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Review Article

Biosensors in Endocrinology- Review Article

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Abstract

Biosensors are classes of sensors in which at least a biological process is used in sensing procedure. They are generally composed of three parts: a sensing element, a transducer, and a signal processor (or detector). They can be categorized by type of sensing materials or by detection techniques. From their invention time up to now, various biological species have been analyzed using variety of biosensors. They have been widely used for environmental, industrial, pharmaceutical and clinical applications in many research papers. Perhaps the number of biosensors which had a chance to commercialize and enter to the market is limited, but by recent developments in science and technology, day-by-day, the number of commercial biosensors are growing. Their importance in clinical medicine can be found in determination of biomarkers for early diagnosis of disease or for control and manage of them in point-of-care devices. Diagnosis and control of many endocrine diseases and metabolic disorders depend strongly on determination of chemicals, hormones and antibodies. A large number of biosensors research studies have focused on determination of these biomolecules. One of the famous commercial biosensor is widely used in management of diabetes is glucometer. They are portable commercial biosensors which measure the amount of glucose in a blood drop. The main challenges in designing biosensors are decrease the limit of detection, increasing the sensitivity and accuracy in an analysis, increasing lifetime and miniaturization. Even scientists are now trying to develop biosensors for non-invasive measurements of biomarkers in saliva or tears.

Keywords: Biosensor, Endocrinology, Point-of-care device, Diabetes, Glucometer

Introduction

Chemical sensors are the most rapidly growing class of sensors in analytical chemistry. A chemical sensor is a device which can produce continuous information about specific chemical properties of its environment. In fact, it can convert a chemical signal to an analytical useful one which then is read or record by an instrument. An ideal sensor produces a certain type of signal which directly corresponds to the concentration of a specific molecule. In general, chemical sensors compose of three components connected in series: a sensing element, a physicochemical transducer and a signal processor (or detector) as shown in Fig. 1. According to the IUPAC definitions (1), biosensors are chemical sensors that use a biological reaction to produce a signal. Like sensors (Fig. 1)

they are composed of three parts. They can be classified according to the type of sensing materials used in sensing process or detection techniques used for producing the signals.

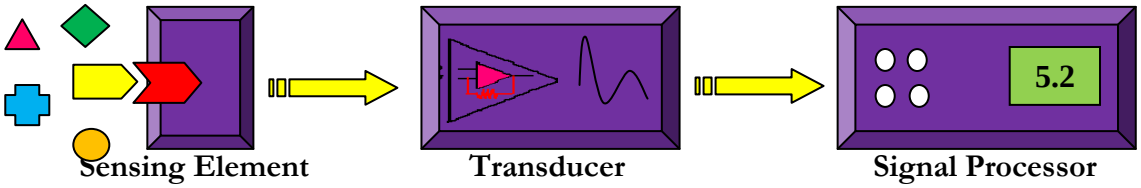

Fig. 1: Schematic diagram of a sensor/biosensor system

Enzymes, antibodies (or antigens), aptamers, DNA sequence, organelles, tissues, whole cells or even microorganisms or bacteria can be used as receptors in the sensing elements of biosensors.

In construction of each biosensor immobilization of the biological elements (small molecules/protein/DNA/even whole cells or microorganism) on to the surface of the electrode (it can be Nobel metals like gold or platin, graphite, polymers) is the most important step. It should be noted that the function of the biomolecules should be kept unchanged after its attachment. Various approaches have been used for the attachment of the biomolecules to the transducer properly. The general methods for this purpose are adsorption, microencapsulation, entrapment, cross-linking, and covalent bonding.

