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BIOSENSORS AND ITS APPLICATIONS

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ABSTRACT

The ability to detect pathogenic and physiologically relevant molecules in the body with high sensitivity and specificity offers a powerful opportunity in early diagnosis and treatment of diseases. Early detection and diagnosis can be used to greatly reduce the cost of patient care associated with advanced stages of many diseases. However, despite their widespread clinical use, these techniques have a number of potential limitations. For example, a number of diagnostic devices have slow response times and are burdensome to patients. Furthermore, these assays are expensive and cost the health care industry billions of dollars every year. Therefore, there is a need to develop more efficient and reliable sensing and detection technologies. A biosensor is commonly defined as an analytical device that uses a biological recognition system to target molecules or macromolecules. Biosensors can be coupled to a physiochemical transducer that converts this recognition into a detectable output signal. Typically, biosensors are comprised of three components: (1) the detector, which identifies the stimulus; (2) the transducer, which converts this stimulus to a useful output; and (3) the signal processing system, which involves amplification and display of the output in an appropriate format. The goal of this combination is to utilize the high sensitivity and selectivity of biological sensing for analytical purposes in various fields of research and technology. Here we review some of the main advances in this field over the past few years, explore the application prospects, and discuss the issues, approaches, and challenges, with the aim of stimulating a broader interest in developing biosensors and improving their applications in medical diagnosis.

INTRODUCTION

A biosensor is an analytical device, used for the detection of an analyte, that combines a biological component with a physicochemical detector. The sensitive biological element (e.g.

tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, etc.) is a biologically derived material or biomimetic component that interacts (binds or recognizes) with the analyte under study. The biologically sensitive elements can also be created by biological engineering. The transducer or the detector element (works in a physicochemical way; optical, piezoelectric, electrochemical, etc.) transforms the signal resulting from the interaction of the analyte with the biological element into another signal (i.e., transduces) that can be more easily measured and quantified. The biosensor reader device with the associated electronics or signal processors that are primarily responsible for the display of the results in a user-friendly way. This sometimes accounts for the most expensive part of the sensor device, however it is possible to generate a user friendly display that includes transducer and sensitive element (holographic sensor). The readers are usually custom-designed and manufactured to suit the different working principles of biosensors.

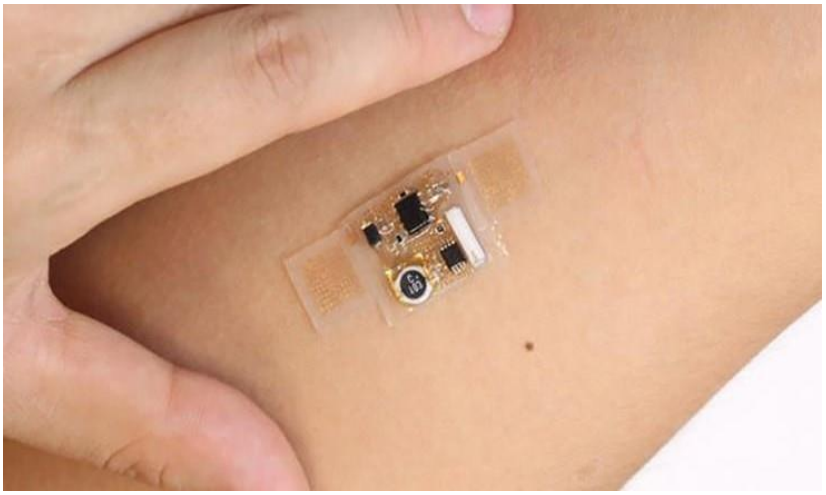

Figure: A Biosensor.

BIOSENSOR SYSTEM

A biosensor typically consists of a bio-recognition site, biotransducer component, and electronic system which includes a signal amplifier, processor, and display. Transducers and electronics can be combined, e.g., in CMOS-based micro sensor systems. The recognition component, often called a bioreceptor, uses biomolecules from organisms or receptors modeled after biological systems to interact with the analyte of interest. This interaction is measured by the biotransducer which outputs a measurable signal proportional to the presence of the target analyte in the sample. The general aim of the design of a biosensor is to enable quick, convenient testing at the point of concern or care where the sample was procured.

