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Enzyme Biosensors for Point-of-Care Testing

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Abstract

Biosensors are devices that integrate a variety of technologies, containing biology, electronics, chemistry, physics, medicine, informatics, and correlated technology. Biosensors act as transducer with a biorecognition element and transform a biochemical reaction on the transducer surface directly into a measurable signal. The biosensors have the advantages of rapid analysis, low cost, and high precision, which are widely used in many fields, such as medical care, disease diagnosis, food detection, environmental monitoring, and fermentation industry. The enzyme biosensors show excellent application value owing to the development of fixed technology and the characteristics of specific identification, which can be combined with point-of-care testing (POCT) technology. POCT technology is attracting more and more attention as a very effective method of clinic detection. We outline the recent advances of biosensors in this chapter, focusing on the principle and classification of enzyme biosensor, immobilization method of biorecognition layers, and fabrication of amperometric biosensors, as well as the applications of POCT. A summary of glucose biosensor development and integrated setups is included. The latest applications of enzyme biosensors in diagnostic applications focusing on POCT of biomarkers in real samples were described.

Keywords: enzyme biosensors, point-of-care testing, classification, immobilization, low cost, fabrication of biosensor

1. Introduction

Biosensor is a delicate analytical device that combines a biologically derived sensitive element with a physical transducer that produces a tip or a continuous digital electrical signal (**Figure 1**) that is proportional to the analyte [1].

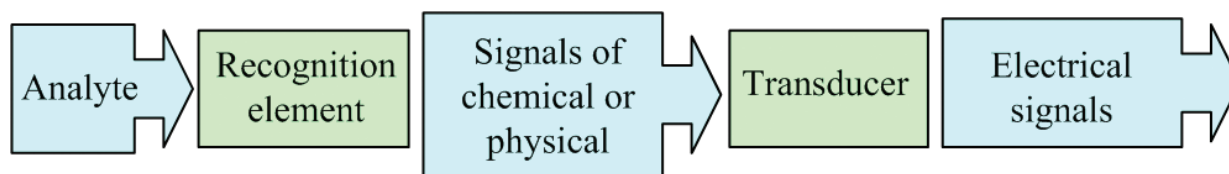


Figure 1. The principle of biosensor.

Biosensor is a new detection technology developed in recent decades. It is an advanced detection method and monitoring method necessary for the development of biological technology. It is also a rapid and micro effective analysis method at the level of material molecules.

Biosensors have become an active research field and demonstrated a very promising prospect, showing important practical value in environmental monitoring, clinical inspection, food and drug analysis, and biochemical analysis, such as rapid and sensitive detection of pathogens and pesticide residue in food and beverage, remote detection of air pollution or heavy metal ion pollution, real-time detection of human blood components and pathogens, long-term monitoring of the health of the human body, as well as the rapid detection of the battlefield weapons, which can be extended to molecular device development, neural network simulation, bionic intelligent devices, and basic research of biological computer [2].

The biosensor has several advantages as the following: (1) the biosensor has good selectivity owing to its high-sensitive molecular recognition element. The biosensor is highly integrated, and the detected components in the sample can be detected directly without the sample pretreatment, and no additional reagents are needed in the determination. (2) The biosensor with small size can be used for continuous monitoring. (3) The biosensor has rapid response and requires only a small amount of sample; as the sensitive material is fixed, it can be used repeatedly. (4) The cost of the sensor and the matched measuring instrument is lower than that of the large analytical instrument, which is very helpful for point-of-care testing (POCT) detection.

Biosensors are devices that are sensitive to biological substances and convert their concentrations to electrical signals. Biosensors act as analytical tools including biologically sensitive material immobilized as recognition element (**Figure 2A**) (including enzyme, antibody, antigen, microorganism, cell, tissue, nucleic acid, and other biologically active substances), physicochemical transducer (**Figure 2B**) (such as the electrochemical electrode, photodiodes, FET, piezoelectric, etc.), and signal amplifying device [1–3]. It has been widely used in biological medicine, drug development and testing, environmental quality testing, and other fields.

The development of enzyme electrode is the most representative and the most studied in biosensor field. Electrochemical biosensors are the largest group of biosensors, which have the earliest development and the most abundant research content. A large number of research achievements have been achieved, and some of them have been widely used [4, 5].

Electrochemical enzyme biosensors have unique advantages in improving selectivity and sensitivity. The microstructure on the surface of the electrochemical biosensor can provide a variety of potential fields that can effectively separate and enrich the analytes, which can

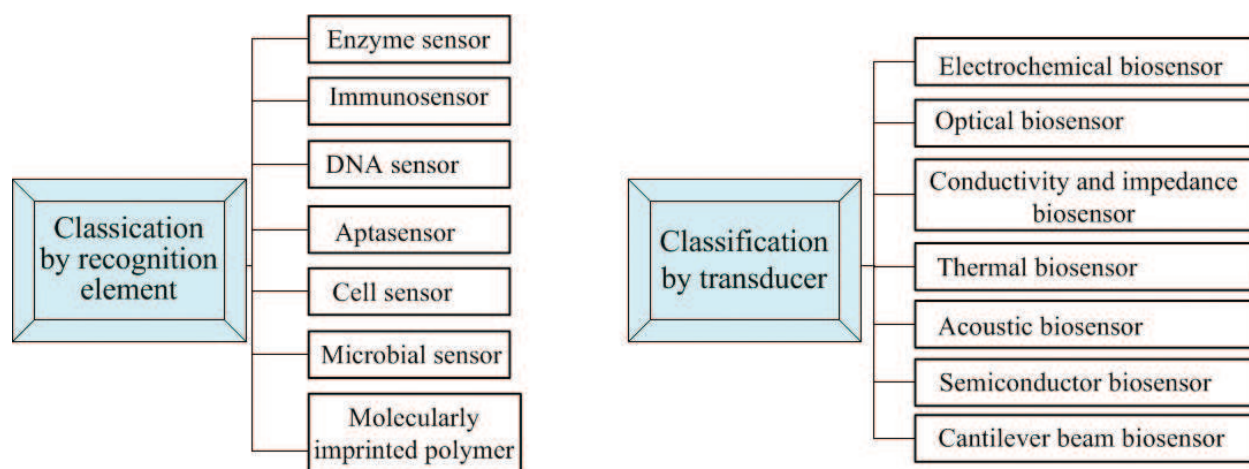


Figure 2. Classification of biosensors.

control the electrode potential, further improve the selectivity, and also combine the sensitivity of the determination method with the selectivity of the chemical reaction of the surface material. The electrochemical enzyme sensor is an ideal system for the integration of separation, enrichment, and selective determination. Among them, the development of amperometric enzyme biosensor is the most representative and the most studied in biosensor field [6, 7]. Biosensors are developing to the following aspects in recent years. (1) Biosensor with low cost, high sensitivity, high stability, and long life can meet the needs of field or on-site sampling and measurement. With the development of biosensor technology, it is necessary to reduce the cost of products and improve the sensitivity, stability, and life. The improvement of these characteristics will accelerate the commercialization and commercialization of biosensors. (2) With the development of microfabrication technology and nanotechnology, biosensors based on MEMS technology has been received a rapid development [7], and biosensors have been further miniaturized. The emergence and application of various portable biosensors will enable people to diagnose diseases at home, so they can directly detect food in the market, which meet the requirements of testing in vivo intracellular detection, online detection, and so on.