Electrochemical, optical, thermal and mass signals can be applied as detection technique in designing biosensors. In optical biosensors, the output transduced signal is light. The biosensors in this group can be designed based on optical diffraction, fluorescence, chemiluminescence, electro-chemiluminescence phenomena. There are some optical biosensors which works based on the phenomenon of surface plasmon resonance. In this technique a thin layer of gold on a high refractive index glass surface absorb laser light and producing electron waves (surface plasmons) on the gold surface. This phenomenon occurs just at a specific angle and wavelength of the incident light. It depends highly on the surface of the gold. Thus, binding of a target analyte to its receptor immobilized on the gold surface produces an analytical signal.

Transduction of the biological signal into an electrical signal can be done by voltammetry, potentiometry and conductometry. Electrochemical biosensors are generally based on enzymatic catalysis of a reaction which produces or consumes electrons (redox enzymes). Because of the considerable simplicity, ease of signal production, miniaturization ability and low cost, electrochemical biosensors receive more attraction in comparison with optical, mass and thermal ones.

Mass and thermal biosensors are known as physical biosensors, they sensitive to the thermal changes or mass changes through a bioreaction. Piezoelectric biosensors are a kind of mass biosensors. They use crystals which undergo an elastic deformation after an electrical potential is applied to them. Alternating potentials can produce standing waves in the crystal at a characteristic frequency. The frequency depends on the elastic properties of the crystal. If a crystal is coated by a biological recognition element, the binding of a large target analyte to its receptor can cause a change in the resonance frequency, and produce an analytical signal. This is a specialized application of the Quartz crystal microbalance works like the mentioned mechanism.

The thermal biosensors work based on absorption or production of heat. Changes in the temperature of the medium in which the reaction takes place produce an analytical signal. They compose of the combination of the immobilized enzyme and temperature sensors (thermistors). After the analyte bind to the enzyme, the heat reaction of the enzyme interaction is measured by thermal sensor and is calibrated by the analyte concentration. The

total heat produced or absorbed in the reaction is proportional to the molar enthalpy and the total number of molecules in the reaction.

From biosensor invention time up to now, various biological species have been analyzed using variety of the biosensors. They have been widely used for environmental, industrial, pharmaceutical and clinical applications in many research papers (2-15). The advantages of biosensors in comparison with other sensitive instrumental techniques are their ability to detect small amounts of analyte, accuracy, easy to use, fast response, cost effectiveness, portability, on-line monitoring, and continuous monitoring.

Since this review article is going to open a new window in clinical medicine for interdisciplinary research studies and its readers are clinical medicine researchers, the experimental and technical concepts in designing biosensors has not been discussed. It is mainly focused on advances of biosensors in diagnosis of endocrine disease and future perspectives.

Biosensors in Clinical Medicine

Biosensor importance in clinical medicine can be found in determination of many biomarkers for early diagnosis of disease or for self-control and manage of them in point-of-care devices (16-23). Today, for rapid diagnosis of some diseases or self-controlling of them by patients biosensors have entered in clinical managements. Clinicians need rapid, cheap, and low-tech devices which operate easily by technicians or patients.

Biosensors in Endocrinology

One of the wide groups of non-communicable diseases is endocrine and metabolic disorder. Endocrine disorders are involving a mixed picture of hyposecretion and hypersecretion because of the feedback mechanisms involved in the endocrine system. Thus, they are complex. For example, most forms of hyperthyroidism are associated with an excess of thyroid hormone and a low level of thyroid stimulating hormone. Diagnosis and control of many endocrine diseases and metabolic disorders depend strongly on determination of chemicals, hormones and antibodies. A large number of biosensors research studies have focused on determination of these important biomolecules.

As an example, an electrochemical biosensor was reported for the sensitive determination of thyroid stimulating hormone (TSH) (24). Microelectromechanical systems (MEMS) technology was used as transducers and TSH protein was detected by antibodies labeled by enzyme linked immunosorbent assay. The TSH concentration could be calculated according to the electrical conductance of interdigitated electrodes. TSH concentrations from 0.02 to 100 mIU/L can be determined by the designed electrochemical biosensor.

Also, there are some reports on cortisol (25), growth hormones (26, 27), sex hormones (28-31), human chorionic gonadotropin (32) biosensors.

