Biosensors

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Biosensors function by coupling a biological sensing element with a detector system using a transducer. They are widely used in diagnostics, pharmaceutical research, fermentation-based industries and environmental and pollution monitoring.

Introduction

Biosensors consist of a biological entity that can be an enzyme, antibody, or nucleic acid that interacts with an analyte and produces a signal that is measured electronically. Each biosensor, therefore, has a biological component that acts as the sensor and an electronic component to transduce and detect the signal. A variety of substances including nucleic acids, proteins (particularly antibodies and enzymes), lectins (plant proteins that bind sugar moieties) and complex materials (organelles, tissue slices, microorganism), can be used as the biological components. In each case it is the specificity of the biological components for an analyte (or group of related analytes) that makes the biomolecules attractive as sensing component. For example, a single strand of DNA can be used as a biomolecular sensor that will hybridize only to its complementary strand under appropriate conditions. The signal, which can be electrical, optical or thermal, is converted by means of a suitable transducer into a measurable electrical parameter such as current or voltage (*Figures* 1a, 1b). Biosensor probes are attaining increasing sophistication because of the fusion of two technologies: microelectronics and biotechnology. Biosensors provide a useful means for measuring a wide spectrum of analytes (e.g., gases, ions and organic compounds, or even bacteria) and are suitable for studies of complex microbial environments.

In 1956, Leland C Clark Jr., who is known as the father of Biosensors, published his definitive paper on the oxygen

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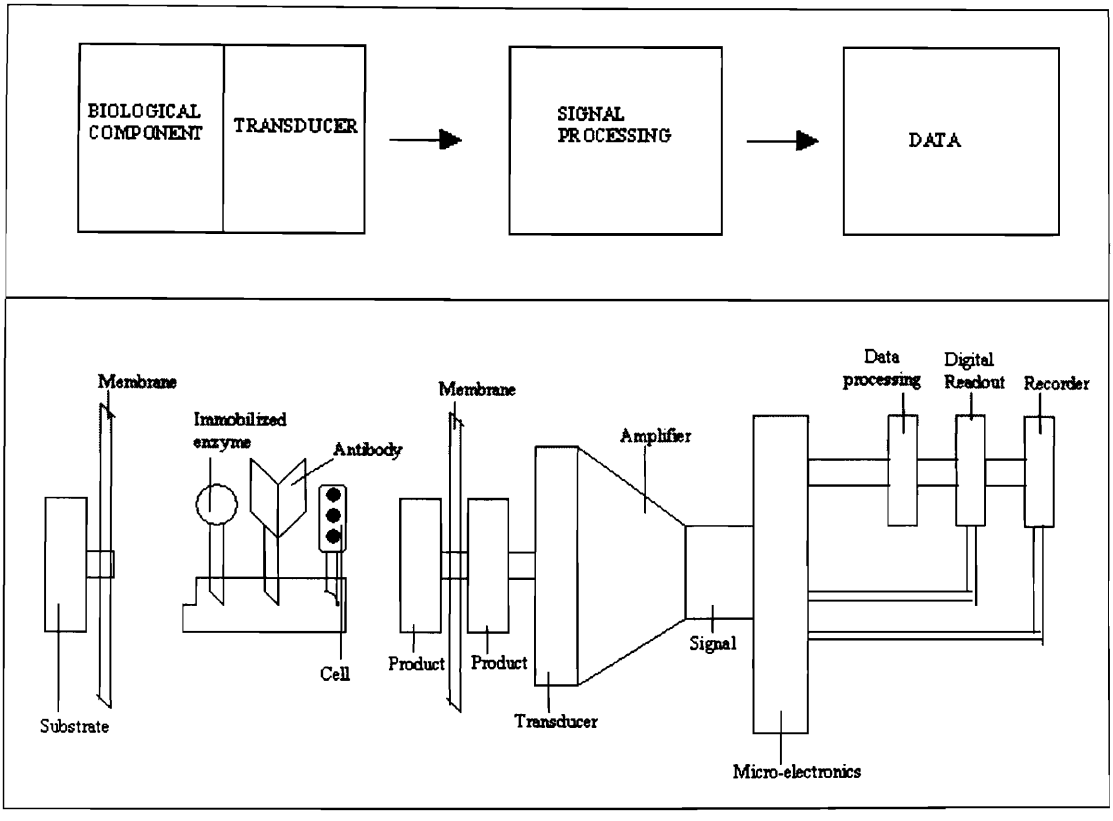