POCT technology has emerged and progressed with the development of computers, biosensors, electronics, and medicine. As a new method of clinical detection, POCT is getting more and more attention. POCT test equipment is portable, easy to operate, and suitable for non-laboratory professionals. Therefore, POCT is fast, sensitive, and free from site conditions. It plays an increasingly important role in medical field, environmental monitoring, and safety monitoring. The advantage of POCT is the immediate determination of the whole blood specimen, without the anticoagulant steps, without specimen preparation, a small amount of specimens, the short specimen turnaround time (TAT), the miniaturized instrument, and the patient's bedside test. The central laboratory test model of multistep and multi-staff was avoided. At present, POCT can include blood gas, electrolytes, blood sugar, renal function, liver function, hemoglobin, and cardiac markers, such as creatine kinase (CK), myoglobin (MYO), troponin (TNI, TNT), and so on. The POCT test project has already met some needs of emergency patients. And, the new POCT detection program is coming out. This shows that the real-time detection result of POCT has opened up a new prospect for clinical testing.

This chapter discusses several topics related to enzyme-based biosensors, including immobilization of recognition element, biosensor miniaturization, and application of biosensors.

2. Enzyme biosensors for POCT

The enzyme biosensor is composed of a sensitive membrane-immobilized enzyme and electrode transducer system, which combined enzyme and electrode together. It has advantages of both the high stability of insoluble enzyme system and the high sensitivity of the electrochemical system. Due to the specificity and the high selectivity of enzyme reaction, the complex samples can be directly determined by the sensor. The enzyme biosensor can be divided into two kinds of electric potential sensor and amperometric sensor. The development of the amperometric enzyme electrode is more widely [6]. The amperometric enzyme electrode is defined as an output of a corresponding current signal was generated by the targets reacting or redox reaction on the electrode, which has a linear relationship with the concentration of the tested substance under certain conditions. The basic biosensor mainly uses oxygen electrode and hydrogen peroxide electrode.

2.1. The enzyme immobilization method

2.1.1. Definition and characteristics of biosensor immobilization technology

Enzyme immobilization technology is of great importance in the development of biosensors. The main purpose of immobilization is to restrict the enzyme and other biologically sensitive elements to a certain space, but it does not interfere with the free diffusion of the analytes. It is one of the key factors that affect the stability (or life span), the sensitivity, and selectivity of the biosensor. The immobilization method can be divided into two kinds: direct method and indirect method. The direct method is the surface of the probe which is directly fixed to the transducer by physical and chemical modification. The direct method is the surface of the probe which is directly immobilized by the biomolecules on the transducer using the physical and chemical modification. This method is to integrate sensitive materials and probes, which is helpful to improve response performance. It is the main research direction of the commercialization of the portable enzyme sensors. The indirect rule is to fix the biological component on a carrier first and then install it on the probe of the sensor. As the sensitive part is independent of the converter, it is conducive to prolonging the service life of the converter and is suitable for long time monitoring. Indirect immobilization has become the main direction of the commercialization of enzyme sensors in the process of online analysis and process control. Compared with the free phase of biological materials, solid biological material has a series of advantages, for example, the thermal stability, the repeated use, no need to be separated and reactive substances in catalytic material after reaction, and the determination of the film life according to the known half-life. The immobilization method plays a decisive role in the performance and usage of the biosensors.

2.1.2. Classification of biosensor immobilization methods

The commonly used immobilization methods of biosensors include adsorption, embedding, covalent bonding, cross-linking, and electrochemical polymerization (**Figure 3**).

(1) Physical adsorption

The biosensor element is immobilized by the physical adsorption or ion binding of non-water-soluble carrier, which is defined as the adsorption method.

These binding forces may be hydrogen bonds, van Edward forces, or ionic bonds and may also play a role in a variety of bonding forms. There are a wide variety of carriers, such as active carbon, hydroxyl limestone, aluminum powder, gold, chitosan, cellulose, and ion exchangers. The firmness of the adsorption is related to the pH, ionic strength, temperature, properties and types of solvent, and the concentration of the enzyme. The enzyme is directly adsorbed on the surface of the electrode. The method is simple, and the enzyme activity is seldom degraded. However, the stability of the enzyme is not good, and this technology is not widely used at present. The more distinctive work is the co-deposition of enzyme and platinum/palladium; therefore, the enzyme is adsorbed during the growth of platinum black or palladium black particles. Platinum black holes have larger surface area, and their affinity for protein can keep the stability of the enzyme [8].

In addition, the adsorption method combined with nanomaterials can also help to stabilize the enzyme activity and improve the life span. The adsorption process is mainly used to prepare enzymes and immune membranes. The adsorption process usually does not require chemical reagents, and it has little effect on the activity of protein molecules. Because the protein molecules are easy to fall off, especially when environmental conditions changed, so they are often used by combining with other immobilization methods.

