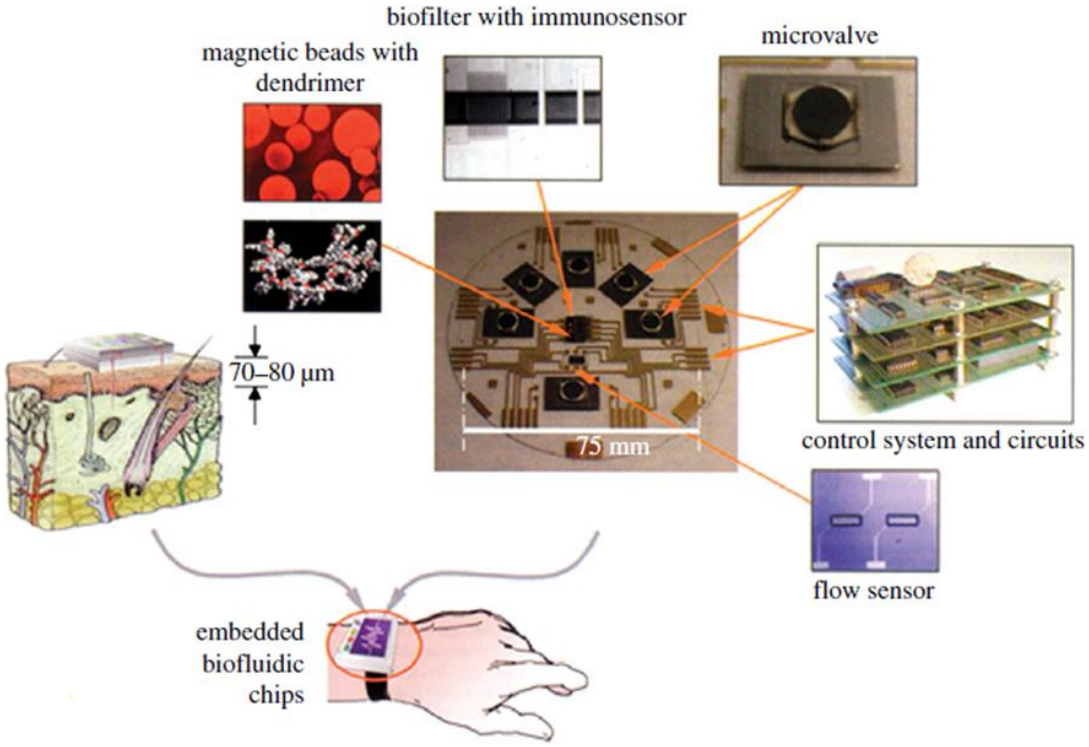

FIGURE 15.9
An example of a microfluidic-based biosensor that can be incorporated onto a wristwatch. The lab-on-a-chip system relies on manipulation of small volumes of fluid in microchannels using microvalves (from reference [46])

The biochip is used to simultaneously analyze a panel of related tests in a single sample, producing a patient profile. The patient profile can be used in disease screening, diagnosis, monitoring disease progression or monitoring treatment. Performing multiple analyses simultaneously, described as multiplexing, allows a significant reduction in processing time and the amount of patient sample required. Biochip Array Technology is a novel application of a familiar methodology, using sandwich, competitive and antibody-capture immunoassays. The difference from conventional immunoassays is that the capture ligands are covalently attached to the surface of the biochip in an ordered array rather than in solution.

Microbial Biosensor

Microorganisms have been integrated with a variety of transducers such as amperometric, potentiometric, conductimetric, luminescence and fluorescence to construct biosensor devices. Since microbial biosensor response, operational stability and long-term use are, to some extent, a function of the immobilization strategy used, immobilization technology plays a very important role and the choice of immobilization technique is critical. Microorganisms can be immobilized on transducer or support matrices by chemical or physical methods. Several reviews papers and book chapters addressing microbial biosensor development have been published [5, 15, 23, 28, 47]. Based on the sensing technique, recent reported microbial biosensors can be classified into two

major groups: electrochemical microbial biosensors and optical microbial biosensors. Amperometric microbial biosensors have been extensively exploited for the determination of biochemical oxygen demand (BOD) for the measurement of biodegradable organic pollutants in aqueous samples [47]. Amperometric microbial biosensors also provide rapid and sensitive tools in health and fermentation applications. The detection of glucose, which is of great interest in the diagnosis of diabetes and quality control of fermentation and food, accounts for about 90% of the entire biosensor market. Several microbial biosensors for glucose detection have been fabricated based on the oxygen consumption of the respiratory activity in the microbes. Generally, the bacteria, which can uptake glucose, also possess the enzyme activity to metabolize other carbohydrates, such as galactose, catechol, mannose, and xylose. Selective biosensors can still be developed for different sugars as long as the bacteria are adapted to the specific analyte in advance through the selective cultivation. The qualitative and quantitative detection of alcohols with high sensitivity, selectivity, and accuracy is required in many fields. Several microorganisms which can metabolize ethanol with the consumption of oxygen, such as G. oxydans, Pichia angusta, and Candida tropicalis have been applied to the construction of ethanol whole-cell biosensors. Potentiometric microbial biosensors detect the amount of analytes by measuring the potential difference between the working electrode and the reference electrode separated by a selective membrane. Recently, a potentiometric biosensor based on the pH electrode modified by permeabilized P. aeruginosa was developed for selective and rapid detection of cephalosporin group of antibiotics [48]. The hydrolysis of cephalosporin, due to the enzyme activity of the microbial layer, was accompanied by the production of protons near the pH electrode. The response came from the change of electric potential difference between the working electrode and the reference electrode. Another potentiometric biosensor for the identification of β-lactam residues in milk was also reported [49].

