Biosensors: A Tutorial Review

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Abstract

As the potential threat of bioterrorism increases, there is great need for a tool that can quickly, reliably and accurately detect contaminating bio-agents in the atmosphere. Biosensors can essentially serve as low-cost and highly efficient devices for this purpose in addition to being used in other day-to-day applications. A biosensor is a sensing device comprised of a combination of a specific biological element and a transducer. A "specific biological element" recognizes a specific analyte and the changes in the biomolecule are usually converted into an electrical signal (which is in turn calibrated to a specific scale) by a transducer. In this article we present the basics of biosensing devices which can serve as an introductory tutorial for readers who are new to this field. Subsequently we provide high-level descriptions of a few representative biosensors as case studies, followed by a brief discussion of the major difficulties the biosensor research communities normally encounter.

1. Introduction

The history of biosensors started in the year 1962 with the development of enzyme electrodes by the scientist Leland C. Clark. Since then, research communities from various fields such as VLSI, Physics, Chemistry, and Material Science have come together to develop more sophisticated, reliable and mature biosensing devices for applications in the fields of medicine, agriculture, biotechnology, as well as the military and bioterrorism detection and prevention.

What is a biosensor? Various definitions and terminologies are used depending on the field of application. Biosensors are known as: *immunosensors*, *optrodes*, *chemical canaries*, *resonant mirrors*, *glucometers*, *biochips*, *biocomputers*, and so on. A commonly cited definition is: "a biosensor is a chemical sensing device in which a biologically derived recognition entity is coupled to a transducer, to allow the quantitative development of some complex biochemical parameter", and also: "a biosensor is an analytical device incorporating a deliberate and intimate combination of a specific biological element (that creates a recognition event) and a physical element (that transduces the recognition event)".

The name "biosensor" signifies that the device is a combination of two parts: (i) a bio-element, and (ii) a sensor-element. The basic concepts of a biosensor's operation can be illustrated with the

help of Fig. 1. A specific "bio" element (say, enzyme) recognizes a specific analyte and the "sensor" element transduces the change in the biomolecule into an electrical signal. The bio element is very specific to the analyte to which it is sensitive. It does not recognize other analytes. Depending on the transducing mechanism used, the biosensors can be of many types such as: (i) Resonant biosensors, (ii) Optical-Detection biosensors, (iii) Thermal-Detection biosensors, (iv) Ion-Sensitive FET (ISFET) biosensors, and (v) Electrochemical biosensors. Details of all these different types will be discussed in this article. The electrochemical biosensors based on the parameter measured can be further classified as: (i) conductimetric, (ii) amperometric, and (iii) potentiometric.

Biosensors can have a variety of biomedical, industry, and military applications as shown in Fig. 2. The major application so far is in blood glucose sensing because of its abundant market potential. However, biosensors have tremendous potential for commercialization in other fields of application as well. In spite of this potential, however, commercial adoption has been slow because of several technological difficulties. For example, due to the presence of biomolecules along with semiconductor materials, biosensor contamination is a major issue.

2. Basic Concepts

As demonstrated in Fig. 1, a biosensor consists of a bio-element and a sensor-element. The bioelement may be an enzyme, antibody, living cells, tissue, etc., and the sensing element may be electric current, electric potential, and so on. A detailed list of different possible bio-elements and sensor-elements is shown in Fig. 3. Different combinations of bio-elements and sensor-elements constitute several types of biosensors to suit a vast pool of applications.

The "bio" and the "sensor" elements can be coupled together in one of the four possible ways demonstrated in Fig. 4: Membrane Entrapment, Physical Adsorption, Matrix Entrapment, and Covalent Bonding. In the membrane entrapment scheme, a semi permeable membrane separates the analyte and the bioelement, and the sensor is attached to the bioelement. The physical adsorption scheme is dependent on a combination of van der Waals forces, hydrophobic forces, hydrogen bonds, and ionic forces to attach the biomaterial to the surface of the sensor. The porous entrapment scheme is based on forming a porous encapsulation matrix around the biological material that helps in binding it to the sensor. In the case of the covalent bonding the sensor surface is treated as a reactive group to which the biological materials can bind.