(2) Embedding method

Embedding enzyme molecules or antibody and immobilizing them in the three-dimensional spatial grid matrix of polymers are defined as the embedding method. The process is generally not necessary for binding reaction with the residues of biological material and rarely changing

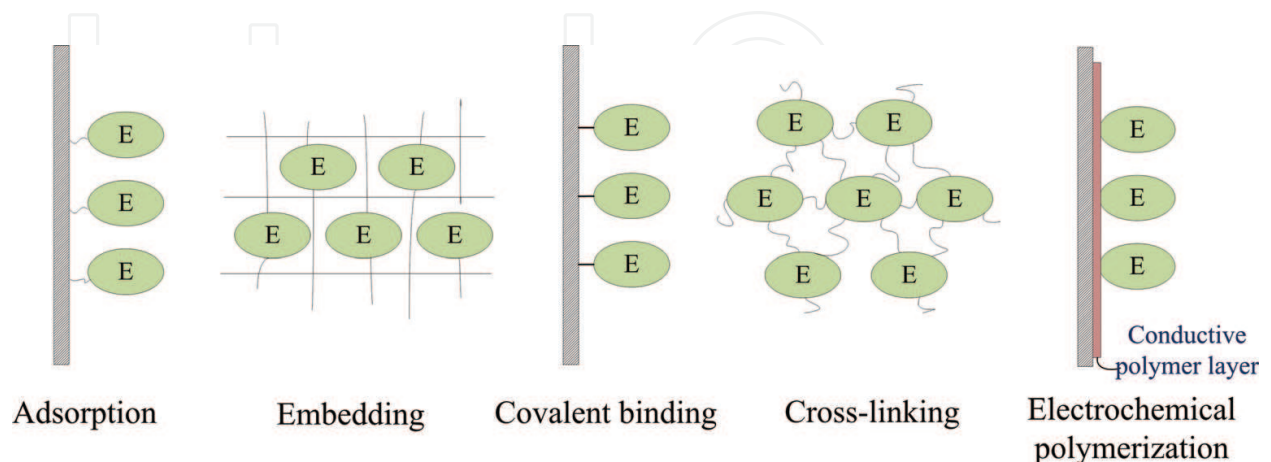


Figure 3. Schematic diagram of enzyme immobilization.

advanced structure of biological active substances; therefore, the loss of biological activity is very small, the pore diameter can be controlled arbitrarily, the encapsulated material is not easy to leak, and the substrate molecules can diffuse freely in the film [9, 10]. The traditional method of encapsulation is to bury the enzyme in the gel or membrane and then immobilize it to the electrode. The main gels are polyacrylic acid amine, polyvinyl chloride (PVC), polyvinyl alcohol (PVA), photosensitive resin, polycarbonate, nylon, acetic acid fiber, and other synthetic polymers and alginate, gelatin, collagen, and other colloidal polymers. This method is generally suitable for the bioactive substances acting on small molecular substrates and products, while the bioactive substances acting on macromolecular substrates and products are too large for mass transfer resistance. The enzyme is easy to leak from the sensing layer, but this method is more suitable for the encapsulation of organelles, lipids, and microbes.

Synthetic polymers, such as Nafion, can be directly embedded on the surface of the enzyme electrode. These films can embed biological molecules, anti-interference and anti-poison and are not easy to leak out, which is suitable for acting as encapsulation and immobilization material. Carbon paste embedding is a method of immobilization of enzyme directly with electrode material (carbon paste and carbon epoxy resin). It is a very common method to prepare biosensors. The carbon paste electrode can not only immobilize enzymes but also immobilize cofactors NAD, media, stabilizers, cells, and tissues. In order to eliminate interference and increase sensitivity, it can also immobilize two enzymes or enzymes in carbon paste.

Photopolymerization has recently been introduced into the gel of immobilized enzyme. The enzyme is dissolved in the mixture solution of polymer monomer and the photoinducer. It can form redox polymer hydrogel-immobilized enzyme when irradiated under the ultraviolet light conditions [10]. These light-induced polymers include polyethylene glycol (PEG), acrylic acid (acrylic acid), vinyl ferrocene, and other monomers.

(3) Covalent bond method

The method of binding the bioactive molecule through the covalent bond to the insoluble carrier is defined as the covalent bond.

The carrier includes the inorganic carrier and the organic carrier. Organic carriers are cellulose and its derivatives, dextran, agar powder, and so on; the inorganic carriers are mainly porous glass, graphite, and so on. It is particularly important to protect the amino acids of the active center during the covalent immobilization of enzymes. If the amino acid residues of the active center are chemically modified during covalent immobilization, the activity of the enzyme will decrease. There are two ways to effectively protect the active center. The first method is to add enzyme substrate or substrate structure analogues or competitive inhibitors before the covalent reaction to protect the active center. The second method is the use of bifunctional reagents, which have a chemoselectivity and a biologically specific source of covalent reactions. This dual selectivity can not only protect the active sites of protein but also realize directional immobilization of the enzyme molecules [11, 12].

The covalent bonding forms include diazotization method, peptide method, alkylating method, amino reaction, etc. The characteristics of covalent bond are strong combination, not easy to fall off, not easy to be biodegraded, and long life for service. The disadvantage of covalent bond is

that the operation procedure is tedious, the enzyme activity may be reduced by chemical modification, and it is difficult to prepare high active immobilized enzyme. Covalent bonding is mostly used for production of enzyme membrane and immune molecular membrane. It usually requires operation at low temperature (0 degrees), low ionic strength, and physiological pH.

(4) Chemical cross-linking method

Cross-linking is a method of using bifunctional or multifunctional reagents, such as glutaraldehyde, to bind the bioactive substances to the transducer directly. The functional groups involved in the coupling of bioactive substances are $-NH_3$, $-COOH$, $-SH$, $-OH$, imidazolyl, phenol base, etc., but these groups cannot be active center groups. Most transducers have to be pretreated before cross-linking to produce their surfaces for coupling groups. The pretreatment can be treated by electrochemical method and plasma treatment. The cross-linking and immobilization method combined with supramolecular self-assembled monolayers is to assemble thiols on the surface of gold electrodes and then immobilize enzymes on self-assembled membranes with bifunctional reagents [13, 14].

This method gives the film a specific function of highly ordered and complete structure, good stability through molecular design and is the highest form of chemically modified electrodes. At present, a fast and sensitive biosensor can be prepared. Cross-linking is widely used in the preparation of enzyme membrane and immune molecular membrane. The operation is simple and the combination is firm. When the enzyme source is more difficult, it is often necessary to add inert protein which is times the enzyme as substrate. The problem of this method is that pH must be strictly controlled during immobilization. It is usually operated near the isoelectric point of protein. The concentration of cross-linking agent should also be carefully adjusted, such as glutaraldehyde itself can cause protein poisoning, usually under 2.5% (volume fraction) concentration. In the cross-linking reaction, the enzyme molecules are inevitably partially inactivated.

(5) Electrochemical polymerization

Electrochemical polymerization is based on electrostatic interaction. In the process of electropolymerization, enzymes act as anions and interact with positively charged polymer skeletons, and enzyme is added to the polymer membrane and directly immobilized on the electrode surface [15].