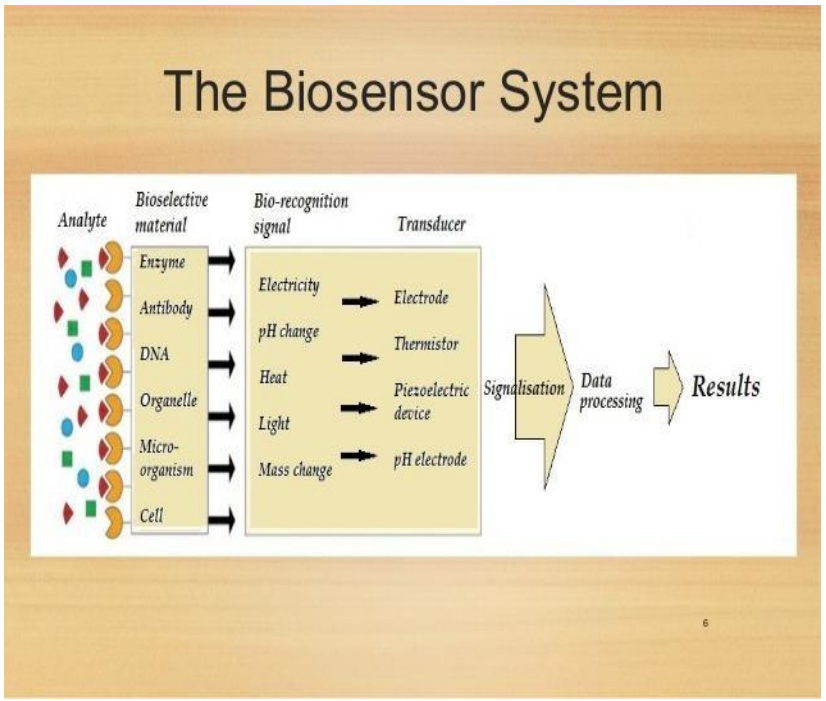

Figure: Biosensor System.

BIORECEPTORS

In a biosensor, the bioreceptor is designed to interact with the specific analyte of interest to produce an effect measurable by the transducer. High selectivity for the analyte among a matrix of other chemical or biological components is a key requirement of the bioreceptor. While the type of biomolecule used can vary widely, biosensors can be classified according to common types bioreceptor interactions involving: anitbody/antigen, enzymes/ligands, nucleic acids/DNA, cellular structures/cells, or biomimetic materials.

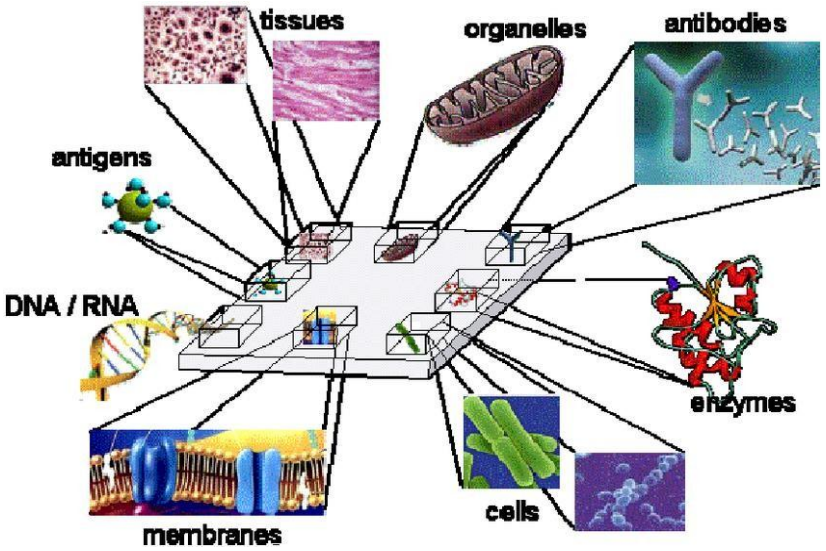

Figure: Bioreceptors.

Antibody/antigen interactions

An immunosensor utilizes the very specific binding affinity of antibodies for a specific compound or antigen. The specific nature of the antibody-antigen interaction is analogous to a lock and key fit in that the antigen will only bind to the antibody if it has the correct conformation. Binding events result in a physicochemical change that in combination with a tracer, such as a fluorescent molecule, enzymes, or radioisotopes, can generate a signal. There are limitations with using antibodies in sensors: 1. The antibody binding capacity is strongly dependent on assay conditions (e.g. pH and temperature) and 2. The antibody-antigen interaction is generally irreversible. However, it has been shown that binding can be disrupted by chemotropic reagents, organic solvents, or even ultrasonic radiation.

