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Integration of biosensors and drug delivery technologies for biomedical applications

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﴿وَلَقَدْ آتَيْنَا دَاوُودَ وَسُلَيْمَانَ عِلْمًا وَقَالَا الْحَمْدُ لِلَّهِ الَّذِي فَضَّلَنَا عَلَى كَثِيرٍ مِنْ عِبَادِهِ الْمُؤْمِنِينَ﴾

صدق الله العظيم

سورة النمل الآية (15)

Dedication

This study is whole-heartedly dedicated to our beloved parents , who have been our source of inspiration and gave us strength when we thought of giving up , who continually provide their moral , spiritual , emotional , and financial support .

To our brothers , sisters , relatives , mentor , friends , and classmates who shared their words of advice and encouragement to finish this study .

The researchers

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The researchers

Abstract

Biosensor-integrated drug delivery systems are innovative devices in the health area, enabling continuous monitoring and drug administration. The use of smart polymer, bioMEMS, and electrochemical sensors have been extensively studied for these systems, especially for chronic diseases such as diabetes mellitus, cancer and cardiovascular diseases as well as advances in regenerative medicine. Basically, the technology involves sensors designed for the continuous analysis of biological molecules followed by drug release in response to specific signals. The advantages include high sensitivity and fast drug release. In this work, the main advances of biosensor-integrated drug delivery systems as new biomedical materials to improve the patients' quality of life with chronic diseases are discussed.

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List of Abbreviations

Abbreviations	Meaning
Ache	Acetylcholinesterase
AP	Alkaline phosphatase
BioMEMS	Bio-micro-electro-mechanical systems
BSA	Bovine serum albumin
ChEt	cholesterol esterase
ChOx	Cholesterol oxidase
CEA	Carcinoembryonic antigen
CRP	C- reactive protein
CVD	cardiovascular diseases
DNA	Deoxyribonucleic Acid
dsDNA	double-stranded Deoxyribonucleic Acid
EDTA	Ethylenediaminetetracitic acid
ELISA	Enzyme-Linked Immunosorbent Assay
EPA	Environmental Protection Agency
HPV	Human Papilloma Virus
IGG	Immunoglobulin G
IGM	Immunoglobulin M
GLDH	glutamic dehydrogenase
Omp85	Outer-membrane protein of 85 kDa
QCM	Quartz crystal microbalance
ROS	reactive oxygen species
ssDNA	Single-stranded Deoxyribonucleic Acid
SPR	surface plasmon resonance
SELEX	Systemic evolution of ligands by exponential enrichment

Chapter One

1.1. Introduction

Biosensor-integrated drug delivery systems have been extensively studied, especially for the treatment of chronic diseases such as cardiovascular diseases (CVD), diabetes mellitus, and cancer, where regular drug administration and continuous monitoring are relevant . The conventional modes of treatment have been associated with serious side effects; thus, over the years, controlled drug delivery systems have been explored as a promising alternative to improve the efficacy and safety by optimizing the duration and kinetics of release .

Biosensors are analytical devices composed of two main components: a bio-recognition element and a transducer . The bio-recognition element of the sensor identifies the target analyte, while a transducer converts the result of the molecular recognition into an electrical signal. Different biomolecules such as enzymes, nucleic acids, antibodies, proteins, and peptides can be used as a bio-recognition element and biosensors can thus be used to detect specific physiochemical changes in the body (associated with the diseases) with high sensitivity and specificity . Biosensors have been widely utilized for diagnostic and imaging , however, they are not originally equipped with therapeutics to treat the diseases. Several studies that merge biosensing and drug delivery concepts have been described in the last few decades . These systems are a special class of biosensor designed for the continuous analysis of biological molecules followed by drug release in response to specific signals. These delivery systems, also known as closed loop delivery systems, have proven to be practical tools by tuning drug release as a function of specific signals associated with physiological and pathological processes .

The closed-loop drug delivery systems usually consist of a monitoring component that senses the surrounding conditions and an actuator component with the capability to trigger drug release. The pairing of the monitor/actuator architecture allows the drug release to be activated at or above a certain signal concentration or threshold, but inhibits such release when the signal level is in normal ranges . A typical example of such systems is the glucose-responsive insulin delivery system, which imitates the pancreatic beta cells to release insulin with a specific dose at a specific time point by responding to the plasma glucose levels .

Many biosensor-integrated drug delivery applications utilizing bio micro-electro-mechanical systems (bioMEMS), electrochemical sensors, and stimulus responsive biopolymers have been described. MEMS are devices with electrical and mechanical components. MEMS designed for biomedical applications are called bioMEMS, which have gained much attention in the biomedical engineering field for biomolecular analyses and sensing. BioMEMS provide many advantages such as short response time, high scalability, and high sensitivity. In bioMEMS, physical, chemical, or biological signals are converted into electrical signals that trigger the drug release. BioMEMS are implanted into the human body and the drug is released according to sensor feedback .

Electrochemical biosensors have electrodes that convert the chemical signal into an electrical signal. Electrochemical sensors can detect various biomolecules in the human body such as glucose, cholesterol, uric acid, lactate, DNA, hemoglobin, blood ketones and have great potential to treat diseases related to imbalances of biomolecules. Electrochemical sensors are mostly used for biosensing applications, with very few studies relating to biosensing integrated drug delivery applications .

Bioresponsive polymers or smart polymers can undergo structural alterations in response to physical, chemical, or biological stimuli. Many microdevices making use of these smart polymers have been described, which respond to external stimuli and deliver drugs when required . These smart polymer-based systems, although not true biosensors (as they lack the signal processing unit), have been widely studied for biosensing integrated drug delivery systems. Attachment of the enzyme glucose oxidase and insulin within a hydrogel, which is responsive to pH changes, is a particularly good example of one such system where the smart polymer acts both as a sensor of glucose concentration and as a drug delivery vehicle for insulin . The emphasis of this chapter is on the design, development and applications of biosensors. Various components that constitute a biosensor as well as the working principle of biosensors will be presented. Moreover, various fields where these devices are used will also be discussed especially medical field and drug delivery.

1.2. Biosensors

A sensor is a device which functions by producing a signal which is proportional to the concentration of a specific (bio) chemical or a set of (bio) chemicals in the presence of a number of interfering species . This is accomplished by means of using biological recognition elements such as enzymes, antibodies, receptors, tissues and microorganisms as sensitive materials because of their excellent selective functionality for target substances.

A successful biosensor is composed of two main components, mainly a biological receptor or sensor element (bio-recognition) and a transducer. A signal processing unit that usually contains a display or printer is normally used in conjunction to a biosensor as depicted in **Figure 1.**

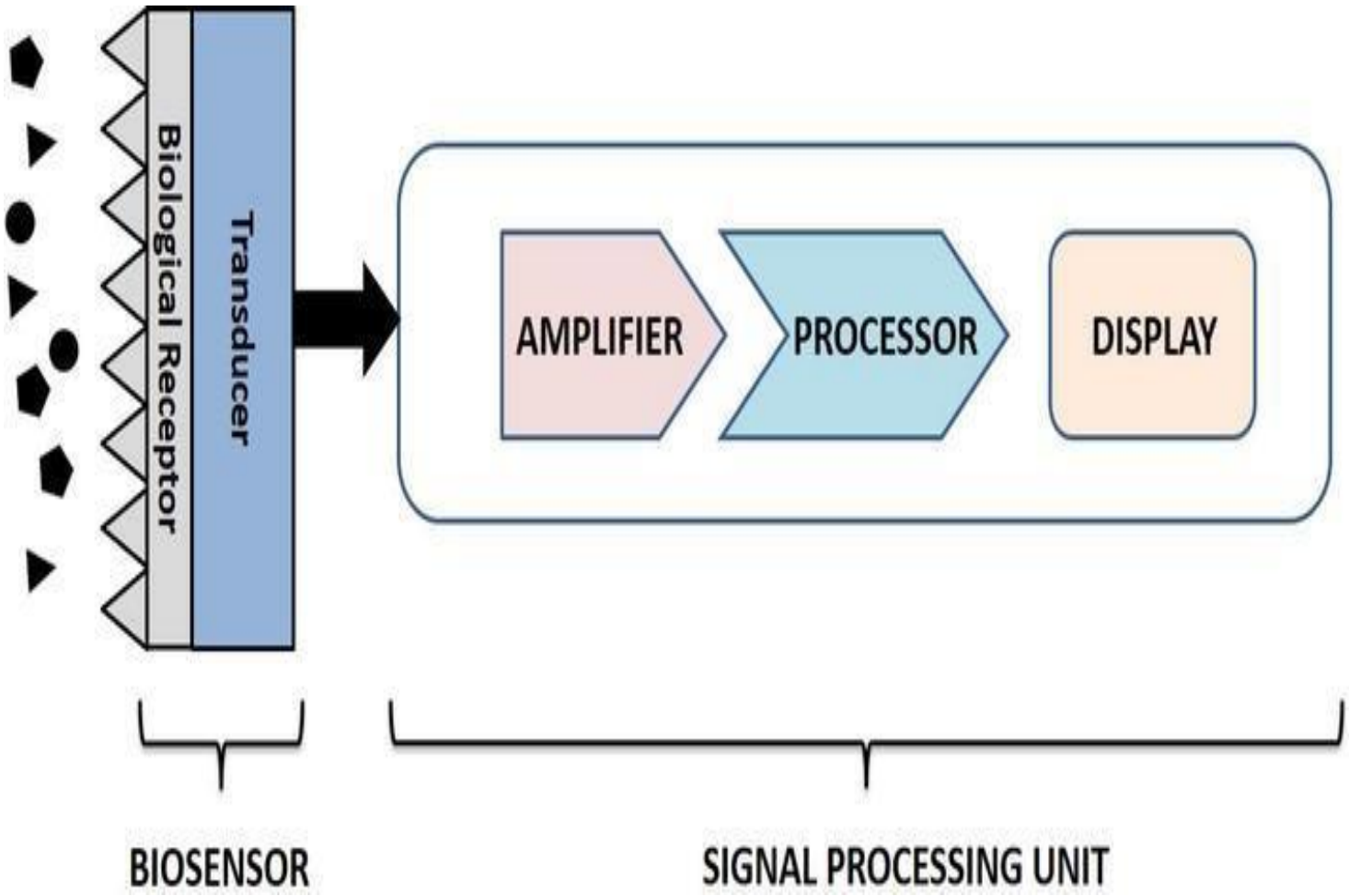


Figure 1.

Biosensor design showing the various components necessary for generating a signal.

1.2.1. Biological receptor (bio-recognition)

This component is also known as a sensor or detector element and is responsible for sensing or detecting the presence and/or the concentration of the target analyte or substance. This is a biological component, which serves as a biochemical receptor that specifically recognizes the target analyte . When the biological receptor interacts with a target analyte, it generates a signal in the form of light, heat, pH, charge or mass change . This material should be highly specific, stable under storage conditions and must be immobilized. Furthermore, the biological receptor should be capable of selectively detecting the target compound or analyte in the test sample. According to Paddle , the biological receptor determines the sensitivity of the entire device through the generation of the physicochemical signal that is monitored by the transducer .