Figure1.
(a) (top): Construction and mode of operation of a biosensor.
(b) (bottom): Schematic outline of biosensor.

electrode in which he described the fabrication of electrochemical sensors. He carried this out by using an enzyme transducer. Clark's oxygen electrode was the enzyme glucose oxidase, entrapped in dialysis membrane. The method of detection is based on the decrease in oxygen concentration that is proportional to glucose concentration.

Guilbault and Montalvo were the first to provide a detailed description of potentiometric enzyme electrode, i.e. the urea sensor, based on urease, immobilized at an ammonium–selective liquid membrane electrode. The use of thermal transducers for biosensors was proposed in 1974 and the new devices were christened thermal enzyme probes. In 1975, Divis suggested that bacteria could be harnessed as the biological element in microbial electrodes for the measurement of alcohol content. Lubbers and Opitz coined the term 'optode' to

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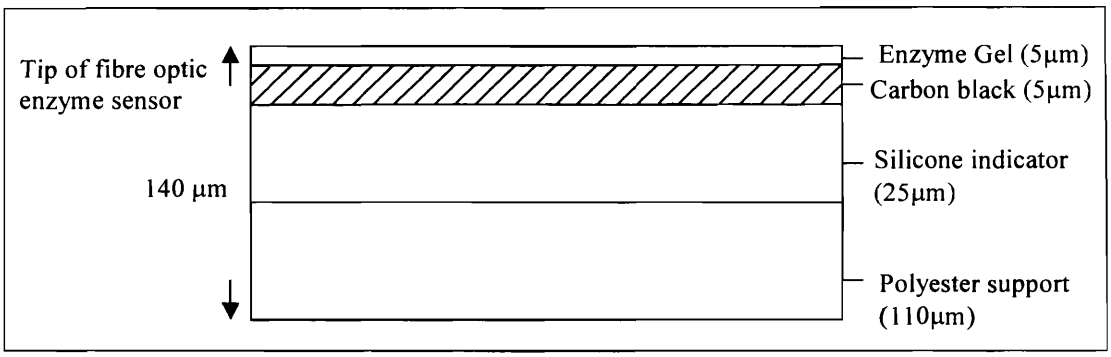

Figure 2. Enzyme optrodes schematic structure.

describe a fiber-optic sensor with an immobilized indicator to measure carbon dioxide or oxygen (*Figure* 2).

Biosensors can be broadly classified as follows, based on the principle involved.

A. Electrochemical Sensors

In this configuration, sensing molecules are either coated onto or covalently bonded to a probe surface. A membrane holds the sensing molecules in place, excluding interfering species from the analyte solution. The sensing molecules react specifically with compounds to be detected, sparking an electrical signal proportional to the concentration of the analyte. The bio-molecules may also respond to an entire class of compounds such as opiates and their metabolites. The most common detection method for electrochemical biosensors involves measurement of current, voltage, conductance, capacitance and impedance.

B. Optical Sensors

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

Luciferase Optical Electrode

A luciferase systems light emitting reaction proceeds via the reaction of molecular oxygen with reduce flavin (FMNH) and aliphatic aldehyde as follows:

$FMNH_2 + RCHO + O_2 \rightarrow FMN + RCOOH + H_2O + h\nu$

The system may be regenerated by supplying FMN reductase together with NAD(P)H.

signal from these devices is accomplished through flexible cables, which can transmit light to the biological component.

Optrodes use fiber optics for performing optical measurement away from the measuring locations (e.g., intra-arterial determination using FIA systems). A powerful and sensitive analytical methodology has been constructed based on the luciferin/luciferase bioluminescence reaction.

