Advances in the Manufacturing, Types, and Applications of Biosensors

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In recent years, there have been significant technological advancements in the manufacturing, types, and applications of biosensors. Applications include clinical and non-clinical diagnostics for home, bio-defense, bio-remediation, environment, agriculture, and the food industry. Biosensors have progressed beyond the detection of biological threats such as anthrax and are finding use in a number of non-biological applications. Emerging biosensor technologies such as lab-on-a-chip have revolutionized the integration approaches for a very flexible, innovative, and user-friendly platform. An overview of the fundamentals, types, applications, and manufacturers, as well as the market trends of biosensors is presented here. Two case studies are discussed: one focused on a characterization technique—patch clamping and dielectric spectroscopy as a biological sensor—and the other about lithium phthalocyanine, a material that is being developed for in-vivo oxymetry.

INTRODUCTION

A biosensor is a device that detects, records, and transmits information regarding a physiological change or the presence of various chemical or biological materials. These biological materials include enzymes, tissues, microorganisms, antibodies, cell receptors, and biologically derived materials.[1] Biosensors also have a mimicking component due to intimate contact with a physio-chemical transducer or transducing microsystems in the environment. In other words, biosensors are analytical devices incorporating biological materials operating via a bio-recognition process, which can be due to their bio-affinity or bio-metabolism. The results of this recognition process will then be converted through a transduction mechanism using transducers, which are the components that convert a biochemical signal into a quantifiable electrical signal. Consequently, the output of this transduction mechanism can be an optical, electrochemical, piezoelectric, thermometric, magnetic, or even calorimetric signal that can be used to quantify the analyte presented. Figure 1 represents the schematic of a biosensor. In Figure 2, the various components of a biosensor are described.

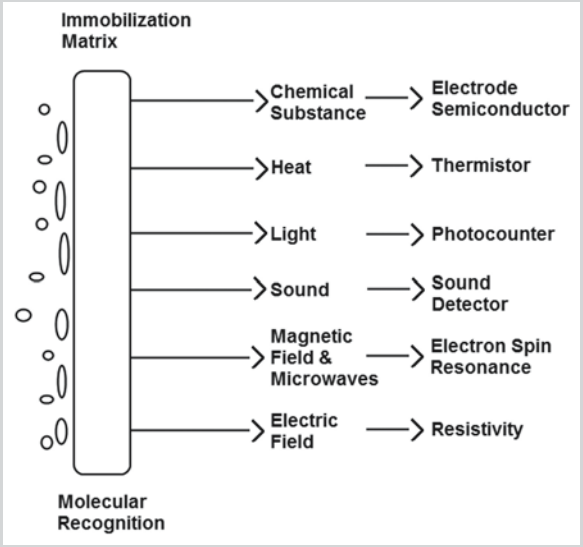

Figure 1. A schematic of a biosensor.

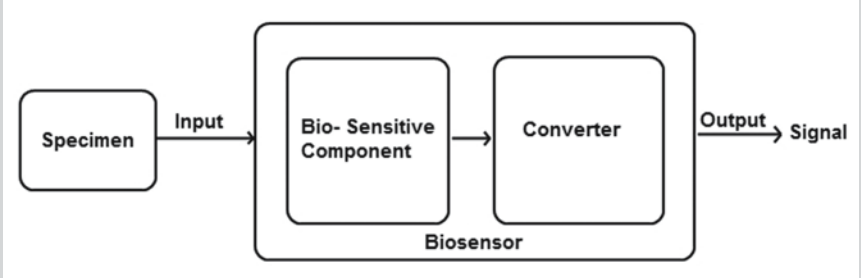

Figure 2. The various components of a biosensor.

Technologically, a biosensor is a probe that integrates a biological component, such as a whole bacterium or a biological product (e.g., an enzyme or antibody) with an electronic component to yield a measurable signal. Biosensors, which come in a large variety of sizes and shapes, are used to monitor changes in environmental conditions. They can detect and measure concentrations of specific bacteria or hazardous chemicals as well as measure acidity levels (pH). Biosensors have found applications in a variety of scenarios. Due to their simplicity, high sensitivity, and potential for real-time and on-site analysis, biosensors have been widely applied in various fields in medical diagnostics, environmental

monitoring and genetics, health care, patient management, food processing, and defense including industrial processes, clinical detection, and environmental control.

