

Biosensor: A Hope for Our Future

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Abstract: 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). In this article we will study about hopes regarding DNA biosensor, Microbial biosensor, Ozone biosensor, Metastatic cancer cell biosensor etc.

Keywords: Biosensor, biological component, enzymes

1. Introduction

A biosensor typically consists of a bio-recognition component, biotransducer component, and electronic system which include a signal amplifier, processor, and display. Transducers and electronics can be combined, e.g., in CMOS-based microsensor 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.

2. 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: antibody/antigen, enzymes/ligands, nucleic acids/DNA, cellular structures/cells, or biomimetic materials.

3. 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 molecules, 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 chaotropic reagents, organic solvents, or even ultrasonic radiation.

4. 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 disulfide 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 favorable 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 favorable 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 disulfide 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.

Surface attachment of the biological elements

An important part in a biosensor is to attach the biological elements (small molecules/protein/cells) to the surface of the sensor (be it metal, polymer or glass). The simplest way is to functionalize the surface in order to coat it with the biological elements. This can be done by polylysine, aminosilane, epoxysilane or nitrocellulose in the case of silicon chips/silica glass. Subsequently, the bound biological

agent may be for example fixed by Layer by layer deposition of alternatively charged polymer coatings. Alternatively three-dimensional lattices (hydrogel/xerogel) can be used to chemically or physically entrap these (whereby chemically entrapped it is meant that the biological element is kept in place by a strong bond, while physically they are kept in place being unable to pass through the pores of the gel matrix). The most commonly used hydrogel is sol-gel, a glassy silica generated by polymerization of silicate monomers (added as tetra alkyl orthosilicates, such as TMOS or TEOS) in the presence of the biological elements (along with other stabilizing polymers, such as PEG) in the case of physical entrapment. Another group of hydrogels, which set under conditions suitable for cells or protein, are acrylate hydrogel, which polymerize upon radical initiation. One type of radical initiator is a peroxide radical, typically generated by combining a persulfate with TEMED (Polyacrylamide gel are also commonly used for protein electrophoresis), alternatively light can be used in combination with a photoinitiator, such as DMPA (2,2-dimethoxy-2-phenylacetophenone). Smart materials that mimic the biological components of a sensor can also be classified as biosensors using only the active or catalytic site or analogous configurations of a biomolecule.

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.

5. 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 given below:

- 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
- Detection of toxic metabolites such as mycotoxins

A common example of a commercial biosensor is the blood glucose biosensor, which uses the enzyme glucose oxidase to break blood glucose down. In doing so it first oxidizes glucose and uses two electrons to reduce the FAD (a component of the enzyme) to FADH₂. This in turn is oxidized by the electrode in a number of steps. The resulting current is a measure of the concentration of glucose. In this case, the electrode is the transducer and the enzyme is the biologically active component.

A canary in a cage, as used by miners to warn of gas, could be considered a biosensor. Many of today's biosensor applications are similar, in that they use organisms which respond to toxic substances at a much lower concentrations than humans can detect to warn of their presence. Such devices can be used in environmental monitoring, trace gas detection and in water treatment facilities.

Many optical biosensors are based on the phenomenon of surface plasmon resonance (SPR) techniques. This utilises a property of and other materials; specifically that a thin layer of gold on a high refractive index glass surface can absorb laser light, producing electron waves (surface plasmons) on the gold surface. This occurs only at a specific angle and wavelength of incident light and is highly dependent on the surface of the gold, such that binding of a target analyte to a receptor on the gold surface produces a measurable signal.

Surface plasmon resonance sensors operate using a sensor chip consisting of a plastic cassette supporting a glass plate, one side of which is coated with a microscopic layer of gold. This side contacts the optical detection apparatus of the instrument. The opposite side is then contacted with a microfluidic flow system. The contact with the flow system creates channels across which reagents can be passed in solution. This side of the glass sensor chip can be modified in a number of ways, to allow easy attachment of molecules of interest. Normally it is coated in carboxymethyl dextran or similar compound.

The refractive index at the flow side of the chip surface has a direct influence on the behavior of the light reflected off the gold side. Binding to the flow side of the chip has an effect on the refractive index and in this way biological interactions can be measured to a high degree of sensitivity with some sort of energy. The refractive index of the medium near the surface changes when biomolecules attach to the surface, and the SPR angle varies as a function of this change.

Light of a fixed wavelength is reflected off the gold side of the chip at the angle of total internal reflection, and detected inside the instrument. The angle of incident light is varied in

order to match the evanescent wave propagation rate with the propagation rate of the surface plasmon polaritons. This induces the evanescent wave to penetrate through the glass plate and some distance into the liquid flowing over the surface.

Other optical biosensors are mainly based on changes in absorbance or fluorescence of an appropriate indicator compound and do not need a total internal reflection geometry. For example, a fully operational prototype device detecting casein in milk has been fabricated. The device is based on detecting changes in absorption of a gold layer. A widely used research tool, the micro-array, can also be considered a biosensor.