This component can be a tissue, microorganism, organelle, cell receptor, enzyme, antibody or nucleic acid etc. These can be grouped into two categories, namely catalytic and non-catalytic receptors . The catalytic group of biological receptors are used in devices intended for continuous monitoring of substances at millimolar or micromollar concentrations. These include enzymes, tissues and microorganisms. The non-catalytic group is used mainly in biosensor devices that measure analytes such as steroids, drugs, and toxins etc. which usually occur at very low concentrations (micro to picomollar range). These are non-reusable devices which can only be used once and discarded thereafter. Such receptors include antibodies, antigens, nucleic acids etc.

1.2.2. Transducer

A transducer forms the second main component in the design of a biosensor. Generally, a transducer is a material that is capable of converting one form of energy to another . In a biosensor, a transducer is responsible for converting the biochemical signal received from the biological receptor, which is a result of the interaction between the target analyte and the biological receptor, into a measurable and quantifiable signal which can be piezo-electrical, optical, electrochemical, etc. The transducer detects and measures the change that occurs during biological receptor – analyte interaction . An example of a transducer is a pH sensor in a glucose biosensor. An enzyme, known as glucose oxidase, is used as a biological receptor which binds glucose and converts it to

gluconic acid in the presence of oxygen. The pH sensor (transducer) then detects the change in pH (due to production of gluconic acid) and converts it into a voltage change . The following features are recommended when a transducer is designed; specificity to the target analyte, analyte concentration range, response time and suitability for practical applications. Ideally, a transducer should be highly specific to the analyte, give measurement at the lowest analyte concentration within the shortest time possible .

1.3. Working principle of a biosensor

As indicated in the aforementioned sections, a biosensor comprises of a biological receptor coupled with a transducer and signal processing unit, and thus operate on the basis of signal transduction. The combination of these components is designed to convert the biological response into a corresponding electrical response and ultimately a measurable output. In simpler terms, biosensors are responsible for the quantitative analysis of a molecule by relating its biological action into a measurable signal . Initially, the molecule of interest in the test sample binds or interacts specifically with the biological receptor, resulting in a physiological change. This further alters the physicochemical properties of the transducer that is in close proximity to the biological receptor. This further leads to a change in the optical or electronic properties of the transducer which is further converted into an electrical signal which is detectable

The signal generated by the transducer can either be a current or voltage, depending on the type of biological receptor. If the output from the transducer is in the form of a current, then this will be converted into an equivalent voltage. Also, the output voltage is usually very low and masked by a high frequency noise signal, which then requires further alterations, processing and amplification through various filters within the signal processing unit. Finally, the output generated from the signal processing unit should be comparable to the biological quantity being measured .

1.4. Important characteristics of biosensors

Owing to the nature of the applications in which biosensors are used in, several characteristics or parameters have to be met when a biosensor is designed. These characteristics define the performance and usefulness of a biosensor.

1.4.1. Sensitivity

This is considered as the most important characteristic of a biosensor. The sensitivity of a biosensor is defined as the relationship between the change in analyte concentration and the intensity of the signal generated from the transducer. Ideally, a biosensor should generate a signal in response to small fluctuations in the concentration of the target analyte. Depending on the application, biosensors are required to detect analytes in the ng/ml or fg/ml concentration ranges. This is usually important for medical applications and environmental monitoring purposes.

1.4.2. Selectivity

This refers to the ability of the biosensor to selectively bind and respond only to the desired analyte, in the presence of other molecules or substances. When a signal or response is generated from interactions with an analyte that is different from the target analyte such is termed a false positive result. This is common in biosensors with poor selectivity, thus failing in clinical applications. Selectivity is a very important feature especially in medical applications where the test sample or sample matrix, usually blood or urine, contains numerous molecules that are quite similar to the target analyte and compete for binding to the biological receptor .

1.4.3. Stability

Stability of the biosensor is a very important characteristic especially for biosensors used for continuous monitoring. This feature determines the ability of the biosensor device to resist change in its performance over a period of time in response to interruptions arising from external factors. These can be in the form of temperature, humidity or other environmental conditions. Such interruptions have the potential to induce inaccuracies in the output signal during measurement, thereby affecting the precision and accuracy of the biosensor device .

1.4.4. Detection limit

A detection limit is defined as the lowest concentration of the target that is able to elicit a measurable signal or response. Ideally, a biosensor should have the lowest detection limit, especially if it is to be used in medical applications where the target analyte might be present at very low concentrations .

1.4.5. Reproducibility

This is also one of the most important features in biosensing, and refers to the ability of the biosensor device to produce matching output signals or results in duplicate experimental runs. The capability of the biosensor to meet this criteria relies on the transducer which is required to perform in a precise and accurate manner .

1.4.6. Response time

This property determines the time it takes for the biosensor to generate a signal or response following the interaction of the biological receptor with the target analyte .

1.4.7. Range or linearity

Biosensor linearity determines the accuracy of the signal obtained, in response to a set of measurements with differing concentrations. This attribute gives insight into the resolution of the biosensor, defined as the minimal change in the target analyte concentration that will elicit a response from the biosensor. This is a very important attribute for a biosensor since most applications require a biosensor to measure a target analyte over wide concentration ranges .

1.5. Classification of biosensors

Biosensors are classified according to their biological receptors or transducer elements. **Figure 2** displays a flowchart illustrating the different types of biosensors based on the biological receptors and transducer elements . Some of the biosensors shown in the figure will be discussed further in subsequent sections.

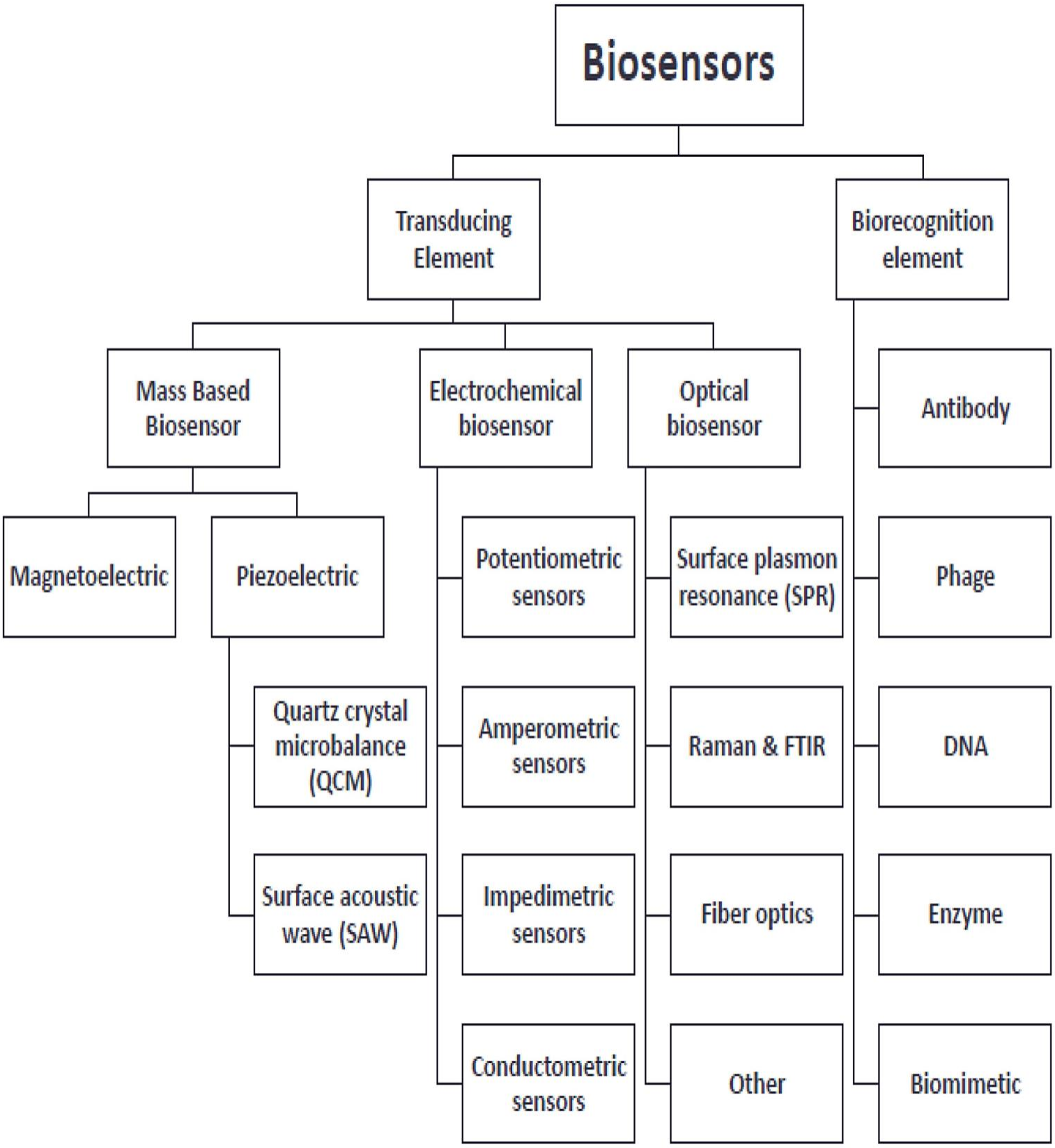


Figure 2.

Flowchart showing the various types of biosensors classified based on their transducing elements and biological recognition elements

1.5.1. Classification based on biological receptors

5.1.1. Enzyme based biosensors

These type of biosensors form the most researched and reported biosensors based on biological receptors . Enzyme biosensors, useful tools for monitoring rapid changes in metabolite levels in real-time, include pure enzyme preparations or biological processes. They have been derived on immobilization processes such as van der Waals forces, ionic or covalent bonding. In 1967, Updike and Hicks successfully developed a working electrode for the detection of glucose levels and this is considered the first biosensor in the world. The well-known enzymatic biosensors today are glucose and urea biosensors. However, glucose biosensors are most popular among researchers and are reportedly the mostly commercialized biosensors. Enzymatic biosensors are known for their prolonged use and reusability due to the fact that enzymes used as biological receptors cannot be consumed. Thus, the detection limit and the lifetime of enzyme based biosensors is greatly enhanced by the stability of the enzyme .

5.1.2. DNA based biosensors

Another group of biosensors based on a biological receptor is DNA biosensors. The most attractive feature of biosensors is the high selectivity of biosensors for their target analytes in a matrix of chemical or biological elements. DNA biosensors, which use nucleic acids as their biological receptors, detect proteins and non-macromolecular compounds that interact with certain DNA fragments known as DNA probes or DNA primers. The interaction observed stems from the formation of stable hydrogen bonds between the double helix nucleic acid strands . Extremely high sensibility and selectivity is needed to maximize the hybridization efficiency and minimize non-specific binding .

1.5.2. Biosensors based on transduction element

5.2.1. Electrochemical biosensors

Electrochemical biosensors, which are the best in the detection of hybridized DNA, DNA binding drugs, glucose concentration, etc., measure the electrical potential difference caused by an interaction between an analyte and the membrane/sensor surface.

5.2.1.1. Conductometric biosensors

Conductometric biosensors measure the electrical conductivity of the solution in the course of a biochemical reaction. When electrochemical reactions produce ions or electrons, the overall conductivity or resistivity of the solution changes. Due to poor signal-to-noise ratio, they are less commonly used in biosensing applications, particularly when the biological receptor used is an enzyme. However, these biosensors remain useful in the detection of affine interactions .