Developing biosensors that can be scaled down to a small footprint with reproducible performance and accurate technical standards is a critical factor for manufacturing. In addition, the cost of manufacturing coupled with factors such as the need for sterilization, repeatability, and time budget for data acquisition and analyses need to be considered.

BIOSENSOR TYPES

There are various types of biosensors based on the principle of detection, including optical, piezoelectric, electrochemical, and thermometric. Optical biosensors, such as Farby–Perot, detect changes in absorbance or fluorescence of an appropriate indicator and changes in the refractive index. Piezoelectric sensors are based on an alternating potential and produce a standing wave in the crystal at a characteristic frequency. This frequency is highly sensitive to the surface properties of the crystal such that, if a crystal is coated with a biological recognition element, the binding of the target analyte to a receptor will produce a change in the resonant frequency. Electrochemical biosensors are based on enzymatic catalysis of a reaction, which produces ions. The sensor substrate contains three electrodes: a reference electrode, an active electrode, and a sink electrode. A counter electrode can also be present as an ion source. The target analyte participates in the reaction that takes place on the active electrode surface, and the ions produced create a potential that is subtracted from that of the reference electrode to yield a signal. Electrochemical biosensors are usually based on potentiometry and amperometry. Thermometric biosensors are constructed by combining enzymes with temperature sensors. When the analyte is exposed to the enzyme, the heat of reaction of the enzyme is measured and is calibrated against the analyte concentration.

The core requirements for a biosensor approach to be valuable in terms of its utility and manufacturing are identification of a receptor that binds to the target of interest; availability of a suitable biological recognition element; and potential for disposable portable detection systems as opposed to laboratory-based techniques in some situations.

See the sidebar for biosensor examples and applications.

BIOSENSOR DEVELOPMENTS

In the mid-1980s, Tuan Vo-Dinh and collaborators[6] at the life sciences division at Oak Ridge National Laboratory were looking for a way to use light to detect cancer-causing agents in groundwater. Their successful results led to the development of a series of fiber-optic-based biosensors. Screen-printing biosensors using enzymes as the bio-catalysts are a platform technology, particularly as specific catalysts or modifications are made or incorporated into the sensing elements. Ink-jet printing technology is a non-contact process that can dispense a well-defined micro-quantity of the

BIOSENSOR EXAMPLES AND APPLICATIONS

Some biosensor examples and applications are:

- Glucose monitoring in diabetes patients; historically, this has been the market driver
- Other medical-related targets
- Environmental applications, for example, the detection of pesticides and river water contaminants
- Remote sensing of airborne bacteria (e.g., in counter-bioterrorist activities)
- Detection of pathogens
- Determining levels of toxic substances before and after bioremediation
- Detection and determining of organophosphate
- Routine analytical measurement of folic acid, biotin, vitamin B12, and pantothenic acid as an alternative to microbiological assay
- Determination of drug residues in food, such as antibiotics and growth promoters, particularly meat and honey
- Drug discovery and evaluation of biological activity of new compounds

An example of a bio-field-effect transistor (BioFET) sensor is shown in Figure A.[3] The gate metal in the silicon-based metal-oxide field-effect transistor (MOSFET) is replaced by a layer of receptor biomolecules. The BioFET works based on the stimulus produced by biomolecular interactions. Figure B shows an example of microfluidic channels in silicon.[3] These microfluidic channels can be integrated with electronics and optics for

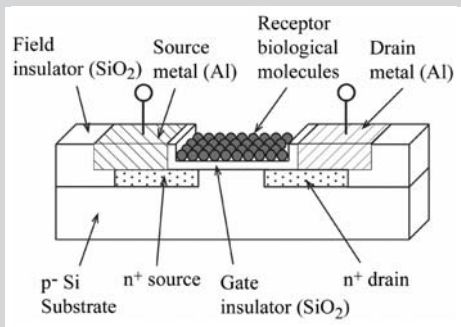

Figure A. A schematic of a BioFET.[2]