Biological biosensors often incorporate a genetically modified form of a native protein or enzyme. The protein is configured to detect a specific analyte and the ensuing signal is read by a detection instrument such as a fluorometer or luminometer. An example of a recently developed biosensor is one for detecting cytosolic concentration of the analyte cAMP (cyclic adenosine monophosphate), a second messenger involved in cellular signaling triggered by ligands interacting with receptors on the cell membrane. Similar systems have been created to study cellular responses to native ligands or xenobiotics (toxins or small molecule inhibitors). Such "assays" are commonly used in drug discovery development by pharmaceutical and biotechnology companies. Most cAMP assays in current use require lysis of the cells prior to measurement of cAMP. A live-cell biosensor for cAMP can be used in non-lysed cells with the additional advantage of multiple reads to study the kinetics of receptor response.

Nanobiosensors use an immobilized bioreceptor probe that is selective for target analyte molecules. Nanomaterials are exquisitely sensitive chemical and biological sensors. Nanoscale materials demonstrate unique properties. Their large surface area to volume ratio can achieve rapid and low cost reactions, using a variety of designs.

Other evanescent wave biosensors have been commercialised using waveguides where the propagation constant through the waveguide is changed by the absorption of molecules to the waveguide surface. One such example, dual polarisation interferometry uses a buried waveguide as a reference against which the change in propagation constant is measured. Other configurations such as the Mach-Zehnder have reference arms lithographically defined on a substrate. Higher levels of integration can be achieved using resonator geometries where the resonant frequency of a ring resonator changes when molecules are absorbed.

Recently, arrays of many different detector molecules have been applied in so called electronic nose devices, where the pattern of response from the detectors is used to fingerprint a substance. In the Wasp Hound odor-detector, the mechanical element is a video camera and the biological element is five parasitic wasps who have been conditioned to swarm in response to the presence of a specific chemical. Current

commercial electronic noses, however, do not use biological elements.

Glucose monitoring

Commercially available glucose 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.

DNA biosensors

In the future, DNA will find use as a versatile material from which scientists can craft biosensors. DNA biosensors can theoretically be used for medical diagnostics, forensic science, agriculture, or even environmental clean-up efforts. No external monitoring is needed for DNA-based sensing devices. This is a significant advantage. DNA biosensors are complicated mini-machines—consisting of sensing elements, micro lasers, and a signal generator. At the heart of DNA biosensor function is the fact that two strands of DNA stick to each other by virtue of chemical attractive forces. On such a sensor, only an exact fit—that is, two strands that match up at every nucleotide position—gives rise to a fluorescent signal (a glow) that is then transmitted to a signal generator.

Microbial biosensors

Using biological engineering researchers have created many microbial biosensors. An example is the arsenic biosensor. To detect arsenic they use the Ars operon. Using bacteria, researchers can detect pollutants in samples.

Ozone Biosensors

Because ozone filters out harmful ultraviolet radiation, the discovery of holes in the ozone layer of the earth's atmosphere has raised concern about how much ultraviolet light reaches the earth's surface. Of particular concern are the questions of how deeply into sea water ultraviolet radiation penetrates and how it affects marine organisms, especially plankton (floating microorganisms) and viruses that attack plankton. Plankton form the base of the marine food chains and are believed to affect our planet's temperature and weather by uptake of CO₂ for photosynthesis.

Metastatic Cancer Cell Biosensors

Metastasis is the spread of cancer from one part of the body to another via either the circulatory system or lymphatic system. Unlike radiology imaging tests (mammograms), which send forms of energy (x-rays, magnetic fields, etc.) through the body to only take interior pictures, biosensors have the potential to directly test the malignant power of the tumor. The combination of a biological and detector element allows for a small sample requirement, a compact design, rapid signals, rapid detection, high selectivity and high sensitivity for the analyte being studied. Compared to the usual radiology imaging tests biosensors have the advantage of not only finding out how far the cancer has spread and checking if treatment is effective, but also are cheaper, more efficient (in time, cost and productivity) ways to assess metastaticity in early stages of cancer. Biological engineering researchers have created oncological biosensors for breast cancer. Breast cancer is the leading common cancer among women worldwide. An example would be a Transferrin-Quartz crystal microbalance (QCM). As a biosensor, quartz crystal microbalances produce oscillations in the frequency of the crystal's standing wave from an alternating potential to

detect nano-gram mass changes. These biosensors are specifically designed to interact and have high selectivity for receptors on cell (cancerous and normal) surfaces. Ideally this provides a quantitative detection of cells with this receptor per surface area instead of a qualitative picture detection given by mammograms.

Seda Atay, a Biotechnology researcher at Hacettepe University, experimentally observed this specificity and selectivity between a QCM and MDA-MB 231 breast cells, MCF 7 cells, and starved MDA-MB 231 cells in vitro. With other researchers she devised a method of washing these different metastatic leveled cells over the sensors to measure mass shifts due to different quantities of transferrin receptors. Particularly, the metastatic power of Breast cancer cells can be determined by Quartz crystal microbalances with nanoparticles and transferrin that would potentially attach to transferrin receptors on cancer cell surfaces. There is very high selectivity for transferrin receptors because they are over-expressed in cancer cells. If cells have high expression of transferrin receptors, which shows their high metastatic power, they have higher affinity and bind more to the QCM that measures the increase in mass. Depending on the magnitude of the nano-gram mass change, the metastatic power can be determined.

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