5.2.1.2. Potentiometric biosensors

Potentiometric biosensors measure changes in pH and ion concentrations resulting from antigen/antibody interactions. Although potentiometric biosensors are the least common of all biosensors, different strategies for the development of these biosensors are found. The working principle relies on the fact that when a 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 analyte. Some of the known potentiometric biosensors include those used for the detection of Neisseria meningitides, Brucella melitensis and Francisella tularensis species .

5.2.1.3. Amperometric biosensors

This is perhaps the most common electrochemical detection method used in biosensors. This high sensitivity biosensor can detect electroactive species present in biological test samples . Amperometric-based biosensors detect the difference in current potentials during redox reactions when antigen/antibody pairing occurs. The most common amperometric biosensors use the Clark oxygen

electrode. Amperometric biosensors have been developed for the indirect detection of E. coli by Nakamura and co-workers .

5.2.1.4. Impedimetric biosensors

Impedimetric-based biosensors monitor changes in impedances upon antigen/antibody interaction. Impedance, which usually employs a circuit bridge as a measurement tool, is well suited for detection of bacteria in clinical specimens, to monitor quality and to detect specific food pathogens. Moreover, these biosensors are useful in controlling industrial microbial processes .

5.2.2. Mass based biosensors

Piezoelectric biosensors are a group of analytical devices working on a principle of affinity interaction recording. A piezoelectric platform or piezoelectric crystal is a sensor part working on the principle of change in oscillations due to mass bound on the piezoelectric crystal surface. Piezoelectric biosensors, which are considered as mass-based biosensors, produce an electrical signal when a mechanical force is applied. An example of piezoelectric biosensor is the quartz crystal microbalance (QCM) model. The working principle of QCM is depicted in **Figure 3.** Quartz crystal microbalance (QCM) is a very popular tool that is used extensively in the electronic industry. Currently, these tools are used as attenuators in electronic devices and they have a typically fundamental mode frequency of 1–20 MHz. Though higher frequencies provide good opportunities for a sensitive assay, QCM with high frequencies have been reported to exhibit several drawbacks such as their fragility and also the technologically demanding equipment needed for their manufacture . The basic material used in the development of the QCM sensor consists of quartz crystal, which is equipped with metal electrodes. A sensitive coating material on the sensor surface is used to enable detection of the target analyte in the environment. An appropriate electronic circuit is necessary to make conversion of the measured quantity to an electrical signal .

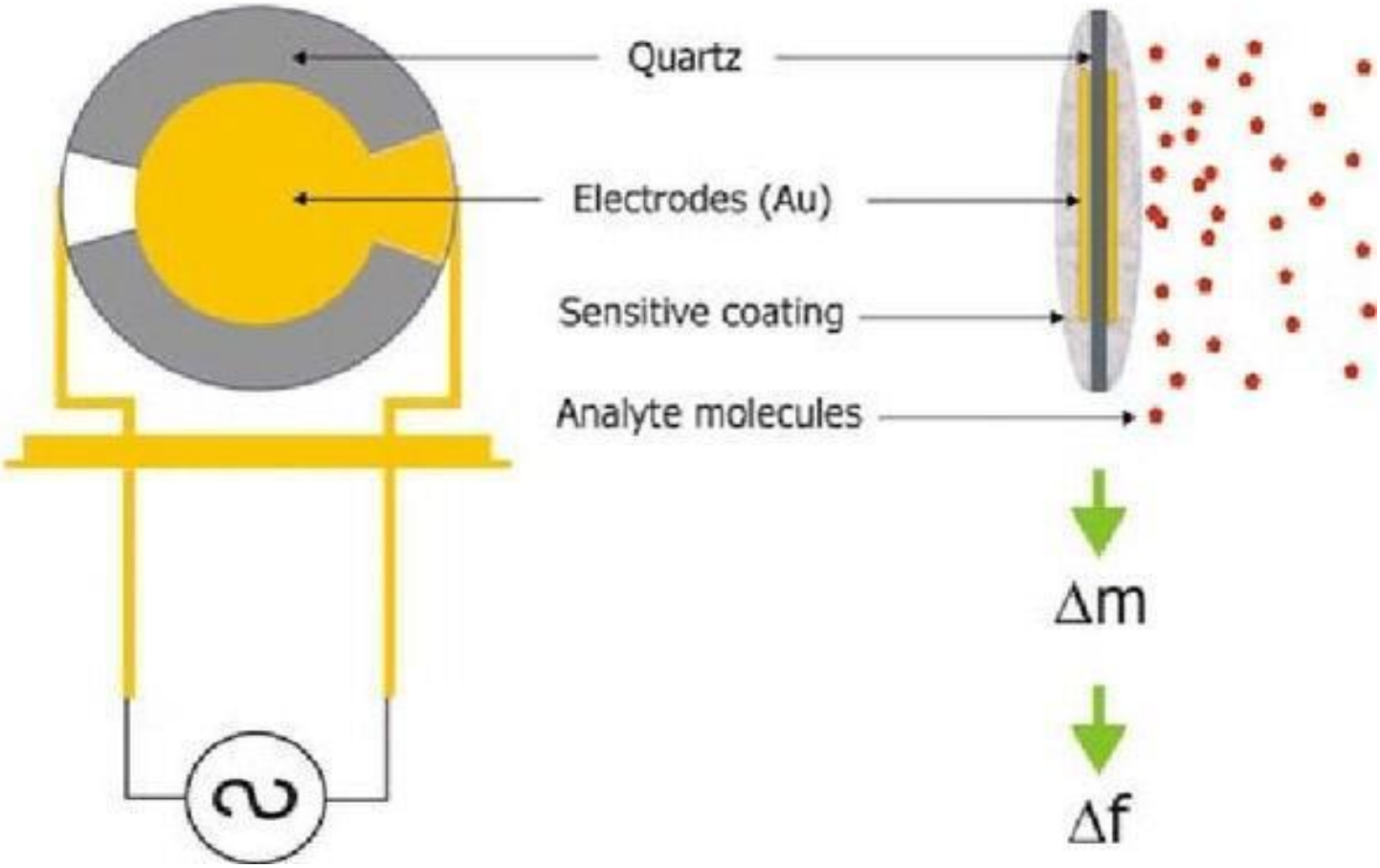


Figure 3.

Basic working principle of Quartz Crystal Microbalance (QCM) sensor

5.2.3. Optical biosensor

Optical biosensors are based on the interaction of a sensing element with electromagnetic radiation. They consist of a light source, as well as numerous optical components to generate a light beam with specific characteristics and to beeline this light to a modulating agent, a modified sensing head along with a photodetector. An optical surface plasmon resonance (SPR) biosensor can detect the refractive index changes on the surface of sensor chips, label-free and in real-time. Although different optical methods such as absorption, fluorescence, luminescence, internal reflection, surface plasmon resonance, or light scattering spectroscopy utilized herein are becoming popular, fluorescence and surface plasmon resonance enabled spectroscopies still remain the most and widely researched and applied methods .

Chapter Two

2.1. Integration of Biosensors with Drug Delivery Systems

Biosensors are the tools that can shape illness treatment by increasing accuracy of diagnosis, illness monitoring and prognosis. The advantages of biosensors are that they are easy to use, inexpensive, rapid, robust and can allow analysis of different biomarkers simultaneously . The other main advantage is that there is no sample preparation since the biosensor can detect the biomarker within a pool of other bimolecular substances and this makes the integration of biosensors with current drug delivery systems feasible. Microneedles are painless minimally invasive drug delivery systems that do not contact with blood thereby reducing infection and risk of device contamination. In drug delivery, these microneedles are used to inject a therapeutic transdermally whilst for biomedical sensing they aid in fluid extraction for analysis. Utilizing such and many other tools the current research in illness management focuses one of its aspects on integration of biosensors with drug delivery systems. Many such systems that have been studied and published are based on responsive drug release, biocompatibility, biofouling, self-regulatory implants and refillable reservoirs .

2.1.1. Bio-Micro-Electro-Mechanical Systems (Bio-MEMS)

The development of Micro-Electro-Mechanical Systems (MEMS) devices is accomplished the process of micro-fabrication, where silicon, glass and plastic are used. The use of MEMS has led to the development of microfluidics which is a field of the design and development of miniature devices that can sense, pump, mix, monitor and control flow of small volumes of fluids .

BioMEMS technology has allowed fabrication of both disposable (external application) and implantable drug delivery systems and diagnostic tools. Solid durable, solid degradable and hollow microneedles can be used for delivery of insulin and for vaccination . Implantable drug delivery microdevices designed by means of BioMEMS technology can reduce conventional implantable drug delivery devices disadvantages. Most implantable drug delivery devices have unintended drug dumping events which cause side effects and reduce patient compliance as this causes health risk to patients . Implant lifetime also affects compliance as this increases cost of implant replacement. These implants have further problems such that the implant drug release rate and drug contents cannot be changed without invasive procedure.

Conventional pumps are usually osmotically, electrolytic or peristaltic driven . By means of BioMEMS, a piezoelectric pump controlled drug delivery system was made for transdermal delivery of insulin by means of using microneedle, which improved precision and accuracy in relation to mechanical controlled pumps . For longer lifetime and improved biocompatibility, the BioMEMS device will require use of biodegradable polymers or compounds that mitigate tissue response to the implant such as antibiotics or anti-inflammatory agents .

2.1.2. Smart Polymers

Smart polymers represent a group of polymers that function in the same manner as biological systems. Stimuli responsive hydrogels can undergo structural changes when exposed to external stimuli such as pH, temperature and ionic changes. The polymers are divided into three groups based on their physical form. Linear free chains in solutions are when the polymer undergoes a reversible collapse after a stimulus is applied, covalently cross-linked reversible gels are when swelling/shrinking are triggered by environmental changes and chain adsorbed/surface-grafted form represent polymers that have reversible swelling/collapse on the surface once a trigger is changed . Similar to affinity biosensors a hydrogel has been designed by grafting an antigen-antibody complex onto polymer network that will lead to competitive binding of the free antigen triggering a change in the network structure of the hydrogel . Figure 4 indicates that the hydrogel regains its primary structure due to shape memory behavior after reversible binding . Such behavior allows long term use of the system unlike affinity biosensors that get saturated over time as reversible binding is not favored. In another approach the entrapment of glucose oxidase within a pH responsive hydrogel (gluconic acid increase due to oxidation of glucose) and attachment of insulin allowed the smart polymers to act as both drug delivery vehicles for insulin in addition to being a biosensor of glucose concentration .