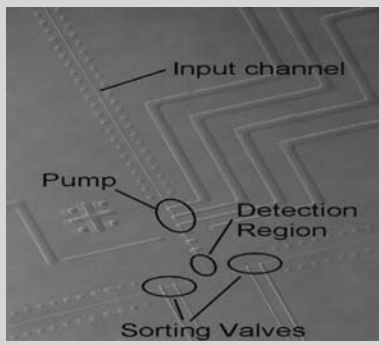

Figure B. Microfluidic channels in silicon.[3]

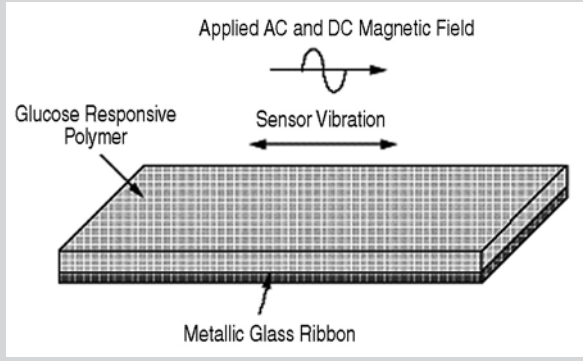

Figure C. A magnetoelastic glucose sensor.[4]

enzyme. While the physics and the approaches of various ink-jet printing may be different, the outcomes of the ink-jet printing are similar in obtaining the well-controlled, micro-size droplets.

Bacteria can be used as biosensors to demonstrate the toxicity of a variety of environmental media including soil, sediment, and water by coupling bacteria to transducers that convert a cellular response into detectable signals.[7] These bacterial biosensors are engineered by pairing a reporter gene that generates a signal with a contaminant-sensing component that responds to chemical or physical change, such as exposure to a specific analyte. When the biosensor is exposed to such a change, the sensing component stimulates the reporter gene through a biochemical pathway in the cell. The reporter gene then produces a measurable response such as emitting visible light, indicating the degree of chemical or physical change.[8–10] Several biosensors have been developed that indicate toxicity of any chemical or physical change; new biosensors are being developed to respond to particular analytes. Such biosensors have been developed for heavy metals and metalloids including arsenic, cadmium, mercury, and lead.[11] The RAPTOR[12] is a portable automated fiber optic biosensor for detection of biological threat agents. It performs rapid (three to ten minute), fluorescent sandwich immunoassays on the surface of short polystyrene optical probes for up to four target analytes simultaneously.

Over the past 3–5 years, optical biosensors have demonstrated the sensitivity required for typical drug candidates and lower molecular weight drug fragments or "needles." The sensitivity for drug needles is poorer than for larger molecular weight drugs as surface plasmon resonance (SPR) measures changes in refractive index that are directly related to the molecular weight of the binding molecule. After more than 20 years of research and development, SPR sensitivity may be approaching theoretical limits in terms of the detection interface sensitivity.

Bioreporters refer to intact, living microbial cells that have been genetically engineered to produce a measurable signal in response to a specific chemical or physical agent in their environment. Bioreporters contain two essential genetic elements, a promoter gene and a reporter gene. The promoter gene is turned on (transcribed) when the target agent is present in the cell's environment. The promoter gene in a normal bacterial cell is linked to other genes that are then likewise transcribed and then translated into proteins that help the cell in either combating or adapting to the agent to which it has been exposed. In the case of a bioreporter, these genes, or portions thereof, have been removed and replaced with a reporter gene. Consequently, turning on the promoter gene now causes the reporter gene to be turned on. Activation of the reporter gene leads to production of reporter proteins that ultimately generate some type of a detectable signal. Therefore, the presence of a signal indicates that the bioreporter has sensed a particular target agent in its environment.

compact chemical and biological sensor circuits, laboratory-on-a-chip diagnostic tools, short analysis times, and small analyte volumes. Figure C shows a magnetoelastic glucose sensor made of an amorphous metallic glass ribbon coated with a thin layer of mass changing glucose responsive polymer. The magnetoelastic material resonates at a characteristic frequency when excited by a magnetic field.