Artificial binding proteins

The use of antibodies as the bio-recognition component of biosensors has several drawbacks. They have high molecular weights and limited stability, contain essential disulphide bonds and are expensive to produce. In one approach to overcome these limitations, recombinant binding fragments (Fab, Fv or scFv) or domains (VH, VHH) of antibodies have been engineered. In another approach, small protein scaffolds with favourable biophysical properties have been engineered to generate artificial families of Antigen Binding Proteins (AgBP), capable of specific binding to different target proteins while retaining the favourable properties of the parent molecule. The elements of the family that specifically bind to a given target antigen, are often selected in vitro by display techniques: phage display, ribosome display, yeast display or mRNA display. The artificial binding proteins are much smaller than antibodies (usually less than 100 amino-acid residues), have a strong stability, lack disulphide bonds and can be expressed in high yield in reducing cellular environments like the bacterial cytoplasm, contrary to antibodies and their derivatives. They are thus especially suitable to create biosensors.

Enzymatic interactions

The specific binding capabilities and catalytic activity of enzymes make them popular bioreceptors. Analyte recognition is enabled through several possible mechanisms: 1) the enzyme converting the analyte into a product that is sensor-detectable, 2) detecting enzyme inhibition or activation by the analyte, or 3) monitoring modification of enzyme properties resulting from interaction with the analyte. The main reasons for the common use of enzymes in biosensors are: 1) ability to catalyse a large number of reactions; 2) potential to detect a

group of analytes (substrates, products, inhibitors, and modulators of the catalytic activity); and 3) suitability with several different transduction methods for detecting the analyte. Notably, since enzymes are not consumed in reactions, the biosensor can easily be used continuously. The catalytic activity of enzymes also allows lower limits of detection compared to common binding techniques. However, the sensor's lifetime is limited by the stability of the enzyme.

TYPES OF BIOSENSORS

Electrochemical sensors

These sensors employ redox reactions to quantify the amount of an analyte. The current flowing through the system or the potential difference between the electrodes as a result of the oxidation and reduction reactions involving the analyte are used for its quantification in the sample. The electrochemical sensors do not suffer from the drawbacks of optical sensors. They have more stable output, have high sensitivity, fast response and suffer from lesser interferences. Also, it is tedious to tag the analyte with any fluorescent label and hence electrochemical measurements are often used for sensing applications. The various electrochemical parameters that could be monitored are:

- Conductimetric measurements, which measures changes in the conductance of the system due to the presence of the analyte.
- Potentiometric measurements, which measures the electrical potential difference between a working and reference electrode. The reference electrode is one whose potential remains invariant during the entire duration of measurement. The working electrode undergoes significant change in its potential even for small changes in the analyte concentration. Potentiometric measurements are also carried out to monitor the accumulation of charge at zero current created by selective binding of the analyte at the electrode surface. The electrode may be selective for certain ions or gases and these include F^-, I^-, CN^-, Na^+, K^+, Ca^{2+}, H^+, NH^{4+}, CO_2, NH_3 etc.
- Amperometric measurements, which involves measuring the current generated by electrochemical oxidation or reduction of electroactive species at a constant applied potential. Fast measurements, sensitivity (ability to sense even 10^{-9}M concentration) and ability to perform measurements on turbid/opaque solutions are its advantages over conventional optical sensors. However, pH-sensing mechanisms require weakly buffered or non-buffered solutions and this is a drawback for this category of sensors.

Electrochemical sensors are one among the most popular sensors. The glucometer that is available for quantifying glucose levels in blood samples is an electrochemical biosensor that is based on the potentiometric principle.

Figure 4.1(a) depicts the principle of a simple electrochemical sensor. The working electrode is a platinum electrode that is in contact with a cellulose acetate and a polycarbonate layer sandwiching an enzyme layer. enzyme catalyses the redox reaction involving the leading to the formation of hydrogen peroxide current flowing through the electrode.