Microbial biosensors have been under extensive investigation over decades. Particularly, some electrochemical and optical microbial biosensors developed for environmental applications have been commercialized. For example, commercial on-line BOD microbial biosensors were available from Biosensores SL Moncofar, Spain and Isco GmbH, Gross Umstadt, Germany. The Green Screen Environmental Monitoring (EM) with a yeast cellular sensing element was designed for the simultaneous detection of genotoxicity and cytotoxicity by Gentronix Ltd., Manchester, UK. In addition, amperometric and bioluminescent whole-cell toxicity biosensors have been developed by Euroclone Ltd., West Yorkshire, UK and Remedios Ltd., Aberdeen, UK [50]. Nevertheless, commercial microbial biosensors are just tips of the iceberg compared to the great amount of academic research on them. The intrinsic disadvantages (slow response, low sensitivity, and poor selectivity) using microorganisms as the biosensing element limit the widespread interest of microbial biosensors on the market. Microbial biosensors typically suffer from the poor selectivity because of the non-specific cellular response to substrates. With the development of biotechnology and the availability of genome sequence for more microorganisms, we can genetically engineer microbes with specific metabolic pathways up-regulated or downregulated, thus providing enhanced selectivity to specific targets. Another way to improve the selectivity of microbial biosensors is to develop microbial sensor arrays. The introduction of analyte to the microbial sensor arrays will generate a finger-printed response pattern. By combining with artificial neural network analysis, the target compound can be identified [47].

NanoBiosensors: Basic concepts & Applications

Advances in nanotechnology have led to the development of nanoscale biosensors that have exquisite sensitivity and versatility. The ultimate goal of nanobiosensors is to detect any biochemical and biophysical signal associated with a specific disease at the level of a single molecule or cell. They can be integrated into other technologies such as lab-on-a-chip to facilitate molecular diagnostics. Their applications include detection of microorganisms in various samples, monitoring of metabolites in body fluids and detection of tissue pathology such as cancer. Their portability makes them ideal for pathogenesis of cancer applications but they can be used in the laboratory setting as well.

The ability to detect disease-associated biomolecules, such as disease-specific metabolites, nucleic acids, proteins, pathogens, and cells such as circulating tumor cells, is essential not only for disease diagnosis in the clinical setting but also for biomedical research involving drug discovery and development. Nanotechnology, with its enhanced sensitivity and reduced instrumentation size, will rapidly improve our current biodiagnostic capacity with respect to specificity, speed, and cost. Reduction in sensor size provides great versatility for incorporation into multiplexed, transportable, portable, wearable, and even implantable medical devices. The integration of nanoscale ultrasensitive biosensors with other medical instruments will open the door to emerging medical fields, including point-of-care diagnostics and ubiquitous healthcare systems. The biomedical application of nanobiosensors is wide; moreover, the future impact of nanobiosensor systems for point-of-care diagnostics will be unmatched. This technology will revolutionize conventional medical practices by enabling early diagnosis of chronic debilitating diseases, ultrasensitive detection of pathogens, and long-term monitoring of patients using biocompatible integrated medical instrumentation.

There are different strategies for creating next generations of nanobiosensor devices: i) the use of a completely new class of nanomaterial for sensing purposes, ii) new immobilization strategies, and iii) the new nanotechnological approaches. In the second part of this chapter, current state-of-the art principles of nanobiosensor systems are discussed along with future perspectives.

Nanomaterials for new biosensing principles

One of the first new nanomaterials to impact on amperometric biosensors was the carbon nanotube (CNT), which was blended into a number of formulations to improve current densities and overall performance of enzyme electrodes and enzyme-labelled immunosensors [51]. Amperometric enzyme electrodes benefited from enhanced reactivity of NADH and hydrogen peroxide at CNT-modified electrodes and aligned CNT ‘‘forests’’ appeared to facilitate direct electron transfer with the redox centers of enzymes. The most widely used nanomaterial in industry overall to date, however, is the silver nanoparticle. These have also been harnessed as a simple electrochemical label in a highly sensitive amperometric immunoassay intended for distributed diagnostics and as an inexpensive solution for immunoassays performed in developing countries. In this electrochemical sandwich immunoassay, silver nanoparticles are used as a robust label, which can be solubilised after the binding reaction has occurred, using thiocyanate, to form a silver chelate. This benign chemistry replaces earlier versions using aggressive chemical oxidants such as nitric acid. Once solubilised, the silver concentration can be very sensitively determined using stripping voltammetry on a single-use screen-printed carbon electrode. The silver colloid aggregates due to the presence of thiocyanate and the negatively charged aggregates are attracted to the positive potential of the carbon electrode during the pre-treatment. Once in direct contact

with the electrode surface, the silver is oxidised at 0.6 V to form soluble silver ions, which are immediately complexed by the thiocyanate and detected by the ensuing anodic stripping voltammetry. Hence, the analyte concentration yields a signal which is directly proportional to the anodic stripping voltammetry peak of silver. In one example, the cardiac marker myoglobin, was measured down to 3 ng mL^{-1}, which was comparable with the conventional enzyme-linked immunosorbent assay (ELISA). Samples volumes of less than 50 mL could be handled and the assay worked in turbid solutions without the need for sample clean up [52].

A variety of other nanoparticle-based strategies have been described in the literature for electrochemical affinity assays [53]. Most recently, nanostructured materials have been used to deliver label-free electrochemical immunoassays. Gooding's group in Australia described a direct electrochemical immunosensor for detection of veterinary drug residues in undiluted milk. They used a displacement assay for with a mixed layer of oligo(phenylethynylene) molecular wire, to facilitate electrochemical communication, and oligo(ethylenelycol) to control the interaction of proteins and electroactive interferences with the electrode surface [54]. More recently, Turner et al. reported on the use of a highly conductive N-doped graphene sheet-modified electrode, which exhibited significantly increased electron transfer and sensitivity towards the breast cancer marker CA 15-3. This label-free immunosensor delivered a low detection limit of 0.012 U mL^{-1} and worked well over a broad linear range of 0.1–20 U mL^{-1} [55].