In Figure D, the schematic of a passive Fabry–Perot interferometer-based biosensor is shown. This sensor is based on the modulation of transverse mode spectra by a biological cell. Different biological cells have different shapes and refractive index profiles. When placed inside an actively or passively excited Fabry–Perot cavity, these cells uniquely modulate the transmission spectra of the resonator. Single-cell spectra obtained using this method qualitatively appear to have characteristics such as the number of modes and mode spacing that can be used to differentiate cells. In Figure E, the various capabilities of optical imaging for biosensor applications are summarized.[5]

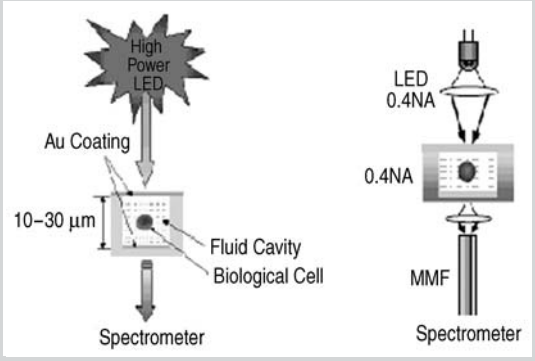

Figure D. A passive Fabry–Perot interferometer based biosensor.

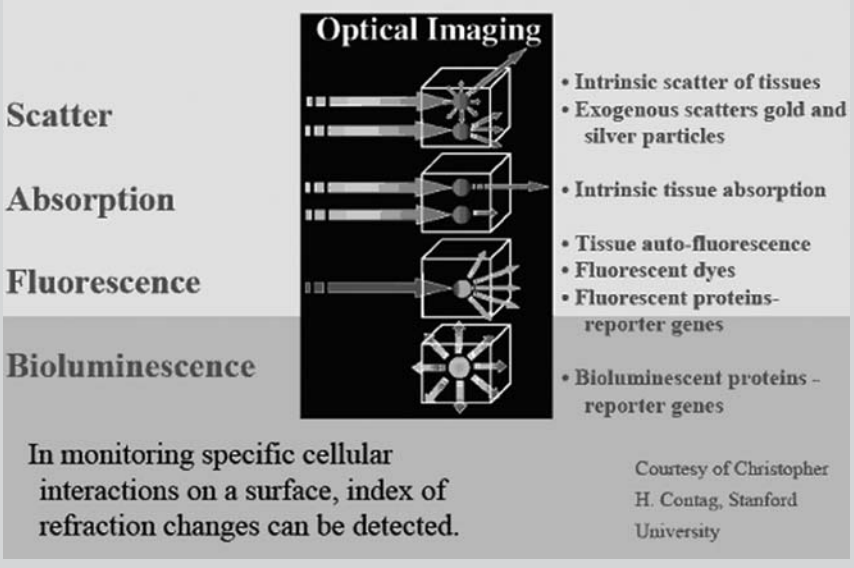

Figure E. Optical imaging techniques for biosensor applications.[5]

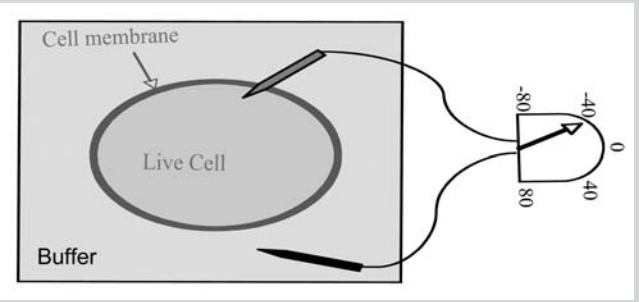

Figure 3. The microelectrode technique: one glass electrode is inserted inside the cell while another glass electrode is kept in the buffer. The potential difference between the inside and outside the cell (also called membrane potential) is recorded.

CASE STUDY I: PATCH CLAMPING AND DIELECTRIC SPECTROSCOPY

Perspective

The ability to detect and identify organisms, as well as to detect changes when different stress parameters are applied to them, plays an important role in life sciences. For example, one of the first steps in drug discovery is to test if the chemical compound enters inside the cell or binds to the membrane. Most of the sensing is based on changes in the membrane potential of live cells.