C. Piezoelectric Sensors

In this mode, sensing molecules are attached to a piezoelectric surface – a mass to frequency transducer – in which interactions between the analyte and the sensing molecules set up mechanical vibrations that can be translated into an electrical signal proportional to the amount of the analyte (*Figure* 3). Example of such a sensor is quartz crystal micro or nano balance.

D. Field Effect Transistor (FET)

This method makes use of an ion-sensitive field effect transistor (ISFET) built on standard technology that produces source, drain and gate regions. The gate uses an ion sensitive membrane that renders ISFET capable of biochemical recognition in the presence of the analyte with the increase in local ion concentra-

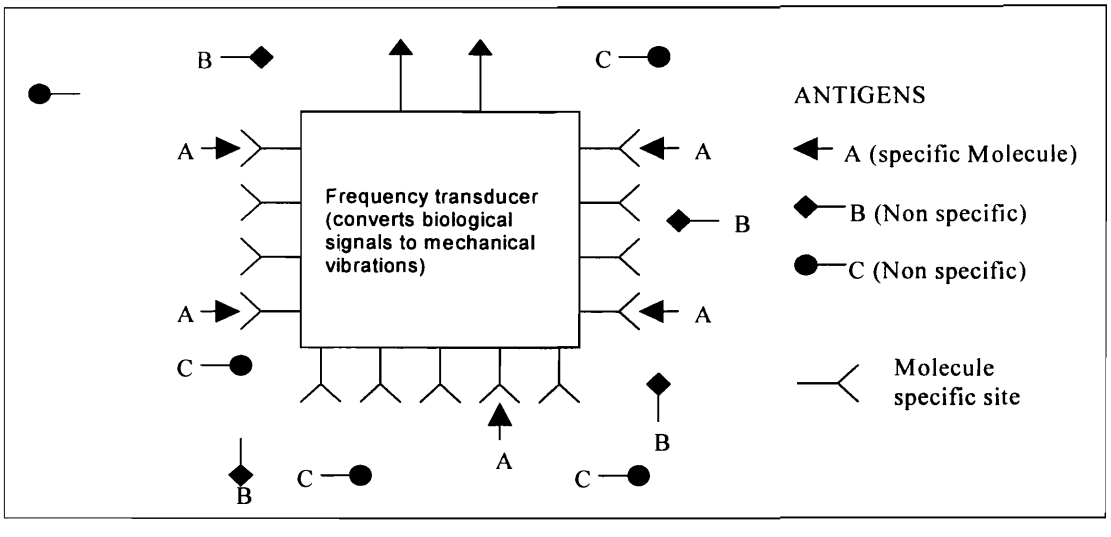

Figure 3. Piezo sensor based on molecular recognition by specific molecule attachment which leads to mechanical vibrations.