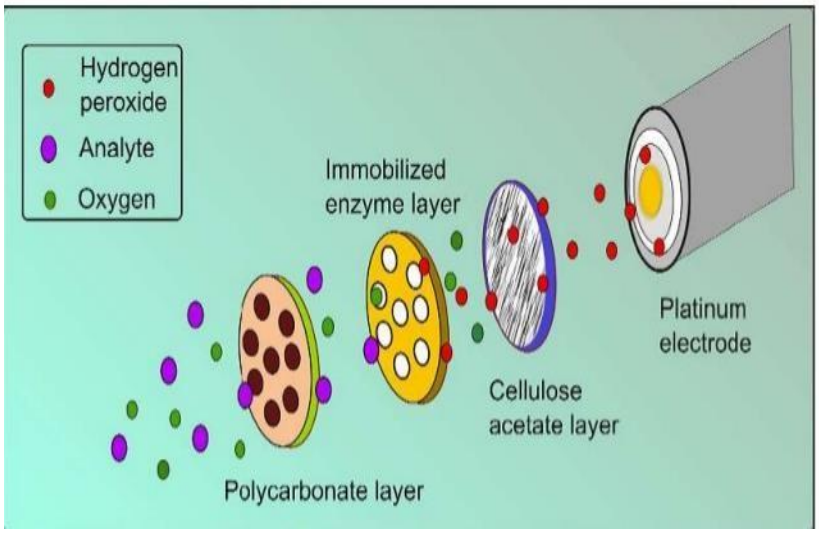

Figure: A schematic representation of an electrochemical sensor.

Use of multiple enzymes instead of a single enzyme good amplification of the signal. employing multiple enzymes was designed using three enzymes namely L dehydrogenase and tyrosine electrode. When the sample is acts upon the L-phenylalanine During this reaction, the co is converted to its reduced form reaction catalysed by salicylate dehydrogenase forming catechol. During this process, NADH is oxidized back to NAD involve in further reactions involving L then oxidized into o-Quinone by detected using Ag/AgCl reference electrode. Through reduction thereby making it available the reactions that are involved in this system. Levels of phenylalanine in biological fluids are phenyl ketoneuria as well as hyper phenylalanine.

The NADH formed serves as the coenzyme for the that acts upon salicylic acid and oxygen that can now The catechol is which is electrochemically Quinone can be converted to catechol. Figure 4.1(b) gives Where can such sensors be employed? High an indicator of a condition known as This sensor can be employed to successfully detect even slight elevation

in the levels of L concept can be employed for other types of enzyme.

Mass-sensitive measurements

Piezoelectric sensors

The mass-sensitive sensors use piezoelectric crystals for detection. Piezoelectric materials are those that produce an electric signal in response to mechanical forces. Commonly employed piezoelectric materials in sensors are the quartz crystals. In these sensors, the crystals are made to vibrate at a specific frequency by the application of electric signal.

The oscillation frequency of the crystal depends on the applied frequency. For sensing application, a 'bio-capture' layer is introduced on the surface of the crystal. The 'bio-capture' layer consists of a biomolecule that will exhibit specific binding with the analyte. Generally, antibodies are the most commonly employed bio-capture molecules and such sensors are referred to as piezoelectric immunosensor. The piezoelectric sensors are among the most sensitive biosensors available. Upon addition of the sample, specific binding occurs between the bio-capture molecules on the sensor and the analyte and consequently a mass change occurs leading to a subsequent change in the oscillation frequency and production of an electric signal that is detected.

The piezoelectric immunosensor have been successfully demonstrated for the ultra- sensitive detection of HIV (human immunodeficiency virus) in samples. Figure 4.2(a) depicts the principle of a typical piezoelectric immunosensor.

- Piezoelectric effect was discovered in 1880 by Jacques and Pierre Curie
- The domestic gas lighter is a common device employing piezoelectric effect
- The first piezoelectric immunosensor was reported in 2005.