Membrane potential is defined as follows. The cells contain a large number of negatively charged molecules and every single cell is surrounded by a membrane. The inside overall negative charge attracts positive charges from the outside, mainly potassium and sodium positive ions. The cell allows most of the potassium ions to enter inside, still maintaining an overall negative charge, but keeps most of the sodium ions outside. This charge distribution gives rise to a sharp potential difference across the membrane. This is known as the membrane potential. Its value can be in a range from 60 mV to a few tens over 100 mV.

The electric field due to such potential differences is enormous due to the very small thickness of the membrane. For example, 100 mV applied across a membrane of thickness of 10 nm is equivalent to an electric field of 10 million V/m. The cell utilizes this electric field to protect itself from unwanted intruders. The electric field also plays an important role in cell functions, such as muscle contraction, cardiac functions, and in the transport of nutrients across the membrane.[13,14]

Patch Clamping

Patch clamping or the microelectrode technique refers to recording changes in the membrane potential of a single cell and forms a vast area of research in life sciences. Mainly, a single cell is attached to the surface and a glass electrode is inserted inside the cell while the second electrode is kept in the buffer solution surrounding the cell. The potential difference between the inside and outside the cell is measured (see Figure 3). When chemically charged particles enter the cell or attach to the membrane, the membrane potential changes. Also, when the membrane's channels are blocked, there is a drop in the absolute value of the membrane potential.

The applications of patch clamping are found in pharmaceutical and medical research. Often, drugs alter the membrane potential of cells by attaching to the membrane, blocking some specific channels (such as painkillers) or by entering inside the cell. Recording the changes in the membrane potential can yield information about the effects of the drugs on cells. Also, changes in the membrane potential have been linked to various diseases, such as Parkinson, epilepsy, and Bartter's syndrome.[15] In addition, many tumor cells can develop multi-drug resistance (MDR) to chemotherapeutics. In these cells, a decrease in the plasma membrane potential has been observed, with increased expression of MDR protein, also known as P-glycoprotein.[16] Many studies are also aimed at measuring the membrane potential of mitochondria,[17] since defects in mitochondrial functions are linked to Alzheimer's disease.[18] An illustration of the various types of patching is given in Figure 4.[19]

Dielectric Spectroscopy

Dielectric spectroscopy has been used in many areas of research including materials science and life sciences. The basic idea in dielectric spectroscopy is that the material to be investigated is placed between two capacitor plates and a voltage is applied across the plates and its impedance is measured. If the material is modeled as a resistance capacitance (RC) circuit, the real part of the impedance is proportional to the conductivity of the material and the imaginary part to its dielectric permittivity. Thus, one can determine the dielectric properties of the material by a simple alternating

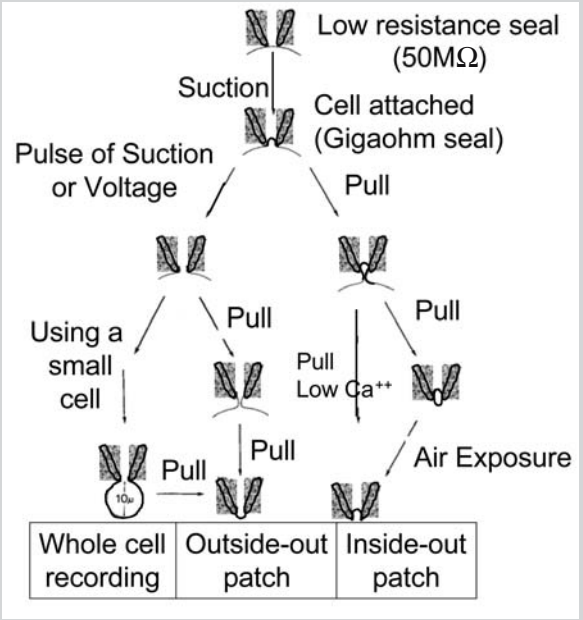

Figure 4. An illustration of the different types of patching. Besides membrane potential, other cell parameters can potentially be recorded by this method, such as the currents associated with single channel recording (from Hamill et al.[19]).

current (ac) measurement. The material to be studied can be a collection of cells, such as in a tissue or cell suspension.