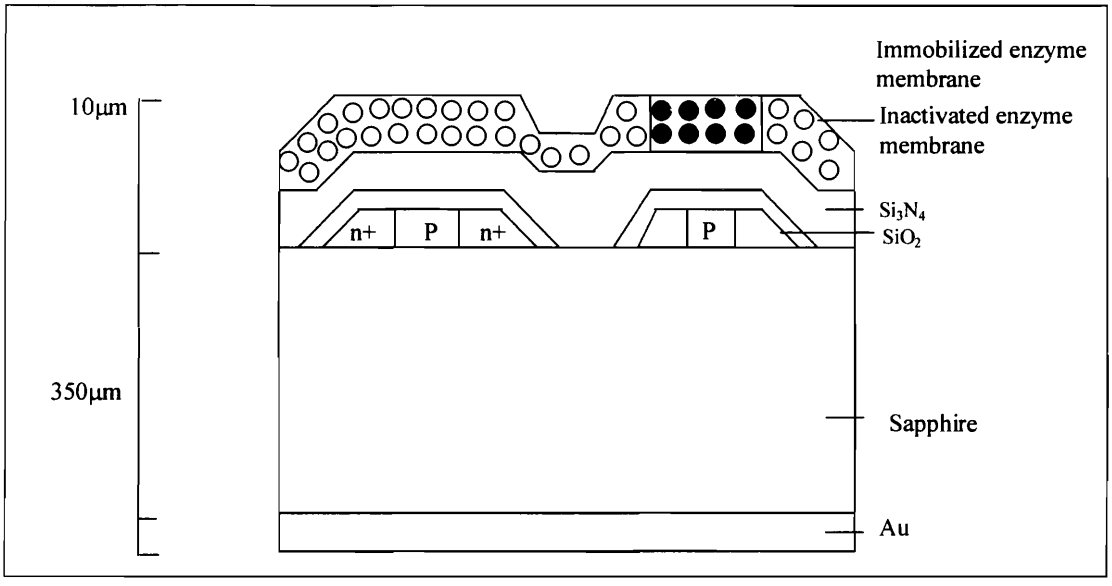

Figure 4. Ion sensitive field effect transistor.

tion. Microelectrodes are created on a silicon nitride surface using vapour deposition method and partially insulated by titanium oxide (*Figure* 4). The hardware component consists of an electrode system that could either be a conventional platinum or silver–silver chloride microelectrode and a field effect transistor with an ion sensitive gate or gas sensing electrode.

Biosensing Method

The essence of the biosensor is matching the appropriate biological and electronic components to produce a relevant signal during analysis. Isolation of the biological component is necessary to ensure that only the molecule of interest is bound or immobilized on the electronic component or the transducer. The stability of the biological component is critical, since it is being used outside its normal biological environment.

Attachment of the biological component to the electronic component is vital for the success of these devices. If the biological component is destroyed in the process of binding or if it binds with the active site unavailable to the analyte, the biosensor will not function. Attachment can be accomplished in a variety of ways, such as covalent binding of the molecule to the detector

(usually through a molecular cross-bridge), adsorption onto the surface entrapment in porous material, or micro encapsulation. Ultra-thin applications of biological material are usually deposited on transducers by using the Langmuir – Blodgett or molecular self-assembly technique.

Types of Biosensors

BOD Sensor: A biosensor consisting of immobilised yeast, *Trichosporon cutaneum* and an oxygen probe was developed for BOD estimation. The BOD biosensor includes an oxygen electrode that consists of a platinum cathode and an alluminium anode bathing in saturated KCl solution and a Teflon membrane. Yeast cells are immobilized on a porous membrane and are trapped between the pores and the Teflon membranes. Oxygen consumption by the immobilized microorganisms causes a decrease in current until a steady state is reached. The BOD biosensor measures BOD at 3-60 mg/L.

Methane Biosensor: This biosensor consists of immobilized methanotrophic bacteria (*Methylomonas flagellata*) in contact with an oxygen electrode. The immobilized bacteria use methane as well as oxygen according to the following reaction.

$$CH_4 + NADH_2 + O_2 \rightarrow CH_3OH + NAD + H_2O,$$

where NAD is nicotinamide adenine dinucleotide and $NADH_2$ is the reduced form of the coenzyme. Oxygen consumption leads to a decrease in current, which is proportional to the methane concentration in the sample.

Ammonia and Nitrate Biosensors: Ammonia biosensor, based on amperometry, consists of immobilized nitrifying bacteria (e.g., *Nitrosomonas europaea*) and a modified oxygen electrode. This biosensor, with a lifetime of approximately 2 weeks was used for ammonia determination in waste waters based on the conversion of nitrate to N_2O by an immobilized denitrifying bacterium *Agrobacterium* sp. The nitrate biosensor has been used to measure nitrate profiles in biofilms in environment samples.

Microbial Biosensor: A microbial sensor consists of a microorganism immobilized on a membrane and an electrode. The principle of a microorganism sensor is based on either the change in respiration or the amount of metabolites produced as a result of the assimilation of substrates by the microorganism. Recently, microbial sensors using thermophilic bacteria have been developed (*Table* 1). They reduce contamination of other microorganisms by the use of high temperature. For example

Table 1. Examples of microbial biosensors.