The typically used bio-element, enzyme is a large protein molecule that acts as a catalyst in chemical reactions, but remains unchanged at the end of reaction. Fig. 5 shows the working principle of enzymes. An enzyme upon reaction with a substrate forms a complex molecule which under appropriate conditions forms the desirable product molecule releasing the enzyme at the end. The enzymes are extremely specific in their action: an enzyme X will change a specific substance A (not C)

to another specific substance B (not D), as illustrated in Fig. 6. *This extremely specific action of the enzymes is the basis of biosensors*.

3. Types of Biosensors

In this section we will discuss some common types of biosensors.

3.1 Resonant Biosensors

In this type of biosensor, an acoustic wave transducer is coupled with an antibody (bio-element). When the analyte molecule (or antigen) gets attached to the membrane, the mass of the membrane changes. The resulting change in the mass subsequently changes the resonant frequency of the transducer. This frequency change is then measured.

3.2 Optical-detection Biosensors

The output transduced signal that is measured is light for this type of biosensor. The biosensor can be made based on optical diffraction or electrochemiluminescence. In optical diffraction based devices, a silicon wafer is coated with a protein via covalent bonds. The wafer is exposed to UV light through a photo-mask and the antibodies become inactive in the exposed regions. When the diced wafer chips are incubated in an analyte, antigen-antibody bindings are formed in the active regions, thus creating a diffraction grating. This grating produces a diffraction signal when illuminated with a light source such as laser. The resulting signal can be measured or can be further amplified before measuring for improved sensitivity.

3.3 Thermal-detection Biosensors

This type of biosensor is exploiting one of the fundamental properties of biological reactions, namely absorption or production of heat, which in turn changes the temperature of the medium in which the reaction takes place. They are constructed by combining immobilized enzyme molecules with temperature sensors. When the analyte comes in contact with the enzyme, the heat reaction of the enzyme is measured and is calibrated against the analyte concentration. The total heat produced or absorbed is proportional to the molar enthalpy and the total number of molecules in the reaction. The measurement of the temperature is typically accomplished via a thermistor, and such devices are known as enzyme thermistors. Their high sensitivity to thermal changes makes thermistors ideal for such applications. Unlike other transducers, thermal biosensors do not need frequent recalibration and are insensitive to the optical and electrochemical properties of the sample. Common applications of this type of biosensor include the detection of pesticides and pathogenic bacteria.

3.4 Ion-Sensitive Biosensors

These are semiconductor FETs having an ion-sensitive surface. The surface electrical potential changes when the ions and the semiconductor interact. This change in the potential can be subsequently measured. The Ion Sensitive Field Effect Transistor (ISFET) can be constructed by covering the sensor electrode with a polymer layer. This polymer layer is selectively permeable to

analyte ions. The ions diffuse through the polymer layer and in turn cause a change in the FET surface potential. This type of biosensor is also called an ENFET (Enzyme Field Effect Transistor) and is primarily used for pH detection.

3.5 Electrochemical Biosensors

Electrochemical biosensors are mainly used for the detection of hybridized DNA, DNA-binding drugs, glucose concentration, etc. The underlying principle for this class of biosensors is that many chemical reactions produce or consume ions or electrons which in turn cause some change in the electrical properties of the solution which can be sensed out and used as measuring parameter. Electrochemical biosensors can be classified based on the measuring electrical parameters as: (1) conductimetric, (2) amperometric and (3) potentiometric. A comparative discussion of these three types of electrochemical biosensors is given in Table 1.

3.5.1 Conductimetric

The measured parameter is the electrical conductance / resistance of the solution. When electrochemical reactions produce ions or electrons, the overall conductivity or resistivity of the solution changes. This change is measured and calibrated to a proper scale. Conductance measurements have relatively low sensitivity. The electric field is generated using a sinusoidal voltage (AC) which helps in minimizing undesirable effects such as Faradaic processes, double layer charging and concentration polarization.

3.5.2 Amperometric

This high sensitivity biosensor can detect electroactive species present in biological test samples. Since the biological test samples may not be intrinsically electro-active, enzymes are needed to catalyze the production of radio-active species. In this case, the measured parameter is current.