Despite using new nanotechnologies for biosensors the application of nanomaterials to bioanalytics in array-type assays or in vivo monitoring is currently a replacement of organic dyes, radioactive or metal labels and contrast agents by metal, oxide or luminescent nanocrystals. Such methods have to be used to investigate metabolic pathways on cellular levels where conventional device-based nanobiosensors have no chance to measure. Using such new labelling nanomaterials the bioanalytical and imaging methods remain mostly unchanged, whereas the tagged or labelled biomolecule is replaced by a bionano-system. The conjugation between biomolecule and nanocrystal is crucial for every bionano-system as it determines the overall biological properties of the conjugate.

Immobilization Strategies at the Nanoscale

Since the development of the first biosensor, biosensors technology has experienced a considerable growth in terms of applicability and complexity of devices. In the last decade this growth has been accelerated due the utilization of electrodes -modified nanostructured materials in order to increase the power detection of specific molecules. Other important feature can be associated with the development of new methodologies for biomolecules immobilization. This includes the utilization of several biological molecules such as enzymes, nucleotides, antigens, DNA, amino acids and many others for biosensing. Moreover, the utilization of these biological molecules in conjunction with nanostructured materials opens the possibility to develop several types of biosensors such as nanostructured and miniaturized devices and implantable biosensors for real time monitoring. The interface between the nanostructure and the biomolecule requires significant attention as it dictates the biosensor performance and sensitivity. Based on the physical and chemical properties of both the nanostructure and the biolmolecule, a number of immobilization methods have been proposed. The key problem during the immobilization is how to fully maintain the biomolecule's conformation and activity. Non-specific biomolecule adsorption onto the nanostructure is the initial stage of the degradation mechanisms that will ultimately compromise the functionality of the biosensor. The different methods of conjugation between nanostructures and biomolecules can be divided into three categories.

The first category includes methods where biomolecules are bound non-covalently to nanoparticles. Therefore, nanoparticles are first derivatized with a chemisorbed monolayer or the capping agent from synthesis to have hydrophobic surfaces. In a second step, these hydrophobic nanoparticles are precipitated and redissolved in water within tensidic micelles. In principle, this method works with all common micelle building agents such as phospholipids and sodium dodecylsulfate. In a final step, biomolecules are coupled covalently to functional groups at the outer sphere of the micelles [56]. A major advantage of this method is that the whole process, from non-polar/polar solvent transfer to the coupling, is relatively easy to perform. The bond between nanoparticles and biomolecules is based on hydrophobic interactions within the micelles. Therefore, the conjugate disintegrates relatively easily.

The second category contains methods in which biomolecules are chemisorbed onto nanoparticles by means of a 'linker'. This can be realized in two variations: first, the biomolecules contain surface-active groups such as, e.g., thiols, and are directly chemisorbed onto the nanoparticles. Second, a bifunctional molecule is chemisorbed onto the nanoparticles and biomolecules are coupled to these molecules in a second step [57], similar to the micelles from the first category.

Chemisorption of thiols onto gold surfaces is well known and as long as the adsorption energy is less than −40 kJ mol^{-1}, this bond has mostly covalent character. However, from a practical point of view, on a longer time-scale these conjugates can disintegrate by desorption, what could become critical for long-term experiments in the range of days.

The third category of coupling methods includes methods in which biomolecules are bound covalently to modified nanoparticles. Therefore, the nanoparticles have to be derivatized with a cross-linked surface shell, which contains binding sites for biomolecules. This cross-linked surface shell could consist of functionalized polymers or inorganic networks like silica. Second, the biomolecules have to be coupled to these surface shells [58]. Such conjugates are very stable due to covalent bonds. The major disadvantage of these methods is the sometimes difficult and costly preparation. Compared to the other categories these methods are recommended when long-term stability of the conjugate is necessary.

In conclusion, it can be stated that different methods of producing bionanosystems with different advantages and disadvantages are available. The major problem with all of them is that the biomolecule is turned into a colloid by attaching it to a nanocrystal. Because colloids have very different 'solubility' from biomolecules, there is always a tendency for coagulation within biological media.

Examples of NanoBiosensors

Nanowire Biosensors

Nanowire biosensors are a class of nanobiosensors, of which the major sensing components are made of nanowires coated by biological molecules such as DNA molecules, polypeptides, fibrin proteins, and filamentous bacteriophages (figure 15.10). A bionanowire is a one-dimensional fibril-like nanostructure, with the diameter constrained to tens of nanometers or less and unconstrained length. Since their surface properties are easily modified, nanowires can be decorated with virtually any potential chemical or biological molecular recognition unit, making the wires themselves analyte independent. The nanomaterials transduce the chemical binding event on their surface into a change in conductance of the nanowire in an extremely sensitive, real time and quantitative fashion. One dimensional nanowires, nanotubes, nanobelts and nanosprings have become the