Substances measured (Class and Example)	Microorganism employed	Detected substance*	Useful concentration measured
Alcohol			
Ethanol	*Trichosporon brassicae* (or *Acetobacter xylinium*)	O_2	Below 22mg litre^{-1}
Amino acid			
L-arginine	*Streptococcus faecalis*	NH_3	$10^{-5}\times5\times10^{-5}$ mol/litre
L-glutamate	*Escherichia coli*	CO_2	10^{-3}–10^{-5} mol/litre
Antibiotics			
Nystatin	Yeast cells	O_2	0.5 to 80 units/ml
Cephalosporin	*Citrobacter freundii*	H^+	Below 22 mg/litre
Co-factors			
NAD^+	*Escherichia coli*/NADase	NH_3	8×10^{-4} to 5×10^{-5} mol/litre
Gases			
Methane	*Methylomonas flagelata*	O_2	Upto 6.6×10^{-3} mol/litre
Organic acids			
Formate	*Clostridium butyricum*	Fuel cell	Upto 1.0 g/litre
Salts			
Nitrate and nitrite	*Azotobacteria vinelandii*	NH_3	8×10^{-4} to 10^{-5} mol/litre
Sugars			
General	Bacteria from human dental plaque	H^+	10^{-4} to 10^{-5} mol/litre
Vitamins			
Nicotinic acid	*Lactobacillus arabinosus*	H+	5×10^{-8} to 5×10^{-6} gm/ml

* Usually detected by potentiometric or amperometric methods

BOD and carbon dioxide sensors are constructed by using thermophilic bacteria isolated from a hot spring.

Microbiosensors have many advantages:

1. They can be implanted in the human body and are suitable for *in vivo* detection.
2. They can be integrated on one chip and are useful for measuring various substrates in a small amount of sample solution simultaneously.
3. Semiconductor fabrication technology can be applied to microbiosensors. It is possible to develop disposable transducers for biosensors through mass production.

Microbiosensors are based on ion sensitive field effect transistor (ISFET) and were first reported by Bergveld (1970). Matsuo *et al.* (1974) improved the ISFET using silicon nitride as the gate insulator to construct micro pH sensitive devices. They show rapid response, low power consumption and low noise.

Urea Sensor: A urea sensor consists of urease immobilized on membrane and a pH electrode. Urease-catalyzed reaction cause pH changes so that ISFET can be used as a transducer. The urea sensor gives the linear relationship between the initial rate of the output gate voltage and the logarithm value of urea concentration in the range 16.7 to 167 mM and can be used for 20 days with slight degradation of the enzyme activity.

Alcohol Sensor: This system consists of membrane bound alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH) and an electron transfer system which can be used in conjunction with an ISFET.

Hypoxanthine and Inosine Sensor: Hypoxanthine is measured on the basis of the reaction catalyzed by xanthine oxidase (XO). The pH change caused by uric acid is detected by using a Si ISFET. The oxidation of hypoxanthine to uric acid by xanthine oxidase is initiated immediately after injection. The response to inosine, however, has a time lag of 90 sec after injection. This

Hypoxanthine and Inosine Sensor

To maintain quality, evaluation of freshness is important in the fish industry. When a fish dies, adenosine 5' triphosphate (ATP) decomposition in the fish meat occurs and adenosine 5' diphosphate (ADP) and adenosine 5' monophosphate (AMP) and related compounds are generated where IMP, HxR, Hx, X and U stands for inosine 5' monophosphate, inosine, hypoxanthine, xanthine and uric acid respectively.There comes the use of hypoxanthine and inosine sensor.

ATP→ ADP→AMP→ IMP→HxR→Hx→X→U,

where IMP, HxR, Hx, X and U stands for inosine 5' monophosphate, inosine, hypoxanthine, xanthine and Uric acid, respectively, consequently, Hx accumulation with an increase in storage time can be used as an indicator of fish meat freshness. Therefore, simple and rapid methods for the determination of Hx and HxR are required in the seafood industry.

phenomenon is attributed to the three-step reaction. On the basis of this time lag the sensor can determine inosine and hypoxanthine simultaneously.