The interaction between polymer, metal, and carbon conductor lays a foundation for immobilization of enzyme on the electrode surface, thereby improving the communication efficiency between oxidation–reduction center and electrode. For conductive polymer membrane, this method can not only control the thickness of the membrane but also control the density of the membrane. But the amount of enzyme is relatively small, the background current is large, and a large amount of enzymes are wasted in the solution of electropolymerization. It is also useful for nonconducting polymer membrane-immobilized enzymes, which are limited in thickness. The oxidoreductase can be doped into polypyrrole, polyaniline, and polyphenol membrane [16, 17].

2.2. Amperometric biosensor for glucose detection using POCT

The amperometric enzyme biosensor is the most representative biosensor. The development of the amperometric enzyme electrode mainly consists of three stages [18, 19]. The classic enzyme

electrode is represented by the Clark enzyme electrode as the first-generation electrochemical sensor (**Figure 4A**). The mediated enzyme electrode is the second-generation electrochemical sensor (**Figure 4B**), which solves the interference of oxygen and the interference of electrode active substances and overcomes the problem of high working potential of electrodes, which are often used for POCT [20–23]. The direct electrochemical enzyme electrode is the third-generation electrochemical sensor (**Figure 4C**), which is mainly used to solve the problem of low-efficiency transmission between enzymes and other biometric elements and electrodes.

2.2.1. The first-generation enzyme electrode (Clark enzyme electrode)

The first generation of enzyme electrodes was first proposed by Clark in 1962, and it was based on oxygen reduction and used glucose oxidase (GOD) as an example to catalyze glucose [22, 24].

The oxidation–reduction reaction occurs on the enzyme layer:



The measurements of peroxide are carried out on the electrode at a moderate anodic potential of +0.6 V (vs. Ag/AgCl):



The most used oxygen electrodes are electrolyzed Clark oxygen electrodes, which are made up of platinum cathode, Ag/AgCl anode, KCl electrolyte, and air permeable membrane. The overpotential of H_2O_2 on both metal and carbon electrodes is higher, generally from +0.6 to +0.8 V (vs. Ag/AgCl). The high detection potential can interfere with the electroactive substances such as antilob acid and uric acid in samples. Various selective osmotic membranes can be used to remove interfering substances or chemically modified electrodes to reduce the overpotential, but their sensitivity is always limited by the concentration of dissolved oxygen in the system.

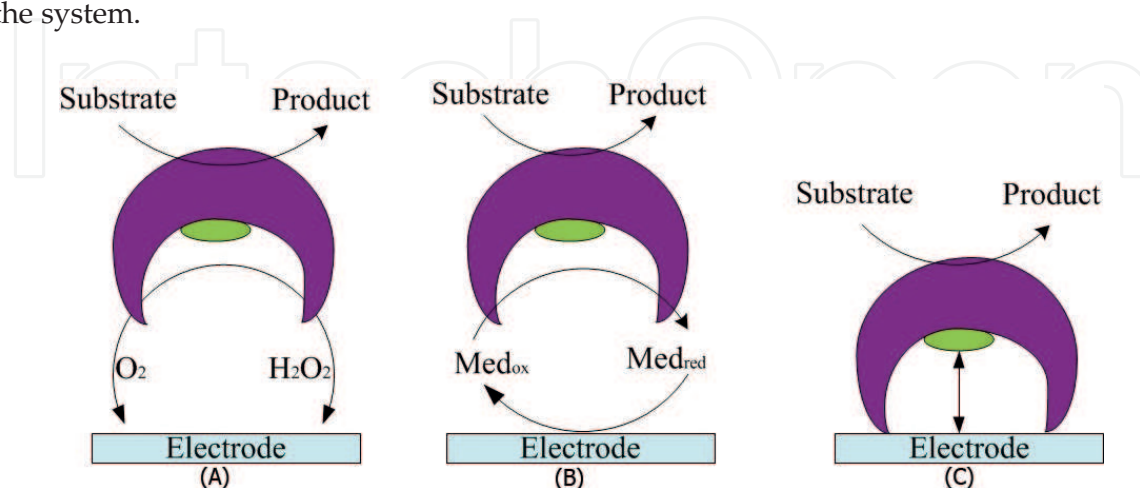


Figure 4. Electron transfer comparison diagram of the three generation amperometric enzyme electrodes, which are (A) first-generation enzyme electrode (Clark enzyme electrode), (B) second-generation enzyme electrode (mediated enzyme electrode), and (C) third-generation enzyme electrode (direct enzyme electrode).

2.2.2. The second-generation enzyme electrode (mediated enzyme electrode)

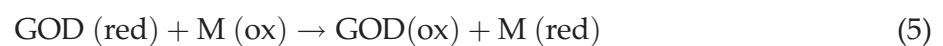
In order to overcome the shortcomings of the first generation of enzyme electrodes, the second generation of enzyme electrodes (mediated enzyme electrode) is widely used nowadays, which is the use of artificial electronic media to solve the problem of transferring electrons. The chemical modification layer was added at the second generation of enzyme electrodes. The chemically modified layers on the electrode are used to expand the range of chemical substances and also to improve the sensitivity of the determination. The modified substrate electrode can be regarded as an improved signal converter, and the modifier becomes an electron transfer medium. The role of electronic media is to promote the electron transfer process, reduce the working potential, and eliminate the interference of other electroactive substances.

In 1984, Cass established a mediated enzyme electrode method [23]. The chemical mediated ferrocene substituted the molecular oxygen as the electron acceptor for the enzymatic reaction and immobilized on the graphite electrode with the glucose oxidase (GOD). This research contributed to the successful development of a printed enzyme electrode in the US MediSense company in 1987. However, due to the difference between the reduced state of the medium and the oxidation state, the leakage occurred, which leads to the gradual decrease of the response activity of the electrode with time.

Dong used Nafion (a cationic resin) to fix ferrocene and then fixed the GOD to the Nafion-ferrocene electrode. Because of the hydrophilic structure and hydrophobic structure of Nafion, Fc/Fc⁺ is retained in the membrane. Therefore, the service life of electrodes is greatly prolonged, and the stability of the mediated enzyme electrode is improved [25, 26]. In commercial products, in order to avoid the problems of dielectric loss and electrode calibration, many enzyme electrodes have been designed to be disposable.