focus of intensive research in biosensing due to their unique properties and their potential for fabrication into high density nanoscale devices. The nanowires can be used for both efficient transport of electrons and optical excitation, and these two factors make them critical to the function and integration of nanoscale devices. In fact, they are the smallest dimension structures that can be used for efficient transport of electrons and are thus critical to the function and integration of these nanoscale devices. Because of their high surface-to-volume ratio and tunable electron transport properties due to quantum confinement effect, their electrical properties are strongly influenced by minor perturbations. One of the excellent candidates for development of enzyme/protein-based biosensors is the CNT, due to its unique electric, electrocatalytic, and mechanical properties. Wang and co-workers employed CNT/Nafion-based electrodes for immobilizing glucoseoxidase (GOx) enzyme for sensitive detection of glucose [59]. This CNT/Nafion composite was prepared by dispersing solubilized CNTs in Nafion solution onto an electrode surface. The CNT-based biosensor offers substantially greater signals, especially at low potential, reflecting the electrocatalytic activity of CNTs. Such low potential operation of the CNT-based biosensor results in a wide linear range and a fast response time. Boron-doped silicon nanowires (SiNWs) have been used to create highly sensitive, real-time electrically based sensors for biological and chemical species [60]. Biotin-modified SiNWs were used to detect streptavidin down to at least a picomolar concentration range. The small size and capability of these semiconductor nanowires for sensitive, label-free, real-time detection of a wide range of chemical and biological species could be exploited in array-based screening and in vivo diagnostics. Furthermore, the highly-ordered nanowires array combined with multiple biorecognition holds the promise of developing multiplexed nanobiosensors. They will be very useful for high-throughput diagnosis and screening. Due to their small size and robustness, the nanowires are a good candidate material for fabricating nanoscale biosensors for in-body biosensing and for making remote-controlled nanbiosensors for environmental monitoring. In general, nanobiosensors based on nanowires such as CNTs show great promise for future applications in health-care testing, disease diagnostics and environmental monitoring.

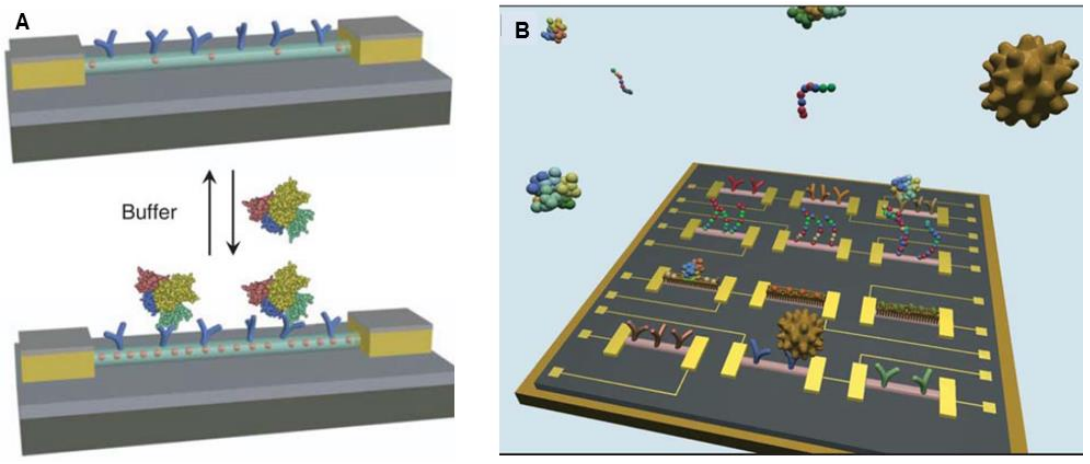

FIGURE 15.10
The image on the left illustrates the sensing principle of nanowire-based biosensing with an FET configuration. The image on the right demonstrates the concept of multi-analyte biosensing with nanowire FET configurations (from reference 61)

Cantilever Biosensors

The best example of the use of nanotransducers is cantilever based biosensors which utilize a micromechanically produced cantilever in a similar manner as for the production of (atomic force microscopy) AFM probes. A several 100 nm thick cantilever is bent due to biosensing interaction on the surface (figure 15.11), which can be optically sensed by a laser. The sensitivity can be tuned down to single molecule interaction analysis [60]. The high sensitivity of microcantilever sensors has proven to be a powerful platform for detecting molecular interactions in a label-free, time resolved manner [63]. By an asymmetrical chemisorption of molecules (i.e., on one side of the microcantilever), the sensors can detect processes in "static" mode by measuring the bending of a microcantilever due to stress formation during the adsorption process; or in "dynamic" mode where the resonant frequency of an oscillating microcantilever shifts due to mass adsorption on its surface. The versatility of the microcantilever technique as a chemical/biological sensor has been demonstrated for vapors, ions, DNA, proteins, antibiotics and pathogenic microorganisms. The mechanical sensitivity of the static mode technique stems from changes in surface stress caused by molecular interactions with the surface (change in the electronic charge distribution of the substrate's surface atoms) and by lateral interactions within the molecular layer (electrostatic forces, structural changes and steric competition) [64]. This sensitivity to structural changes in static mode operation has shown to be particularly suited for measuring binding processes based on conformational changes of molecules attached to the microcantilever's surface such as proteins, DNA or lipid bilayers. Recently, Bumbu et al. [65] applied the static mode technique to study the behavior of poly(methyl methacrylate) brushes that had been polymerized from the silicon surface of a microcantilever sensor, i.e., using a "grafting from" approach. While this allowed the authors to study the in situ swelling and collapse of poly(methyl methacrylate) brushes, the kinetics of brush formation could not be monitored in real-time. The driving impetus behind this work is to apply microcantilever sensors operated in static mode to study in real-time i) the kinetic aspects of surface PEGylation, and ii) conformational changes in the PEG layer over a timescale of tens of minutes in situ.

In the last decade, several research groups observed that microcantilevers can transduce a number of different signal domains, e.g. mass, temperature, heat, electromagnetic field, stress, into a mechanical deformation: either a bending or a change in the resonance frequency, with a resolution which is orders of magnitude higher than that achievable with macroscopic structures. Over the last years, the number of applications of these sensors has shown a fast growth in diverse fields, such as genomic or proteomic, because of the biosensor flexibility, the low sample consumption, and the non pretreated samples required.