3.5.3 Potentiometric

In this type of sensor the measured parameter is oxidation or reduction potential of an electrochemical reaction. The working principle relies on the fact that when a ramp voltage is applied to an electrode in solution, a current flow occurs because of electrochemical reactions. The voltage at which these reactions occur indicates a particular reaction and particular species.

4. Glucose Biosensors

The most commercially successful biosensors are amperometric glucose biosensors. These biosensors have been made available in the market in various shapes and forms such as glucose pens, glucose displays, etc.

The first historic experiment that served as the origin of glucose biosensors was carried out by Leland C. Clark. He used platinum (Pt) electrodes to detect oxygen. The enzyme *glucose oxidase* (GOD) was placed very close to the surface of platinum by physically trapping it against the electrodes with a piece of dialysis membrane. The enzyme activity changes depending on the

surrounding oxygen concentration. Fig. 7 shows the reaction catalyzed by GOD. Glucose reacts with glucose oxidase (GOD) to form gluconic acid while producing two electrons and two protons, thus reducing GOD. The reduced GOD, surrounding oxygen, electrons and protons (produced above) react to form hydrogen peroxide and oxidized GOD (the original form). This GOD can again react with more glucose. The higher the glucose content, more oxygen is consumed. On the other hand, lower glucose content results in more hydrogen peroxide. Hence, either the consumption of oxygen or the production of hydrogen peroxide can be detected by the help of platinum electrodes and this can serve as a measure for glucose concentration.

Disposable amperometric biosensors for the detection of glucose are also available. The typical configuration is a button-shaped biosensor consisting of the following layers: metallic substrate, graphite layer, isolating layer, mediator modified membrane, immobilized enzyme membrane (GOD), and a cellulose acetate membrane. This biosensor uses graphite electrodes instead of platinum electrodes (as originally used by Clark). The isolating layer is placed on the graphite electrodes which can filter out certain interfering substances (ascorbic acid, uric acid) while allowing the passage of hydrogen peroxide and oxygen. The mediator modified membrane helps in keeping the GOD membrane attached to the graphite electrode when the electrochemical reaction takes place at a specific applied potential. The cellulose acetate outer layer placed over the GOD membrane also provides a barrier for interfering substances. The amperometric reading of the biosensor (current versus glucose concentration) shows that the relationship is linear up to a specific glucose concentration. In other words current increases linearly with glucose concentration, hence it can be used for detection.

The current and future applications of glucose biosensors are very broad due to their immediate use in diabetic self-monitoring of capillary blood glucose. These types of monitoring devices comprise one of the largest markets for biosensors today and their existence has dramatically improved the quality of life of diabetics.

5. A Biosensor to Monitor Cell Morphology

Another type of biosensor can be used to monitor cell morphology in tissue culture environments. The sensing principle used is known as Electric Cell-substrate Impedance Sensing (ECIS). In this process, a small gold electrode is immersed in a tissue culture medium. When cells get attached and spread on the electrodes, the impedance measured across the electrodes changes. This changing impedance can be used for understanding the cell behavior in the culture medium.

The *attachment* and *spreading* behavior of the cells are important factors for this type of biosensor. Cancerous cells can usually grow and reproduce (*mitosis*) freely in a medium without being attached to any substrate/surface. Normal cells, on the other hand, need to be attached to a surface before they

grow. After attachment the shape of the cells becomes flat and no longer remains spherical. Fig. 8 demonstrates this cell behavior in a tissue culture medium.

The principle of measurement is as follows: The cells are grown on gold electrodes. The electrodes are immersed in a tissue culture medium which works as electrolyte. A voltage is applied through a resistance and the magnitude and phase of the voltage are measured with a lock-in-amplifier. Since the current is constant, the measured magnitude and phase can be assumed to be proportional to impedance (resistance and capacitance). After some time, it is found that the resistance and capacitance values fluctuate very often. This happens when cells are alive and moving. This type of biosensor has several advantages: It is less time consuming compared to conventional methods, it is possible to automate and quantify cell morphology measurements, and the fluctuating pattern can be used as signature for a cell.