Electron mediator refers to the transfer of electrons generated from the enzyme reaction process to the surface of the electrode, so that the electrodes can generate corresponding molecular electric conductors. The most basic requirement for mediator is low redox potential and high electrochemical reaction rate, and the reaction is reversible [27]. Cyclic voltammetry is usually used to investigate the electrochemical properties of the mediator.

The electron transfer mechanism of glucose oxidase catalyzed by glucose is acted as an example and described as the following:



Media has been developing rapidly in the past 10 years, the species is also increasing, and at present, the electron transfer mediator according to the fixed method is divided into three types [28, 29] (1) the simple electron transfer medium, (2) the electron transfer mediator covalent bond with enzyme, and (3) the electron transfer medium covalent bond with the polymer.

(1) Simple electron transfer medium

A simple electron transfer medium refers to the existence of an electron transfer medium in the form of a monomer in the application, including the oxidation–reduction pair and the organic conductor salt. Ferrocene and its derivatives [30–33], and quinone and its derivatives [34], are widely used as electron transfer mediators. The glucose biosensor was constructed by Lange [30], using a glassy carbon electrode as the substrate, and immobilized on the electrode with glucose oxidase, ferrocene, and polyamide gel. The sensor response principle is that GOD (FAD) catalyzes the oxidation of glucose and itself is reduced to GOD(FADH₂), the GOD (FADH₂) was oxidized by ferrate into GOD(FAD), and reduced ferrocene was oxidized into high iron state on electrode. The oxidation current produced by the oxidation of two ferrocenes on the electrode is related to the concentration of glucose. Two ferrocenes here have the electron transfer between the GOD(FADH₂) and the electrode.

However, the simple electron transfer mediator acts as effective electron transfer between enzyme redox active center and electrode, overcomes the limitations of O₂ or H₂O electrode in direct electrochemical reduction or oxidation, and greatly improves the biosensor performance in response to speed, detection sensitivity, and anti-interference ability. However, simple electron transfer mediators have encountered some difficulties in practical applications, such as dissolution or partial dissolution of mediators, diffusion of mediators away from electrode surface, and so on. Because of the loss of mediators, the stability and service life of biosensors are affected, which limits the clinical application of such biosensors as subcutaneous probes and for long time online analysis. However, the simple electron transfer medium has an irreplaceable advantage; that is, the electron transfer speed is fast.

(2) Electron transfer mediator covalent bond with enzyme

In order to prevent the loss of the electron transfer medium from the electrode surface due to dissolving and other reasons, the electron transfer medium can be chemically bonded to the enzyme. Bonding can not only better fix the electron transfer mediator but also make the redox active center of the enzyme more closely contact with the electron transfer mediator, which is more conducive to the electron transfer [35–40]. For example, Degani and Heller [41, 42] have successfully made glucose biosensors, which are chemically bonded to glucose oxidase by electron transfer mediator ferrocene derivative and ammonia ruthenium. The electron transfer mediator is chemically bonded with enzyme, which successfully prevents the loss of electron transport mediator, improves the stability of biosensor, and prolongs the service life. However, the catalytic activity of the enzyme is reduced after chemical modification, which will also reduce the sensitivity of the biosensor [43].

(3) The electron transfer medium covalent bond with the polymer

In recent years, great progress has been made in the research of bonding of electron transfer mediator and suitable polymer in order to prevent the loss of electron mediator and overcome the shortcomings of reducing enzyme activity after mediator and enzyme binding. A more typical example is the bonding of ferrocene and its derivatives to a siloxane polymer. A new type of ferrocene-modified siloxane polymer was synthesized by Hale [44]. The glutamate biosensor was prepared by combining this polymer with glutamate oxidase. Niwa [45] et al.

made a complex sensor of horseradish peroxidase and glutamate oxidase based on the complexes of polypyridine and osmium. The sensor has the characteristics of fast response, strong anti-interference ability, large current response, and high stability. The electrochemical behavior of the cations, such as Os(bpy)₂PVPCl, is also proposed by Han [46]. In addition to the electron transfer mediator bonded to the siloxane polymer with ethylene oxide, silicon can also be bonded to the polymer polypyrrole and polypyridine. The electron transfer mediator covalently bonded to a polymer medium, insoluble in water; it is not easy to spread out the surface of the electrode. The biosensor made of it avoids the pollution of the sample solution and improves the stability of biosensor by prolonging the life of biosensor.

2.2.3. The third-generation enzyme electrode (direct enzyme electrode)

The biosensor performance of the third-generation enzyme electrode is realized by the direct electrocatalysis of the enzyme on the electrode. It is named as a non-reagent sensor. It takes advantage of the direct electron transfer between enzyme and electrode and does not need to add other media reagents to reduce operation steps. It is a real reagent-free biosensor. Glucose oxidase is used as an example to illustrate the reaction mechanism on enzyme film and electrode separately:



The implementation of the enzyme's direct electrochemistry is of great significance for the development of a non-media enzyme sensor. Because the enzyme molecule belongs to protein and has large molecular weight, the active center of enzyme molecule is deeply embedded in the interior of the molecule, and it is easy to deform after adsorption on the electrode surface. Therefore, it is difficult to directly transfer electrons between enzyme and electrode. Theoretically, the direct electron transfer process between enzyme and electrode is closer to the original model of biological redox system, which lays the foundation for revealing the mechanism of biological redox process. Therefore, although the study of this direction is very difficult, it is still the direction of development in this field. In addition, the combination of nano-modification technology, biological simulation, and molecular imprinting technology can provide power for the development of direct electrochemical measurement [47].

2.3. Fabrication of amperometric enzyme biosensor and POCT detection

POCT detector is usually combined with biosensors. A biosensor coupled to a specific biological detector (such as enzyme, antibody, or nucleic acid probe) to a transducer is used for direct determination of target analytes without separation from matrix. It embodies the combination of enzyme chemistry, immunochemistry, electrochemistry, and computer technology. It can be used to carry out ultramicroanalysis of the analyte in the organism's fluid.

At present, more and more factories in the world are developing POCT devices [48]. The main products are hematology analyzer, electrolysis analyzer, and blood gas analyzer, and the analytical performance of some selected commercial POCT devices was shown in **Table 1**.