The presence of the membrane potential has a specific effect on the dielectric behavior of cell suspensions, namely the appearance of the so-called alpha dispersion in the low-frequency part of the dielectric dispersion curves.[20] Figure 5 illustrates the typical reaction of a live cell to external electric fields. It also shows the typical profile of the dielectric dispersion curves for live cell suspensions. When an external electric field is applied, the charges surrounding the membrane move, creating an electric dipole. In the alpha regime, the dipole is very large, leading to an extremely high dielectric permittivity.

The dielectric permittivity of a suspension of live cells can be 10^6 times larger than that of vacuum.[21] Due to relatively small mobility, the charges cannot follow the electric field at higher frequencies. The dielectric curve drops at some point into another regime, called the beta plateau. The dielectric permittivity is still much larger than that of the substances found in the cell and its value is typically in the hundreds. The existence of the beta plateau is due to the presence of the membrane. At even higher frequencies, typically above 10^5 Hz, the dispersion curve drops again into another regime, called the gamma plateau. At these frequencies, the molecular structures become important and the value of the dielectric permittivity becomes comparable to that of the substances found in the solution.

The capability of dielectric spectroscopy is that it can distinguish between different effects at a different range of frequencies. For example, in the low frequency range, the effect of the membrane potential is dominant (Figure 6), so one can determine the membrane potential from a simple dielectric measurement.[21] Figure 6 shows that, when the membrane potential increases, the dielectric permittivity of the cells increases only at low frequencies (up to 10^4 in figure). At higher frequencies, in the beta regime, the dielectric properties of the membrane play a dominant role. In the beta regime, the thicker the membrane, the lower the dielectric response. Actually, because of these reasons, the beta regime of the dispersion curves is used to study the binding of proteins to the membrane.

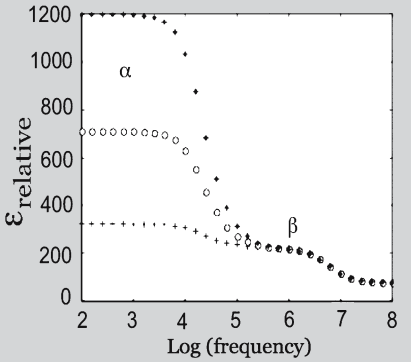

Figure 6. Theoretical dispersion curves for different values of the cell membrane potential: 50 mV (+), 100 mV (o), and 150 mV (*).

CASE STUDY II: LITHIUM PHTHALOCYANINE FOR IN-VIVO OXYMETRY

Background

Lithium phthalocyanine (LiPc) is a narrow bandgap semiconductor with an energy gap of 0.2 eV and a high-frequency dielectric constant equal to 6. In recent years, this material has gained notoriety, both as bulk and in thin film form on various substrates, as an oxygen sensor for biological applications.[22,23] Lithium phthalocyanine (LiPc) is one of the most widely studied organo-metallic compounds due to its unique magnetic and electrical properties. It exhibits three distinct crystalline polymorphs, designated as a-, b-, and x-form, whose structural, magnetic, and electrical properties have been well characterized in single crystals, powders, and thin films. Oxidation of Li2Pc in acetonitrile or in acetone leads to the x form of LiPc which shows an important sensitivity to oxygen. LiPc powders can be formed by electrochemical oxidation of Li2Pc in acetonitrile and acetone or by a new electrochemical synthesis method.

A notable problem in the use of the LiPc has been that it is very difficult to control the reproducibility of the composition and sensitivity of the material to oxygen. Different batches of preparations tend to yield crystals with wide variations in the oxymetry properties. Hence, it has become important to investigate the mechanism of the synthesis and properties, with relevance to its performance as an oxymetry probe, so that quality control can be established for its routine usability.

It has been shown that, as the crystal size is reduced, its sensitivity to oxygen is greatly increased. Thus, it is feasible to fine-tune the oxymetry characteristics of this material over a range of partial pressure of O_2 and sensitivity by controlling the size of the particles. Similar to single crystals, the microcrystalline powders show high stability and reproducibility in aqueous and physiological environments. The results demonstrate that LiPc microcrystalline powder can provide accurate and repetitive measurements of oxygen concentrations in any region of pathophysiological interest.