6. DNA Detection

The category of biosensors used for DNA detection is also known as biodetectors. The objective is to isolate and measure the strength of single DNA–DNA or antibody–antigen bonds, which in turn helps in detecting and characterizing single molecules of DNA or antigen. In one method, multiple copies of the sample DNA are created using polymerase chain reaction (PCR). On the other hand, FABS (Force Amplified Biological Sensor), BARC (Bead Array Counter), and FDA (Force Differentiation Assay) biosensors can perform many such measurements in a single easy operation. In these cases magnetic microbeads are used to pull on DNA–DNA or antibody–antigen bonds with a known force, and the strengths of the presumed bonds are tested by observing with a micromechanical sensor (FABS), or with a magnetoresistive sensor (BARC) whether the beads detach from the surface. This kind of biosensor is extremely useful in the detection of Antrax, Ricin, Botulinum and other pathogens.

The FABS is needed for monitoring the concentration of various biological agents that may possibly be present in the environment. FABS is designed in such a way that it is fully automated, compact and rugged, and can be implemented remotely. The assay is also a rapid process as it may warn of some kind of potential threat to human health. FABS can detect various biologically active materials like toxins, proteins, viruses, and bacteria, in low concentrations. To accomplish this it uses a sandwich assay technique, in which antibodies against a particular protein, virus, or bacterium are covalently bound to a solid surface. The sample solution flows over the surface, and the antibodies capture the virus present in the sample. Next, super paramagnetic beads, also coated with an antibody against the virus, flow through the liquid and bind to the analyte. After washing away excess beads, a number of beads remain bound to the surface through the virus. By determining the number of beads, the concentration of virus in the original sample is calculated.

The biodetectors are used to identify a small concentration of DNA (of microorganisms like viruses or bacteria) in a large sample. This relies on comparing sample DNA with DNA of known

microorganisms (probe DNA). Since the sample solution may contain only a small number of bioorganism molecules, multiple copies of the sample DNA need to be created for proper analysis. This is achieved by the help of *polymerase chain reaction* (PCR). PCR starts by splitting samples of double-helix DNA into two parts by heating it. If the reagents contain proper growth enzymes, then each of these strands will grow the complementary missing part and form the double-helix structure again. This happens when the temperature is lowered. Thus, in one heating/cooling cycle the amount of sample DNA is doubled (one cycle time is one about minute). Typically, 25-40 cycles are needed to produce approximately a billion copies. This amount is sufficient for optical detection. While the PCR is busy in copying DNA, identification also could be made possible using fluorescent DNA probes.

In general, PCR is very power consuming because of the successive heating/cooling cycles which take about 30 minutes. It was previously not possible to fabricate portable battery operated biodetectors which can do PCR. However, using MEMS (Micro Electro Mechanical Systems) such kinds of biodetectors (which are basically lab-on-a-chip systems) have been developed. In these MEMS based devices the amount of reagent used is scaled down. The advantages of this type of biosensor are: (i) many times faster than conventional PCR. (ii) more efficient in the number of DNA copies produced, (iii) easily designed to use small volumes, and (iv) economical.

7. A Holographic Biosensor for Screening Pancreatic Disorders

Holograms are photographs of 3D impressions on the surface of light. To make a hologram one needs to photograph light waves. When an object wave meets a reference wave, a standing wave pattern of interference is created which can be photographed, thus creating a hologram. A hologram is generally recorded on silver halide film. The film consists of a base material of glass or plastic. On top of it there is a photoactive layer called *emulsion*. This emulsion layer is made up of gelatin (a colorless / yellowish protein). Silver and halide materials float in the gelatin layer. They chemically react to form silver halide molecules. When light energy travels into the gelatin, it is transferred to the silver halide molecule.

Biosensors which use holograms as the sensing element have been produced. This biosensor can have potential applications in screening pancreatic disorders at lower price. The bioelement used is *bovine pancreatic trypsin inhibitor* (BPTI) which is an enzyme. To screen pancreatic disorders *trypsin* needs to be detected in duodenal fluid or stool sample. By proper use of BPTI, trypsin detection can be made possible.