Manufacturer	System	Target analytes	Sample volume (μL)
Bayer	RAPIDLab 800	pO_2 , pCO_2 , pH, electrolytes, glucose, lactate	140–175
Nova Biomedical	Xpress, Nova 16	Blood gases, electrolytes, glucose, lactate, urea nitrogen, creatinine, hematocrit	85–190
Radiometer	ABL 725 ABL 77	pO_2 , pCO_2 , pH, Na^+ , K^+ , Ca^{2+} , glucose, lactate	80–135
Instrumentation Laboratory	Synthesis 1745 GEM Premier 3000	pO_2 , pCO_2 , pH, Na^+ , K^+ , Ca^{2+} , Hct, glucose, lactate	135
Abbott Diagnostics	i-STAT	pO_2 , pCO_2 , pH, electrolytes, Hct, urea nitrogen, glucose, lactate, creatinine	65–95
Agilent Technologies	IRMA	pO_2 , pCO_2 , pH, electrolytes, Hct, glucose, lactate	125
Roche	OMNI 9	pO_2 , pCO_2 , pH, electrolytes, Hct, Hb, urea nitrogen, glucose, lactate, creatinine	40–161
Yellow Springs Instruments	2300 Stat Plus	Glucose, lactate	120

Electrolytes: Na^+ , K^+ , Ca^{2+} , Cl^- .

Table 1. Electrochemical sensors in commercial systems for critical care and POCT.

There are two kinds of handheld and portable products. Handheld products (**Figures 5, 6**) are small in size, such as mobile phones, automatic calibration, and less blood collection. Analysis items can be preset, records can be stored, maintenance is free, and quality control procedures are completed in 1 min. The practical significance of such products is that they can be simplified and routinely used for blood analysis, such as body temperature and blood pressure, under the premise of clinical diagnosis and treatment.

Blood glucose monitoring is the most common application for enzyme biosensor. The design and structure of a disposable electrochemical enzyme sensor, as well as the use of the media, are determined by the concentration and sampling method of the analytes. Cost and convenience must be taken into account in structural design. Therefore, the most commonly used single parameter amperometric enzyme sensor is a double-electrode system or a tri-electrode system.

2.3.1. The fabrication of disposable biosensor with dual electrodes modified by ferrocene

The electrode arrays (**Figure 6**) were fabricated with the vacuum sputtering technology through sputtering gold on polycarbonate (PC) plastic substrate material. The two-electrode system was made up of a working electrode (1×2 mm) and a pseudo-reference/counter electrode (1×1 mm). The channel region where reaction took place and bonding pads were defined by two-piece insulating plastic layer. The volume of capillary-fill region channel was $3 \mu\text{L}$. The gold working and counter electrodes were deposited to form nanoporous platinum layer. The electrodes were then activated by the method of plasma cleaning, and significant improvements of hydrophilicity and electroactivity were achieved by the simple treating. $3 \mu\text{L}$ of the reagent (containing enzyme, potassium ferricyanide, BSA, and 0.1% triton) was added

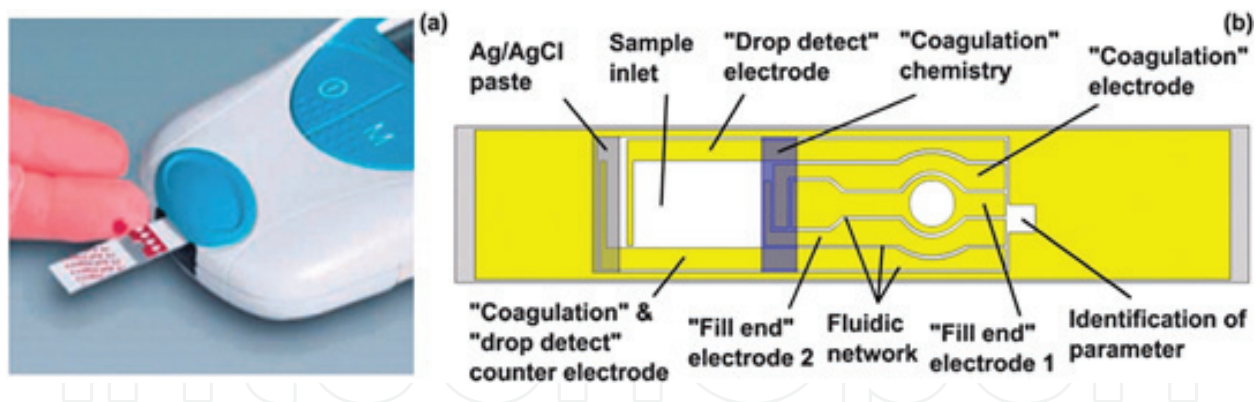


Figure 5. The biosensor and handheld POCT device for blood coagulation test. (a) Handheld POCT meter. (b) the disposable biosensor for blood coagulation with the design of the microfluidic structure and four electrodes by electrochemical detection (from [49, 50]).

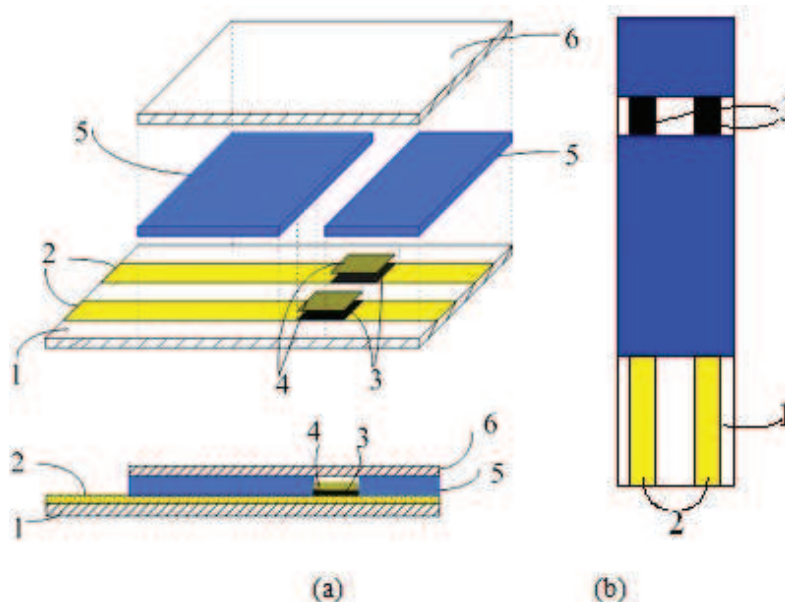


Figure 6. Schematic diagram of disposable biosensor with dual electrodes. Three-dimensional structure schematic diagram. (b) Planar graph of the biosensor. (1) The plastic substrate, (2) lead, (3) the reaction region modified by Pt-black, (4) dry reagent, (5) insulating double-sided adhesive, and (6) the cover layer (from [51]).

on the channel region of the electrodes and dried at 25°C. After the reagent was immobilized on the capillary-fill channel, a covering sheet was coated on the insulation layer to prevent the electrode from staining. The dry strip/biosensor was stored at room temperature. During the experiments, a potential of 0.2 V vs. reference/counter electrode was employed. 3 µL serum doses were added on the biosensor through capillary effect. The whole blood samples were determined with biosensors combined with handheld test meter.