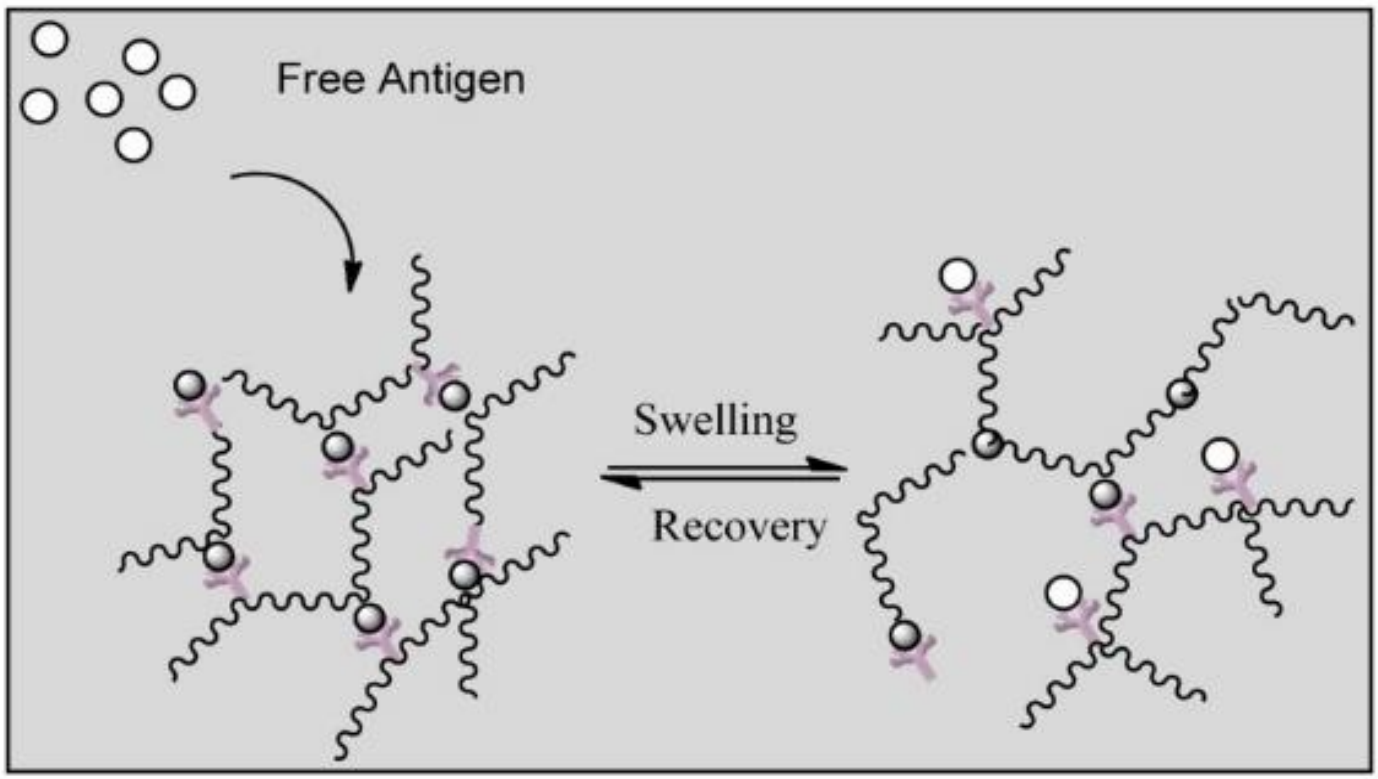
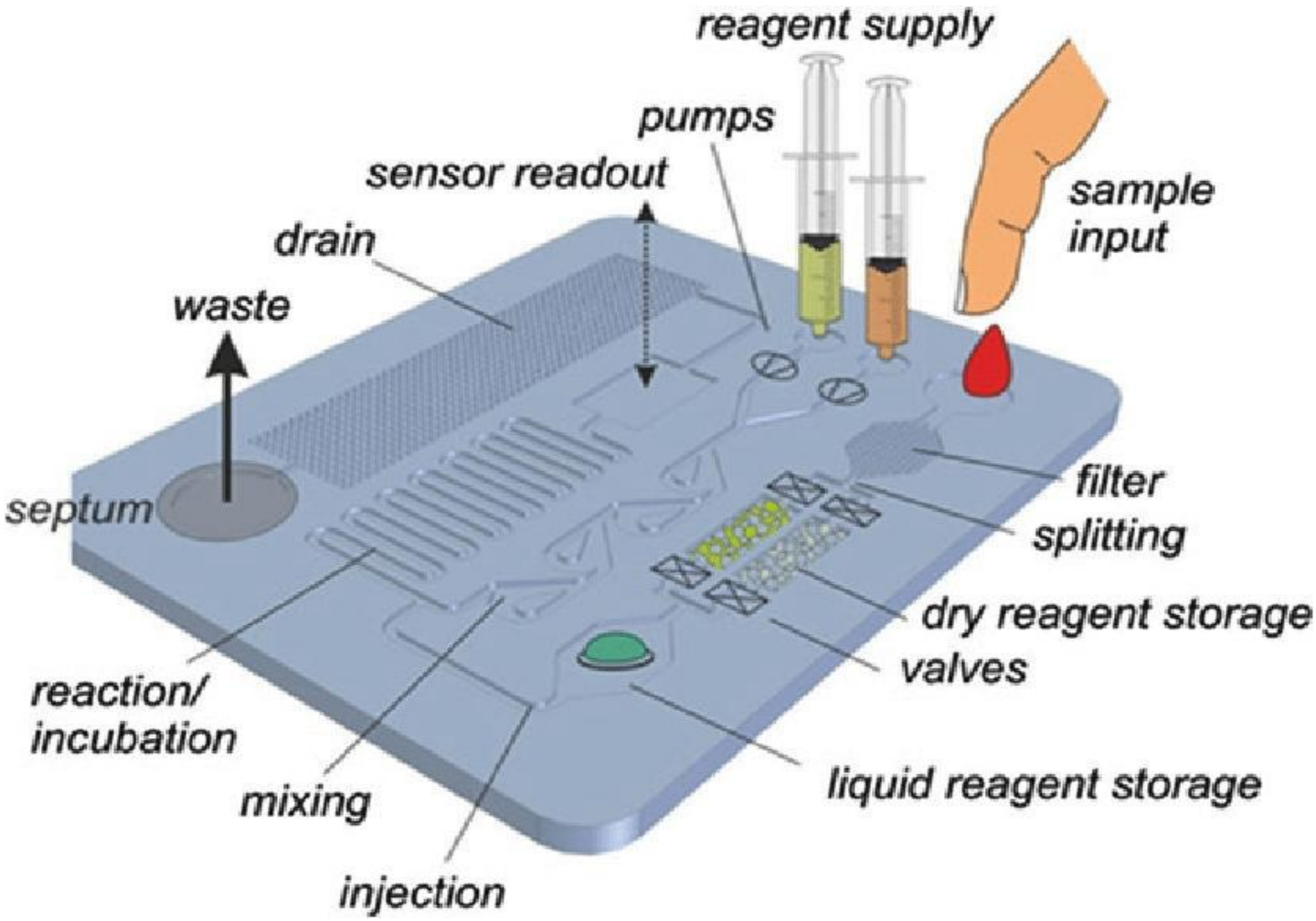


Figure 4. *Reversible antigen responsive hydrogel*

2.1.3. Lab-on-A-Chip

Lab-on-a-chip systems are increasing rapidly as they have significant benefits in different fields of health care and environmental affairs . These benefits include rapid data analysis, improved analysis and portability of the devices. This allows individuals to monitor their own health sparing them from visiting a physician. Since technologies such as lap-on-a-chip can generate data comparable to a laboratory conducted data; this allows point of care diagnosis and treatment. Incorporation of a micro-reservoir drug depot, micro-pump, valves, and sensors

onto BioMEMS devices allowed responsive and controlled release of drug. Controlled release is required as many drugs delivered through conventional modes of delivery leads to low bioavailability with low concentration and increase toxicity when high drug concentration is released or accumulates over time. A controlled-release microchip has been created that use silicon wafers and different drug depots for single and multiple drug release. Integration of biosensors and drug delivery can be achieved by adding drug loaded hydrogels, biosensors, and other features that are responsive to the local environment that ultimately allows pharmaceutical devices to operate in a more closely integrated manner with the biological surroundings with limited scientist intervention (Figure 5).



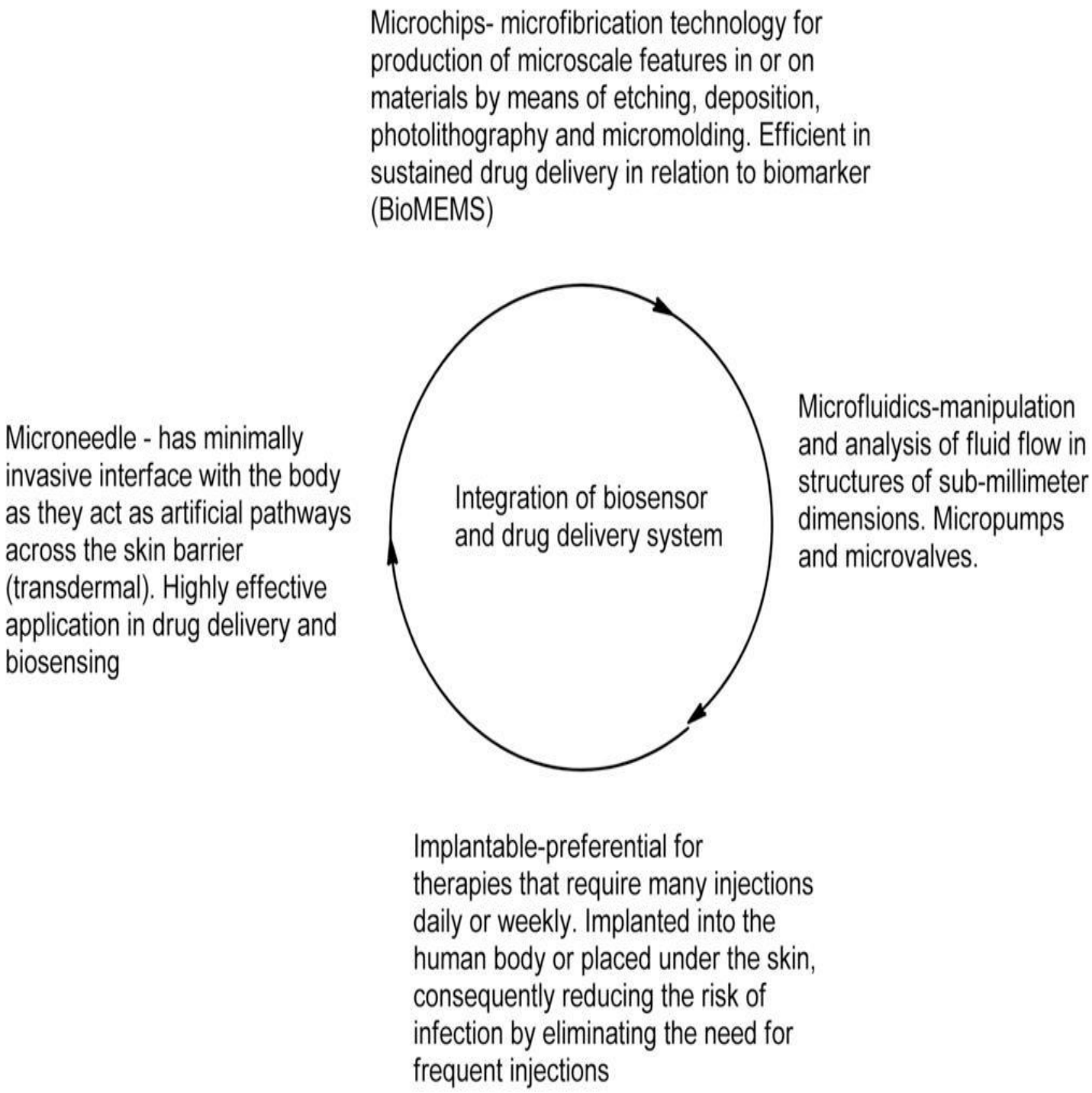


Figure 5. *Technologies for integration of biosensors and drug delivery systems.*

2.2. Applications of biosensors

Conventional analysis requires the samples to be sent to a laboratory for testing. These methods allow the highest accuracy of quantification and the lowest detection limits, but are expensive, time consuming and require the use of highly trained personnel. Due to the above drawbacks, there has been a great interest in the technology of biosensors. There has been a phenomenal growth in the field of biosensor development in recent years with emerging applications in a wide range of disciplines. These include environmental monitoring, disease detection, food safety, defence, drug discovery and many more as depicted in Figure 6 below. A summary of the few and selected representatives and examples of developed applications of biosensors is given below.