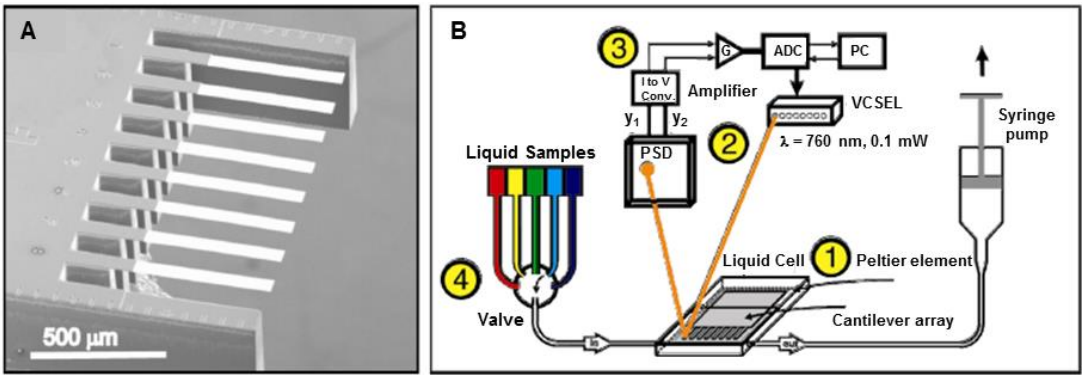

FIGURE 15.11
(A) Scanning electron microscope image of a silicon microcantilever array consisting of eight cantilevers and two sidebars. (B) Schematic drawing of the sensor instrument: 1) the measurement cell with a mounted microcantilever array, 2) optical-read out system comprising vertical cavity surface emitting lasers (VCSELs) and a position sensitive detector (PSD), 3) data processing and acquisition, 4)valve selector connected to liquid samples (from reference 63)

Ion-channel based sensing

Biological ion channels are water-filled subnanosized pores formed by protein molecules in the membranes of all living cells. Ion channels play a crucial role in living organisms by selectively regulating the bflow of ions into and out of a cell thereby controlling the cell's electrical and biochemical activities. New generations of nanobisensors have been developed in which the conductance of a population of molecular ion channels is switched by the recognition event. The approach mimics biological sensory functions and can be used with most types of receptor, including antibodies and nucleotides. The technique is very flexible and even in its simplest form it is sensitive to picomolar concentrations of proteins. The sensor is essentially an impedance element whose dimensions can readily be reduced to become an integral component of a microelectronic circuit. It may be used in a wide range of applications and in complex media, including blood. These uses might include cell typing, the detection of large proteins, viruses, antibodies, DNA, electrolytes, drugs, pesticides and other low-molecular-weight compounds [66, 67].
The active elements of the ion-channel switch comprise a gold electrode to which is tethered a lipid membrane containing gramicidin ion channels linked to antibodies (figure 15.12). The molecular structure of the tethered membrane results in an ionic reservoir being formed between the gold electrode and the membrane. The ionic reservoir can be accessed electrically through connection to the gold electrode. In the presence of an applied potential, ions flow between the reservoir and the external solution when the channels are conductive. The ion current is switched off when mobile channels diffusing within the outer half of the membrane become crosslinked to antibodies immobilized at the membrane surface. This prevents them forming dimers with channels immobilized within the inner half of the membrane. The number of dimers is measured from the electrical conduction of the membrane. The switch has a high gain; a single channel facilitates the flux of up to a million ions per second. A quantitative model of the biosensor has been verified experimentally. The detection of analytes possessing multiple recognition sites is performed using the structure shown schematically in figure 15.12. This structure is assembled using a combination

of sulphur–gold chemistry and physisorption. The membrane consists of lipids and channels, some immobilized on the gold surface and some diffusing laterally within the plane of the membrane. The antibodies on the mobile channels scan an area of the order of 1 μm^2 in less than 5 minutes. Thus with a low density of channels and a high density of immobilized antibodies, each channel can access up to 10^3 more capture antibodies than if the gating mechanism were triggered by a directing binding of analyte to the channels. The speed and sensitivity of the biosensor response may be adjusted in direct proportion to the number of binding sites accessible to each mobile channel. This allows for quantitative detection of analyte from sub-picomolar to micromolar concentrations in less than 10 minutes.

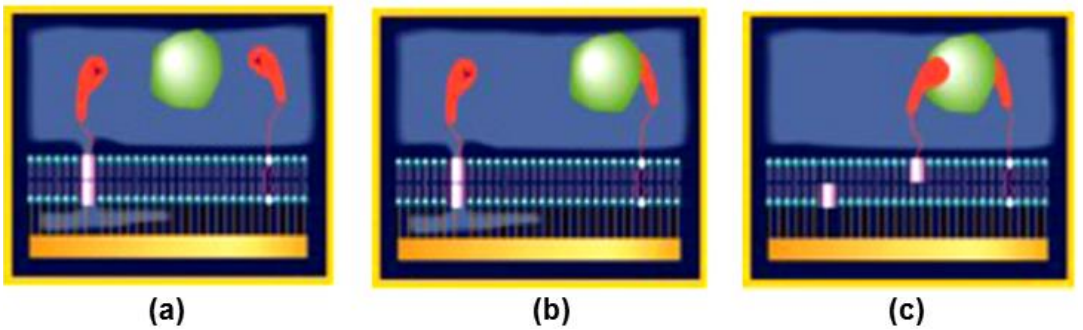

FIGURE 15.12
Large analyte transduction mechanism. The binding of analyte (green) to the antibody fragments (Fab) (red) causes the conformation of gramicidin A to shift from conductive dimers to nonconductive monomers. This causes a loss of conduction of ions across the membrane. The scale can be visualized by the fact that the tethered lipid bilayer is 4 nm thick (from reference 67)

Several companies/research groups have developed biosensors based on synthetic lipid monolayers and bilayers. For example, OhmX Corporation is currently developing a reagentless biosensor system using self-assembled monolayers tethered to a gold surface for the electronic detection of biomarkers in clinical samples. Stochastic signal analysis has been employed by Bayley's group at Oxford and has made substantial contributions in advancement of ion channel biosensors.