Extensive studies have shown that the electron paramagnetic resonance (EPR) spectrum of the paramagnetic center in solid LiPc exhibits a partial pressure of oxygen (pO_2)-dependent line width. The temperature dependence of the EPR intensity of LiPc directly corresponds to its spin susceptibility (see Figure 7). With decreasing temperature, the intensity increases to a maximum at around 30K and then decreases to a finite value at low temperatures. This sensitivity of its EPR spectra to oxygen content has made it a very viable candidate for in-vivo oxymetry.[22,23]

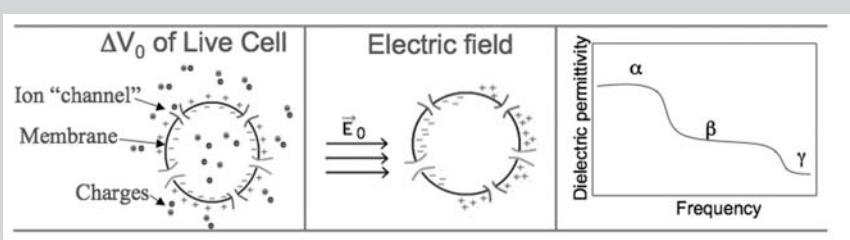

Figure 5. The behavior of live cells in electric fields.

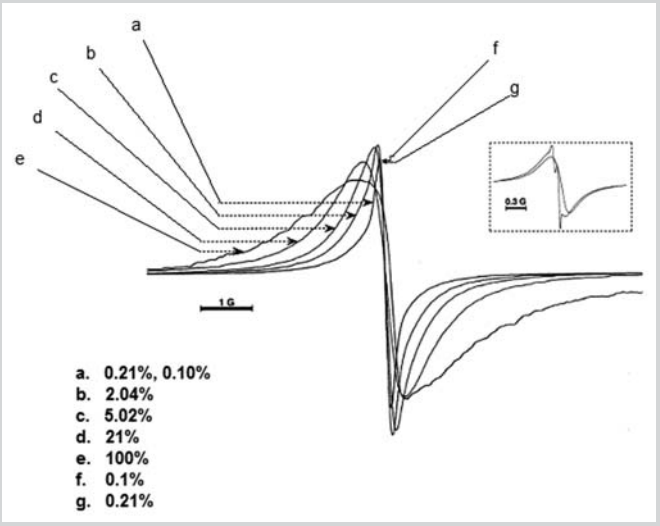

Figure 7. The continuous wave (cw) X-band EPR spectra of an oxygen-dependent LiPc batch (electrochemically synthesized at a constant potential of 0.35 V using a platinum gauze working electrode) under different pO_2 levels. The inset shows the spectra of an LiPc batch (electrochemically synthesized at a constant potential of 0.15 V using a coiled working electrode) that is predominantly composed of oxygen-insensitive crystals of LiPc at 0% O_2 and 21% O_2 (compressed air); the broad and narrow lines correspond to the oxygen-insensitive and oxygen-sensitive crystals, respectively.[22]

Biosensor Market—Manufacturers, Potential, and Drivers

Rapid scaling down in size, high throughput, low power dissipation, ease of calibration, and low cost will be the key factors that will quicken expanded commercialization of biosensors to penetrate several untapped markets. Manufacturers have successfully integrated biosensor technology with leading-edge integrated circuit and wireless technology in high-end applications. Recently, there has been a tremendous push for molecular foundries, electronic printing, and disposable sensors. In this context, biosensors have the potential to overcome most of the disadvantages of conventional methods.

Novel integration technologies, such as magnetic field-assisted assembly,[24,25] will assist in developing methodologies for implementing schemes such as lab-on-a-chip for use as biosensors. This coupled with self-assembled enzyme aggregates prepared from magnetic iron oxide nanoparticles[26] will have significant utility in biosensor applications.