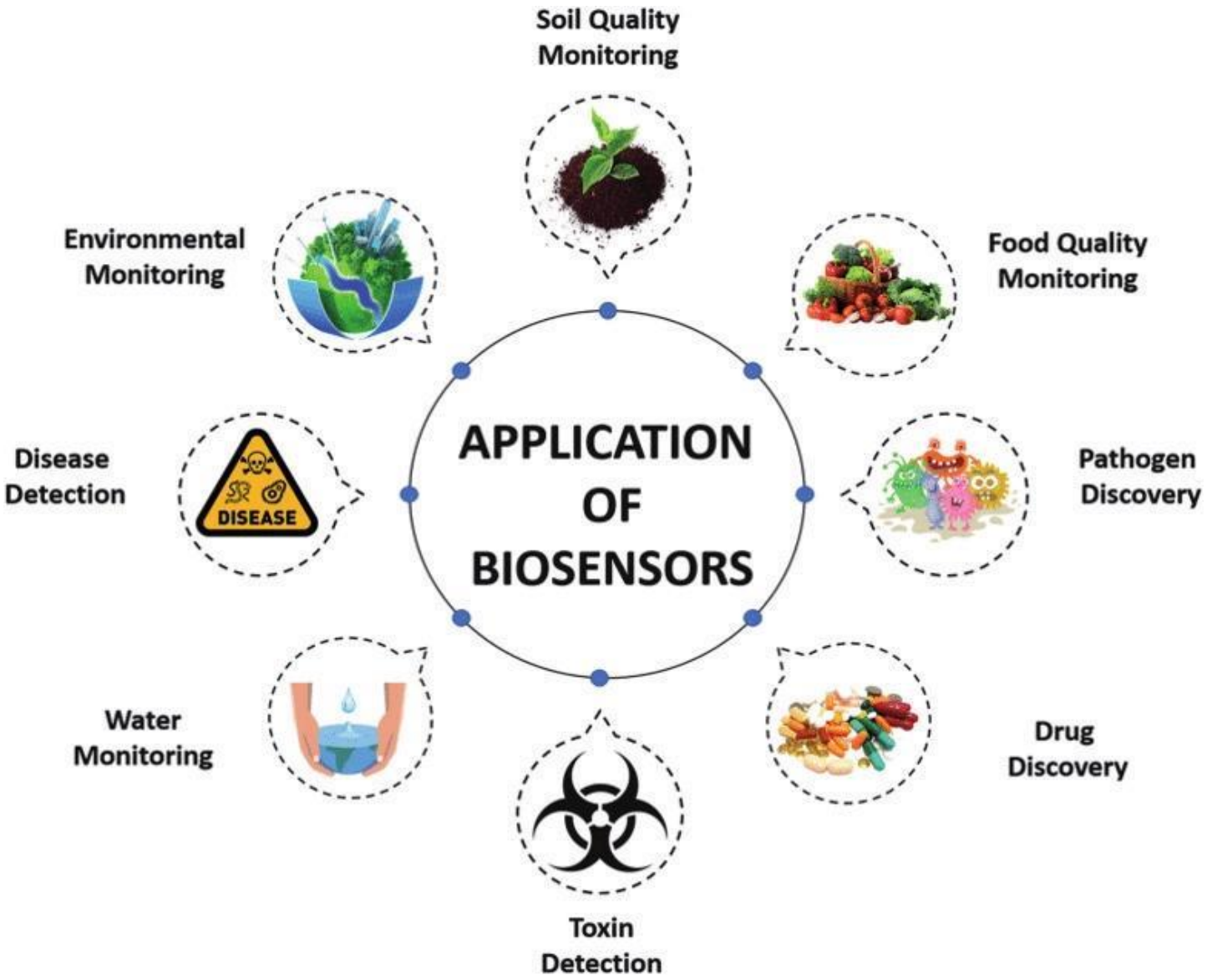


Figure 6. *Various applications where biosensors have been used.*

2.2.1. Food industry

Biosensors have been used extensively in the food industry for quality control and assurance purposes. These include applications in the agricultural field during crop production and also during food processing. Quality control remains a major part of food production and is responsible for the production of healthy food with a prolonged shelf life and also complies with regulations. Biosensors have been used as on-line or at-line quality sensors that make it possible for quality sorting, automation and reduction of production cost and production time. Also, biosensors have been developed to detect particular compounds in foods. These devices detect chemicals or biological agents that contaminate food or might indicate the presence of unwanted substances in food. Moreover, biosensors have been developed for monitoring and estimating cross-contamination of surfaces and food products .

2.2.2. Environment

Environmental pollution has an impact on human health and can therefore compromise the quality of life. Depending on the purpose, sensitive and selective methods are needed for both quantitative and qualitative determination of target analytes. Biosensors have found widespread use in environmental monitoring for the detection of chemical agents, organic pollutants, potentially toxic elements and pathogens that might pose a health hazard. Biosensors such as immunosensors, aptasensors, genosensors and enzymatic biosensors are amongst the most preferred for environmental monitoring. These are known to use antibodies, aptamers, nucleic acids and enzymes as biological receptors. For example, a biosensor was developed to detect pesticides such as organophosphate and carbamate and also monitor their effects on the environment. Biosensors detect pollutants by measuring colour, light, fluorescence or electric current .

2.2.3. Medical field

2.2.3.1. Biosensors for diabetes applications(glucose measuring)

Glucose can be monitored by invasive and non-invasive technologies. Glucose biosensor was the first reported biosensor and after that a great number of different glucose biosensors were developed, including implantable sensors for measuring glucose in blood or tissue. Glucose sensors are now widely available as small, minimally invasive devices that measure interstitial glucose levels in subcutaneous fat . Requirements of a sensor for in vivo glucose monitoring include miniaturization of the device, long-term stability, elimination of oxygen dependency, convenience to the user and biocompatibility. Long-term biocompatibility has been the main requirement and has limited the use of in vivo glucose sensors, both subcutaneously and intravascular, to short periods of time. Diffusion of low-molecular-weight substances from the sample across the polyurethane sensor outer membrane results in loss of sensor sensitivity. In order to address the problem, microdialysis or ultrafiltration technology has been coupled with glucose biosensors. The current invasive glucose monitors commercially available use glucose oxidase-based electrochemical methods and the electrochemical sensors are inserted into the interstitial fluid space. Most sensors are reasonably accurate although sensor error including drift, calibration error, and delay of the interstitial sensor value behind the blood value are still present . The glucose biosensor is the most widely used example of an electrochemical biosensor which is based on a screen-printed amperometric disposable electrode. This type of biosensor has been used widely throughout the world for glucose testing in the home bringing diagnosis to on site analysis.

Non-invasive glucose sensing is the ultimate goal of glucose monitoring and the main approaches being pursued for glucose sensor development are: near infrared spectroscopy, excreted physiological fluid (tears, sweat, urine, saliva) analysis, microcalorimetry, enzyme electrodes, optical sensors, sonophoresis and iontophoresis, both of which extract glucose from the skin . Despite the relative ease of use, speed and minimal risk of infection involved with infrared spectroscopy, this technique is hindered by the low sensitivity, poor selectivity, frequently required calibrations, and difficulties with miniaturization. Problems surrounding direct glucose analysis through excreted physiological fluids include

a weak correlation between excreted fluids and blood glucose concentrations. Exercise and diet that alter glucose concentrations in the fluids also produce inaccurate results . The desire to create an artificial pancreas drives for continued research efforts in the biosensor area. Nevertheless, the drawbacks of in vivo biosensors must be solved before such an insulin modulating system can be achieved.

2.2.3.2. Biosensors for cardiovascular diseases applications

Biosensors for cholesterol measurement comprise the majority of the published articles in the field of cardiovascular diseases. In the fabrication of cholesterol biosensor for the estimation of free cholesterol and total cholesterol, mainly cholesterol oxidase (ChOx) and cholesterol esterase (ChEt) have been employed as the sensing elements (Fig. 7). Electrochemical transducers have been effectively utilized for the estimation of cholesterol in the system . Based on number and reliability of optical methods, a variety of optical transducers have been employed for cholesterol sensing, namely monitoring: luminescence, change in color of dye, fluorescence and others . Other cardiovascular disease biomarkers are also quantified. CRP measurement rely mainly on immunosensing technologies with optical, electrochemical and acoustic transducers besides approaches to simultaneous analytes measurement. Silva et al. (2010) incorporated streptavidin polystyrene microspheres to the electrode surface of SPEs in order to increase the analytical response of the cardiac troponin T and Park et al. (2009) used an assay based on virus nanoparticles for troponin I highly sensitive and selective diagnostic, a protein marker for a higher risk of acute myocardial infarction. Early and accurate diagnosis of cardiovascular disease is crucial to save many lives, especially for the patients suffering the heart attack. Accurate and fast quantification of cardiac muscle specific biomarkers in the blood enables accurate diagnosis and prognosis and timely treatment of the patients. It is apparent that increasing incidences of cardiovascular diseases and cardiac arrest in contemporary society denote the necessity of the availability of cholesterol and other biomarkers biosensors. However, only a few have been successfully launched in the market. One of the reasons lays in the optimization of critical parameters, such as enzyme stabilization, quality control and instrumentation design. The efforts directed toward the development of cardiovascular disease biosensors have resulted in the commercialization of a few

cholesterol biosensors. A better comprehension of the bioreagents immobilization and technological advances in the microelectronics are likely to speed up commercialization of the much needed biosensors for cardiovascular diseases.