The two-electrode system biosensor is suitable for detection of analytes with mM concentration, which is simple in preparation, simple in driving system, and low in cost. Many of the blood glucose and lactate biosensors in commercial systems adopt the two-electrode design.

2.3.2. The fabrication of biosensor with tri-electrodes modified by Os polymer mediator

The electrode structure of the sensor (**Figure 7**) is different for different parameters and analytes. For the biosensors used for the detection of the analytes with low concentration, the classical tri-electrode structure is adopted.

The electrode arrays were fabricated with the vacuum sputtering technology through sputtering gold on polycarbonate (PC) substrate material. Each three-electrode system was made up of a working electrode with surface of 3 mm^2 , a counter electrode, and a reference electrode with surface of 6 mm^2 . The channel region where reaction took place and bonding pads were defined by two-piece insulating plastic layer. Nanoporous platinum layer was deposited on the gold working and counter electrodes. The gold electrodes were first cleaned with plasma cleaner and then were electroplated in a solution containing H_2PtCl_6 (hexachloroplatinate); then, the electrodes were washed with distilled water and dried in desiccator. The $\text{Ag}|\text{AgCl}$ layer was electrochemically formed on the reference electrode. On the surface of the freshly prepared silver electrode, silver chloride coating was formed by anodizing for 30 s in 0.1 M HCl solution using 10 mA/cm^2 current density. The electrodes were then activated by the method of plasma cleaning, and significant improvements of hydrophilicity and electroactivity were achieved. Then $1 \text{ }\mu\text{L}$ PVP-Os-HRP redox polymer was added on the surface of the working electrodes, and the polymer was allowed to dry overnight under ambient conditions. $3 \text{ }\mu\text{L}$ of the enzyme reagent was added on the channel region of the electrodes and dried at 25°C . After the reagent was immobilized on the capillary-fill channel, a covering sheet was coated on the insulation layer to prevent the biosensors from staining. Finally, the electrode arrays were cut in pieces, and dry biosensors were stored at 4°C .

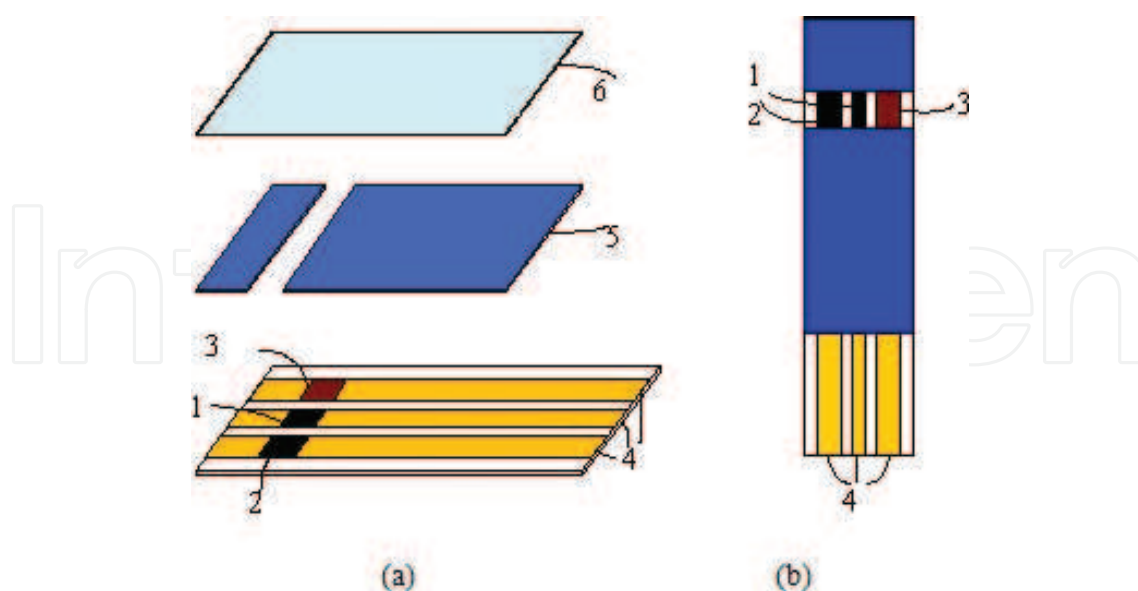


Figure 7. Schematic diagram of disposable biosensor with tri-electrodes. (a) Three-dimensional structure schematic diagram. (b) Planar graph of the biosensor. (1) Working electrode, (2) counter electrode, (3) $\text{Ag}|\text{AgCl}$ reference electrode, (4) lead line, (5) insulating double-sided adhesive, and (6) the cover layer (from [52, 53]).

During the amperometric experiments, a potential of 0.1 V vs. integrated Ag|AgCl reference electrode was employed. The dry reagent biosensor combined with handheld meter was used to determine analytes in blood. The osmium redox polymer medium covalently combined on the electrode reduces the working potential of the sensor and reduces the interference of other active substances.

The disposable biosensors have the advantages of easy handling, quick test in several minutes, friendly to untrained users. In order to realize multiparameter detection and more accurate measurement, more complex biosensors are designed and developed. The biosensors designed by i-STAT (Figure 8) have prestorage of two kinds of reagents (dry and liquid), using the airbag driving mode, which further improves the precision and linearity of the detection.