The total global market for biosensors and bioelectronics is expected to grow from $6.96 billion in 2006 to $8.2 billion in 2009, at an average annual growth rate of about 6.3% (see Figure 8). Glucose sensors accounted for nearly all of the market in 2003. Sales of other bioelectronic devices are projected to increase significantly over the next five years. Biomedical and life sciences applications account for 99% of the market, with environmental monitoring and remediation applications a distant second.[27]

A partial list of some of the manu-

Table I. A Partial List of International Manufacturers of Biosensors

Manufacturer	Biosensor	Application
Applied Enzyme Technology, Pontypool, U.K.	Ammonia biosensors	Monitoring sewage effluent
BioDot Ltd., Huntingdon, U.K.	BioJet Plus Nanoliter dispenser	Environmental control and vision inspection systems
BiomedLab Co., Ansan, South Korea	DNA chips, TB chip	Detection and genotyping of human Palillonavirusus; screening TB
Biozyme Laboratories, Blaenavon, U.K.	Purified enzymer biosensors	Glucose oxidase preparations
Chemel AB, Lund, Sweden	SIRE	Food beverage, pharmaceutical, and forest industries
DuPont Ltd., Bristol, U.K.	DuPont biosensors	Medical monitoring, diagnostics, drug delivery
Ercon Inc., Wareham, Massachusetts, USA	Biosensor	Food and beverage testing and environmental sensor
Eco Chemie B.V., Utrecht, The Netherlands	Amperometer	Life science and pharmaceutical research
Elsevier Ltd, Oxford, U.K.	Biosensors	Bio-chemical research
Ercon Inc., Wareham, Massachusetts, USA	Electrochemical sensors	Detection, quantification, and monitoring of bodily fluid and environmental constituents and in other healthcare diagnostics and therapeutics
Gwent Electronic Materials (GEM), Pontypool, U.K.	Biosensors	Wide variety of industries. GEM designs electrodes from drawing concepts to screen designs and the final printed sensors
Institute of Bioscience, Cranfield, U.K.	ISBT biosensors	Healthcare, environmental, food, and defense
Plam Instruments, Houten, The Netherlands	PalmSens	Electroanalytical techniques for amperometric and voltammetric sensor
Uniscan Instruments Ltd., Buxton, U.K.	PG580 Digital scanning system, biosensor	Applications in miniature scale, general
Wales Trade International, Reforest, U.K.	Biosensors	Applications in miniature scale, general
Philips Medical Systems, Bothell, Washington, USA	Molecular Diagnostics	Optical detection, magnetic biosensors, fluidics without moving parts
SmartPill, Buffalo, New York, USA	SmartPill GI Monitoring System	Gastroenterology

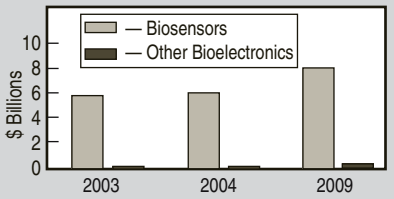

Figure 8. The growth of the biosensor industry.[27]

facturers of biosensors in presented in Table I.

Some of the key market drivers will include the following technologies:

- Detection of drug delivery, characterization of cancer cells/AIDS virus, selection of the ideal drug, and targeted drug delivery for treatment of diseases such as cancer and AIDS.
- Frictionless nanofluidics platform that will be lossless and contamination free, and requires transport, distribution, and mixing of nanoliter volumes.
- Radio-frequency-triggered drug delivery systems that will use plasmon-enhanced biosensor array and will require quantitative assessment of biomolecules with high accuracy, selectivity, and sensitivity.
- Radio-frequency triggered drug delivery systems that will perform drug delivery through coated magnetic nanoparticles contained in the targeted tumor using a magnetic field.
- Biosensor devices such as the SmartPill pH.p capsule[28] that can measure pressure, pH, and temperature from within the entire gastrointestinal tract using miniaturized on-board sensor technology; the SmartPill pH.p capsule assists in the evaluation of motility disorders, such as gastroparesis.
- Devices to detect biological agents such as botulinum toxins that can contaminate water and anthrax that can destroy the fundamental global infrastructure.
- Innovative multidisciplinary approaches including printed intelligence[29] that will integrate mass manufacturing methods such as ink-like innovative material technology and functionalities created from electronics, biotechnology, chemistry, optics, optoelectronics, or their combinations.

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