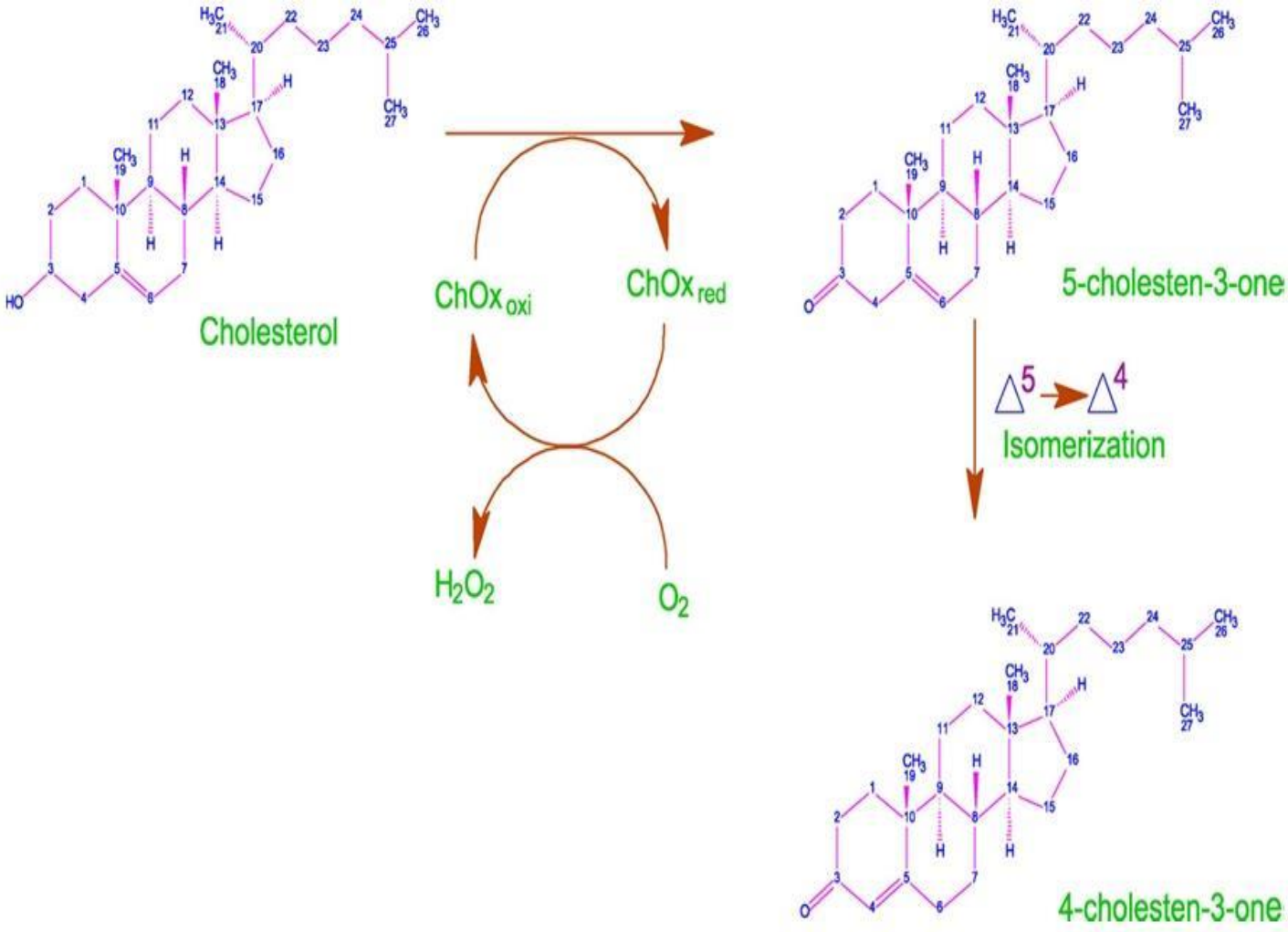


Fig. 7. *Pathway of cholesterol oxidase enzyme reaction*

2.2.3.3. Biosensors for cancer applications

Existing methods of screening for cancer are heavily based on cell morphology using staining and microscopy which are invasive techniques. Furthermore, tissue removal can miss cancer cells at the early onset of the disease. Biosensor-based detection becomes practical and advantageous for cancer clinical testing, since it is faster, more user-friendly, less expensive and less technically demanding than microarray or proteomic analyses. However, significant technical development is still needed, particularly for protein based biosensors. For cancer diagnosis multi-array sensors would be beneficial for multi-marker analysis. A range of molecular recognition molecules have been used for biomarker detection, being antibodies the most widely used. More recently, synthetic (artificial) molecular recognition elements such as nanomaterials, aptamers, phage display peptides, binding proteins and synthetic peptides as well as metal oxides materials have been fabricated as affinity materials and used for analyte detection and analysis . Antibodies (monoclonal and polyclonal) have been applied in cancer diagnostics tests targeting cancer cells and biomarkers. Polyclonal antibodies can be raised against any biomarker or cells and with the introduction of high throughput techniques, applying these molecules in sensors has been successful. The use of monoclonal antibodies however, results in more specific tests. The drawbacks include that monoclonal antibodies are more difficult to maintain and can be more expensive than polyclonal antibodies . Replacing natural biomolecules with artificial receptors or biomimics has therefore become an attractive area of research in recent years. The advantages of using these molecules are that they are robust, more stable, less expensive to produce and can be modified easily to aid immobilization on the sensor surface as well as adding labels as the maker for detection . Those molecules can be synthesized after a selection from combinatorial libraries with higher specificity and sensitivity when compared to the antibody molecule . For cancer biomarkers analysis, bioaffinity based electrochemical biosensors are usually applied to detect gene mutations of biomarkers and protein biomarkers. Electrochemical affinity sensors based on antibodies offer great selectivity and sensitivity for early cancer diagnosis and these include amperometric, potentiometric and impedimetric/conductivity devices. Amperometric and potentiometric transducers have been the most commonly used, but much attention in recent years has been devoted to

impedance based transducers since they are classified as label-free detection sensors. However, much of the technology is still at the research stage . Besides based on antibodies, electrochemical devices have been developed based on DNA hybridization and used for cancer gene mutation detection. In this type of device a single stranded DNA sequence is immobilized on the electrode surface where DNA hybridization takes place . ELISA based assays conducted on the electrode surface are the most frequently used techniques for cancer protein markers analysis, such as CEA. In this method the antibody (or antigen) is labeled with an enzyme such as horseradish peroxidase (HRP), or alkaline phosphatase (AP) and these will then catalyze an added substrate to produce an electroactive species which can then be detected on an electrochemical transducer. Electrochemical detection of rare circulating tumor cells has the potential to provide clinicians with a standalone system to detect and monitor changes in cell numbers throughout therapy, conveniently and frequently for efficient cancer treatment . Many commercially available platforms use fluorescence labels as the detection system. However, the instruments used for signal readout are usually expensive and are more suitable for laboratory settings. As an example the Affymetrix gene chip (Affymetrix Inc., Santa Clara, USA) can be used for screening cancer and cancer gene identification. Other biosensor platforms such as grating couplers, resonant mirrors and surface plasmon based systems have also been used for cancer biomarkers diagnosis. These are classified as label-free and real-time affinity reaction detection systems. Different SPR based biosensors have been developed for cancer markers detection based on the above optical systems. Recently, microcantilever based sensors have also been applied for early-stage diagnosis of hepatocellular carcinoma . In spite of the achieved development in cancer biosensing, the point-of-care testing is not yet available. In order to achieve this goal challenges must be overcome such as: development of reproducible biomarker assays; improvement in recognition ligands; development of multi-channel biosensors; advances in sample preparation; device miniaturization and integration; development of more sensitive transducers; microfluidics integration; advanced manufacturing techniques and cost reduction

2.3. Biosensors for detection and identification of infectious diseases

2.3.1. Biosensors for detection of pathogenic virus

Viruses are infectious agents that may be responsible for several diseases in humans, including Human Papilloma Virus (HPV) , dengue virus and hepatitis virus .There are 100 genotypes of HPV virus and some of them are associated with cancer, especially in the cervix and anus . The methods used for the diagnosis have limitations, such as low specificity . To overcome this disadvantage, Huang and coworkers described a highly sensitive electrochemical biosensor based on DNA probes for Human Papillomavirus (HPV), using a glassy carbon electrode functionalized with graphene, gold nanorods and polymeric film. They used electrochemical impedance spectroscopy and 1,10-phenanthroline ruthenium dichloride ($Ru(phen)_3^{2+}$)as redox indicator, amplifying the electrochemical signal. The biosensor described proved to be efficient in the viral DNA detection, specifically detecting the target in human serum samples with a detection limit of 4.03 x 10-14 M . Nasirizadeh et al developed a genosensor using gold electrodes, thiolated oligonucleotides specific for HPV and monitored the interaction of hematoxylin with dsDNA formed after the hybridization process. They used the techniques of cyclic voltammetry and differential pulse voltammetry, observing a remarkable difference between the voltammetric signals in different samples after hybridization. The linear relationship with the concentration of DNA target varied from 12.5 to 350.0 nM and the detection limit was 3.8 nM .

Another infectious disease caused by a DNA virus that infects hepatocytes of the liver is hepatitis B. Hepatitis B virus infection can harm the liver, with high risk of death from liver cirrhosis and cancer . During the chronic phase of the disease, monitoring is crucial, since it prevents the development of progressive diseases, such as cirrhosis and liver failure, as well as hepatocellular carcinoma. As an alternative to the traditional methods, Castro and coworkers developed an electrochemical biosensor for the detection of a specific DNA sequence of the hepatitis B virus, using graphite electrodes modified with poly (4-aminophenol), differential pulse voltammetry as detection technique and ethidium bromide as

hybridization label (Figure 8). They showed that this device was effective for diagnosis in the serum of infected patients and had a detection limit of 2.61 nM

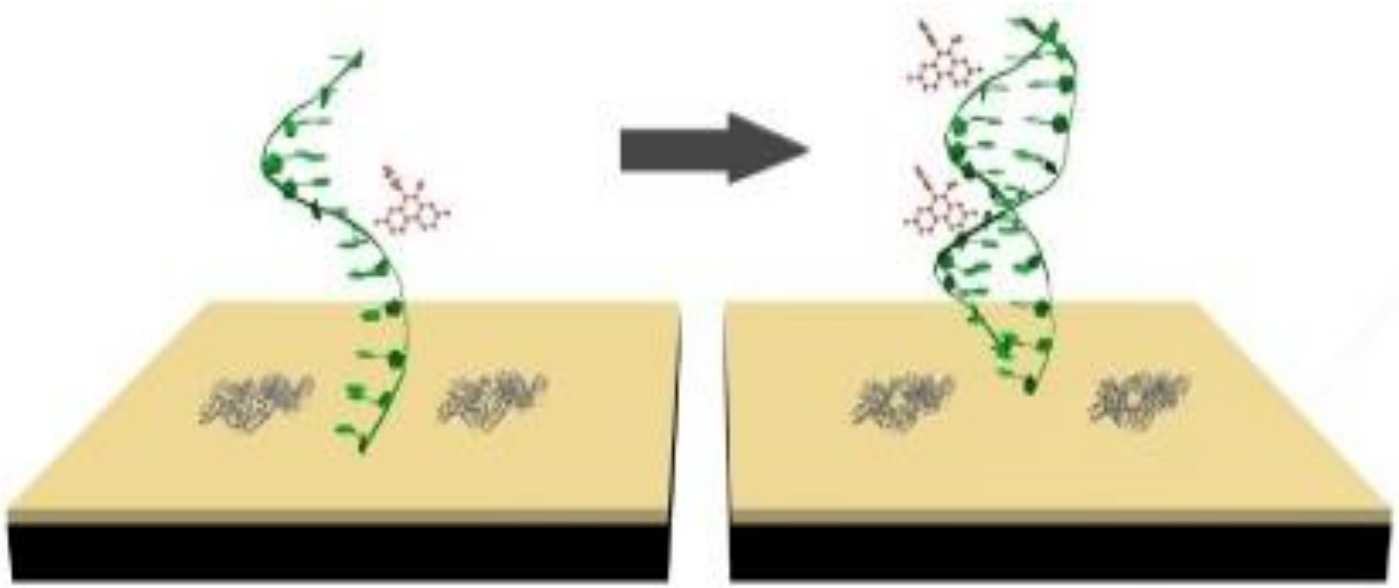


Figure 8*:* Example of genossensor for detection of a specific DNA sequence before (left) and after (right) the binding of the target . A specific DNA oligonucleotide was immobilized on a graphite/poly (4-aminophenol) surface and it was blocked with BSA. Then, the complementary DNA target was applied. Ethidium bromide was used to discriminate the single-stranded and double-stranded DNA.