Glucose and Carbon Dioxide Sensors using Micro-oxygen Electrode

The Glucose Sensor

The glucose sensor is fabricated by immobilizing glucose oxidase (GOD) on the membrane of the oxygen electrode. The glucose sensor responds as soon as the glucose solution is injected into the buffer solution and reaches a steady state in 5 to 10 min. The sensor responds almost linearly for glucose concentration between 0.2 and 2mM, which is comparable to conventional glucose sensors.

A microbial CO_2 sensor using this oxygen electrode was constructed by Suzuki *et al.* The autotrophic bacterium S17, which can grow with only carbonate as the carbon source was used. Bacterial cells are immobilized on a micro oxygen electrode. The sensitive area of the oxygen electrode is immersed in 0.2% sodium alginate solution containing S17 whole cells, then removed and immediately immersed in 5% $CaCl_2$ solution to form bacteria immobilized calcium alginate gel. The response

time is 2 to 3 min. Carbon dioxide was supplied by acidification of $NaHCO_3$, the concentration of which can be related to CO_2 concentration. Linear relationship is obtained between the current decrease and $NaHCO_3$ concentration in the range 0.5 to 3.5mM. The lowest detection limit is 0.5mM $NaHCO_3$ within the margin of the noise amplitude (*Figure* 5).

Integrated Multibiosensor

These biosensors are based on the ISFETs and electrodes, coated with enzyme immobilized membrane. An enzyme-immobilized membrane should be precisely deposited onto a gate region or small working electrode. Different enzyme membranes can be prepared without mixing.

Applications

Biosensors have many uses in clinical analysis, general health care monitoring, veterinary and agricultural applications, industrial processing and monitoring, and environmental pollu-

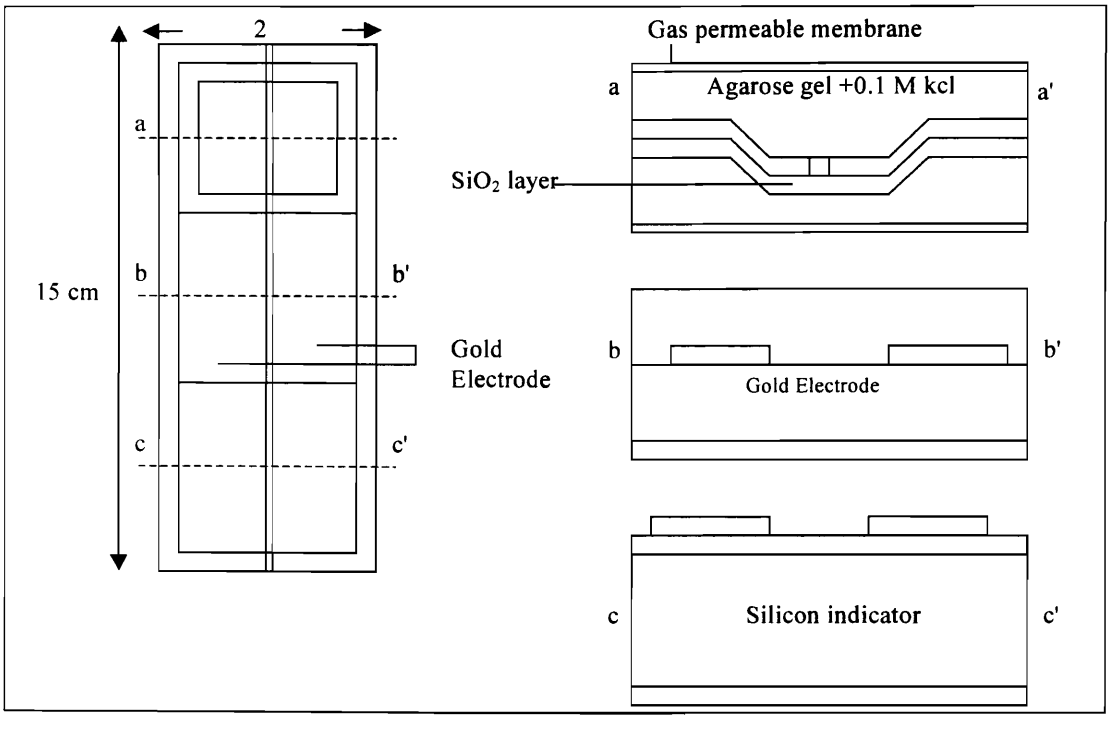

Figure 5. Structure of micro oxygen electrode showing different parts.