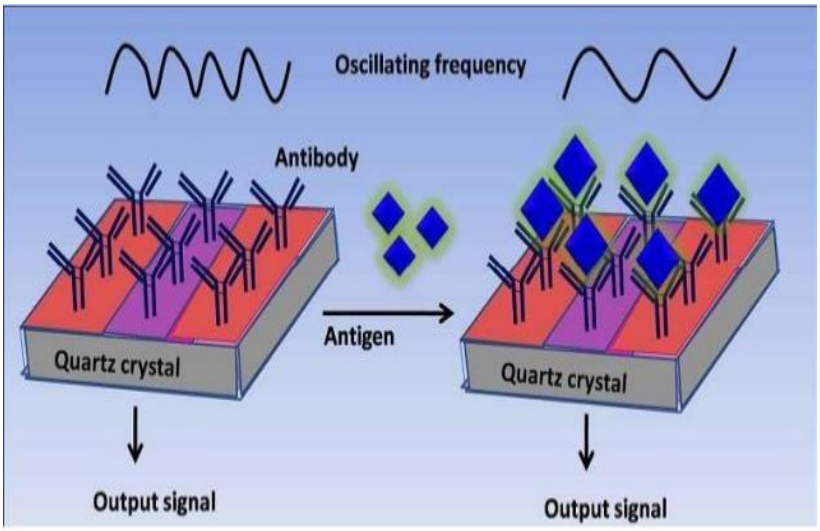

Figure: Piezoelectric immunosensor.

Surface Plasmon resonance (SPR)

Plasmons are described as plasmons oscillate at a particular frequency characteristic of the material. Surface plasmons are those species of plasmons whose oscillations are confined to the material. Generally, gold or silver surfaces are preferred for use in SP sensors. When electromagnetic radiation is allowed to fall on a metal surface (gold or silver), at a particular angle of incidence matches the frequency of the vibrations resulting in surface plasmon resonance). This resonant angle depends upon the refractive index of the medium, which in turn is determined by the local mass density on the metal surface. surface of the metal film is modified with the capture molecule on addition of the sample, specific binding occurs between the capture molecule and its ligand leading to a change in the mass and hence a change in the This change can be quantified for depicts a schematic representation of a SPR-based sensor.

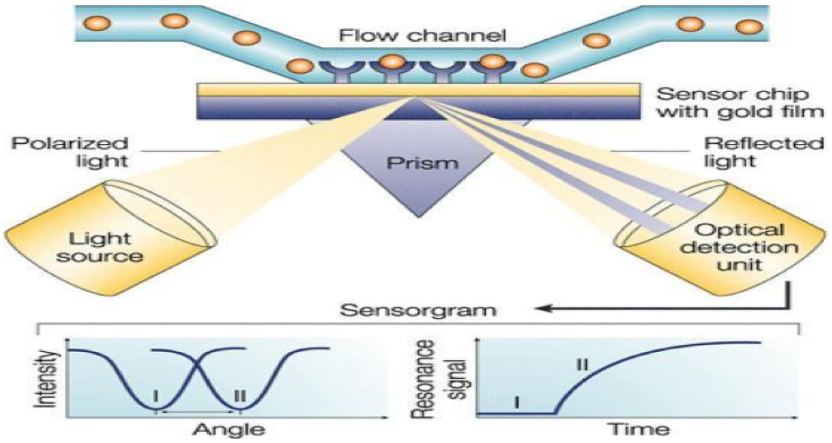

Fig.: An SPR-based immunosensor.

Calorimetric Sensors

Calorimetric sensors involve the measurement of heat that is generated. These sensors typically utilize thermistors that transform electrical signal. The analyte is made up of a calorimetric sensor. The entire sensing set-up is surrounded by generated during the reaction. The analyte is the substrate for the enzyme. Figure 4.3(a) shows a typical set-up of a calorimetric sensor.

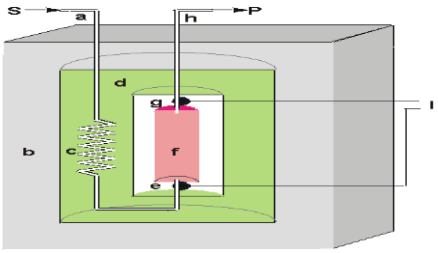

Fig.: A calorimetric sensor.

BIOTRANSDUCER

Biosensors can be classified by their biotransducer type. The most common types of biotransducers used in biosensors are 1) electrochemical biosensors, 2) optical biosensors, 3) electronic biosensors, 4) piezoelectric biosensors, 5) gravimetric biosensors, 6) pyroelectric biosensors.