The fabrication of the ion-channel switch (ICS) biosensor has several interesting properties that make it an appealing case study. The ICS biosensor incorporates a self-assembled monolayer providing enhanced stability. The tethered bilayer permits 2-D diffusion of gramicidin channels that provides a remarkable gating mechanism. Since gramicidin has a terminal ethanolamine group that permits a range of chemistries, the biosensor may be prepared for use with a wide range of receptors to detect many different analytes. The ICS sensing mechanism does not require washing (unlike an ELISA assay), provides large transduction amplification (millions of ions for every channel dimerization), and a high detection sensitivity since a single channel can diffuse and identify analyte molecules bound to many capture sites. The ICS biosensor also provides an objective electrical readout that is intrinsically digital. The digital output permits the use of sophisticated statistical signal processing algorithms to estimate the type and concentration of analyte [67].

NanoBiosensors in Nanomedicine

Nanomedicine involves cell-by-cell regenerative medicine, either repairing cells one at a time or triggering apoptotic pathways in cells that are not repairable. Multilayered nanoparticle systems are being constructed for the targeted delivery of gene therapy to single cells. Cleavable shells containing targeting, biosensing, and gene therapeutic molecules are being constructed to direct nanoparticles to desired intracellular targets. Therapeutic gene sequences are controlled by biosensor-activated control switches to provide the proper amount of gene therapy on a single cell basis. The central idea is to set up gene therapy "nanofactories" inside single living cells. Molecular biosensors linked to these genes control their expression. Gene delivery is started in response to a biosensor detected problem; gene delivery is halted when the cell response indicates that more gene therapy is not needed. Cell targeting of nanoparticles, both nanocrystals and nanocapsules, has been tested by a combination of fluorescent tracking dyes, fluorescence microscopy and flow cytometry. Intracellular targeting has been tested by confocal microscopy. Successful gene delivery has been visualized by use of GFP reporter sequences. DNA tethering techniques were used to increase the level of expression of these genes. Integrated nanomedical systems are being designed, constructed, and tested in-vitro, ex-vivo, and in small animals. While still in its infancy, nanomedicine represents a paradigm shift in thinking – from destruction of injured cells by surgery, radiation, chemotherapy to cell-by-cell repair within an organ and destruction of non-repairable cells by natural apoptosis.

Conclusion

Simple, easy-to-use measurement devices for a diverse range of biologically relevant analytes have an intuitive appeal as portable or pocket-sized analysers, and this has driven the diverse range of applications reported in the literature. However, both historical precedent and a critical analysis of potential markets leads to an indisputable conclusion that healthcare is and will continue to be the most important area for the application of biosensors. The maintenance of health is one of the most laudable technological objectives challenging science and technology and diagnosis is an essential prerequisite for treatment and prevention of disease. Moreover, related applications of biosensors, such as the maintenance of food safety and environmental monitoring can be aligned with this central objective. The developing world has a desperate need for robust diagnostics that can be deployed in the field by both healthcare professionals and volunteers. Infectious diseases account for around a quarter of worldwide deaths, although they are projected to decline as a percentage of total deaths over the coming 20 years, as other cause become more prevalent. In developing countries we are faced with diseases of poverty such as HIV/AIDS and tuberculosis, where the former kills 1.8 million people each year and the latter still affects around a third of the world’s population and accounts for an estimated 1.4 million deaths, according to the WHO (2012), although the incidence has been falling globally at a rate of 2.2% in recent years. In addition there are 2.5 million deaths from diarrheal infections and almost 800 000 from malaria. Of the estimated 57 million global deaths in 2008, 36 million (63%) were due to non-communicable diseases.

Technology needs to offer more economic solutions and distributed diagnostics enabled by biosensors and enhanced by consumer products available over-the-counter are a key part of the solution. This is also commercially attractive, with in vitro diagnostics already worth an estimated US$40 billion per year. While glucose biosensors for diabetes have had the most profound effect on

disease management to date, biosensors for other metabolites promise utility for other non-communicable diseases such as kidney disease, which is increasingly being recognised as an emerging problem in a rapidly ageing population. Multifarious affinity biosensors have been described to detect cardiac disease markers such as creatine kinase and troponin, while cancer markers and single cell cancer detection have attracted considerable recent literature.

We hope that this brief overview has illustrated that biosensors have achieved considerable success both in the commercial and academic arenas and that the need for new, easy-to-use, home and decentralized diagnostics is greater than ever. The enormous success of the glucose sensor serves as a model for future possibilities and should not overshadow the multifarious other applications that this versatile technology can address. Emerging science, driving new sensors to deliver the molecular information that underpins all this, includes the development of semi-synthetic ligands that can deliver the exquisite sensitivity and specificity of biological systems without the inherent instability and redundancy associated with natural molecules. Currently aptamers, affibodies, peptide arrays and molecularly imprinted polymers are particularly promising research directions in this respect. Chances of success are enhanced by the potential utility of some of these materials for novel therapeutic, antimicrobial and drug release strategies, since these complimentary areas will drive investment in these approaches.