2.3.2. Biosensors for detection of pathogenic bacteria

Pathogenic bacteria are important targets for detection in several fields, such as medicine and food safety. Different approaches have been developed for the detection of pathogenic bacteria, since these microorganisms contribute to globally important diseases, such as tuberculosis, leprosy and meningitis . Tuberculosis is caused by the pathogenic bacteria Mycobacterium tuberculosis and is currently the leading infectious cause of death, undoubtedly representing a global public health priority . According to the World Health Organization, in 2013 approximately 5.7 million cases of tuberculosis were reported worldwide. In recent years, many biosensors platforms have been developed for tuberculosis based on different biological recognition elements and various transducers. Liu et al. developed an electrochemical genosensor for M. tuberculosis based on the immobilization of a specific sequence of the IS6110 gene using a reduced graphene oxide-gold nanoparticle-modified electrode as a sensing platform and gold nanoparticles–polyaniline as a tracer label for amplification. The linear response of the sensor was 1 x 10-15 to 1 x 10-9M.

Other detection systems have been reported, such as Surface Plasmon Resonance (SPR), an optical detection technique that has been widely used for the development of genosensors for M. tuberculosis , and immunoassays .

Another disease caused by bacteria of the genus Mycobacterium is leprosy, a chronic disease caused by Mycobacterium leprae. According to WHO, in 2013 about 215,000 cases of leprosy were reported in the world, and the early diagnosis is important to interrupt transmission and prevent severe damage to patients . Afonso et al. developed an electrochemical genosensor based on the immobilization of a specific single-stranded DNA oligonucleotide on a graphite electrode modified with poly (4-aminophenol). The system target was M. leprae and the linear range of detection was from 0.35 to 35.0 ng µL-1. In addition, a fast and quantitative test for leprosy was developed by immobilizing two specific antigens on nitrocellulose membranes to detect IgM and IgG antibodies .

Meningitis can be caused by various pathogens, such as bacteria, fungi, viruses and parasites. Among the bacteria species that can cause meningitis, the most common are Streptococcus pneumoniae, Neisseria meningitidis, Haemophilusinfluenzae, Listeria monocytogenes and Streptococcus . Particularly, Neisseria meningitidis has the potential to cause large epidemics.

Among the studied targets for detection, there is Omp85, a virulence gene that codes for a conserved outer membrane protein of N. meningitidis. Reddy et al. described the development of an immunosensor using the quartz crystal microbalance as transducer and antibodies against the cell surface outer membrane protein 85 of N. meningitides as biological recognition element (Figure 4). In addition, an electrochemical genosensor was developed using specific oligonucleotides for this virulent gene immobilized on screen-printed gold electrodes and the sensor sensitivity was 2.6 ($\mu A/cm^2$)/ng .

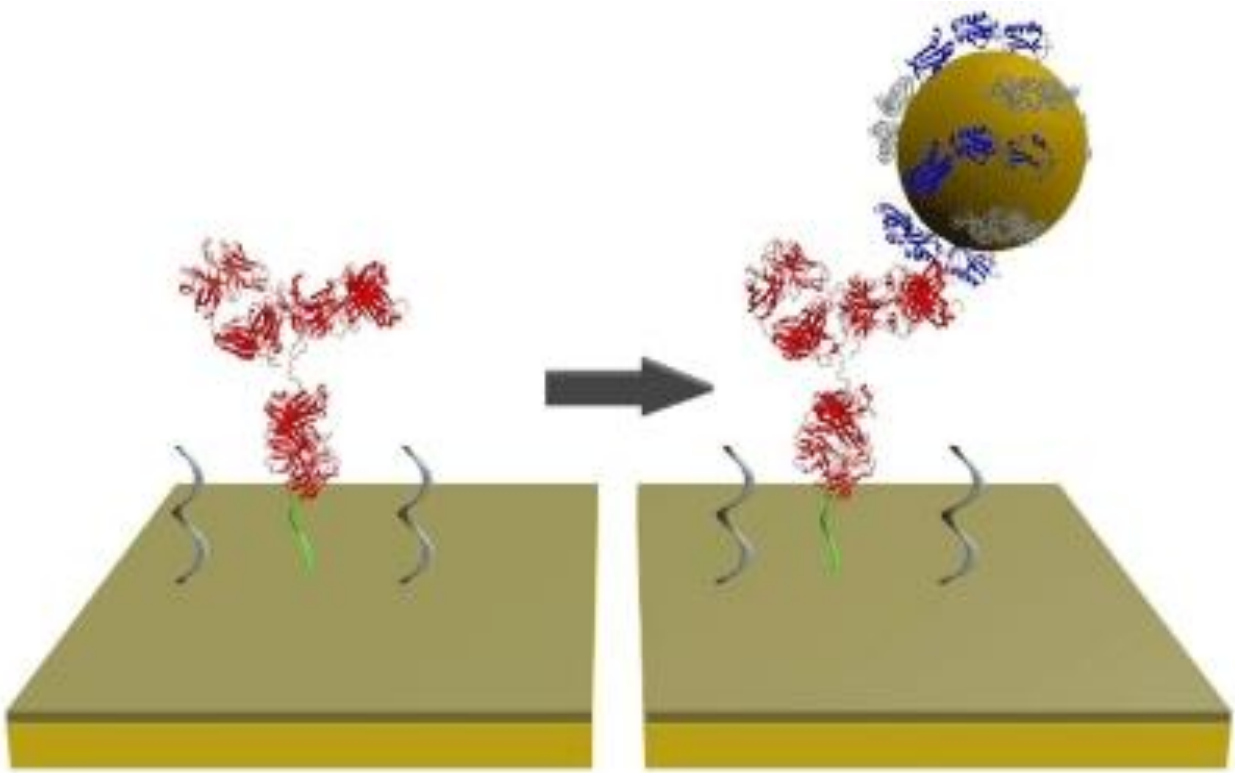


Figure 9: Example of immunosensor for detection of meningococcal antigen before (left) and after (right) the binding of the target . A gold electrode was modified with polyvinylidenedifluoride thin film deposition. Antibodies were directionally orientated by interaction with protein A and the surface was blocked with casein. Next, gold nanoparticles conjugated with the target antigen and BSA was applied to the surface, in order to allow the antigen-antibody interaction.

2.3.3. Biosensors for detection of pathogenic protozoan

Protozoa are one of the main classes of parasites that cause diseases in humans. A wide variety of approaches have been applied to the development of biosensors for the diagnosis of protozoan-caused diseases such as malaria, leishmaniasis, American trypanosomiasis (Chagas disease) and toxoplasmosis. Malaria is transmitted to humans by the bite of more than thirty species of female anopheline mosquitoes. The etiologic agent is a protozoan of genus Plasmodium. Five species, P. falciparum, P. vivax, P. malariae, P. ovale, and P. knowlesi, are known to affect humans . According to the WHO, in 2013 about 48 million cases of malaria were reported worldwide, with 584,000 deaths .

Various biomarkers have been used to malaria diagnosis .Histidine-rich protein-II is produced and secreted by the parasite during its growth and development and it has been widely used for the development of electrochemical and optical immunosensors . Biosensors based on the immobilization of aptamers with high affinity for lactate dehydrogenase, another biomarker for malaria, has been reported in the literature using electrochemical and colorimetric transducers. Reddy et al. describes the development of Plasmodium lactate dehydrogenase-specific ssDNA aptamers by SELEX using magnetic beads. The selected aptamers were characterized and used for the construction of an aptamer-based electrochemical sensor able to discriminate malaria positive samples from non-infected sample (Figure 10). In addition, Ittarat et al. described a genosensor based on quartz crystal microbalance to differentially diagnose malaria infection by either P. falciparum or P. vivax .

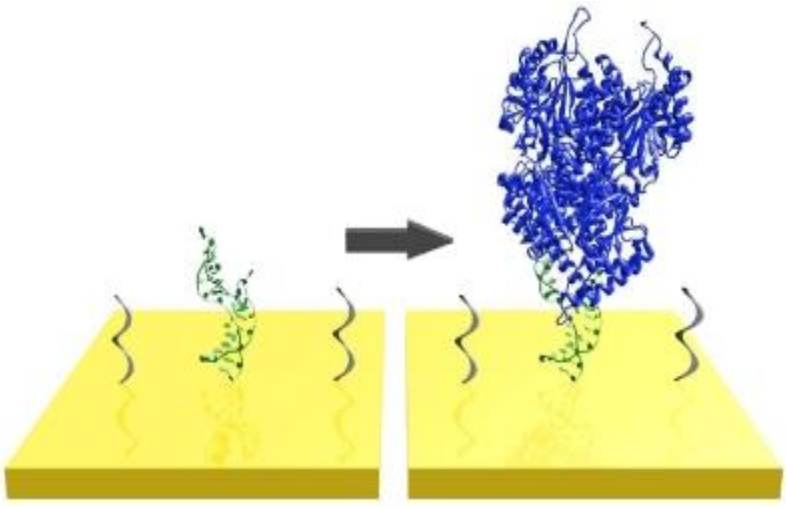


Figure 10: Example of aptasensor for the diagnosis of malaria before (left) and after (right) the binding of the target. The scheme illustrates a specific thiol-modified aptamer immobilized on a gold electrode, together with spacer molecules. Next, the protein target was applied for interaction with the aptamer probe.

Leishmaniasis is a tropical disease caused by an intracellular parasite of the genus Leishmania. The vector of transmission is the sandfly, which may deposit one of the 20 disease-causing protozoan species during blood ingestion. Clinical presentation depends on the complex interplay between the host cell-mediated immune response, and the specific protozoa and vector species. There are four generally accepted classifications of clinical disease: cutaneous, diffuse cutaneous, mucocutaneous and visceral leishmaniasis . According to the WHO, in 2013 about 215 thousand cases of leishmaniasis were reported worldwide. Among the immunosensors reported, Sousa et al. developed a new fluorescence-based immunosensor that comprised magnetic polymer microspheres coated with recombinant antigens, to improve the detection of anti-Leishmania infantum specific antibodies in the serum of infected dogs. Souto et al. described the development of an immunosensor for anti-L. infantum antibodies based on detection by SPR technique.