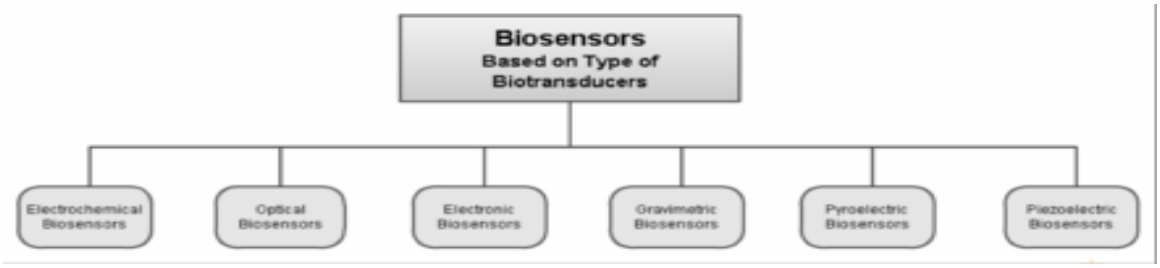

Figure: Classification of Biosensors based on type of biotransducer.

PLACEMENT OF BIOSENSORS

The appropriate placement of biosensors depends on their field of application, which may roughly be divided into biotechnology, agriculture, food technology and biomedicine.

In biotechnology, analysis of the chemical composition of cultivation broth can be conducted in-line, on-line, at-line and off-line. As outlined by the US Food and Drug Administration (FDA) the sample is not removed from the process stream for in-line sensors, while it is diverted from the manufacturing process for on-line measurements. For at-line sensors the

sample may be removed and analysed in close proximity to the process stream. An example of the latter is the monitoring of lactose in a dairy processing plant. Off-line biosensors compare to bioanalytical techniques that are not operating in the field, but in the laboratory. These techniques are mainly used in agriculture, food technology and biomedicine.

In medical applications biosensors are generally categorized as in vitro and in vivo systems. An in vitro biosensor measurement takes place in a test tube, a culture dish, a microtiter plate or elsewhere outside a living organism. The sensor uses a bioreceptor and transducer as outlined above. An example of an in vitro biosensor is an enzyme-Conductimetric biosensor for blood glucose monitoring. There is a challenge to create a biosensor that operates by the principle of Point-of-care testing, i.e. at the location where the test is needed. The elimination of lab testing can save time and money. An application of a POCT biosensor can be for the testing of HI virus in areas, where it is difficult for patients to be tested. A biosensor can be sent directly to the location and a quick and easy test can be used.

APPLICATIONS

There are many potential applications of biosensors of various types. The main requirements for a biosensor approach to be valuable in terms of research and commercial applications are the identification of a target molecule, availability of a suitable biological recognition element, and the potential for disposable portable detection systems to be preferred to sensitive laboratory-based techniques in some situations. Some examples are glucose monitoring in diabetes patients, other medical health related targets, environmental applications e.g. the detection of pesticides and river water contaminants such as heavy metal ions, remote sensing of airborne bacteria e.g. in counter-bioterrorist activities, remote sensing of water quality in coastal waters by describing online different aspects of clam ethology (biological rhythms, growth rates, spawning or death records) in groups of abandoned bivalves around the world, detection of pathogens, determining levels of toxic substances before and after bioremediation, detection and determining of organophosphate, routine analytical measurement of folic acid, biotin, vitamin B12 and pantothenic acid as an alternative to microbiological assay, determination of drug residues in food, such as antibiotics and growth promoters, particularly meat and honey, drug discovery and evaluation of biological activity of new compounds, protein engineering in biosensors, and detection of toxic metabolites such as mycotoxins.

Glucose monitoring

Commercially available gluocose monitors rely on amperometric sensing of glucose by means of glucose oxidase, which oxidises glucose producing hydrogen peroxide which is detected by the electrode. To overcome the limitation of amperometric sensors, a flurry of research is present into novel sensing methods, such as fluorescent glucose biosensors.

Interferometric reflectance imaging sensor

The interferometric reflectance imaging sensor (IRIS) is based on the principles of optical interference and consists of a silicon-silicon oxide substrate, standard optics, and low-powered coherent LEDs. When light is illuminated through a low magnification objective onto the layered silicon-silicon oxide substrate, an interferometric signature is produced. As biomass, which has a similar index of refraction as silicon oxide, accumulates on the substrate surface, a change in the interferometric signature occurs and the change can be correlated to a quantifiable mass. Daaboul et al. used IRIS to yield a label-free sensitivity of approximately 19 ng/mL. Ahn et al. improved the sensitivity of IRIS through a mass tagging technique.