When the hologram is illuminated by white light constructive interference gives a characteristic spectrum having spectral peaks described by the “Bragg equation”. The characteristic spectrum is dependent on the gelatin matrix of the hologram. If gelatin molecules of hologram film are protease degraded the characteristic spectrum changes. This change is specific to the type of degradation. The reflected light from the hologram is detected by spectrograph and CCD detectors at intervals of some

minutes and are analyzed for peak wavelength and reflectivity change with time. The major advantage of this biosensor is that very small trypsin levels can be detected within a 60 minute period.

8. Conclusions

In this paper we have discussed various biosensors in detail. The survey initially introduces the basic concepts of the biosensor. A high level overview of different types of biosensors is also given. Working principles, constructions, advantages, and applications of many biosensors are presented. In addition we should point out that during the last two decades, advances in microelectromechanical systems (MEMS) have given rise to a whole new class of biosensors which involve the transduction of mechanical energy and are based on mechanical phenomena.

The authors would like to mention that there are various technical difficulties for which some solutions exist, but still more research efforts are needed in order to find better alternatives. Some of them are: (a) contamination: bioelements and chemicals used in the biosensors need to be prevented from leaking out of the biosensor over time (serious issue for non disposable ones), (b) immobilization of biomolecules: to avoid contamination, biomolecules are attached to the transducer as strongly as possible, but the problem with this is that the behavior of enzymes when absorbed on the surface is not well understood (reaction of enzymes in free solutions is better understood, (c) sterilization: if a sterilized probe is used some sensor's biomolecules may be destroyed whereas if non-sterile probes are used some compromises are needed, (d) uniformity of biomolecule preparation: fabrication of biosensors that can reproduce results need such uniformity, (e) selectivity and detection range: should be more selective and the detection range should be large, (f) cost: research should be focused on the development of low-cost biosensors. At present, with the threat of bioterrorism omnipresent, the development of faster, reliable, accurate, portable and low-cost biosensors has become more important than ever.

Acknowledgements

The authors would like to acknowledge feedback on the contents of this article by Dr. Shekhar Bhansali, Department of Electrical Engineering, University of South Florida, Tampa, FL.

Read more about it

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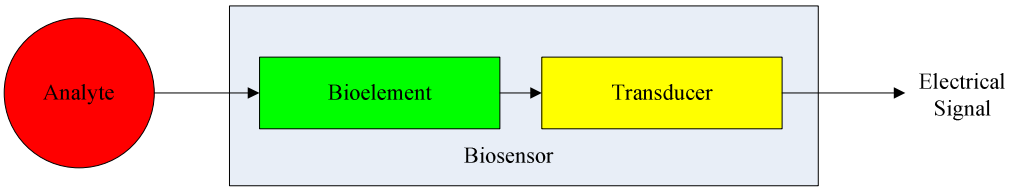

Fig. 1: A Schematic Representation of Biosensors

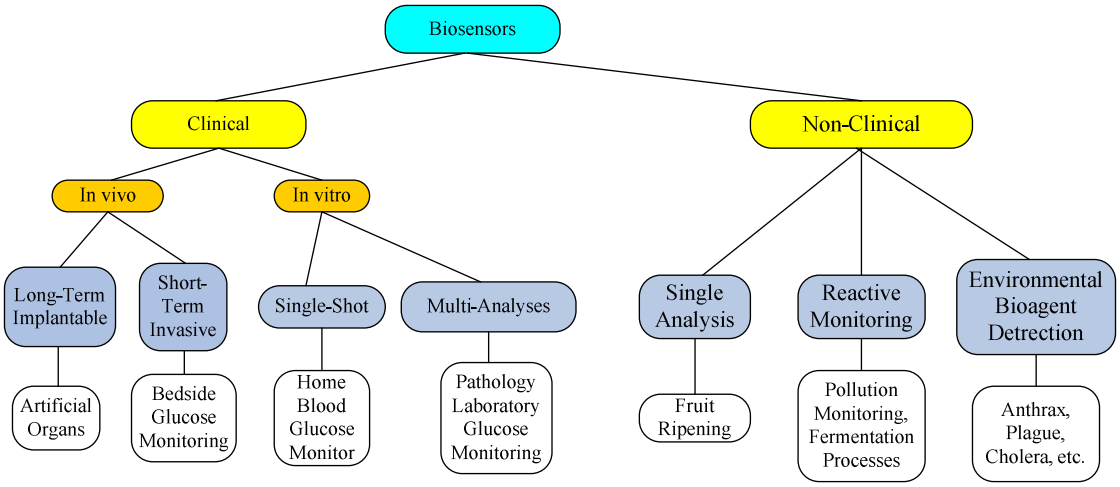

Fig. 2: Potential Applications of Biosensors.