Nucleic Acid Biosensor

It depends on the ability of a single stranded nucleic acid to hybridize with another fragment of DNA by complementary base pairing. Technological innovation is introduced in the manner in which the nucleic acid oligomer is attached to the surface of the detector and the manner in which the hybridized nucleic acid is detected and transduced into a measurable signal. Ammonia derivatised oligonucleotides can be detected can be attached to glass (SiO_2) surfaces such as fiber-optic cables, glass beads or microscopic slides through covalent bonding with a chemical linker.

A nucleic acid biosensor that utilizes evanescent wave technology by using short fragments of nucleic acids that are small enough to reside within the field of the evanescent wave. They were able to detect fluorescent – labeled DNA hybridizing to their complementary immobilized probes in a flow cell. Fluorescence was monitored and reported as a change in the output voltage. Nucleic acid biosensors are potentially useful in the field of rapid DNA sequencing as well as in clinical applications.

The Biosensor described by Eggers *et al.* integrates microelectronics, molecular biology and computational sciences in an optical electrode format. Their device can detect hybridization and report on the spatial address of the hybridization signal on a glass surface or a silicon wafer, to which the DNA probes are attached. Several different DNA oligomers can be attached to the optical electrode at different locations. The DNA on the biosensor is then hybridized to DNA that is free in solution. The free DNA must be labeled, usually with a fluorescent, luminescent, and radioisotope decay ^{32}P signal. The signal is detected by charged coupled device (CCD), which is extremely sensitive. The computer identifies the location of the affected pixels and forms the signals into a recognizable array not only in this technology suitable for rapid DNA sequencing, but it is also applicable to the rapid detection of many different gene sequences from DNA extracted from a consortium.

tion control. The advantages are likely to include low cost, small size, quick and easy use, as well as a sensitivity and selectivity greater than the current instruments.

The advent of cheap, user-friendly biosensors will revolutionize the practice of healthcare monitoring and enables more in-depth diagnosis on a metabolic basis. The introduction of suitable biosensors would have considerable impact in the following areas:

Clinical and Diagnostic Applications: Bench top biosensors of the electrochemical variety are used now in clinical biochemistry laboratories for measuring glucose and lactic acid. A key feature of this is the ability for direct measurement on undiluted blood samples. Consumer self-testing, especially self-monitor-

Suggested Reading

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ing of blood components is another important area of clinical medicine and healthcare to be impacted by commercial biosensors. Current methods are based on colorimetric dry reagent chemistry often in conjunction with a portable reflectance meter.

Biosensors offer the potential of reusable systems and other advantages by employing electrochemical detection rather than colour changes to help alleviate the problems of those with poor eyesight (some of them diabetics who are often heavy users of biosensors for glucose determination). Reusable sensors also permit calibration and quality control unlike the present disposable sticks where only one measurement can be carried out. Such testing will improve the efficiency of patient care, replacing the often slow and labour intensive present tests. It will bring clinical medicine closer to bedside, facilitating rapid clinical decision-making. Examples of potential biosensor forms and their uses in diagnostic medicine are:

Industrial Applications: Along with conventional industrial fermentation producing materials, many new products are being produced by large-scale bacterial and eukaryotes cell culture. The monitoring of these delicate and expensive processes is essential for minimizing the costs of production; specific biosensors can be designed to measure the generation of a fermentation product.

***Environmental Monitoring*:** Environmental water monitoring is an area in which whole cell biosensors may have substantial advantages for combating the increasing number of pollutants finding their way into the groundwater systems and hence into drinking water. Important targets for pollution biosensors now include anionic pollutants such as nitrates and phosphates.

The area of biosensor development is of great importance to military and defense applications such as detection of chemical and biological species used in weapons.