Besides the above mentioned hormones, determination of insulin is also of great importance in endocrinology. Insulin is a polypeptide hormone which is secreted by pancreatic cells. It plays a key role in regulation of glucose homeostasis. In type I diabetes its deficiency is observed and in type II diabetes, obesity and some metabolic disorders resistance to insulin can be seen. Thus, its determination can be important in clinical diagnosis or control of diabetes. Recently, Xu et al. have reported an ultrasensitive electrochemical and label-free biosensor for insulin determination in blood serum with a detection limit of 1.2 pM. The transducing surfaces, based on readily prepared, antibody modified, polyethylene glycol monolayer modified polycrystalline gold surfaces, respond in a highly specific and re-useable manner to the target in up to 50% blood serum insulin (33).

Glucose is one of the most important biochemical compounds that monitoring its concentration is very important in diagnosis and management of diabetes (34-37).

One of the famous commercial biosensor is widely used in management of diabetes is glucometer. They are portable commercial biosensors which measure the amount of glucose in a blood drop.

There are some reports on determination of other chemical compounds which are valuable in diagnosis of endocrine disease or metabolism disor-

ders including cholesterol (38-42), and triglyceride (43-48).
In addition, some studies reported biosensors for determination of endocrine disruptors such as phenolic compounds (49-51).
Neurotransmitters are signaling biomolecules which secrete from neurons and endocrine cells. In a recent study, the use of biosensor cells to detect neurotransmitter release from endocrine cells in real-time has been describes (52). The muscarinic acetylcholine (ACh) receptors M3 from Chinese hamster ovary cells were used as ACh biosensors to record ACh release from human pancreatic islets. The ACh biosensor was loaded with the Ca^{2+} indicator Fura-2 and pressed against isolated human pancreatic islets and makes the detection of ACh release possible. In fact, the Ca^{2+} signal generated in the biosensor cell reveals the release of a neurotransmitter. There are other reports on acetylcholine biosensors (53-56).
Bone turnover markers are useful biomarkers in understanding bone physiology and assessing the action of medications. They can be also used to assess fracture risk and to determine the response of bone to treatment. Bone turnover markers actually reflect the metabolic activity of bone during the modeling and remolding phases. In a study by Yun et al. biosensor based on label-free immunosensing for the detection of the C-terminal telopeptide bone turnover marker from type-1 collagen has been reported (57). In this study, a self-assembled monolayer of dithiodipropionic acid was deposited on a gold electrode. Then streptavidin and biotinylated anti-human C-terminal telopeptide antibody were conjugated on the self-assembled monolayer. Electrochemical impedance spectroscopy with DC bias was used as detection technique and a detection limit of 50 ng/mL was obtained.

Marketing of biosensors

Perhaps the number of biosensors which had a chance to commercialize and enter to the market is limited, but by recent developments in science and technology, day-by-day, the number of biosensors comes from the bench of research laboratory to the market are growing.
The main points should be considered for a biosensor to be commercialized are ability to identify a target molecule, availability and price of a suitable biological recognition element, shelf-life, the potential for disposable portable detection systems, the whole cost of the package, simplicity of the usage. One of the high consuming biosensor which is widely available in drugstores is pregnancy test device. It detects hCG protein in urine. Interpretation and data analysis in this qualitative sensor can perform fast and easily by the user.
A biosensor should have the following properties which can be commercialized:

- Having high accuracy and precision
- Having small size to be portable
- Having low cost for mass production
- Having self calibrating
- Having user friendly operation system
- Having wide applications

Commercial Available Biosensors in Endocrinology

Some biosensors have been commercialized and are available in the market to help diagnosis or manage the endocrine disease. One of the important examples of using commercialized biosensors is glucometers which are used in diabetes by patients. Diagnosis, screening, long-term management of patients with diabetes needs glucose monitoring.
Diabetes mellitus is the most common endocrine disorder which deals with carbohydrate metabolism. In long term diabetes causes morbidity and mortality and also a major health problem for most countries. Unfortunately, the prevalence of diabetes is increasing yearly very fast. World Health Organization (WHO) has declared the number of persons with diabetes worldwide in 2000 are approximately 171 million, and it will increase to 366 million by 2030 (58). Blood glucose monitoring is a valuable biomarker in the management and control of diabetes. Patients need to check the level of blood glucose concentrations one to six times a day to manage their diabetes. Thus, glucose biosensors can play a essential roles in life of millions of people.