Toxoplasmosis is caused by the parasite Toxoplasma gondii, an obligate intracellular protozoan, capable of infecting humans. Most infections are asymptomatic or take a mild form, characterized by fever, malaise and lymphadenopathy. However, in cases of immune deficiency or when the parasite is congenitally acquired, it may cause serious illness and even death . The diagnosis and genetic characterization of T. gondii infection is crucial for monitoring, prevention and control of toxoplasmosis. Traditional approaches for the diagnosis of toxoplasmosis include molecular and imaging techniques . Most biosensors for toxoplasmosis described in the literature are based on immunoassays for the detection of anti-T. gondii antibodies. An agglutination-based piezoelectric immunoassay was developed for directly detecting anti-T. gondii immunoglobulins in infected rabbit serum and blood. The proposed technique is based on the specific agglutination of antigen-coated gold nanoparticles (10 nm diameter), in the presence of the corresponding antibody, which causes a frequency change monitored by a piezoelectric device. The developed system is sensitive to dilution ratios of anti-T. gondii antibody as low as 1:5500 .

Ding et al. developed an electrochemical biosensor based on an enzyme-catalyzed amplification. T. gondii antigen was immobilized on the surface of a gold electrode in order to bind anti-toxoplasma IgG, and this was followed by the addition of anti-toxoplasma IgG horseradish peroxidase conjugate. The

transduction methods were quartz crystal microbalance, electrochemical impedance spectroscopy and cyclic voltammetry, with a detection limit of 1:9600 in dilution ratio.

2.4. Biosensors for Determination of Heavy Metals in Water

2.4.1. Mechanism of heavy metal toxicity

Metals and metalloid ions can be divided into three groups according to their toxicity. The first group includes metals (metalloids) that are toxic at extremely low concentration, such as lead, cadmium, and mercury. “Metals of the second group (arsenic, bismuth, indium, antimony and thallium) are less toxic, i.e., they are toxic only in higher concentrations. The third group includes metals (metalloids) of essential importance, such as copper, zinc, cobalt, selenium and iron, which are necessary for different chemical and biochemical processes in the body, and are toxic only above a certain concentration.” Concentration window “of these heavy metals is somewhere between toxic and maximum permissible limits” . Table 1 gives critical concentrations of some heavy metals in natural waters according to EPA .

Metal	**Max. allowable concentration (µg/ml)**
Mercury	0.002
Arsenic	0.5
Lead	0.5
Copper	0.6
Cadmium	0..04
Zinc	5

Table 1.

Critical concentrations of some heavy metals in natural waters according to EPA

The toxic effects of heavy metals can be the result of changes in numerous physiological processes at the cellular or molecular level caused by the inactivation of the enzyme. It can also occur as a result of the blocking of functional groups of metabolically important molecules or by replacing the essential elements and disturbing the integrity of the membrane. A rather frequent consequence of heavy metal poisoning is the production of reactive oxygen species (ROS) due to interference with the transport activities of electrons, especially the chloroplast membrane . This increase in ROS exposes cells to oxidative stress that leads to peroxidation of lipids, biological damage of macromolecules, membrane decay, and DNA splitting .

They can penetrate into the organism in elemental form, in salt form, or as organometallic compounds, wherein the process of absorption, distribution, deposition, and elimination depends on the form in which the metal is present. Metals are very toxic because they are either in ionic form or within the compound, soluble in water, and easily absorbed by living organisms .

The mobility of heavy metals in water is particularly affected by the pH of water, the presence of hydrated forms of Mn and Fe, the concentration of carbonates and phosphates, as well as the content of organic matter. In addition, if the medium is very acidic and increased redox potential, the mobilization of Cu and Pb occurs, and under the reduction conditions, the hydroxides Mn and Fe are mobilized.

Anthropogenic sources of heavy metals have emerged with the development of society. For example, the release of metal from the dishes causes contamination of food and water with metals.

2.4.2. Application of biosensors in detection and monitoring of heavy metals

The unique biosensor features make them widely applicable in the field of water quality control, from the point of view of detecting and determining the concentration of heavy metals. The use of biosensors for individual or continuous measurements is dependent on the type of biologically active element. Since biological compounds such as cholesterol, glucose, urea, etc. are generally not electroactive, the combination of reactions is needed for obtaining an electroactive element, which leads to a change of current intensity .

The factors which determine the choice of a suitable physical or chemical immobilization method are physicochemical properties of the analyte, nature of the chosen biosensing element, the type of used transducer, and the operating conditions of biosensor. Antibody-based biosensors can be used as an alternative approach for the detection of metal ions, due to antibody features such as high specificity and binding affinity for antigens harmful for the organism. Detection mechanism of these devices is based on antibody-metal ion complex formation. The resulted response of their immunochemical interaction is converted by a transducer to measurable values and processed to readable values. Antibodies are capable for antigen detection in very low concentrations , but if their cross-reactivity is high, they can yield false-positive results of an assay of heavy metals in water .

A monoclonal antibody that recognizes 16 different metal-EDTA complexes has been produced and evaluated in terms of its binding affinity. The obtained results showed that the antibody has a maximum binding affinity for cadmium and mercury-EDTA complexes. In the inhibition immunoassay where the measurement of Cd2+ in water samples was carried out using monoclonal antibodies firmly bound to the cadmium-EDTA complex, but not to EDTA without metal , the biosensor showed satisfactory insensitivity to cations Ca2+, Na2+, and K1+ it encountered and achieved a reliable measurement in the presence of 1 mM of excess Fe3+, Mg2+, and Pb2+ .

Monoclonal antibodies were used to detect Pb2+ without labeling, in a localized surface plasmon resonance-based optical biosensor . The results of the experiment showed that at optimal monoclonal antibody immobilizing conditions, absorbability increased to 12.2% for detecting 10–100 ppb Pb(II)-EDTA complex with a limit of detection of 0.27 ppb.

Kulkarni et al. were the first to develop acid phosphatase-based fluorescence biosensor for the analysis of heavy metal ions Hg2+, Cr2+, and Cu2+. Increased concentration of metal ions resulted in increased enzyme inhibition and therefore decreased fluorescence. The enzyme was stable for more than 2 months at 4°C . They also observed that mixture of heavy metal ions exhibit positive effect on the performance of biosensor.

The urease enzyme has been widely investigated as a possible biocomponent in heavy metal detection biosensors. Urease has been tested single and in combination with other enzymes. Electrochemical biosensor based on urease and glutamic dehydrogenase (GLDH) was developed for detecting heavy metals in water samples . Also, a disposable potentiometric biosensor based on pure urease was developed, with the ability to detect copper and silver at sub-ppm level. For the detection of Pb and Cd in liquid samples, biosensors based on the combination of urease and acetylcholinesterase (Ache) were developed as a biocomponent with a detection limit of 1 ppb in water samples. It is known that ions of heavy metals inhibit alkaline phosphatase which was used for forming the biosensor with alkaline phosphatase as a biocomponent. It was found that the sensitivity of the developed biosensor to Cd2+ and Zn2+ was 10 ppb, whereas, with regard to ion Pb2+, there was no significant inhibition.

Capacitance protein-based biosensor using synthetic phytochelatins (ECs) was developed for the detection of heavy metal ions (Cd2+, Cu2+, Hg2+, Pb2+, and Zn2+), and the results of the experiments showed a lower sensitivity for all metal ions except for Zn2+ compared to systems based on SmtA and MerR, which can be explained by conformational changes in the protein, taking into account that the change in capacitance is function of the resulting change in protein conformation .

In cell-based biosensors, bioelement is fused with reporter gene. The detection mechanism is based on the activation of the reporter gene upon the contact between bioreceptor and target analyte, yielding an output measurable signal that is a correlation with bioavailable concentration of heavy metal.

Various cell-based biosensors have been used for the detection of heavy metals in water due to their ease of production and field testing, the ability to perform fast single measurement, as well as continuous measurements, and the ease of identifying bioavailable concentrations of toxicants that allows estimation of effects that heavy metals have on living organisms.

The advantage of bacterial cells is resistance to environmental conditions that could destroy the sensory element if exposed to them, supplying it with a relatively stable environment. Due to specific metabolic pathways used in microorganisms, compared to isolated enzymes, microbial sensors have the potential for more selective analysis of heavy metals which cannot be measured by simple enzyme reactions .

In order to be available for any sensing mechanism that is based inside the cell, there is a need for analytes to be able to enter the cell via diffusion, nonspecific uptake, or active transport. Alternative approaches are implemented in the cases when membrane permeability for an analyte is not sufficient. These approaches include allocation of the recognition element to the outside of the cell or the introduction of an appropriate transport mechanism for importing the analyte .

A large number of studies in which performances of whole cell-based biosensors were tested have utilized electrochemical and optical transducers. For detection of heavy metal ions (Cd2+, Cu2+, Fe3+, Hg2+, Pb2+,and Zn2+) at concentrations of 10µM, a mammalian heart cells-based biosensor was developed , with excellent performance in terms of frequency selection, amplitude and duration of detection within 15 min.

Biosensor, based on immobilized engineered bacteria Alcaligenes eutrophus (AE1239) and optical transducer, was utilized for monitoring the bioavailable copper ions in synthetic water samples, wherein the lowest limit of detection was 1 µM .

2.5. Conclusions

Biosensors continue to offer solutions and control of various processes across a range of applications. As technology advances, new methods that will result in the development of even better biosensors are emerging, and these seek to address all limitations associated with these devices. The development of biosensors revolves around their sensitivity, specificity, cost effectiveness and ability to detect small molecules. This is mostly determined by the right combination of a biological receptor and a transducer element, components which form the basis of a biosensor.

According to the World Health Organization, cardiovascular diseases are the leading cause around the World for an estimated 12 million deaths. Diabetes mellitus is however categorized on a pandemic level where its prevalence in Africa ranges between 1 and 20%. The increase in chronic respiratory diseases is often under diagnosed due to limited diagnostic resources. The cause in children is mainly due to allergens and pollutants which can be monitored and controlled. Due to low availability and accessibility of drugs and diagnostic tools, these diseases continue to increase. Integration of biosensors with drug delivery builds the design of implantable pharmacy which can operate as a closed loop system. This will offer continuous diagnosis, treatment and prognosis without vast data processing and specialist intervention. Point of care treatment moving from lab-on-a-chip technology to implantable chips which interacts with drug reservoirs, will increase compliance of patients who require continuous monitoring as in case of chronic diseases such as diabetes, lupus, osteoarthritis, rheumatoid arthritis, cancer, Cystic fibrosis, asthma and Parkinson's disease, coronary heart illness and AIDS. Implantable sensors are expected to interface with the body's biochemistry which will provide a critical link between diagnosis and therapeutics. Thus allowing continuous monitoring of analyte concentration and rapid analysis before major physiochemical outburst can occur such as hypertension. However, the creation of biosensor integrated drug delivery system requires a closed loop monitoring of the device. The use of implants in a BioMEMS category can provide a continuous drug supply at a specified time interval to allow better illness management without any denting intervention. Illnesses such as diabetes and coronary heart diseases, asthma, and arthritis require a responsive treatment since physiochemical changes may occur anytime.

In general, integration of biosensors and drug delivery systems offers patients a chance for self-monitoring which will improve illness management since all information in respect to their medical problems may be continuously monitored and maintained. Early detection of chronic illnesses such as cancer will therefore offer better and effective therapeutic treatments, while illness monitoring is applicable to common chronic illness such as diabetes and cardiovascular diseases which are increasing at an alarming rate in developing countries. By designing an implantable biosensor which will function as a “lab on a chip” will facilitate rapid illness management since the patients are in control of the health status. This may further be optimized by including multiple drugs in the implant reservoir for better illness management, thus preventing any further complication that may occur during self-regulatory therapeutic treatment .

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