References

1. Arnold M.A. Meyerhoff M.E. Recent Advances in the Development and Analytical Applications of Biosensing Probes. Crit. Rev. Anal. Chem. 1988; 20: 149–196.
2. Belkin, S., 2003. Curr. Opin. Microbiol. 6, 206–212.
3. Eggins, B.R., 2002. Chemical Sensors and Biosensors. John Wiley, Chichester.
4. Wilson, G.S., Gifford, R., 2005. Biosens. Bioelectron. 20, 2388–2403.
5. Wilson, J.S., 2005. Sensor Technology Handbook. Elsevier, Amsterdam/Boston.
6. Schubert, F.; Wollenberger, U.; Scheller, F.W.; Mu¨ller, H.G. Artificially Coupled Reactions with Immobilized Enzymes: Biological Analogs and Technical Consequences. In Bioinstrumentation and Biosensors; Wise, D.L., Ed.; Marcel Dekker, Inc.: New York, 1991; 19.
7. Clark, L.C.; Lyons, C. Electrode systems for continuous monitoring cardiovascular surgery. Ann. N. Y. Acad. Sci. 1962, 102, 29–45.
8. Amine, A., Mohammadi, H., Bourais, I., Palleschi, G., 2006. Biosens. Bioelectron. 21, 1405–1423.
9. Kissinger, P.T., 2005. Biosens. Bioelectron. 20, 2512–2516.
10. Lazcka, O., Del Campo, F.J., Munoz, F.X., 2007. Biosens. Bioelectron. 22, 1205–1217. Thévenot DR, Toth K, Durst RA, Wilson GS (1999) Pure. Appl. Chem. 71: 2333—2348.
11. Dorothee Grieshaber;, Robert MacKenzie;, Janos V¨or¨os and Erik Reimhult; Sensors 2008, 8, 1400-1458 ISSN 1424 8220 2008
12. Madhura Nayak, Akhil Kotian, Sandhya Marathe, Dipshikha Chakravortty. Detection of microorganisms using biosensors—A smarter way towards detection techniques. Biosensors and Bioelectronics 25 (2009) 661–667
13. Ibtisam E. Tothill , Biosensors for cancer markers diagnosis, Seminars in Cell & Developmental Biology 20 (2009) 55–62
14. Joseph Wang, Electrochemical Glucose Biosensors, Chem. Rev. 2008, 108, 814-825
15. Thevenot, D. R.; Toth, K.; Durst, R. A.; Wilson, G. S. Electrochemical biosensors: recommended definitions and classification. Biosensors & Bioelectronics 2001, 16(1-2), 121–131.

16. Electrochemical Sensors, Biosensors and Their Biomedical Applications, Xueji Zhang, Huangxian Ju, Joseph Wang, Academic Press is an imprint of Elsevier, 2008.
17. Jiawang Ding , Wei Qin, Potentiometric sensing of nuclease activities and oxidative damage of single stranded DNA using a polycation-sensitive membrane electrode, Biosensors and Bioelectronics 47 (2013) 559–565
18. Juan C.Vidal, Laura Bonel , AlbaEzquerra , Susana Hernández , Juan R. Bertolín, Carlota Cubel , Juan R Castillo, Electrochemical affinity biosensors for detection of mycotoxins: A review, Biosensors and Bioelectronics 49 (2013) 146–158
19. Kauffmann JM, Guilbault GG: Potentiometric enzyme electrodes. Bioprocess Technol. 15: 63–82, 1991.
20. D. Purvis, O. Leonardova, D. Farmakovsky, and V. Cherkasov, An ultrasensitive and stable potentiometric immunosensor. Biosens. Bioelectron. 18, 1385–1390 (2003)
21. Buerk, D. Biosensors. Theory and Applications. Technomic Publishing, Lancaster, 1993.
22. Bakker, E.; Pretsch, E. Potentiometric sensors for trace-level analysis. Trac-Trends in Analytical Chemistry 2005, 24(3), 199–207.
23. Chaubey, A.; Malhotra, B. D. Mediated biosensors. Biosensors & Bioelectronics 2002, 17(6-7), 441–456.
24. Wang, J. Electrochemical biosensors: Towards point-of-care cancer diagnostics. Biosensors & Bioelectronics 2006, 21(10), 1887–1892.
25. Miroslav Pohanka, Petr Skládal. Electrochemical biosensors – principles and applications. J. Appl. Biomed. 6: 57–64, 2008
26. Kueng, A.; Kranz, C.; Mizaikoff, B. Amperometric atp biosensor based on polymer entrapped enzymes. Biosensors & Bioelectronics 2004, 19(10), 1301–1307.
27. Li, J. P.; Peng, T. Z.; Peng, Y. Q. A cholesterol biosensor based on entrapment of cholesterol oxidase in a silicic sol-gel matrix at a prussian blue modified electrode. Electroanalysis 2003, 15(12), 1031–1037.
28. Harsh Sharma, Raj Mutharasan, Review of biosensors for foodborne pathogens and toxins, Sensors and Actuators B 183 (2013) 535–549
29. M.N. Velasco-Garcia. Optical biosensors for probing at the cellular level: A review of recent progress and future prospects. Seminars in Cell & Developmental Biology 20 (2009) 27–33
30. B. Liedberg, C. Nylander, I. Lunstr · · om, Sens. Actuators 4, (1983) 299.
31. X.D. Hoa, A.G. Kirk, M. Tabrizian, Biosens. Bioelectron. 23 (2007) 151.
32. J. Homola, Chem. Rev. 108 (2008) 462.
33. G.G. Nenninger, P. Tobiˇska, J. Homola, S.S. Yee, Sens. Actuators B Chem. 74 (2001) 145.
34. J.T. Hastings, IEEE Sens. J. 8 (2008) 170.
35. J. Guo, P. Keathley, J. Hastings, Opt. Lett. (2008).
36. R. Slav´ık, J. Homola, Sens. Actuators B Chem. 123 (2007) 10.
37. T. Hien Nguyen, Sheila A. Hardwick, Tong Sun, and Kenneth T. V. Grattan. ntrinsic Fluorescence-Based Optical Fiber Sensor for Cocaine Using a Molecularly Imprinted Polymer as the Recognition Element. IEEE SENSORS JOURNAL, VOL. 12, NO. 1, JANUARY 2012. 255-260
38. A.D. Kersey, M.A. Davis, H.J. Patrick, M. LeBlanc, K.P. Koo, C.G. Askins, M.A. Putnam, E.J. Friebele, J. Light wave Technol. 15 (1997) 1442.
39. Xudong Fan, Ian M. White, Siyka I. Shopova, Hongying Zhu, Jonathan D. Suter, Yuze Sun. Sensitive optical biosensors for unlabeled targets: A review. analytica chimica acta 6 2 0 (2 0 0 8) 8–26
40. Kerstin Länge & Bastian E. Rapp & Michael Rapp. Surface acoustic wave biosensors: a review. Anal Bioanal Chem (2008) 391:1509–1519