Variety of glucose biosensors have been developed from construction of the first glucose biosensor by Leland C. Clark (59) up to now. The Clark glucose biosensor worked based on a platinum (Pt) electrode and detect oxygen. Glucose oxidase (GOD) was placed very close to the surface of the platinum electrode by physically trapping with a piece of dialysis membrane. The enzyme activity changes the surrounding oxygen concentration. During the past 50 years, glucose biosensor technology developed very fast till point-of-care devices, continuous glucose monitoring systems and non-invasive glucose monitoring systems are constructed.

Glucometers generally use the glucose oxidase (or dehydrogenase) enzyme to measure the blood glucose content. The available devices can work based on optical or electrochemical detection systems. First-generation glucose biosensors worked based on the use of natural oxygen substrate and detection of the produced hydrogen peroxide (60). Main problem in the first-generation of glucose biosensors was that to have a high selectivity, the amperometric measurement of hydrogen peroxide should be done in a high operation potential.

This problem was solved by using mediated glucose biosensors, called second-generation glucose biosensors. In this type non-physiological electron acceptors (as redox mediators) was used that were able to carry electrons from the enzyme to the surface of the working electrode (61). A reduced mediator is formed instead of hydrogen peroxide. It was then reoxidized at the electrode surface to produce the signal and regenerate the oxidized form of the mediator. A variety of electron mediators, such as ferrocene, ferricyanide, quinines, tetrathialfulvalene, tetracyanoquinodimethane, thionine, methylene blue, and methyl viologen were used to improve biosensor performance (61). After that, third-generation glucose biosensors were introduced. They were reagentless biosensors and worked based on direct electron transfer between the enzyme and the electrode surface without mediators. Instead of mediators with high toxicity, organic conducting materials based on charge-transfer complexes were used. Hence, third-generation glucose biosensors were able to be used in implantable, needle-type devices for continuous in vivo monitoring of blood glucose (61). Moreover the absence of mediators provides the biosensors with superior selectivity.

Future Perspective

The main challenges in designing biosensors are decrease the limit of detection, increasing the sensitivity and accuracy in an analysis, increasing lifetime and miniaturization. Even scientists are now trying to develop biosensors for non-invasive measurements of biomarkers in other fluids of the body.

Non-Invasive biosensors

Non-invasive biosensors are the devices which are able to determine the analytes or metabolites in other fluids of the living organism other than blood without hurting it. Such biosensors are important in the life of patients who have to control the important biomolecules like glucose or urea daily.

Commercial available glucose biosensors (glucometers) which are used as point-of-care devices needs a drop of blood from the finger. Long-term using the lancet of the glucometers in diabetic people makes it a painful job. Pain-free determination of blood glucose can improve the life quality of diabetes patients, and help them to better control the disease and keeping them away from physiological complications.

Determination of glucose using other fluidic of the body such as a sample of tears or saliva is a new and interesting topic in non-invasive methods (62-67).

A contact lens based biosensor for in situ monitoring of tear glucose was constructed and tested (67). Biocompatible 2-methacryloyloxyethyl phosphorylcholine polymer and polydimethyl siloxane were used as the matrix material. A flexible Pt working electrode and a Ag/AgCl reference/counter electrode, which were formed by micro-electro-mechanical systems (MEMS) technique was used in this biosensor. The electrode at the sensing region was modified with glucose oxidase. The proposed biosensor showed a good relationship between the output current and glucose

concentration in a range of 0.03-5.0 mM, with a correlation coefficient of 0.999.