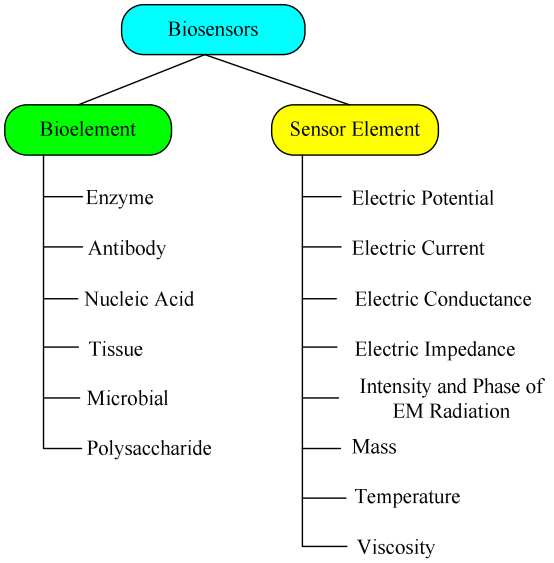

Fig. 3: Elements of Biosensors

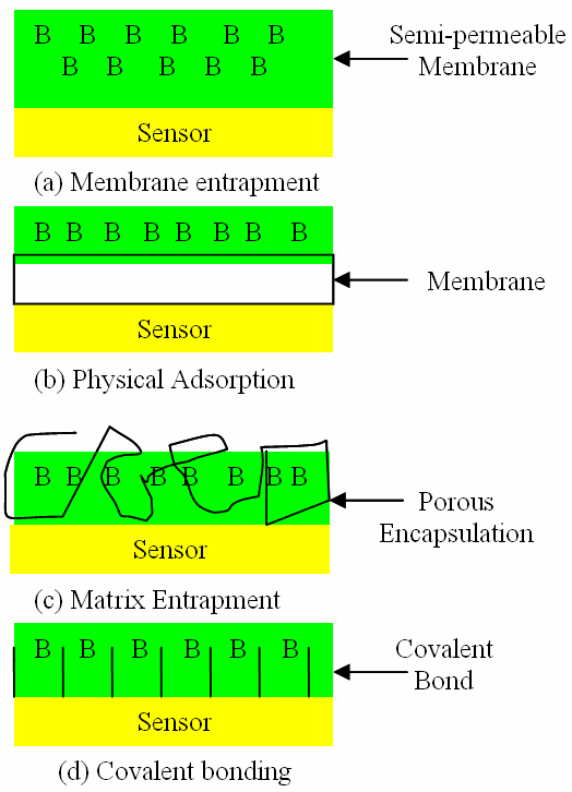

Fig. 4: Biomaterial-Sensor Coupling

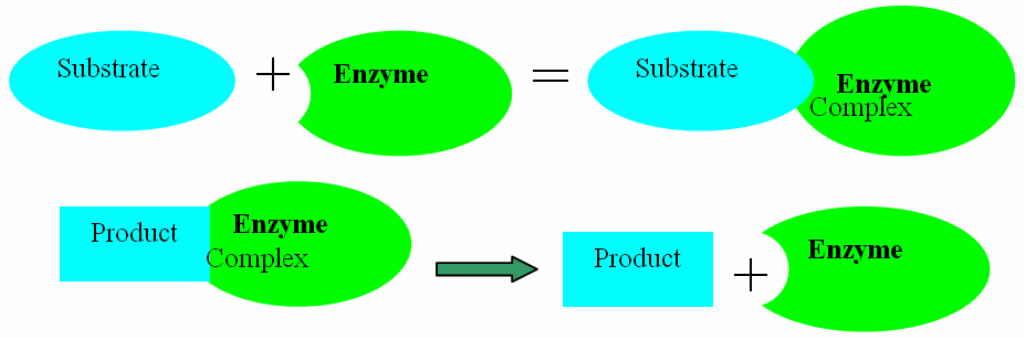

Fig. 5: Working Principle of Enzymes.