Since initial publication, IRIS has been adapted to perform various functions. First, IRIS integrated a fluorescence imaging capability into the interferometric imaging instrument as a potential way to address fluorescence protein microarray variability. Briefly, the variation in fluorescence microarrays mainly derives from inconsistent protein immobilization on surfaces and may cause misdiagnoses in allergy microarrays. To correct from any variation in protein immobilization, data acquired in the fluorescence modality is then normalized by the data acquired in the label-free modality. IRIS has also been adapted to perform single nanoparticle counting by simply switching the low magnification objective used for label-free biomass quantification to a higher objective magnification. This modality enables size discrimination in complex human biological samples. Monroe et al. used IRIS to quantify protein levels spiked into human whole blood and serum and determined allergen sensitization in characterized human blood samples using zero sample processing. Other practical uses of this device include virus and pathogen detection.

Food analysis

There are several applications of biosensors in food analysis. In the food industry, optics coated with antibodies are commonly used to detect pathogens and food toxins. Commonly, the light system in these biosensors is fluorescence, since this type of optical measurement can greatly amplify the signal.

A range of immuno- and ligand-binding assays for the detection and measurement of small molecules such as water-soluble vitamins and chemical contaminants (drug residues) such as sulfonamides and Beta-agonists have been developed for use on SPR based sensor systems, often adapted from existing ELISA or other immunological assay. These are in widespread use across the food industry.

CONCLUSION

The past decade has seen great advancements in the field of biosensor along many fronts. This dynamic tool has been applied in many area of life science research, health care, environmental, food and military application. Biosensor technology has received heightened interest over the past decade, since it is a promising candidate for lower detection limit with rapid analysis time at relatively low cost. However, the review shows that there is a lot of studies have been undertaken using indirect measurement with simple clean buffer solution instead direct measurement for in situ real-sample monitoring which is more vital. Technological advances have provided us with the tools and materials needed to construct biochip which integrated with microfluidic system, probe, sampler, detector, amplifier and logic circuitry. This biochip is a promising candidate for label free, reagentless, real time monitoring, miniaturization and low cost application. For medical application, this cost advantage will allow the development of extremely low cost, disposable biochips that can be used for in-home medical diagnostics of diseases without the need of sending samples to a laboratory for analysis which time consuming.

FUTURE PERSPECTIVES AND RESEARCH CHALLENGES

The goal of implantable complete devices comprising of sensors, instrumentation, signal processing, power and wireless data transmission remains in the future, but substantial progress continues to be made. Obviously, such complete devices will require new ways of thinking about the other key components, apart from the sensors. Batteries remain the most likely technology and power harvesting or other approaches remain research topics. Battery form is important and the ability to mass manufacture, or scale-up lab-based technology is critical. Recent progress has been reported for the Finnish-developed ‘Enfucell softBattery’ based on Zn-MnO_2-$ZnCl_2$ robust chemistry which is now commercially available in a suitable size (0.7 mm thick, the smallest is 42X60 mm), in a 10–90 mAh capacity and manufactured using reel-to-reel processes along with a 1–2-year shelf life. A device has been reported that is fabricated from materials described by the authors as “completely edible” though more

fastidious diners may baulk at silver nanowires and poly(glycerol-co-sebacate)-cinnamate. Graphene probably has more potential as a power source component than sensor though the way forward may be metal-free and an all polymer PEDOT device has been reported Cheap, printable flexible displays are also an active research area.

Electrochromic displays on PET substrates using organic transistors have been described consisting with demonstration of an 88 pixel display manufactured using solution processing based on standard printing and coating System integration remains the 'holy grail' but is still rarely reported, presumably because of the skill mix required for implementation transcends traditional disciplinary boundaries and the engineering problems remain significant on all aspects, ranging from biocompatibility and sensor stability (probably the most difficult challenge), through to power management, wireless data transmission and presentation of the data in a clinically meaningful form. A complete implantable device the Nano-tera i-IronIC has recently been reported by de Micheli (from EPFL) at the DATE13 conference. At only 14 mm long, it consists of five sensors and a radio transmitter. The device is implanted just below the skin with an external 0.1 W battery patch. Peer-reviewed publication is eagerly awaited to see if the science lives up to the press release. There continues to be major advances in biosensors for biomedical application. Whilst we now have a better understanding of long-standing problems of poor biocompatibility of typical sensor materials and poor stability of implanted devices, there are no magic bullets and the interface between the sensor and the biology remains the major problem.