41. Sauerbrey, G. Verwendung von schwingquarzen zur wägung dünner schichten und zur mikrowägung. Zeitschrift Fuer Physik, Vol. 155 (1959) 206-222
42. Ogi, H.; Nagai, H.; Fukunishi, Y.; Hirao, M. & Nishiyama, M. 170MHz electrodeless quartz crystal microbalance biosensor: capability and limitation of higher frequency measurement. Analytical Chemistry, Vol. 81 (2009) 8068-8073
43. Guilbault, G.; Lubrano, G. Anal. Chim. Acta 1973, 64, 439.
44. Monošik R, Streďansky M, Greif G, Šturdik E (2012) Cent. Eur. J. Chem. 10: 157—184.
45. Fluorescence Imaging Principles and Methods, Handbook 63-0035-28 [Online], Amersham Biosciences, 2002.
46. SHAURYA PRAKASH, MARIE PINTI AND BHARAT BHUSHAN. Phil. Trans. R. Soc. A 370, 2269–2303. 2012
47. Liang Su, Wenzhao Ji, Changjun Hou, Yu Lei. Microbial biosensors: A review. Biosens. Bioelectron. 26 (2011) 1788-1799.
48. Kumar, S., Kundu, S., Pakshirajan, K., Dasu, V.V., 2008. Appl. Biochem. Biotechnol. 151 (2–3), 653–664.
49. Ferrini, A.M., Mannoni, V., Carpico, G., Pellegrini, G.E., 2008. J. Agric. Food Chem. 56, 784–788.
50. Farre, M., Brix, R., Barcelo, D., 2005. TrAC: Trends Anal. Chem. 24, 532–545.
51. J. Wang, Electroanalysis, 2005, 17, 7–14.
52. M. Szymanski, A. P. F. Turner and R. Porter, Electroanalysis, 2010, 22, 191–198.
53. Anthony P. F. Turner. Biosensors: sense and sensibility. Chemical Society Reviews, Volume 42, 2013, 3175–3648
54. S. M. Khor, G. Liu, J. R. Peterson, S. G. Iyengar and J. J. Gooding, Electroanalysis, 2012, 23, 1797–1804.
55. H. Li, J. He, Q. Wei, S. Li and A. P. F. Turner, Biosens. Bioelectron., 2012, DOI: 10.1016/j.bios.2012.11.037.
56. Dubertret B, Skourides P, Norris D, Noireaux V, Brivanlou A and Libchaber A 2002 In vivo imaging of quantum dots encapsulated in phospholipid micelles Science 298 1759–62
57. Bruchez M, Moronne M, Gin P, Weiss S and Alivisatos A P Semiconductor nanocrystals as fluorescent labels Science 281 2013–6
58. Taton T A, Lu G and Mirkin C A 2001 Two-color labeling of oligonucleotide arrays via size-selective scattering of nanoparticle probes J. Am. Chem. Soc. 123 5164–5
59. 18. Wang J, Musameh M, Lin YH. J. Am. Chem. Soc 2003;125:2408
60. Cui, Y. Wei, Q. Park, H. Lieber, CM. Nanowire nanosensors for highly sensitive and selective detection of biological and chemical species. Science.293:12, 89 – 92 (2001).
61. Patolsky, F.; Timko, B. P.; Zheng, G. F.; Lieber, C. M. Nanowire-based nanoelectronic devices in the life sciences. MRS Bulletin 2007, 32(2), 142–149,
62. Zhang J, Lang H P, Bietsch A, Huber F, Certa U, G¨untherodt H-J, Hegner M and Gerber Ch 2006 Rapid and label-free nanomechanical detection of biomarker
63. Natalija Backmann, Natascha Kappeler, Thomas Braun, François Huber, Hans-Peter Lang, Christoph Gerber and Roderick Y. H. Lim. Sensing surface PEGylation with microcantilevers. Beilstein J. Nanotechnol. 2010, 1, 3–13.
64. Fritz, J. Analyst 2008, 133, 855–863. doi:10.1039/b718174d
65. Pera, I.; Fritz, J. Langmuir 2007, 23, 1543–1547. doi:10.1021/la0624337
66. B. A. Cornell, V. L. B. Braach-Maksvytis, L. G. King, P. D. J. Osman, B. Raguse, L. Wieczorek & R. J. Pace. Abiosensor that uses ion-channelswitches. NATURE VOL 387 5 JUNE 1997

67. Vikram Krishnamurthy, 3 Fellow, IEEE, Sahar Moradi Monfared, and Bruce Cornell. Ion-Channel Biosensors—Part I: Construction, Operation, and Clinical Studies. IEEE TRANSACTIONS ON NANOTECHNOLOGY, VOL. 00, NO. 00, 2010