Noninvasive glucose monitoring using Wavelength-Modulated Differential Laser Photothermal Radiometry (WM-DPTR) in human skin in vitro in the mid-infrared range has been reported (68). Glucose measurements in human blood serum diffused into a human skin sample (1 mm thickness from abdomen) in the physiological range of 21-400 mg/dl showed high sensitivity and accuracy for use in wide clinical detection requirements.

Continues glucose monitoring systems would offer an improved control of diabetes in providing real-time data of an internal insulin release system. Two types of continuous glucose monitoring systems are currently available a blood glucose monitoring and subcutaneous glucose monitoring (61). Because of the surface contamination of the electrode by proteins and coagulation factors and the risk of thromboembolism, most of these systems do not measure blood glucose directly. Therefore, subcutaneously implantable needle-type electrodes measuring glucose concentrations in interstitial fluid have been developed, which reflect the blood glucose level (61).

One of the examples of these continuous systems was GlucoWatch Biographer, manufactured by Cygnus, Inc. (Redwood City, CA, USA). It was the first transdermal glucose sensor approved by the US FDA. This watch-like device was based on transdermal extraction of interstitial fluid by reverse iontophoresis. Although the idea was an innovation, it never widely accepted in the market because of the long warm up time, false alarm, inaccuracy, skin irritation and sweating. It was withdrawn in 2008 (61).

Since some studies also showed the correlation between blood glucose level and saliva glucose, extensive efforts have been made in the development of non-invasive glucose devices using saliva and tears (69). Tears and saliva attracted more interests among the other fluids. However, the concentration of glucose in these fluids is too low to be measured by the common glucometers.

Beside the glucose monitoring, determination of cortisol in saliva is another interesting field of biosensor research in endocrinology. There are some reports on determination of saliva cortisol with a flow-filtered, portable surface plasmon resonance biosensor system (70), chemiluminescence biosensor (71), immunosensor (72).

Application of nanotechnology in Biosensors

The main challenge in designing of more sensitive non-invasive biosensors is finding a way to lower the limit of detection and increase the signal to noise ratio. An increase of the signal-to-noise ratio is still required for all non-invasive devices.

Recent advances in nanotechnology and nanomaterials cause remarkable developments in designing sensor and biosensor and solve the above problems. After finding the properties of nanomaterials in biosensor systems, various nanostructures were used in construction of the chemical biosensors. During recent years, many nanostructures such as quantum dots, nanoparticles, nanocantilever, nanowire and nanotube are used in construction of biosensors (73-77).

One of the challenges in designing future glucose biosensor seems to be miniaturization for implantation purposes. Development of the miniaturized biosensors that can be implanted to improve the patient compliance and quality of life can be possible by nanotechnology.

Conclusion

Biosensors are analytical useful devices for fast, portable and simple detection of biomolecules. Now days, various biological species have been analyzed using variety of biosensors. They have been widely used for environmental, industrial, pharmaceutical and clinical applications in many research papers. Some of the biosensors find a chance to commercialize and enter to the market. Recent advances in science and technology, causes the number of commercial biosensors are growing. Glucometers which determine glucose concentration in blood is one the widely used biosensor in diabetes. Diagnosis and control of many other endocrine diseases and metabolic disorders de-

pend strongly on determination of chemicals, hormones and antibodies. A large number of biosensors research studies have focused on determination of these biomolecules. The main challenges in designing new biosensors are decreasing the limit of detection, increasing the sensitivity and accuracy in an analysis, increasing lifetime and miniaturization. Even scientists are now trying to develop biosensors for non-invasive measurements of biomarkers in saliva or tears. Using different aspect of nanotechnology in immobilizing the bioreceptors on the surface of the electrodes, these goals have been achievable.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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