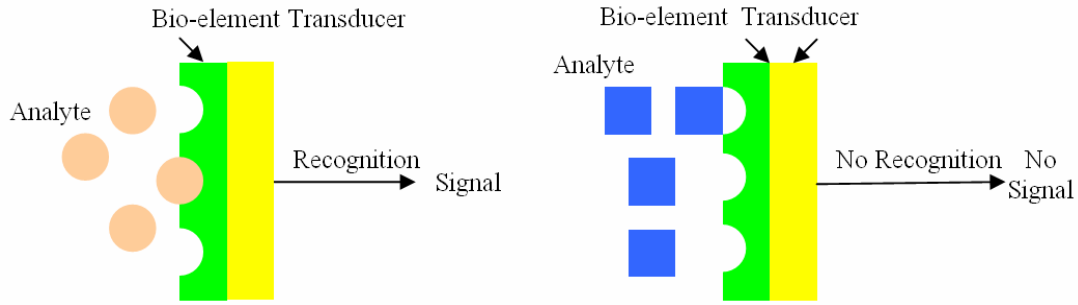

Fig. 6: Specificity of Enzymes is the Basis of Biosensors.

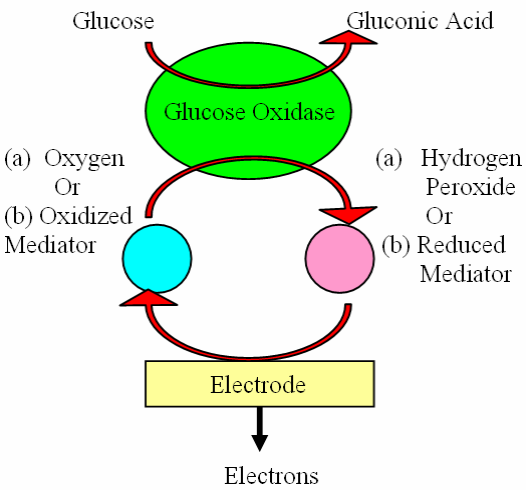

Fig. 7: Clark's Experiment

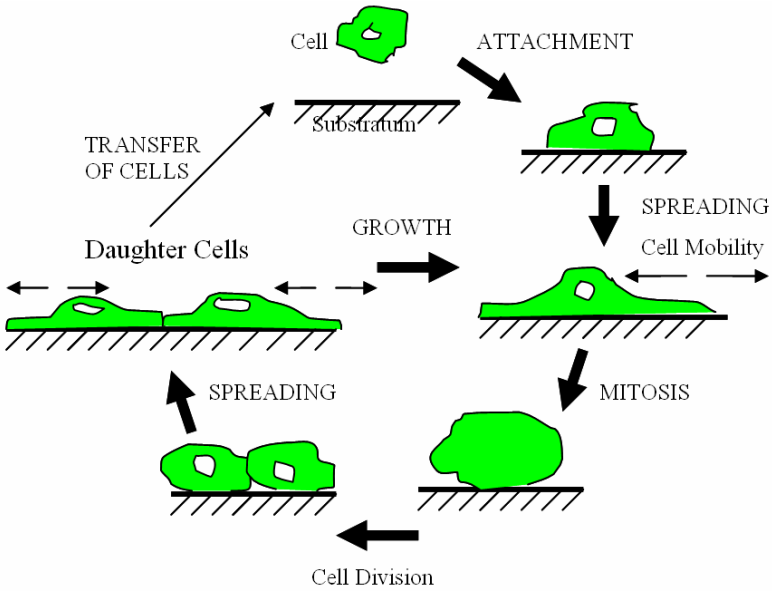

Fig. 8: A Cell in Tissue Culture Medium.