2.4. Continuous real-time in vivo monitoring

Physiological diseases and various physiological activities of human are often identified as biochemical substances. So far, the determination of these identification substances is mainly in vitro. In general, in vitro determination can satisfy the diagnosis of the disease and the basic judgment of some physiological states [55]. However, the monitoring and treatment of some sudden and severe diseases are best to be monitored in real time. Therefore, the measurement

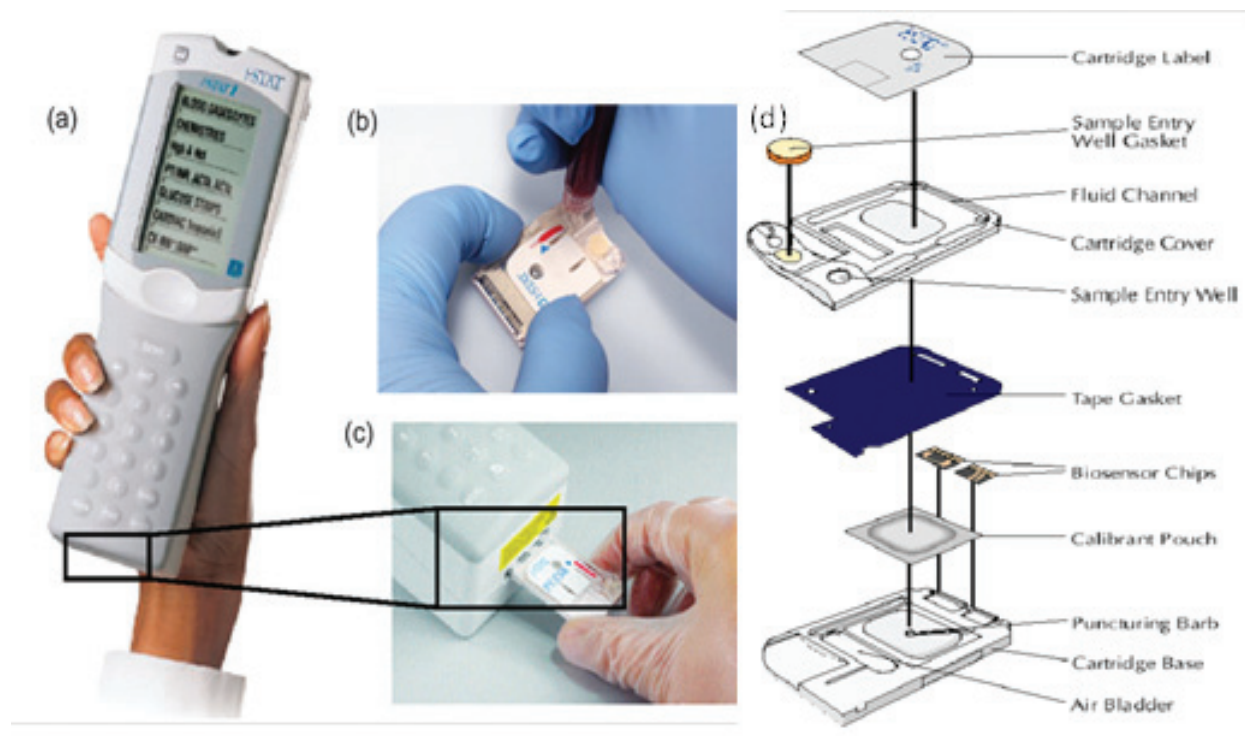


Figure 8. The integrated biosensor and handheld reader of i-STAT analyzer. (a) the portable i-STAT analyzer. (b) the disposable biosensor with integrated structure; 65–95 μL whole blood sample is filled by capillary forces and pressure force. (c) the biosensor is loaded into the meter, and the samples is pushed by pressure and capillary forces for electrochemical detection. (d) the fabrication of the biosensor (from [50, 54]).

of in vivo in the biosensor has its special significance both in the clinical monitoring of the patients and in the basic physiological research [56–58].

The internal environment is very complex, not as easy to control as in vitro environment. After implantation of biosensor, it is important not only to ensure normal performance parameters of biosensors but also to ensure that implanted sensor systems cannot pose any danger to patients or subjects. Therefore, we should not only face some special technical problems but also follow strict regulations on medical instrument management and obtain experimental licenses. Biosensor for in vivo monitoring needs to be implanted into the body (such as blood vessels, cerebral cortex, subcutaneous tissue) and faces many complex environments, including the tissue response, biocompatibility, disinfection problems, oxygen interference, and other technical issues.

Many companies and research institutes carry out the research on in vivo sensor system in recent years. Several glucose sensor systems that continuously monitor the blood glucose level under the subcutaneous have been commercially developed and applied. Nowadays, these blood glucose sensors belong to this kind of “percutaneous,” which directly pierce the fine-needle sensor into the skin, and the base and data part are fixed on the skin. The CGMS probe sensor from Medtronic company has three layers containing the semipermeable membrane, glucose oxidase, and platinum electrode [59]. Among them, the platinum electrode of the Medtronic probe is the tri-electrode probe system, which can reduce the interference of the impurity to the current signal. The CGMS probe was implanted subcutaneously for 3 days in the patient’s abdomen (**Figure 9**). The probe generates electrical signals by chemical reaction with glucose in the subcutaneous intercellular fluid of the patient. The electrical signal is proportional to the concentration of blood sugar, but it is delayed for about 20 minutes. The Abbott freestyle continuous glucose monitoring system contains implantable needle biosensor and wireless reading device. The implantable needle biosensor adopted the same principal with the size of length 5 and 0.4 mm diameter on a coin-sized sticker (**Figure 10**), long term for 14 days sticking on the upper arm [60]. When we want to check blood sugar, the blood glucose meter is close to the circle sticker, which is accurate and convenient. The Eversense Continuous Glucose Monitoring (CGM) developed by Senseonics medical company has been approved by



Figure 9. The Medtronic needle sensor and reader.



Figure 10. The Abbott continuous glucose monitoring sensor.

the EU management department for CE. The Eversense system implanted a needle-type sensor subcutaneously into the upper arm of the patient for a period of 90 days [61].

The new sensing technology improves the value of biological analysis and improves the level of diagnosis and monitoring of disease. These smaller, more easy-to-use new devices not only broaden the scope of diagnosis but also provide more effective methods for monitoring special conditions.

3. Conclusion

Biosensor has the characteristics of cross disciplinary integration. The development trends of biosensors are miniaturization, multifunction, integration, and intelligence. The introduction of some new frontier technology such as microelectronics, nanotechnology, and microelectromechanical technology (Microelectronic Mechanism) is applied to the biomedical sensor to develop a new generation of biosensor with low cost, high sensitivity, high reliability, high life and bionic function, achieving rapid and accurate test of trace components in the sample. At the same time, the biomedical sensor has developed from planar two-dimensional to three-dimensional microelectronic mechanical system sensors. With the development of computer software and hardware technology, sensor technology will also develop, and the biological system of closed-loop control will be possible. In the future, biosensors will be widely used in many fields, such as food detection, medical care, disease diagnosis, environmental monitoring, fermentation industry, and so on.

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